Hypertension

ORIGINAL ARTICLE

Attenuated Relationships Between Indexes of Volume Overload and Atrial Natriuretic Peptide in Uncontrolled, Sustained Volume-Dependent Primary Hypertension

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BACKGROUND: Whether systolic blood pressure (SBP) control in sustained volume-dependent primary hypertension is associated with blunted ANP (atrial natriuretic peptide) relationships with indexes of volume load is unknown.

METHODS: Systemic hemodynamics (central pressure, echocardiographic aortic velocity and diameter measurements in the outflow tract), circulating ANP concentrations (ELISA assays) and glomerular and tubular function (24-hour urine collections [n=519]) were determined in a community of African ancestry (n=772).

RESULTS: As compared with those with a controlled SBP, those with an uncontrolled SBP (n=198) showed lower ANP concentrations ($P<0.005$) despite higher stroke volume and cardiac output ($P<0.0001$) and renal differences consistent with enhanced fluid retention. In those with a controlled SBP, fractional Na^+ excretion ($FeNa^+$; $P<0.0005$) and creatinine clearance (glomerular filtration rate; $P<0.005$) were inversely associated with ANP concentrations independent of confounders. Moreover, in those with a controlled SBP, stroke volume and cardiac output ($P<0.0001$) were independently and positively associated with ANP concentrations. In addition, in those with a controlled SBP, ANP concentrations were independently and inversely associated with systemic vascular resistance (SVR; $P<0.0001$) and aortic characteristic impedance (Zc; $P<0.005$). By contrast, in those with uncontrolled SBP, no relationships between either stroke volume ($P>0.25$), cardiac output ($P>0.29$), $FeNa^+$ ($P>0.77$), or glomerular filtration rate ($P>0.47$) and ANP concentrations were noted. Furthermore, in those with an uncontrolled SBP, no relationships between ANP concentrations and SVR or Zc were observed ($P>0.34$).

CONCLUSIONS: In a population where primary hypertension is strongly volume-dependent, those with an uncontrolled SBP have an attenuated relationship between ANP and both renal and hemodynamic indexes of volume overload and the vascular effects of ANP. **(*Hypertension*. 2023;80:147–159. DOI: 10.1161/HYPERTENSIONAHA.122.19637.)** • **Supplemental Material**

Key Words: aortic impedance ■ atrial natriuretic peptide ■ hypertension ■ systemic flow ■ systemic vascular resistance

Renal mechanisms are well recognized as being central to the pathophysiology of both primary and secondary forms of hypertension.[1–3] However, renal effects in hypertension have largely been considered to translate into sustained increases in blood pressure (BP) through vasopressor rather than through volume

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ORIGINAL ARTICLE

NOVELTY AND RELEVANCE

What Is New?

The relationship between atrial natriuretic peptide (ANP) concentrations and renal-related indexes of volume overload in sustained volume-dependent primary hypertension is unknown.

What Is Relevant?

In a population of African ancestry with prevalent and sustained volume-dependent primary hypertension, we determined whether an attenuated ANP relationship with renal-related increases in circulating volume (indexed by indexes of renal fluid retention and increases in systemic flow), is associated with an uncontrolled systolic blood pressure (SBP). We showed that indexes of renal fluid retention (decreased $FeNa^+$ and glomerular filtration rate) and consequent volume-dependent higher systemic blood flow (stroke volume and cardiac output) were strongly and independently associated with ANP concentrations in individuals with a controlled SBP. Moreover, in individuals with a controlled SBP, ANP concentrations were strongly and independently associated with beneficial vascular changes (lower systemic vascular resistance [SVR] and Zc and higher total arterial compliance [TAC]). In comparison, however, in individuals with an uncontrolled SBP, despite indexes of an enhanced renal fluid retention and systemic flow compared with individuals with controlled SBP, ANP concentrations were lower, and no relationships between either indexes of renal fluid retention or systemic flow and ANP were detected. Furthermore, in individuals with an uncontrolled SBP, SVR and Zc were higher and TAC lower, and no relationships between ANP and either SVR, Zc, or total arterial compliance were noted. Importantly, a lower Na^+ intake (as indexed from 24-hour urine samples) was noted in individuals with uncontrolled SBP compared with those with a controlled SBP.

Clinical/Pathophysiological Implications

An attenuated ANP response to a volume load may play a major role in producing low-renin, renal-related, sustained volume-dependent primary hypertension. As current antihypertensive therapy, including the use of diuretic agents, has no proven ability to produce sustained decreases in volume overload, targeting ANP may be an essential approach to managing uncontrolled hypertension in some populations.

Nonstandard Abbreviations and Acronyms

ANP	atrial natriuretic peptide
BP	blood pressure
GFR	glomerular filtration rate
LV	left ventricular
MAP	mean arterial pressure
SBP	systolic blood pressure
SVR	systemic vascular resistance
TAC	total arterial compliance

or flow-dependent effects.[2] Indeed, in primary hypertensives at a community level, older studies demonstrate relationships between BP and systemic flow only at a younger adult age.[4,5] However, more recent studies show a marked contribution of increases in stroke volume (SV) and peak aortic flow to primary hypertension in some populations.[6–8] Importantly, these flow-dependent mechanisms of primary hypertension may account for a striking proportion of uncontrolled systolic blood pressure (SBP) across the full adult age range.[6,7] In addition, these flow-dependent mechanisms are also strongly associated with renal tubular changes[9] and show marked heritability.[10] Moreover, increases in systemic flow explain the decreases in plasma renin concentrations that frequently occur in primary hypertension in some populations.[6] As current therapy, including therapy with diuretic agents, has no proven ability to produce sustained reductions in circulating volume,[11] these data may explain the often poor response to several classes of antihypertensive agents in groups with a high prevalence of low-renin hypertension.[12–14] In order to develop effective therapeutic strategies to manage persistent increases in systemic flow, the mechanisms responsible for sustained increases in systemic flow require elucidation.

Several compensatory mechanisms are well recognized as contributing to the maintenance of a normal blood volume.[1] Abnormalities of several of these mechanisms, which shift the pressure-natriuresis relationship, are thought to contribute to renal-related increases in BP.[2,3] Although ANP (atrial natriuretic peptide) is a well-established modifier of the pressure-natriuresis relationship,[15] the role of ANP in primary human hypertension is uncertain. Although an attenuated ANP response has been reported to occur in sodium (Na^+)-sensitive hypertension,[16] this may be the consequence of a lack of ability to sustain part of the stimulus for ANP release (volume-dependent mechanical stretch of the atria). Indeed, Na^+-sensitive hypertension occurs in association with increases in systemic vascular resistance and not through sustained increases in blood volume and systemic flow.[2,17] However, whether a similar blunted ANP response occurs in sustained renal-related, volume-dependent primary hypertension under conditions of usual salt intake, is unknown. Although Na^+ intake contributes to increases in BP in populations with prevalent

volume-dependent primary hypertension,[18] this effect is explained by alterations in aortic impedance and not through increases in systemic flow.[19] Nonetheless, the ANP response to the volume overload in sustained volume-dependent primary hypertension has not been determined. Hence, in the present study, we compared the relationships between renal-related increases in systemic flow and circulating ANP concentrations in those with a controlled versus uncontrolled SBP in a population of African ancestry with prevalent and sustained volume-dependent primary hypertension.[6,7,9,10] We also compared the relationships between ANP and the vascular determinants of BP in those with uncontrolled versus controlled SBP. Uncontrolled SBP was employed to identify those in whom increases in systemic flow contribute markedly to uncontrolled hypertension in this population.[7]

METHODS

Study Group

The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval number: M02-04-72 and renewed as M07-04-69, M12-04-108, M17-04-01 and M22-03-93). Participants gave informed, written consent. The data are available from the corresponding authors upon reasonable request. The present study design has previously been described.[6,7,9,10,18–20] Briefly, families of Black African descent (Nguni and Sotho chiefdoms) with siblings older than 16 years of age were randomly recruited from the South West Township (SOWETO) of Johannesburg, South Africa. In the present substudy, 772 participants had high quality velocity measurements in the outflow tract. Complete 24-hour urine collections for the evaluation of glomerular and tubular function were available in 519 of these participants.

Clinical, Demographic, Anthropometric, and Laboratory Information

A questionnaire was administered to obtain demographic and clinical data.[20] Height and weight were measured using standard approaches and participants were considered to be overweight if their body mass index was ≥25 kg/m^2 and obese if their body mass index was ≥30 kg/m^2. Laboratory blood tests of renal function, liver function, blood glucose, lipid profiles, hematological parameters, and percentage glycated hemoglobin were performed. Diabetes was defined as the use of insulin or oral glucose lowering agents or an glycated hemoglobin value >6.5%. High quality office brachial BP measurements were obtained in the seated position and after 5 minutes of rest, by a trained nurse-technician using a standard mercury sphygmomanometer as previously described.[20] The mercury sphygmomanometer approach, rather than oscillometric devices, was employed, as this was specified in guidelines at the time of initiating the study (2001). Importantly, the accuracy (determined in Bland-Altmann analysis) of this approach as compared with ambulatory oscillometric monitoring is high, and the ability to detect target organ damage is similar.[20] After at least an 8-hour fast, the mean of 5 measurements obtained at least 30 seconds apart was taken as office BP. Hypertension was defined as a mean office SBP≥140 mm Hg or diastolic BP≥90 mm Hg or the use of antihypertensive medication. Uncontrolled SBP was defined as an SBP≥140 mm Hg in primary analysis and ≥130 mm Hg in secondary (sensitivity) analysis. An SBP ≥140 mm Hg was identified for primary analysis as this threshold was generally employed worldwide at the time of initiating the study (2001). However, an SBP threshold of 130 mm Hg was also included in the current analysis, as several countries now accept 130 mm Hg as the target threshold. The following groups were therefore defined: Normal BP (SBP<140 or <130 mm Hg and not receiving antihypertensive therapy); controlled SBP<140 mm Hg (irrespective of antihypertensive therapy); uncontrolled SBP≥140 mm Hg (irrespective of antihypertensive therapy); controlled SBP<130 mm Hg (irrespective of antihypertensive therapy); uncontrolled SBP≥130 mm Hg (irrespective of antihypertensive therapy).

All blood samples were obtained after at least an 8 hour fast, in the supine position after 10 minutes of rest in the morning between 10:00 and 12:00 hours. Plasma concentrations of ANP were measured using an enzyme-linked immunosorbent assay (Elabscience Biotechnology, Inc, Houston, TX). The assay has a lower detection limit of 4.69 pg/mL and intra-assay and inter-assay coefficients of variation ranging from 4.52% to 6.34% and 5.51% to 5.86%, respectively. Plasma renin and serum aldosterone concentrations were measured as previously described.[6]

Hemodynamic Assessments

Hemodynamic parameters were determined from noninvasive central arterial pressure measurements derived from peripheral pulse wave analysis, the assessment of aortic velocity and diameter in the outflow tract, and the use of left ventricular (LV) dimension assessments in the 4 chamber view (echocardiography).[6,7,9,10,19] After participants had rested for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm) pulse were recorded by applanation tonometry during an 8-second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc, Houston, TX) interfaced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty. Ltd, West Ryde, New South Wales, Australia). The pulse wave was calibrated by manual measurement (auscultation) of brachial systolic and diastolic BP taken immediately before the recordings. The peripheral pressure waveform was converted into a central aortic waveform using a validated generalized transfer function incorporated in SphygmoCor software. Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was <80 mV, were discarded.

Immediately after central arterial pressure waveforms were obtained, aortic velocity and diameter measurements were acquired by an experienced observer (A.J.W.) in participants in the left lateral decubitus position using an Acuson SC2000 Diagnostic ultrasound system (Siemens Medical Solutions, USA, Inc).[6,7,9,10,19] Velocity waveforms were obtained in the 5-chamber view. High quality velocity assessments were

identified as those with a smooth velocity waveform, a dense leading (outer) edge and a clear maximum velocity. Aortic diameter measurements were obtained just proximal to the aortic leaflets in the long axis parasternal view. The largest diameter recorded in early systole was used to construct the aortic flow waveform. Aortic flow waveforms were generated from aortic velocity and diameter measurements. Taking care to avoid any overshoot of the image, the leading (outer) edge or the most dense, or brightest portion of the spectral image of the velocity waveform was outlined using graphics software. The velocity waveform and aortic diameter measurements were employed to construct a flow waveform where flow=velocity×aortic root cross-sectional area calculated from diameter assessments.

Hemodynamic Calculations

SV was calculated from the product of the velocity-time integral and aortic root cross-sectional area. SV was also determined from the difference between end diastolic and end systolic volumes calculated using the biplane Simpson approach in a 4-chamber view.[6] Cardiac output (CO) was calculated from SV×heart rate. Systemic vascular resistance was calculated from mean arterial pressure (MAP)/CO, assuming that right atrial pressure=0 mm Hg, and where MAP was determined from the arterial pressure wave using SphygmoCor software.[6,7] Total arterial compliance (TAC) was calculated as SV/aortic PP. In line with the recommendation that either time or frequency domains may be employed for the assessment of characteristic impedance (Zc),[21] Zc was determined in the time domain using approaches previously described by groups from both Framingham and Ghent[22,23] and validated against invasive pressure measurements.[24] The volume flow waveform was paired with central arterial pressure waveforms by aligning the foot (t_0) of the respective signal averaged waveforms. The point at which flow achieves 95% of its peak (t_{Q95}) was identified. The corresponding pressure change between t_0 and t_{Q95} was determined. Characteristic impedance was calculated as the ratio of change in pressure to change in flow in the window t_0 to t_{95}.

Glomerular and Tubular Function and Na+ Intake

Glomerular and tubular function and Na^+ intake were determined as previously described.[25,26] Timed urine samples were obtained for a 24-hour period after discarding the urine obtained immediately before the start of the collection period. The mean (±SD) time period of urine collection was 24.5±1.5 hours (range, 24.0–31.1 hours). Urinary and serum Na^+ and creatinine concentrations were measured. Creatinine concentrations were measured using the Advia Chemistry systems (Siemens) with calibration traceable to isotope dilution mass spectrometry. The quality of urine samples was determined by constructing regression relations between 24-hour urine creatinine and body weight and 24-hour urine volume and age in gender-specific groups. Based upon the 95% CIs for each group, a 24-hour urine sample was considered acceptable if 24-hour urine creatinine (mmol) was >3.5 and <35 for males and >3.5 and <30 for females. Samples with urine volumes <300 mL/day were also assumed to be incomplete urine collections. We estimated glomerular filtration rate (GFR) by computing creatinine clearance as $(U_{creat}\times V)/S_{creat}$, where U_{creat} and S_{creat} represent the urinary and serum concentrations of creatinine respectively and V the urine volume in mls/minute.[24] GFR was expressed per body surface area. Fractional sodium excretion ($FeNa^+$) was determined as the clearance of Na^+/clearance of creatinine where the clearance of Na^+ was computed as $(U_{Na+}\times V)/S_{Na+,}$ where U_{Na+} and $S_{Na}{}^+$ represent the urinary and serum concentrations of Na^+, respectively and V the urine volume in mls/minute.[25] Sodium intake was assessed from 24-hour urine samples and determined from 24-hour Na^+ excretion.[26]

Data Analysis

Database management and statistical analyses were performed with SAS software, version 9.4 (The SAS Institute, Cary, NC). Continuous variables are expressed as mean (±SD). Dichotomous variables are expressed as percentages. Circulating ANP concentrations were logarithmically transformed to improve the distribution of the data. To identify independent relationships, multivariate adjusted linear regression analysis was performed. Adjustments were for age, sex, regular alcohol intake, regular tobacco intake, body mass index, diabetes, treatment for hypertension and MAP (for Zc). Linear regression analysis was performed as linear relationships with ANP showed the best fit for both $FeNa^+$ and GFR or hemodynamic variables in comparison to various nonlinear relationships. Importantly, irrespective of the type of relationship (linear, exponential, power or logarithmic) fitted, no significant associations (P>0.42) between ANP and either $FeNa^+$, GFR, or hemodynamic variables were observed in the participants with uncontrolled SBP. As the majority of treated hypertensives were receiving low dose hydrochlorothiazide (12.5–25 mg per day, 91.8%), with few receiving additional agents, adjustments for class of agent were not performed. As treatment for hypertension may nevertheless affect relationships between ANP and hemodynamic or renal factors, sensitivity (secondary) analysis was conducted in untreated participants. As more women than men volunteered for the study, sensitivity analysis was also conducted in women and men separately. To ensure that data obtained in those participants with complete urine collections was similar to that obtained in all participants, relationships between ANP and hemodynamic variables were determined in all participants and as sensitivity analysis in those with complete urine collections. To ensure that differences in relationships between ANP and hemodynamic or renal factors were not due to the impact of age-related changes in confounders, sensitivity (secondary) analysis was conducted in age- and sex-matched untreated participants with normal (SBP<130 mm Hg) or uncontrolled (SBP≥130 mm Hg) SBP.

RESULTS

Participant Characteristics

Table 1 shows the general characteristics of the study sample and of those with either uncontrolled or controlled SBP. A high proportion of participants had hypertension and a significant proportion of hypertensives were not receiving antihypertensive medication. Table S1 shows the characteristics of age- and sex-matched untreated

Table 1. Characteristics of Study Participants

Characteristic	Total group	Controlled SBP <140 mm Hg	Uncontrolled SBP ≥140 mm Hg
Sample size (% women)	772 (67.9)	574 (67.9)	198 (67.7)
Age, y	46.3±18.1	41.6±17.0	60.0±13.5*
Body mass index, kg/m^2	29.7±7.7	28.9±7.7	31.9±7.4*
% overweight/% obese	25.9/44.0	27.9/38.5	20.2/60.1*
Hypertensive, %	47.3	29.1	100*
Treated hypertensives, %	26.9	20.2	46.5*
% with controlled BP	64.6	86.9	0*
Diabetes, %	13.3	10.8	20.7†
Regular smoking, %	15.3	14.6	17.2
Regular alcohol, %	19.7	20.2	18.2
SBP, mm Hg	128±12	118±12	158±18*
Diastolic BP, mm Hg	83±12	79±9	94±14*
MAP, mm Hg	99±15	93±10	118±13*
SV (outflow tract), mls/beat	81.4±35.5	79.8±36.8	95.1±38.6*
SV (outflow tract), mLs/beat.BSA	45.5±19.6	44.7±20.1	53.9±23.2*
SV (LV dimensions), mLs/beat	81.9±27.8	80.5±28.4	94.5±31.7*
CO (outflow tract), L/min	5.48±2.62	5.33±2.56	6.56±2.88*
CO (outflow tract), L/min.BSA	3.16±1.51	2.99±1.42	3.60±1.60*
CO (LV dimensions), l/min	5.48±2.00	5.39±2.01	6.46±1.92*
SVR (outflow tract), mm Hg.min/L	20.7±9.0	20.5±9.0	22.8±11.8†
SVR (LV dimensions), mm Hg.min/L	20.4±8.3	20.2±8.7	22.8±10.2†
Characteristic impedance (Zc), dynes.cm^{-5}	85.3±44.1	80.0±37.2	105.3±50.1*
TAC, mm Hg/mls	2.45±1.05	2.79±1.34	1.94±0.91*
Heart rate, beats/min	68±12	68±12	67±12
Plasma ANP, pg/mL	32.2±22.8	35.8±30.1	28.8±16.4†
Serum Na^+,‡ mmol/L	138±3	138±3	138±3
Serum creatinine,‡ µmol/L	72±22	71±16	73±19
Urinary Na^+ excretion,‡ mmol/h	4.36±2.66	4.59±2.79	3.70±2.13†
Urinary creatinine excretion,‡ mmol/h	0.36±0.24	0.38±0.24	0.31±0.21†
Creatinine clearance‡ (GFR), mls/min per 1.73 m^2	103±31	110±29	85±25*
Fractional Na^+ excretion‡ ($FeNa^+$), %	0.75±0.44	0.77±0.46	0.69±0.34

Data are shown as mean±SD or proportions. Outflow tract, data calculated from measurements in the left ventricular (LV) outflow tract; LV dimensions, data calculated from measurements of left ventricular dimensions. ANP indicates atrial natriuretic peptide; BP, blood pressure; CO, cardiac output; GFR, glomerular filtration rate; MAP, mean arterial pressure; SBP, systolic blood pressure; SV, stroke volume; SVR, systemic vascular resistance; and TAC, total arterial compliance.

*P<0.0001 vs controlled SBP.

†P<0.005.

‡Indicates urinary data that met with prespecified quality control criteria in 519 participants (n=382 and 137 with controlled and uncontrolled SBP values, respectively).

participants with either normal or uncontrolled SBP. Importantly, these 2 groups did not differ with respect to confounders (Table S1).

Circulating ANP Concentrations and Glomerular or Tubular Function or Na^+ Intake

As compared with participants with a controlled SBP (Figure 1; Figure S1) or normal SBP (Figure S2), with adjustments for confounders, participants with an uncontrolled SBP (Figure 1; Figures S1 and S2) had a lower GFR independent of $FeNa^+$ and a diminished $FeNa^+$ independent of GFR. Although Na^+ intake was lower in those with uncontrolled SBP (Table 1), the differences in $FeNa^+$ in those with an uncontrolled SBP were noted either before (data not shown) or after (Figure 1; Figure S1) adjustments for Na^+ intake. Similarly, in comparison to an age- and sex-matched untreated group with normal SBP, participants with an uncontrolled SBP had a lower GFR and $FeNa^+$ (Table S1).

Independent of confounders, and in the same regression model, both $FeNa^+$ and GFR were inversely

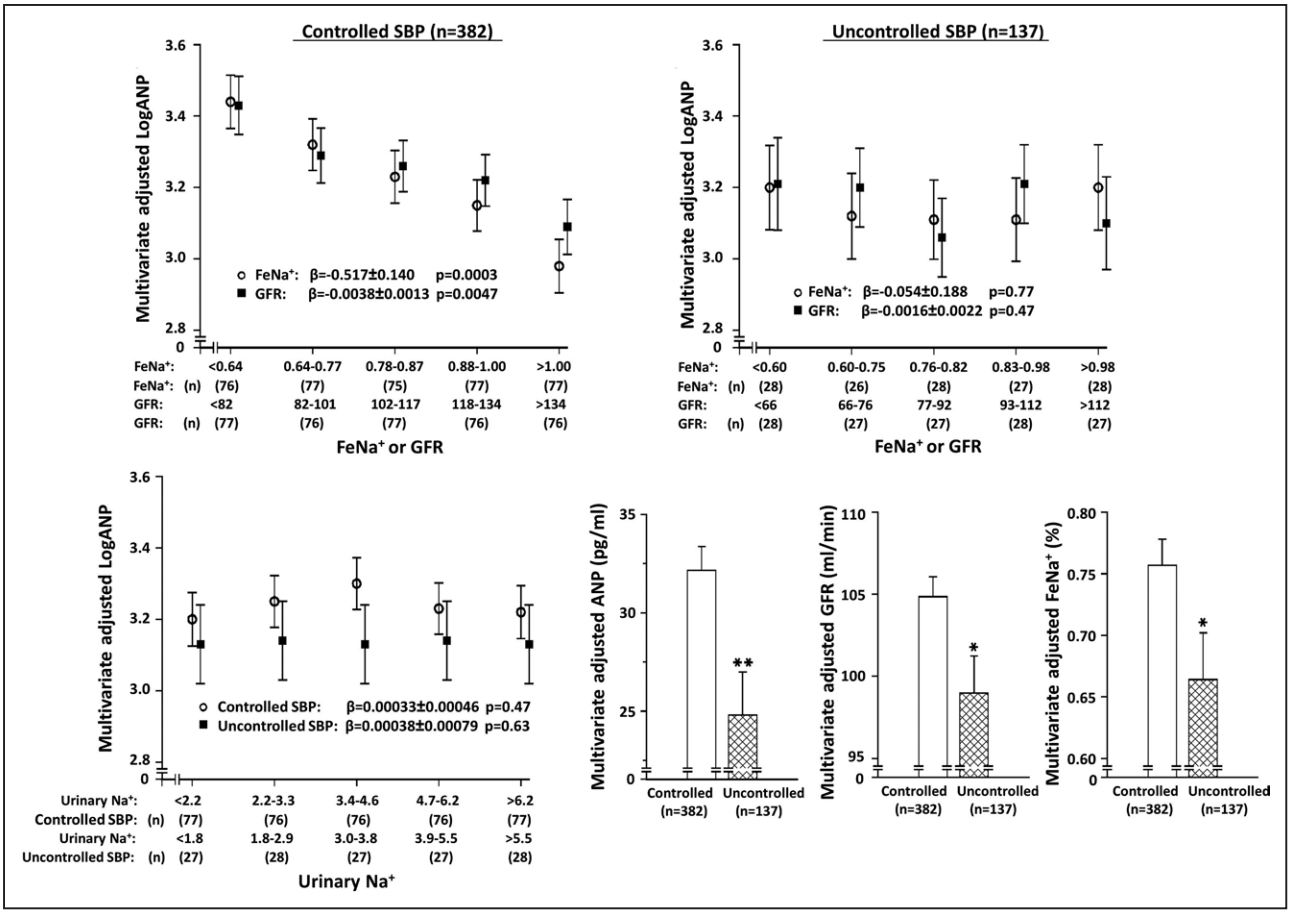

Figure 1. Multivariate adjusted ANP (atrial natriuretic peptide) concentrations across quintiles of fractional sodium excretion (FeNa⁺), glomerular filtration rate (GFR, creatinine clearance), and Na⁺ intake (24-hour Na⁺ excretion) in those with a controlled (systolic blood pressure [SBP]<140 mm Hg) or an uncontrolled (SBP≥140 mm Hg) systolic blood pressure (see Table 2 for SBP< or ≥130 mm Hg).

Lower right show multivariate adjusted differences between groups. Adjustments for relationships and for differences between groups are for age, sex, body mass index, regular smoking, regular alcohol intake, diabetes mellitus, and treatment for hypertension. In addition, relationships with FeNa⁺ are adjusted for GFR and with GFR are adjusted for FeNa⁺ and differences in FeNa⁺ between groups are further adjusted for 24-hour Na⁺ intake. *P<0.05, **P<0.005 for comparisons between those with or without a controlled SBP.

associated with circulating ANP concentrations in the total sample (Table 2) and in those with a controlled SBP (Figure 1; Table 2) or normal SBP (Table S2). Importantly, when adjusted for confounders, there was no relationship between either FeNa⁺ (P=0.78), or GFR (P=0.45) and an interaction between age and ANP. Thus, age is not a modifier of ANP-GFR or ANP-FeNa⁺ relations. The relationship between glomerular or tubular function and ANP concentrations was similar in those with a treated and controlled SBP as it was as in those with a normal SBP (Table S3). Importantly, in regression analysis, the relationship between ANP and GFR was independent of FeNa⁺ and the relationship between ANP and FeNa⁺ was independent of GFR (Figure 1; Table 2; Tables S2 and S3). Moreover, the relationship between ANP and FeNa⁺ was not attributed to Na⁺ intake-FeNa⁺ relations as Na⁺ intake was not associated with FeNa⁺ (P=0.48 in all participants, P=0.24 in those with a controlled SBP and P=0.75 in those with an uncontrolled SBP), and no relationships between Na⁺ intake and ANP concentrations were noted (Figure 1; Table 2). Moreover, further adjustments for Na⁺ intake did not affect the relationship between FeNa⁺ and ANP concentrations (β-coefficients, controlled SBP [<140 mm Hg]=−0.529±0.141, P<0.0005, uncontrolled SBP [≥140 mm Hg]=−0.065±0.184, P=0.72). Despite the differences in glomerular and tubular function in those with uncontrolled versus controlled SBP (Figure 1; Table 1; Figure S1) or normal SBP (Figure S2), and the relationships between glomerular and tubular function and ANP concentrations in the whole group (Table 2), participants with an uncontrolled SBP had lower circulating ANP concentrations than those with a controlled SBP (Figure 1; Table 1; Figure S1) or normal SBP (Figure S2; Table S1). The multivariate adjusted differences in ANP concentrations (pg/ml) given in Figure 1 were similarly noted in all participants (controlled SBP=36.2±1.2, uncontrolled SBP=28.3±2.1, P<0.005) and not only in

Table 2. Multivariate Adjusted Relationships Between Indexes of Glomerular or Tubular Function or Sodium (Na^+) Intake and ANP Concentrations

	All participants		Controlled SBP (<130 mm Hg)		Uncontrolled SBP (≥130 mm Hg)	
ANP versus	β-coeff.±SEM	*P* Value	β-coeff.±SEM	*P* Value	β-coeff.±SEM	*P* Value
Model 1 (total sample, n=519)			n=288		n=231	
$FeNa^+$	−0.430±0.111	<0.0002	−0.499±0.162	<0.002	−0.162±0.170	=0.34
GFR	−0.0035±0.0011	<0.005	−0.0038±0.0015	<0.01	−0.0026±0.0019	=0.16
Model 2 (untreated, n=373)			n=244		n=129	
$FeNa^+$	−0.459±0.139	<0.002	−0.480±0.172	<0.005	−0.201±0.267	=0.45
GFR	−0.0035±0.0013	<0.01	−0.0033±0.0016	<0.05	−0.0021±0.0021	=0.35
Model 3 (women, n=347)			n=193		n=154	
$FeNa^+$	−0.415±0.143	<0.005	−0.527±0.205	<0.01	−0.0032±0.2000	=0.99
GFR	−0.0036±0.0015	<0.02	−0.0041±0.0020	<0.05	−0.0031±0.0022	=0.15
Model 4 (men, n=172)			n=96		n=76	
$FeNa^+$	−0.474±0.184	<0.02	−0.597±0.266	<0.05	−0.164±0.334	=0.63
GFR	−0.0037±0.0018	<0.05	−0.0038±0.0022	=0.09	−0.0029±0.0035	=0.41
Model 5 (total sample, n=519)			n=288		n=231	
$FeNa^+$	−0.318±0.108	<0.005	−0.436±0.162	<0.01	−0.122±0.169	=0.47
24-h Na^+ exc	−0.000002±0.00031	=0.99	−0.000040±0.00043	=0.93	−0.00011±0.00046	=0.82
Model 6 (total sample, n=519)			n=288		n=231	
24-h Na^+ exc	−0.00031±0.00039	=0.43	−0.00017±0.00043	=0.69	−0.000064±0.00053	=0.90

For data on controlled versus uncontrolled SBP defined according to thresholds of 140 mm Hg, see Figure 1. Untreated refers to participants not receiving antihypertensive therapy; 24-h Na^+ exc, 24-h urinary Na^+ excretion as an index of Na^+ intake. Adjustments are for age, sex (except in sex-specific groups), BMI, regular smoking, regular alcohol intake, diabetes mellitus, and treatment for hypertension (except in untreated participants). ANP indicates atrial natriuretic peptide; BMI, body masss index; GFR, glomerular filtration rate; and SBP, systolic blood pressure.

those with complete urine samples. These differences in ANP concentrations were explained by differences in the relationships between ANP and glomerular and tubular function in those with uncontrolled as compared with a controlled SBP or normal SBP. Indeed, while glomerular and tubular function were strongly and independently associated with ANP concentrations in those with a controlled SBP (Figure 1; Table 2) or normal SBP (Table S2), no independent relationships were noted in those with an uncontrolled SBP (Figure 1; Table 2; Table S2). The differences in the ANP-glomerular or tubular function relationships between those with controlled SBP versus uncontrolled SBP were not due to the impact of age-related changes in confounders, as ANP was similarly associated with $FeNa^+$ and GFR in age- and sex-matched untreated participants with normal SBP but not in those with uncontrolled SBP (Table S4). Importantly, the lack of relationship between ANP concentrations and glomerular or tubular function in those with an uncontrolled SBP was not explained by relationships assessed over a different range of $FeNa^+$ or GFR values as that noted in the group with a controlled SBP (Figure 1, top).

In contrast to independent relationships noted between ANP concentrations and glomerular or tubular function, neither plasma renin nor serum aldosterone concentrations were independently associated with glomerular or tubular function (Table S5). Moreover, independent relationships between ANP concentrations and renal function were unaffected by adjustments for plasma renin and serum aldosterone concentrations (Table S5).

Circulating ANP Concentrations and Systemic Flow

As compared with participants with a controlled SBP (Table 1; Figure 2; Figure S3) or normal SBP (Figure S4), with adjustments for confounders participants with an uncontrolled SBP had a markedly higher SV and CO (Table 1; Figure 2; Figures S3 and S4). These differences in SV and CO were noted in the total sample and in those with complete urine samples (Figure S5). The differences in systemic flow were noted irrespective of whether SV was derived from velocity and diameter measurements in the LV outflow tract or from LV diameter measurements (Table 1; Figure 2; Figures S3 and S4). Similarly, in comparison to an age- and sex-matched untreated group with normal SBP, participants with an uncontrolled SBP had higher SV and CO (Table S1). Importantly, with adjustments for confounders no independent relationship between an interaction between ANP and age was associated with either SV (P=0.95) or CO (P=0.86). Thus, age is not a modifier of ANP-systemic flow relations.

In keeping with a volume overload explaining higher ANP concentrations, independent of confounders, SV and CO were positively (directly) associated with circulating ANP concentrations (Table 3; Table S6). The

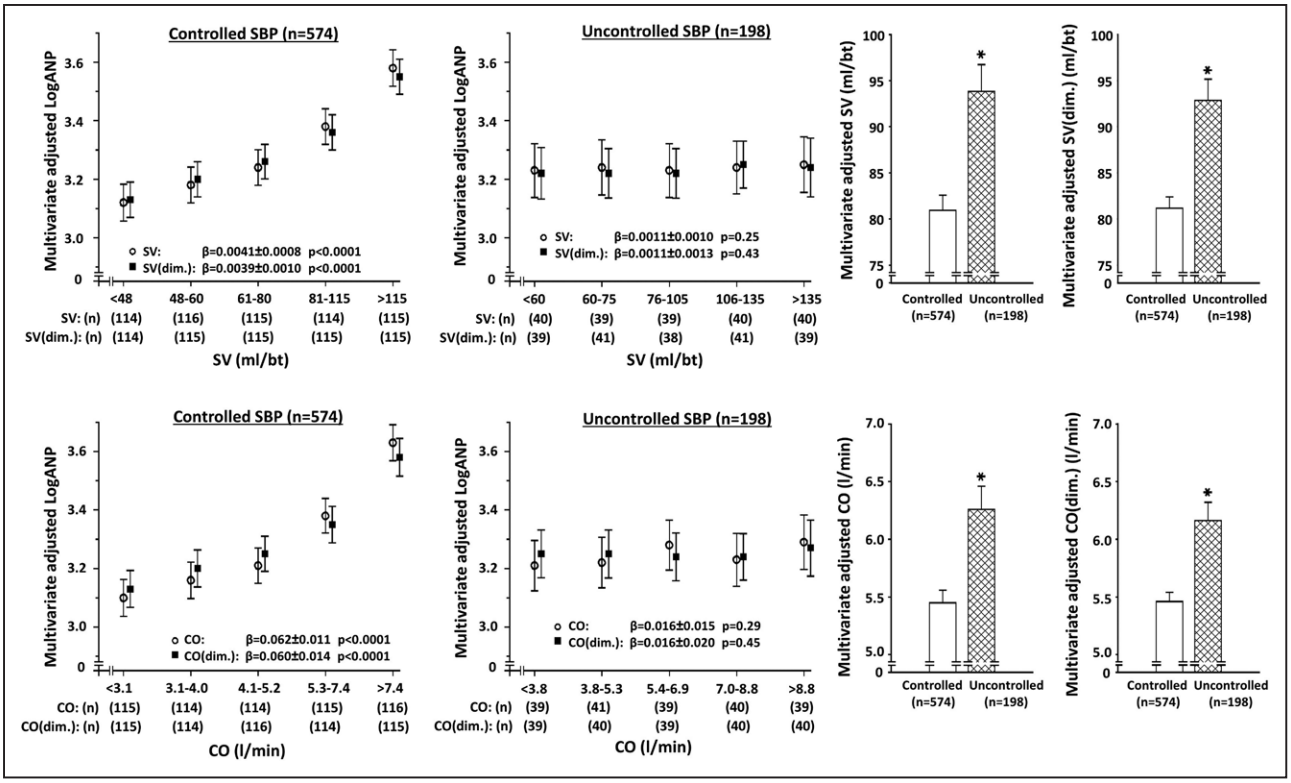

Figure 2. Multivariate adjusted ANP (atrial natriuretic peptide) concentrations across quintiles of indexes of volume overload, including stroke volume (SV) and cardiac output (CO) in those with a controlled (systolic blood pressure [SBP] <140 mm Hg) or an uncontrolled (SBP≥140 mm Hg) systolic blood pressure (left; see Table 3 for SBP< or ≥130 mm Hg).
Right shows multivariate adjusted differences between groups. Data shown are SV and CO determined from both left ventricular outflow tract measurements and dimensions. See Table 1 for additional abbreviations. Adjustments for relationships and for differences between groups are for age, sex, body mass index, regular smoking, regular alcohol intake, diabetes mellitus, and treatment for hypertension. *P<0.0001 for comparisons between those with or without a controlled SBP.

relationship between systemic flow and ANP concentrations was similar in those with a treated and controlled SBP as it was as in those with a normal SBP (Table S7). These relationships were also noted to occur irrespective of the methods employed to derive these values (Table 3; Table S6). No relationships between ANP concentration and heart rate were noted (P=0.54). The direct relationships between SV or CO and ANP concentrations were similar irrespective of whether SV or CO were unindexed or were expressed per body surface area (data not shown). However, while systemic flow (an index of volume overload) was strongly and independently associated with ANP concentrations in those with a controlled SBP, no independent relationships were noted in those with an uncontrolled SBP (Figure 2; Table 3; Table S6). The relationships between SV or CO and ANP concentrations in those with a controlled, but not uncontrolled SBP, were noted in the whole group as well as in those with complete urine samples (Figure S5). The differences in ANP-SV or CO relationships between those with controlled SBP versus uncontrolled SBP were not due to the impact of age-related changes in confounders, as ANP was similarly associated with SV and CO in age- and sex-matched untreated participants with normal SBP but not in those with uncontrolled SBP (Table S8). Importantly, the lack of relationship between ANP concentrations and systemic flow in those with an uncontrolled SBP was not explained by relationships assessed over a different range of ANP concentrations as that noted in the group with a controlled SBP (Figure 2, left).

Circulating ANP Concentrations and Vascular Determinants of BP

As compared with participants with a controlled SBP (Table 1; Figure 3; Figure S3) or normal SBP (Figure S4), with adjustments for confounders participants with an uncontrolled SBP had a higher systemic vascular resistance and Zc and a lower TAC (Table 1; Figure 3; Figures S3 and S4). These differences in systemic vascular resistance (SVR), Zc, and TAC were noted in the total sample and in those with complete urine samples (Figure S5). Similarly, in comparison to an age- and sex-matched untreated group with normal SBP, participants with an uncontrolled SBP had higher Zc and SVR and lower TAC (Table S1). Importantly, with adjustments for confounders, no independent relationship between an interaction between ANP and age was associated with either SVR (P=0.74), or Zc (P=0.36). Thus, age is not a modifier of ANP-vascular function relations.

Table 3. Multivariate Adjusted Relationships Between Hemodynamic Determinants of Blood Pressure and Circulating ANP Concentrations

	β-coefficient (±SEM)	*P* Value	β-coefficient (±SEM)	*P* Value
ANP versus	Total sample (n=772)		Untreated (n=564)	
SV*	0.0033±0.0006	<0.0001	0.0038±0.0007	<0.0001
SV†	0.0034±0.0008	<0.0001	0.0040±0.0010	<0.0001
CO*	0.0503±0.0092	<0.0001	0.0608±0.0111	<0.0001
CO†	0.0514±0.0120	<0.0001	0.0603±0.0153	<0.0001
SVR*	−2.375±0.574	<0.0001	−2.487±0.635	<0.0002
SVR†	−2.288±0.442	<0.0001	−2.213±0.496	<0.0001
Zc	−7.837±2.164	<0.0005	−8.285±2.382	<0.0005
TAC	0.268±0.067	<0.0001	0.293±0.079	<0.0005
	Women (n=524)		Men (n=248)	
SV*	0.0033±0.0008	<0.0001	0.0032±0.0010	<0.005
SV†	0.0035±0.0010	<0.0002	0.0036±0.0012	<0.002
CO*	0.0481±0.0112	<0.0001	0.0515±0.0173	<0.005
CO†	0.0482±0.0116	<0.0001	0.0559±0.0206	<0.01
SVR*	−2.304±0.683	<0.001	−3.072±0.969	<0.005
SVR†	−2.027±0.554	<0.0005	−2.903±0.742	<0.0002
Zc	−7.248±2.612	<0.01	−8.566±4.050	<0.05
TAC	0.322±0.082	<0.0001	0.284±0.132	<0.05
	Controlled SBP (<130 mm Hg)		Uncontrolled SBP (≥130 mm Hg)	
n=	443		329	
SV*	0.0043±0.0009	<0.0001	0.0014±0.0008	=0.062
SV†	0.0045±0.0012	<0.0001	0.0016±0.0011	=0.136
CO*	0.0668±0.0133	<0.0001	0.0194±0.0115	=0.093
CO†	0.0685±0.0172	<0.0001	0.0202±0.0173	=0.244
SVR*	−2.704±0.687	<0.0001	−1.426±1.180	=0.228
SVR†	−2.475±0.561	<0.0001	−1.403±0.844	=0.098
Zc	−5.989±2.250	<0.01	−2.375±4.539	=0.600
TAC	0.328±0.090	<0.0005	0.096±0.093	=0.304

Untreated refers to participants not receiving antihypertensive therapy. Adjustments are for age, sex (except in sex-specific groups), BMI, regular smoking, regular alcohol intake, diabetes mellitus, and treatment for hypertension (except in untreated participants). ANP indicates atrial natriuretic peptide; BMI, body mass index; CO, cardiac output; SBP, systolic blood pressure; SV, stroke volume; SVR, systemic vascular resistance; and TAC, total arterial compliance.

*Calculated from outflow tract measurements.

†Calculated from left ventricular dimensions.

Independent of confounders, circulating ANP concentrations were inversely associated with both SVR and Zc and positively associated with TAC in the community sample (Table 3). The relationship between ANP concentrations and Zc was independent of MAP but not aortic root diameter (*P*=0.43). The inverse relationships between ANP concentrations and SVR were similar irrespective of whether SVR was determined from SV measurements obtained from LV outflow tract velocity and diameter measurements or from LV dimension measurements (Table 3). While ANP concentrations were independently associated with SVR, Zc and TAC in those with a controlled SBP (Figure 3; Table 3) or normal SBP (Table S6), no independent relationships were noted in those with an uncontrolled SBP (Figure 3; Table 3; Table S6). The relationships between either SVR or Zc and ANP concentrations were similar in those with a treated and controlled SBP as in those with a normal SBP (Table S7). The relationships between ANP concentrations and SVR, Zc and TAC in those with a controlled, but not uncontrolled SBP, were noted in the whole group as well as in those with complete urine samples (Figure S6). The relationships between ANP concentrations and SVR, Zc and TAC in those with a controlled, but not uncontrolled SBP, were not due to the impact of age-related changes in confounders, as ANP was similarly associated with SVR, Zc and TAC in age- and sex-matched untreated participants with normal SBP but not in those with uncontrolled SBP

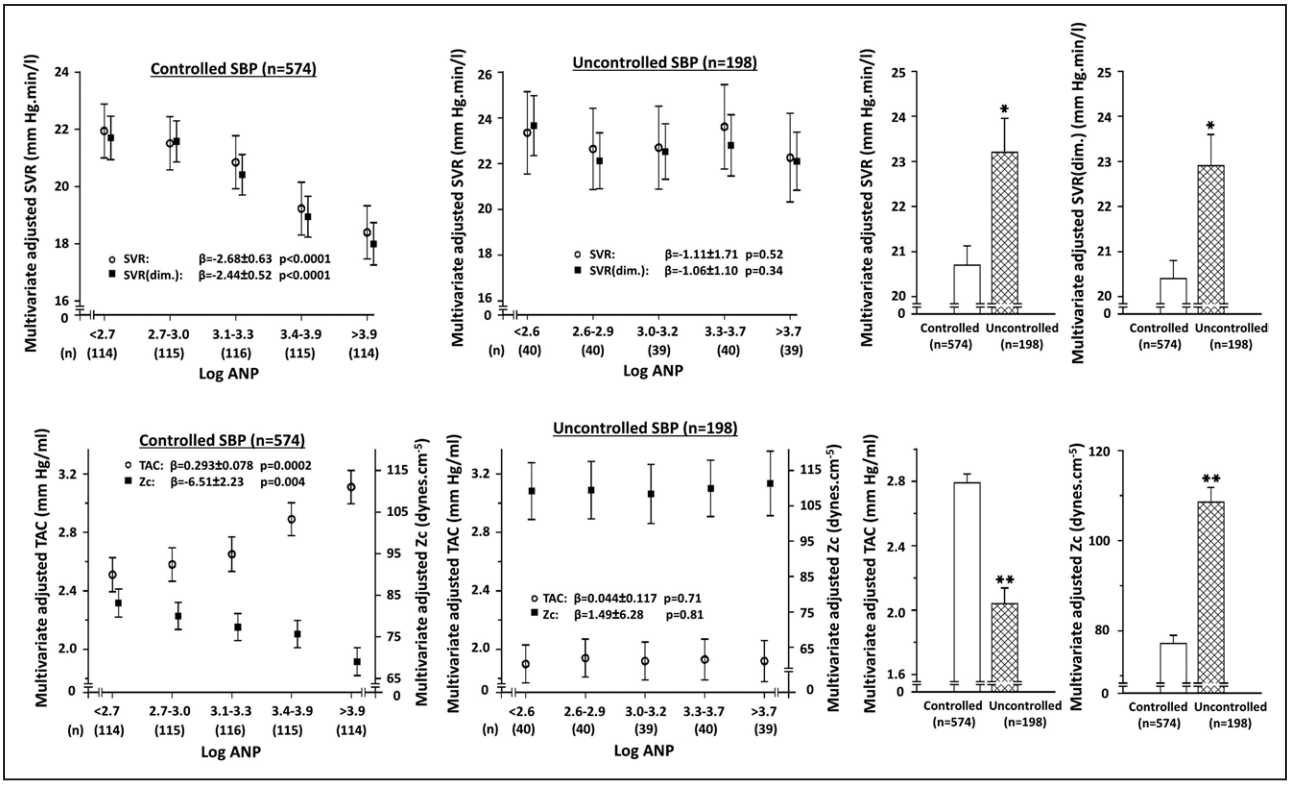

Figure 3. Multivariate adjusted indexes of vascular function across quintiles of ANP (atrial natriuretic peptide) concentrations in those with a controlled (systolic blood pressure [SBP] <140 mm Hg) or an uncontrolled (SBP≥140 mm Hg) systolic blood pressure (left; see Table 3 for SBP< or ≥130 mm Hg).

Right shows multivariate adjusted differences between groups. Data shown are systemic vascular resistance (SVR) derived from stroke volume determined from both left ventricular outflow tract measurements and dimensions. Adjustments for relationships and for differences between groups are for age, sex, body mass index, regular smoking, regular alcohol intake, diabetes mellitus, treatment for hypertension, and mean arterial pressure (for Zc). *P<0.005, **P<0.0001 for comparisons between those with or without a controlled SBP. SVR indicates systemic vascular resistance; TAC, total arterial compliance; and Zc, aortic characteristic impedance,

(Table S8). Of note, the lack of relationship between ANP concentrations and vascular function in those with an uncontrolled SBP was not explained by relationships assessed over a different range of ANP concentrations as that noted in the group with a controlled SBP (Figure 3, left).

DISCUSSION

In the present study, we evaluated whether an attenuated relationship occurs between circulating ANP concentrations and indexes of renal-related increases in circulating volume in individuals with uncontrolled SBP in a community with prevalent and sustained volume-dependent primary hypertension.[6,7,9] We show that indexes of renal fluid retention (decreased $FeNa^+$ and GFR, independent of each other and of 24-hour Na^+ excretion) and a consequent volume-dependent higher systemic blood flow (SV and CO) are strongly and independently associated with ANP concentrations in those of the community sample with a controlled SBP. However, in those with an uncontrolled SBP, an enhanced renal fluid retention and an elevated systemic flow were noted, but ANP concentrations were attenuated and no relationship between either indexes of renal fluid retention or systemic flow and ANP concentrations were detected. Moreover, ANP concentrations were strongly and independently associated with beneficial vascular changes (decreased SVR and Zc and increased TAC) in those of the community sample with a controlled SBP. However, in those with an uncontrolled SBP, SVR and Zc were higher and TAC diminished, and no relationship between ANP and either SVR, Zc or TAC were noted. The attenuated relationships between ANP and indexes of renal fluid retention, volume overload (SV and CO) or vascular effects in those with an uncontrolled SBP, occurred despite a lower Na^+ intake (as indexed from 24-hour urine samples) as compared with those with a controlled SBP.

A blunted ANP response has previously been reported to occur in those with an increased BP following a Na^+ load (salt-sensitive hypertension).[16] However, whether a similar attenuation of ANP release occurs in response to a volume overload in the absence of a Na^+ load in sustained volume-dependent hypertension, is unknown. In this regard, salt-sensitive hypertension produces sustained increases in BP through an enhanced SVR and

not volume-dependent increases in systemic flow.[2,17] Thus, in salt-sensitive hypertension, the extent of the stimulus for ANP release (that component produced by mechanical stretch of the atria due to a volume overload) does not persist. However, when this stimulus does persist in those with sustained volume-dependent hypertension, the impact on ANP release has as yet not been reported. In the present study, we show for the first time that in a community with prevalent and sustained volume-dependent hypertension, as compared with those with a normal SBP, those with an uncontrolled SBP have lower ANP concentrations despite renal and hemodynamic differences which suggest an increase in volume load. Moreover, we demonstrate that despite the strong and independent relationships between indexes of renal fluid retention or systemic flow and ANP concentrations in those with a controlled SBP, these relationships are attenuated in those with an uncontrolled SBP. Importantly, these alterations in ANP and the relationships with indexes of volume overload in those with an uncontrolled SBP occurred in the absence of a greater Na^+ intake.

In the present study, in those with an uncontrolled SBP, attenuated relationships between ANP concentrations and vascular measures of resistance to flow were also noted. In those with a controlled SBP, circulating ANP concentrations were inversely associated with not only systemic vascular resistance but also proximal aortic characteristic impedance (Zc) (independent of MAP). The relationships with Zc were, however, dependent on aortic root diameter and hence are likely to be attributed to alterations in aortic smooth muscle tone, rather than to a decrease in proximal aortic stiffness. In this regard, vasodilators, although able to decrease Zc through reductions in distending pressures (MAP), have never been demonstrated to produce an additional effect mediated by an impact on proximal aortic diameter. These relationships may therefore in-part explain the beneficial actions of agents that increase circulating ANP concentrations in hypertension or heart failure.[27]

The present study suggests that part of the sustained volume-overload that occurs in persistent renal-related, volume-dependent hypertension is mediated by an attenuated release of ANP from the atria, together with a reduced vascular response to ANP in the circulation. These data may therefore explain the particularly striking antihypertensive response noted to neutral endopeptidase inhibitors, which increase circulating ANP concentrations by preventing the metabolism of ANP, in a population in Africa.[28] Agents that increase circulating ANP concentrations and which have a high degree of bioavailability with modest side effect profiles[27] are presently available. Thus, further studies are required to assess whether such agents are able to reduce the volume overload and the increases in systemic flow noted to drive increases in SBP specifically in sustained renal-related, volume-dependent hypertension. In this regard, current diuretic agents have no proven ability to produce sustained reductions in circulating volume or systemic flow.[11]

There are several limitations to the present study. First, the present study was a cross-sectional study and hence relationships reported on are not necessarily cause and effect. Further studies are therefore required to determine whether agents that increase circulating ANP concentrations reduce systemic flow in sustained volume-dependent primary hypertension, and thereby, contribute to maintaining SBP control. Second, ANP concentrations were determined from serum samples obtained at a single time of day and under the same basal conditions. In this regard, ANP concentrations are determined by several variables, which alter during the course of the day. Whether similar relationships are observed at alternative times over a 24-hour period with different levels of activity, position or dietary habits, requires further study. However, the strong relationships between ANP concentrations and glomerular or tubular function determined from 24-hour urine samples suggests that appropriate ANP responses to a volume load over at least a day were identified. Third, the present study was conducted in 1 ethnic group. Therefore, the present study may not be translatable to alternative ethnic groups. However, studies assessing the hemodynamic determinants of hypertension over the full adult age range have been limited to European and African populations. In this regard, renal mechanisms are thought to play a major role in any population either derived from or currently existing in equatorial regions.[29] Importantly, these studies should be conducted in population samples obtained over the full adult age range, as the sustained volume-dependence of hypertension noted in the present population is strongly age-dependent.[6] Last, although we have performed sex-specific analysis in the whole group, the sample of men with either a controlled or uncontrolled SBP was insufficiently large to perform sex-specific analysis in these 2 groups separately using SBP thresholds of 140 mm Hg. Thus, the results may apply to women only. However, we were able to show comparable results in men and women when using SBP thresholds of 130 mm Hg.

PERSPECTIVES

Although a blunted ANP response to a Na^+ load has been demonstrated to occur in Na^+-sensitive hypertension,[16] this may be explained by the limited ability to sustain part of the stimulus for ANP release (decrease in the volume-induced mechanical stretch of the atria). Indeed, Na^+-sensitive hypertension is associated with persistent increases in systemic vascular resistance, rather than sustained volume-dependent increases in systemic flow.[2,17] In the present study, we therefore evaluated whether a decreased relationship between circulating

ANP concentrations and indexes of renal-related volume overload are associated with an uncontrolled SBP in a population with prevalent and sustained, volume-dependent primary hypertension.[6,7,9] We demonstrate for the first time that the higher circulating ANP concentrations noted to occur in those with a controlled SBP in relationship to increases in volume load (as evidenced by renal indexes of fluid retention and increases in systemic flow), are attenuated in those with an uncontrolled SBP and markedly higher indexes of volume load. Moreover, we show that the relationships noted between circulating ANP concentrations and reduced vascular resistance (lower SVR and Zc and higher TAC) in those with a controlled SBP, are similarly significantly attenuated in those with an uncontrolled SBP. These data suggest that an attenuated ANP response to a volume load may play a major role in producing low-renin, renal-related, sustained volume-dependent primary hypertension. As current therapy, including the use of diuretic agents, has no proven ability to produce sustained decreases in volume overload in hypertension, the present study suggests that targeting ANP may be an essential approach to managing uncontrolled hypertension in some populations.

ARTICLE INFORMATION

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Disclosures

None.

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