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PRECLINICAL RESEARCH

Cardiac Versus Renal Response to Volume Expansion in Preclinical Systolic Dysfunction With PDEV Inhibition and BNP

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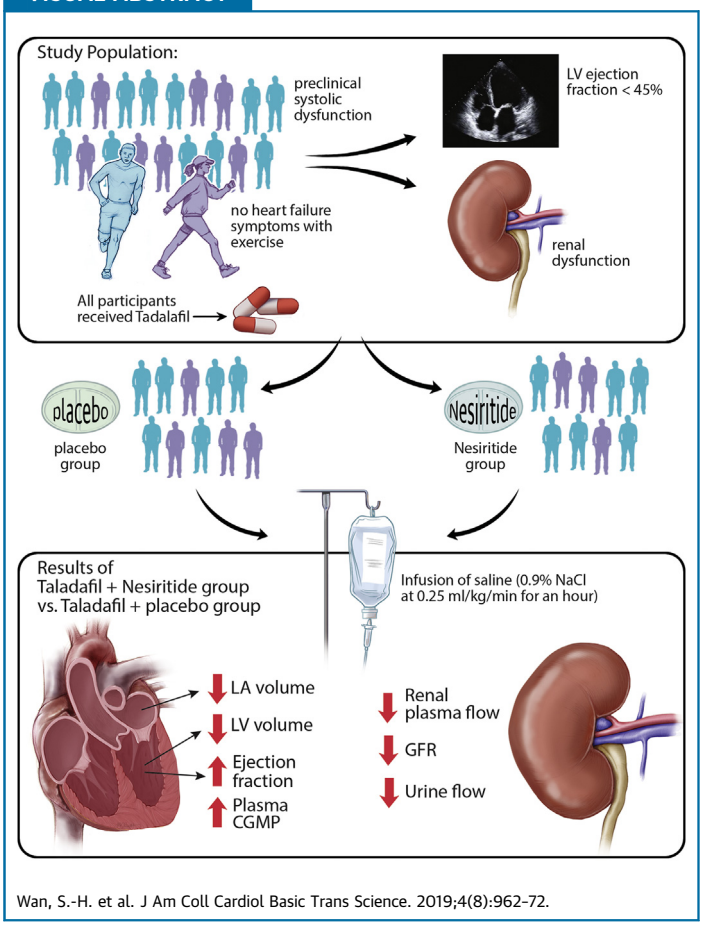
VISUAL ABSTRACT



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HIGHLIGHTS

- **In preclinical systolic dysfunction, defined as left ventricular systolic dysfunction with no heart failure signs or symptoms, impairment in cardiorenal response to volume expansion may lead to symptomatic heart failure. Rescue of this impaired process in preclinical disease may prevent development of symptomatic heart failure.**
- **In preclinical systolic dysfunction, inhibition of phosphodiesterase-V in combination with exogenous B-type natriuretic peptide administration results in improved cardiac function but worsened renal function in response to acute volume expansion.**

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- **Future studies are needed to further define the physiological effects and long-term outcomes of phosphodiesterase-V inhibition and exogenous BNP administration. Understanding the cardiorenal effects and outcomes of combination phosphodiesterase-V with exogenous B-type natriuretic peptide may affect the clinical management of patients with preclinical systolic dysfunction and renal dysfunction.**

SUMMARY

Impaired cardiorenal response to acute saline volume expansion in preclinical systolic dysfunction (PSD) may lead to symptomatic heart failure. The objective was to determine if combination phosphodiesterase-V inhibition and exogenous B-type natriuretic peptide (BNP) administration may enhance cardiorenal response. A randomized double-blinded, placebo-controlled study was conducted in 21 subjects with PSD and renal dysfunction. Pre-treatment with tadalafil and subcutaneous BNP resulted in improved cardiac function, as evidenced by improvement in ejection fraction, left atrial volume index, and left ventricular end-diastolic volume. However, there was reduced renal response with reduction in renal plasma flow, glomerular filtration rate, and urine flow. (Tadalafil and Nesiritide as Therapy in Pre-clinical Heart Failure; NCT01544998) (J Am Coll Cardiol Basic Trans Science 2019;4:962–72) © 2019 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

ABBREVIATIONS AND ACRONYMS

ACC = American College of Cardiology

AHA = American Heart Association

ANP = atrial natriuretic peptide

BNP = B-type natriuretic peptide

cGMP = cyclic guanosine monophosphate

GFR = glomerular filtration rate

HF = heart failure

LAVI = left atrial volume index

LVEF = left ventricular ejection fraction

LVEDV = left ventricular end-diastolic volume

LVESV = left ventricular end-systolic volume

NP = natriuretic peptide

PDEV = type V phosphodiesterase

PSD = preclinical systolic dysfunction

RPF = renal plasma flow

SC = subcutaneous

VE = acute saline volume expansion

Heart failure (HF) remains a major cause of morbidity and mortality worldwide, with >1 million hospitalizations and >$30 billion in health-related costs in the United States annually (1). Preclinical systolic dysfunction (PSD) represents a continuum in the spectrum of HF with reduced ejection fraction and is characterized by systolic dysfunction with absence of signs and symptoms of HF (American College of Cardiology/American Heart Association [ACC/AHA] stage B HF). It is increasingly recognized that PSD is common in the general population and associated with cardiorenal dysfunction and progression to symptomatic HF and mortality (2,3). The St Vincent's Screening To Prevent Heart Failure (STOP-HF) trial demonstrated that early screening and identification is important among those with stage B HF to prevent progression to symptomatic HF and improve outcomes (4).

Natriuretic peptides (NPs) have important physiological functions. Deficiency is associated with HF and fluid retention, and therapies with natriuretic-based therapies may be important in preventing progression along the HF spectrum (5). Cyclic 3′-5′-guanosine monophosphate (cGMP) is the second messenger of the NP system, is metabolized by type V phosphodiesterase (PDEV), and plays an important role in the preservation of myocardial, vascular, and renal function in PSD and HF (6). Previous animal studies have supported the therapeutic role of PDEV inhibitors in cardiac dysfunction (7).

We recently reported that the cGMP signaling pathway is impaired in subjects with PSD, characterized by decreased glomerular filtration rate (GFR) and renal blood flow (RBF), with an attenuated renal

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

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cGMP response to acute volume expansion (VE) (8). Attenuation of renal cGMP generation may be secondary to renal PDEV upregulation, as observed in experimental HF. Furthermore, renal PDEV upregulation may lead to the attenuation of renal cGMP generation in response to both endogenous and exogenous NP. Experimental animal studies have shown that long-term PDEV inhibition enhances cardiorenal response to exogenous B-type natriuretic peptide (BNP) by inhibiting cGMP degradation (9,10). Furthermore, animal studies have demonstrated synergistic effects of combination therapy with PDEV inhibition and exogenous BNP administration. Although PDEV inhibitors are clinically approved for erectile dysfunction and pulmonary hypertension, and nesiritide (recombinant human BNP) is approved for acute decompensated HF, the cardiorenal effects of combining PDEV inhibitors and BNP in humans have not been tested.

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The objective of our study was to assess, for the first time in subjects with PSD and renal dysfunction, whether combination tadalafil (a long-acting PDEV inhibitor) and BNP, in response to acute VE, will enhance cardiorenal response compared with tadalafil alone. Combination therapy with tadalafil and BNP may have potential for rescuing cardiorenal impairment and preventing progression to symptomatic HF.

METHODS

STUDY DESIGN. We used a double-blinded, placebo-controlled, crossover study protocol to compare cardiorenal responses to acute saline VE after tadalafil and subcutaneous (SC) placebo versus tadalafil and SC BNP administration in subjects with PSD and renal dysfunction. This study was approved by the Mayo Foundation Institutional Review Board and was performed at the Clinical Research Unit at Saint Mary's Hospital, Mayo Clinic (Rochester, Minnesota). Written informed consent was obtained from all participants.

STUDY POPULATION. Twenty-five patients who met criteria for PSD (AHA stage B HF) and renal dysfunction (estimated GFR between 30 and 90 ml/min) were enrolled. Four subjects were excluded: 1 participant had GFR <30 ml/min on renal function reassessment; 1 participant withdrew consent before the study; 2 participants were unable to participate due to difficulty in obtaining venous and bladder access (**Figure 1**). Twenty-one subjects were randomized to receive tadalafil and SC placebo or tadalafil and SC BNP before VE. Cardiac and renal assessment, including transthoracic echocardiogram, urine, and plasma analysis, was performed at baseline and 60 min after VE. All patients returned for a second visit and underwent the crossover arm of the study. Sample size calculation is shown in Supplemental Table 1.

INCLUSION CRITERIA. Inclusion criteria were the following: ejection fraction <45%; no current or previous diagnosis of HF; not on loop diuretics; renal dysfunction (creatinine clearance between 30 and 90 ml/min using the Modification of Diet in Renal Disease formula); and minimal distance >450 m on 6-min walk test in the absence of mechanical limitations. Cardiovascular medications were at stable doses for at least 2 weeks before study entry.

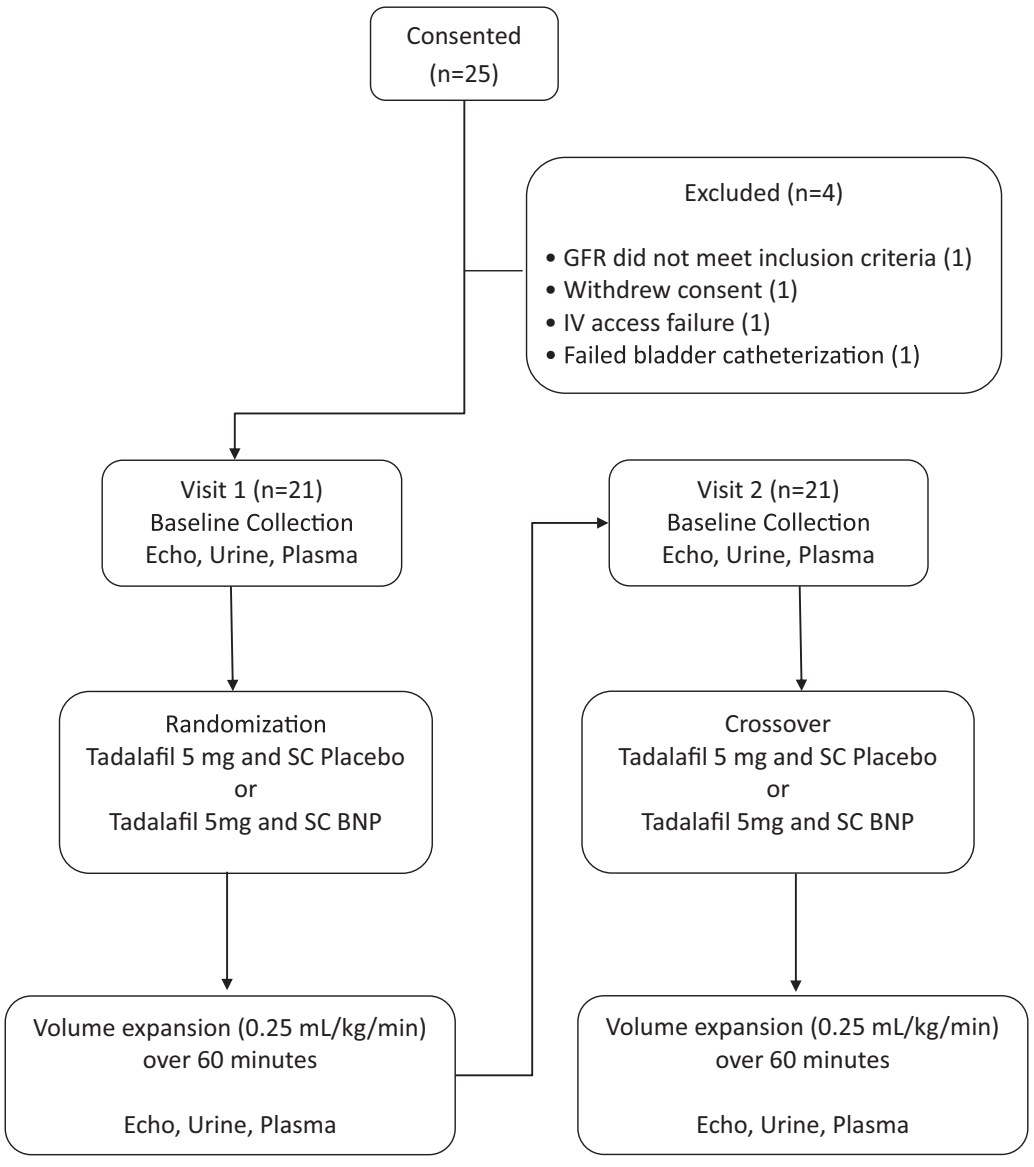
ECHOCARDIOGRAPHIC ASSESSMENT. Echocardiographic images were obtained from standard acoustic windows according to the recommendations of the American Society of Echocardiography (11). Ventricular volumes were assessed by biplane Simpsons method of discs, and a 2-dimensional ejection fraction was obtained. Left ventricular (LV) diastolic function filling pressures were assessed by mitral inflow pulsed-wave Doppler examination and tissue Doppler imaging of the mitral annulus. All echocardiographic data were obtained by a certified sonographer and interpreted by H.H.C., who was blinded to the assigned treatment.

STUDY PROTOCOL. Before study initiation, subjects were stabilized for 1 week on a low-salt diet (120 mEq sodium/day). Baseline hematology and biochemistry laboratory tests, 6-min walking test, vital signs, and a physical examination were obtained. Subjects who met inclusion criteria were recruited and admitted to the Clinic Research Unit at St. Mary's Hospital, Mayo Clinic Center for Translational Science Activities (CTSA), Rochester, Minnesota, 1 day before the study date (**Figure 1**).

On the study day, subjects received their regular medications, except for diabetic therapies that were postponed until the first meal after the last renal clearance measurement. Subjects were orally hydrated with 10 ml/kg of water to ensure sufficient urinary flow.

Subjects were placed into a supine position for 1 h. During the first 15 min, 2 standard intravenous catheters were placed, 1 in each arm, for infusion and blood sampling. Iothalamate and para-aminohippurate were administered, followed by urinary and blood measurements, including urinary flow

FIGURE 1 **Study Design**



BNP = B-type natriuretic peptide; GFR = glomerular filtration rate; SC = subcutaneous.

(ml), urinary sodium excretion (mEq/min), urinary cGMP excretion (pmol/min), blood sodium (mEq/l), and cGMP (pmol/ml). Renal clearances and venous blood samples were obtained at 30 and 60 min, respectively. Subjects were monitored by electrocardiography, and blood pressures were obtained. An echocardiogram was obtained for left atrial (LA) and LV volumes, as well as systolic and diastolic function.

Subjects were then randomized to receive oral tadalafil 5 mg + SC placebo or tadalafil 5 mg + SC BNP 10 microgram/kg (Scios, Mountain View, California). After a 15-min lead-in period, a 30-min renal clearance and blood sample were repeated. An acute saline load was administered (0.9% sodium chloride at 0.25 ml/kg/min for 1 h), and every 30 min, renal and blood samples were obtained. Immediately after the end of the acute volume expansion, an echocardiogram was performed. Subjects returned at least 1 week later for the crossover portion of the study.

TABLE 1 Baseline Characteristics of the Study Population

	Subjects (n = 21)
Age (yrs)	67.5 ± 14.3
Female	4 (19)
Heart rate (beats/min)	62.7 ± 5.9
Blood pressure (mm Hg)	
Systolic	124.7 ± 16.6
Diastolic	71.6 ± 9.3
Body weight (kg)	87.5 ± 14.7
Body mass index (kg/m^2)	29.1 ± 5.0
GFR (ml/min)	66.4 ± 12.0
Diabetes mellitus	3 (14)
Coronary artery disease	14 (67)
Myocardial infarction	7 (33)
Hypertension	9 (43)
ACEI or ARB	18 (86)
Beta-blocker	19 (90)
Thiazide diuretic agents	7 (33)
LV ejection fraction (%)	40.3 ± 8.9
LV end-systolic volume (ml)	122.4 ± 42.2
LV end-diastolic volume (ml)	199.7 ± 48.4
LV end-systolic diameter (cm)	4.4 ± 0.5
LV end-diastolic diameter (cm)	5.7 ± 0.4
LA volume index (ml/m^2)	82.1 ± 21.1
RV systolic pressure (mm Hg)	28.0 ± 5.4
E/e′ (medial)	14.7 ± 9.1
ANP (pg/ml)	71.4 ± 45.4
BNP (pg/ml)	146.5 ± 107.1
Aldosterone (ng/dl)	3.7 ± 2.7
Angiotensin II (pg/ml)	3.3 ± 2.3

Values are mean ± SD or n (%).

ACEI = angiotensin-converting enzyme inhibitor; ANP = atrial natriuretic peptide; ARB = angiotensin receptor blocker; BNP = B-type natriuretic peptide; cGMP = cyclic guanosine monophosphate; E/e′ = E velocity/e′ velocity; GFR = glomerular filtration rate; LA = left atrium; LV = left ventricular; RV = right ventricular.

NEUROHORMONAL, ELECTROLYTE, AND RENAL ASSESSMENT. Plasma atrial natriuretic peptide (ANP), BNP, aldosterone, angiotensin II, and urine cGMP were measured by radioimmunoassay as previously described (8). Plasma and urine concentration of iothalamate and para-amino-hippurate, as well as creatinine, were measured by the Mayo Core Renal laboratory.

STATISTICAL METHODS. Continuous variables are presented as mean ± SD and discrete variables as frequency (proportion). Comparisons between the 2 treatment groups (tadalafil and SC placebo, and tadalafil and SC BNP) were made using the Student's *t*-test for normally distributed continuous variables, the rank-sum test for continuous variables with a skewed distribution, and the Pearson chi-square test for independence of categorical variables. Comparisons within groups (between visit 1 and visit 2) were made using a paired Student's t-test. The relationship between continuous variables was assessed using Pearson correlation coefficients. For all analyses, statistical significance was accepted as $p < 0.05$. Statistical analyses were completed with SAS 9.4 (SAS Institute Inc, Cary, North Carolina).

RESULTS

Baseline characteristics of the study population before acute VE are shown in Table 1. The proportion of women in the study population was 19%, and the average body mass index was 29.1 ± 5.0 kg/m^2. The prevalence of known coronary artery disease and previous myocardial infarction were 67% and 33%, respectively. Hypertension was present in 43% of subjects, and 86% were taking an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, 90% a beta blocker, and 33% a thiazide diuretic. Echocardiographic parameters showed that the average LV ejection fraction (LVEF) was 40.3 ± 8.9%, LV end-diastolic volume (LVEDV) was 199.7 ± 48.4 ml, medial E/e′ was 14.7 ± 9.1, and right ventricular systolic pressure was 28.0 ± 5.4 mmHg. Although plasma ANP and BNP were mildly elevated, plasma aldosterone and angiotensin II levels were within the normal range, which is consistent with PSD (12).

RESPONSE TO ACUTE VOLUME EXPANSION. Tadalafil and SC placebo. Subjects randomized to receive tadalafil and SC placebo had no significant change in systolic blood pressure or heart rate with acute saline VE compared with baseline (Table 2). In response to VE, pre-treatment with tadalafil and SC placebo resulted in increased LVEDV (209.8 ml vs. 196.1 ml; $p = 0.006$) and right ventricular systolic pressure (30.6 mm Hg vs. 28.1 mm Hg; $p = 0.037$), with no change in LVEF (39.4% vs. 38.9%; $p = 0.551$). With VE, ANP and plasma cGMP increased, aldosterone decreased, whereas BNP and angiotensin II remained unchanged. Renal response to VE, as assessed by renal plasma flow (RPF) (364.1 ml/min vs. 302.6 ml/min; $p = 0.023$), urine flow (7.1 vs. 4.4 ml/min; $p < 0.001$), and sodium excretion (245.1 mEq/min vs. 159.0 mEq/min; $p < 0.001$), was higher than baseline in the subjects pre-treated with tadalafil and SC placebo. GFR tended to be higher (82.6 ml/min vs. 72.7 ml/min; $p = 0.081$) in response to VE.

Tadalafil and SC BNP. Subjects treated with tadalafil and SC BNP had lower systolic blood pressure (112.4 mm Hg vs. 124.7 mm Hg; $p < 0.001$) and higher heart rate (62.4 beats/min vs. 58.9 beats/min;

TABLE 2 Clinical Outcomes in Tadalafil and SC Placebo Versus Tadalafil and SC BNP at Baseline and After Normal Saline VE

	Tadalafil and SC Placebo			Tadalafil and SC BNP		
Variable	Baseline (n = 21)	VE (n = 21)	p Value (baseline vs. VE)	Baseline (n = 21)	VE (n = 21)	p Value (baseline vs. VE)
Systolic BP (mm Hg)	120.9 ± 18.3	118.3 ± 14.4	0.248	124.7 ± 14.2	112.4 ± 13.7	<0.001
Diastolic BP (mm Hg)	68.4 ± 10.0	62.9 ± 8.6	0.007	69.3 ± 8.9	60.3 ± 8.3	<0.001
Heart rate (beats/min)	59.6 ± 8.6	61.4 ± 9.0	0.235	58.9 ± 8.4	62.4 ± 9.2	0.018
GFR (ml/min)	72.7 ± 33.6	82.6 ± 24.2	0.081	79.2 ± 38.3	67.9 ± 32.8	0.015
Renal plasma flow (ml/min)	302.6 ± 152.3	364.1 ± 122.8	0.023	333.9 ± 158.7	258.9 ± 133.2	0.022
Urine flow (ml/min)	4.4 ± 2.7	7.1 ± 2.8	<0.001	5.5 ± 3.3	5.3 ± 4.0	0.842
Sodium excretion (mEq/min)′	159.0 ±78.8	245.1 ± 112.7	<0.001	204.4 ± 134.2	246.2 ± 130.5	0.250
Urinary cGMP excretion (pmol/min)	796.4 ± 428.9	824.9 ± 506.2	0.671	852.9 ± 481.7	3,419.8 ± 1,993.9	<0.001
LVEF (%)	38.9 ± 7.7	39.4 ± 7.6	0.551	38.8 ± 9.0	43.1 ± 7.8	<0.001
LV end-diastolic volume (ml)	196.1 ± 46.3	209.8 ± 49.0	0.006	199.1 ± 47.0	177.2 ± 46.1	0.005
LV end-systolic volume (ml)	119.7 ± 37.5	122.3 ± 39.1	0.625	127.6 ± 39.7	101.1 ± 37.0	<0.001
Cardiac output (l/min)	4.6 ± 0.8	5.1 ± 0.8	<0.001	4.6 ± 1.0	5.0 ± 1.0	0.095
E/e′	15.0 ± 7.7	14.3 ± 6.7	0.144	14.5 ± 8.6	12.8 ± 5.6	0.275
RVSP (mm Hg)	28.1 ± 7.9	30.6 ± 7.0	0.037	27.8 ± 5.7	26.8 ± 9.8	0.546
LAVI (ml/m^2)	85.8 ± 26.6	92.2 ± 23.6	0.112	86.2 ± 14.7	76.4 ± 16.9	0.017
ANP (pg/ml)	92.9 ± 81.5	113.1 ± 100.7	0.044	71.4 ± 45.4	56.0 ± 38.5	0.079
BNP (pg/ml)	144.5 ± 97.5	151.8 ± 112.5	0.208	146.5 ± 107.1	1297.5 ± 1380.9	<0.001
cGMP (pmol/ml)	4.0 ± 2.1	5.4 ± 3.3	0.014	3.9 ± 2.0	18.9 ± 13.6	<0.001
Aldosterone (ng/dl)	4.6 ± 3.3	2.7 ± 1.0	0.005	3.7 ± 2.7	3.5 ± 1.8	0.850
Angiotensin II (pg/ml)	3.8 ± 3.8	3.3 ± 2.4	0.528	3.3 ± 2.3	3.8 ± 2.3	0.248

Values are mean ± SD.

BP = blood pressure; LAVI = left atrial volume index; LVEF = left ventricular ejection fraction; RVSP = right ventricular systolic pressure; SC = subcutaneous; VE = volume expansion; other abbreviations as in Table 1.

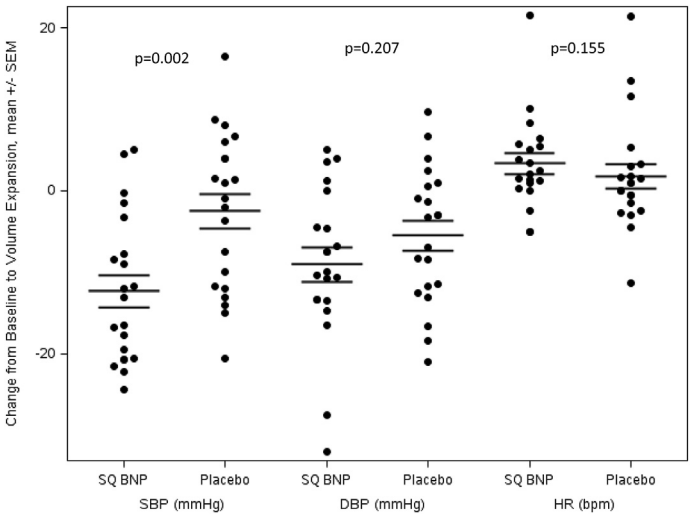
p = 0.018) with VE compared with baseline (Table 2). Pre-treatment with tadalafil and SC BNP resulted in increased LVEF (43.1% vs. 38.8%; p < 0.001) and decreased LVEDV (177.2 ml vs. 199.1 ml; p = 0.005), left ventricular end-systolic volume (LVESV) (101.1 ml vs. 127.6 ml; p < 0.001), and left atrial volume index (LAVI) (76.4 ml/m^2 vs. 86.2 ml/m^2; p = 0.017). Plasma cGMP increased with VE (18.9 pmol/ml vs. 3.9 pmol/ml; p < 0.001), whereas ANP, aldosterone, and angiotensin II remained unchanged. There was a decrease in RPF (258.9 ml/min vs. 333.9 ml/min; p = 0.022) and GFR (67.9 ml/min vs. 79.2 ml/min; p = 0.015) with VE. Pre-treatment with tadalafil and SC BNP resulted in no change in urine flow or sodium excretion in response to VE.

TADALAFIL AND SC BNP VERSUS TADALAFIL AND SC PLACEBO. Hemodynamic parameters. Pre-treatment with tadalafil and SC BNP resulted in a greater reduction in systolic blood pressure from baseline to VE (−12.3 ± 9.0 mm Hg vs. −2.5 ± 9.7 mm Hg; p = 0.002), with trends for greater reduction in diastolic pressure (−9.0 ± 9.4 mm Hg vs. −5.5 ± 8.3 mm Hg; p = 0.207) and a greater increase in heart rate (3.3 ± 5.8 mm Hg vs. 1.8 ± 6.8 mm Hg; p = 0.155) compared with tadalafil and SC placebo (Figure 2).

Echocardiographic parameters. Changes in echocardiographic parameters and pre-treatment with tadalafil and SC BNP versus tadalafil and SC placebo with VE response are shown in Figure 3. With VE, subjects randomized to tadalafil and SC BNP versus tadalafil and SC placebo had a greater increase in LVEF (5.8 ± 4.7% vs. 0.6 ± 4.3%; p = 0.002), with decreases in LAVI (−8.1 ± 12.1 ml/m^2 vs. 5.8 ± 14.1 ml/m^2; p = 0.005), LVEDV (−19.9 ± 24.5 ml vs. 12.5 ± 17.4 ml; p < 0.001), and LV end-systolic volume (LVESV) (−24.6 ± 21.1 ml vs. 1.4 ± 12.5 ml; p < 0.001). With VE, subjects randomized to tadalafil and SC BNP had a reduction in right ventricular systolic pressure (−1.3 ± 6.8 mm Hg vs. 2.3 ± 3.1 mm Hg; p = 0.130) compared with tadalafil and SC placebo, but this did not reach statistical significance.

Renal neurohormonal and physiological parameters. Tadalafil and SC BNP, compared with tadalafil and SC placebo, resulted in a decrease in urine flow with VE (−0.2 ± 4.6 ml/min vs. 2.6 ± 1.8 ml/min; p = 0.015), GFR (−12.7 ± 21.2 ml/min/1.73 m^2 vs. 9.4 ± 22.7 ml/min/1.73 m^2; p = 0.003), and RPF (−69.1 ± 124 ml/min vs. 54.5 ± 98.3 ml/min; p = 0.001). A higher urinary cGMP excretion response to VE was observed in the tadalafil and SC BNP versus tadalafil

FIGURE 2 Blood Pressure and Heart Rate Response to VE in Tadalafil and SC Placebo Versus Tadalafil and SC BNP

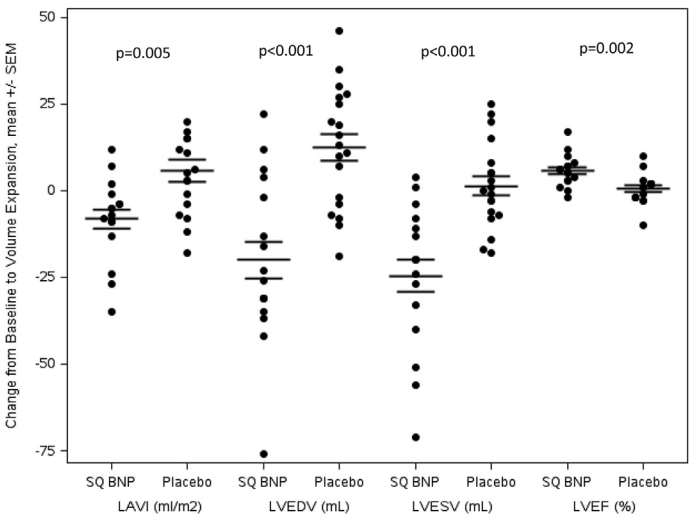


DBP = diastolic blood pressure; HR = heart rate; SBP = systolic blood pressure; VE = volume expansion; other abbreviations as in Figure 1.

and SC placebo group (2,567 ± 1,888 ml/min vs. 34 ± 355 ml/min; p < 0.001) (Figure 4).

With VE, higher plasma cGMP levels were observed with tadalafil and SC BNP versus tadalafil and SC placebo groups (15.3 ± 13.2 pmol/ml vs. 1.4 ± 2.4 pmol/ml; p < 0.001) (Table 3). ANP was decreased in the tadalafil and SC BNP versus tadalafil and SC placebo group (−15.1 ± 35.3 pg/ml vs. 20.3 ± 40.8 pg/ml; p = 0.007). There was no significant change in aldosterone and angiotensin II levels in response to VE in both groups (Table 3).

FIGURE 3 Echocardiographic Parameters and Response to VE in Tadalafil and SC Placebo Versus Tadalafil and SC BNP



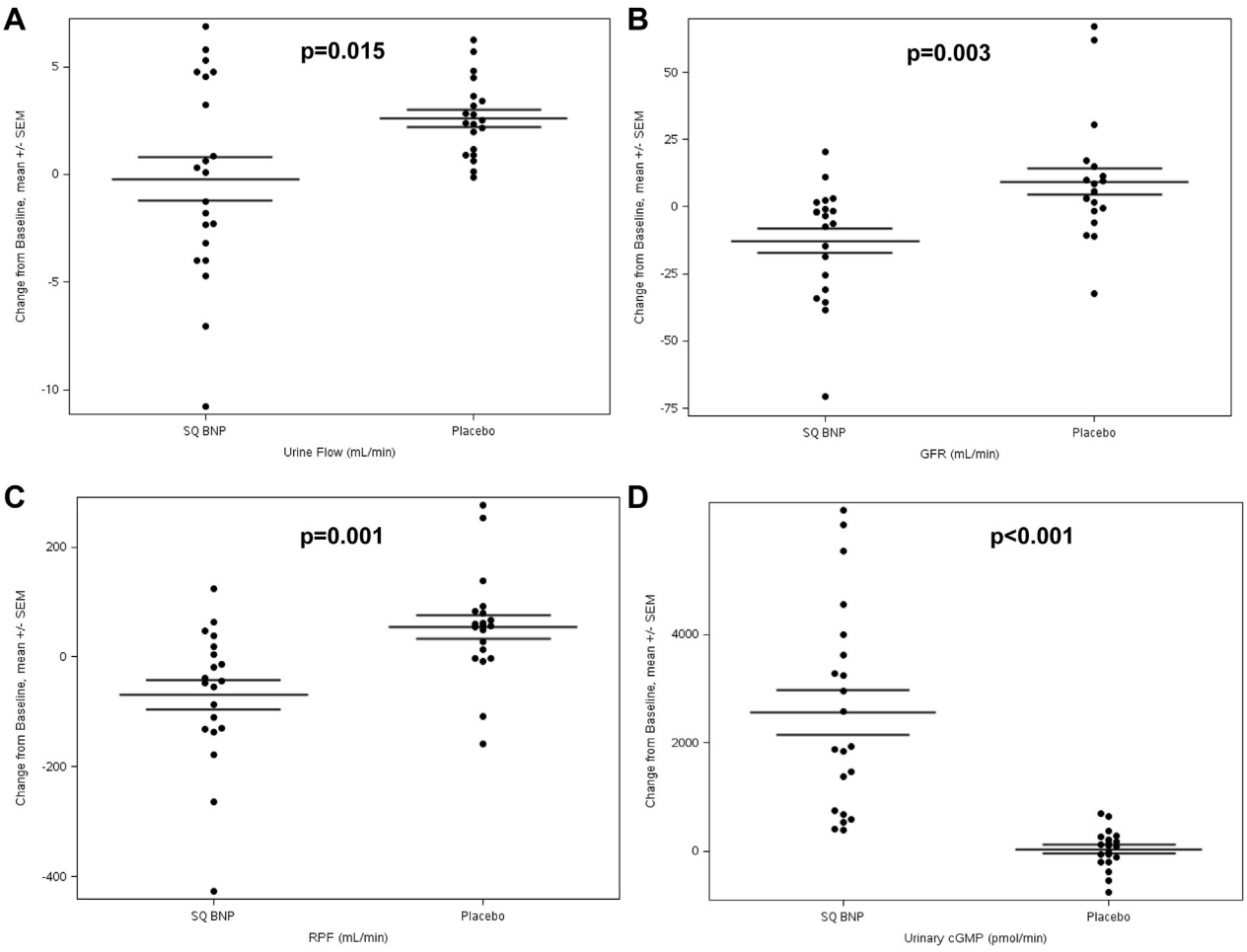
LAVI = left atrial volume index; LVEDV = left ventricular end-diastolic volume; LVESV = left ventricular end-systolic volume; LVEF = left ventricular ejection fraction; other abbreviations as in Figure 2.

Subgroup analysis (eGFR < 60 ml/min vs. eGFR ≥ 60 ml/min). We performed subgroup analyses for estimated GFR <60 ml/min versus estimated GFR ≥60 ml/min for blood pressure, echocardiographic parameters (including LAVI, LVEDV, LVESV, and EF), and renal parameters (including urine flow, RPF, and urinary cGMP). The results compared the tadalafil and SC placebo versus tadalafil and SC BNP subgroups. The additional analyses demonstrated that those with estimated GFR ≥60 ml/min had statistically worsened renal outcomes (urine flow, GFR, RPF) in the BNP versus placebo groups. Among those with an estimated GFR <60 ml/min, there was no statistically significant difference. However, because the sample size in this subgroup was small (n = 4 in each group) and there did appear to be a trend for worsened renal outcomes in the BNP versus placebo groups, as demonstrated by GFR and RPF, we could not exclude the possibility of an association between BNP and worsened renal outcomes among those with a reduced estimated GFR. Further investigation with a larger population is necessary to determine susceptibility to deterioration of renal function when BNP is combined with tadalafil (Supplemental Table 2).

We also evaluated for a correlation between baseline GFR and outcomes. There was a negative correlation between baseline GFR and RPF, and a trend toward negative correlation for urine flow and GFR (p = 0.05 as cutoff for statistical significance) (Supplemental Table 3).

Correlation between change in blood pressure and renal outcomes. We conducted additional analyses in which the change in BP was plotted against the renal parameters (including urine flow, GFR, RPF, and urinary cGMP) in each treatment group to determine the degree with which BP reduction contributed to the results. In both the placebo and BNP groups, there was no significant association between change in blood pressure and renal outcomes. However, this was likely due to the small sample size, and therefore, further investigation with a larger population is needed to determine if greater reduction in blood pressure from a combination of BNP and PDEV inhibition may be related to worse renal outcomes (Supplemental Figure 1).

FIGURE 4 Renal Parameters and Response to VE in Tadalafil and SC Placebo Versus Tadalafil and SC BNP



(A) Urine flow. **(B)** GFR. **(C)** Renal plasma flow (RPF). **(D)** Urinary cyclic guanosine monophosphate (cGMP). Other abbreviations as in Figures 1 and 2.

ADVERSE EVENTS. None of the subjects who received tadalafil and SC placebo had adverse events. In the treatment group, after administration of tadalafil and SC BNP, 2 subjects (10%) experienced nausea and/or vomiting on the first morning of the study, 1 (5%) had transient chest discomfort that resolved spontaneously, and 1 (5%) had hypotension that subsequently resolved with saline infusion.

DISCUSSION

The present study was the first-in-human study to define the acute cardiorenal effects of combination tadalafil and SC BNP in response to VE in subjects with PSD and renal dysfunction. Based on our preclinical studies, we hypothesized that the combination of tadalafil and SC BNP would enhance the cardiorenal response to VE in subjects with PSD with renal dysfunction versus tadalafil alone. In this cohort of subjects with PSD and renal dysfunction, pre-treatment with tadalafil and SC BNP before VE resulted in improved cardiac function compared

TABLE 3 Humoral Outcomes in Tadalafil and SC Placebo Versus Tadalafil and SC BNP, Change From Baseline to After Normal Saline VE

Variable	Tadalafil and SC Placebo (n = 21)	Tadalafil and SC BNP (n = 21)	p Value
ANP (pg/ml)	20.3 ± 40.8	−15.1 ± 35.3	0.007
BNP (pg/ml)	7.3 ± 25.1	1,201.7 ± 1,382.1	<0.001
cGMP (pmol/ml)	1.4 ± 2.4	15.3 ± 13.2	<0.001
Aldosterone (ng/dl)	−1.8 ± 2.7	−0.1 ± 3.2	0.073
Angiotensin II (pg/ml)	−0.5 ± 3.7	0.6 ± 2.0	0.108

Values are mean ± SD.
Abbreviations as in Tables 1 and 2.

with tadalafil and SC placebo, as evidenced by a greater increase in LVEF and reduction in LAVI, LVESV, and LVEDV. Plasma ANP was decreased in the tadalafil and SC BNP versus tadalafil and SC placebo group, which suggested a decrease in cardiac filling pressure in response to VE in the tadalafil and SC BNP group. Pre-treatment with tadalafil and SC BNP before acute VE resulted in increased cGMP levels. However, the favorable cardiac response to VE with tadalafil and SC BNP was associated with an attenuated renal response. Specifically, renal responses to acute VE after pre-treatment with tadalafil and SC BNP, including RPF, GFR, and urine flow, were reduced compared with tadalafil and SC placebo.

PSD, or asymptomatic LV dysfunction, is considered ACC/AHA stage B HF (13). Although the exact definition of LV systolic dysfunction varies in different trials, it is generally defined as LVEF <40% to 50%. It is a common entity that affects approximately 3% to 6% of the general adult population of the United States, and it is more common among men and those with cardiovascular comorbidities (e.g., coronary artery disease and hypertension) (2,14). PSD is associated with increased mortality and cardiovascular events, and the annual incidence of progression from PSD to symptomatic HF is approximately 5% to 20% (14–16). Neurohormonal activation plays an important role in the progression along the HF spectrum. The neurohumoral substudy of the SOLVD (Studies of Left Ventricular Dysfunction) cohort demonstrated a significant increase in neurohumoral activation in the symptomatic cohort compared with the prevention cohort (i.e., PSD) (12). The neurohumoral profile of the present cohort was similar to those in the SOLVD prevention cohort, with a mild activation of the NP system without activation of the renin angiotensin aldosterone system.

Impaired sodium excretion to sodium load is a pathognomonic feature of clinical congestive HF (17). With normal physiology, when renal perfusion and sodium delivery to the kidneys is reduced, renin is released, which eventually stimulates aldosterone, which results in sodium retention. Those with symptomatic HF have excessive renin-angiotensin-aldosterone activation, and subsequent salt retention and inability to excrete sodium load, which leads to increased intra- and extravascular volume (18). This leads to typical HF symptoms, including dyspnea and edema. We previously reported that there is impaired renal response to VE in patients with PSD compared with normal subjects (8). Renal cGMP activation was paradoxically decreased with attenuated urinary sodium excretion in subjects with PSD when exposed to acute VE compared with normal subjects. However, when exogenous SC BNP was administered before acute VE, the patients with PSD experienced an increase in urinary cGMP and natriuresis similar to subjects without underlying cardiac or renal dysfunction (19,20). Potential mechanisms for impaired renal response to acute VE in patients with PSD include decreased plasma ANP levels, upregulation of PDEV that leads to greater degradation of cGMP, down-regulation of NPs in the kidney, and upregulation of neutral endopeptidases, which leads to disruption of the homeostasis of NPs with downstream effects on the renal system.

Phosphodiesterase-5 is ubiquitous in the body, and PDEV inhibitors are currently approved by the Food and Drug Administration (FDA) for the management of erectile dysfunction and pulmonary hypertension. PDEV metabolizes cGMP, a second messenger that leads to vascular smooth muscle relaxation and subsequent vasodilatation. Renal cGMP plays an important role in modulating GFR and natriuresis. The effects of long-term PDEV inhibition in experimental HF and its influence on enhancing the renal actions of exogenous BNP with maximizing the cGMP system in an animal model have been previously described (9). The renal effect of BNP and PDEV inhibition were synergistic compared with BNP monotherapy, which suggested that PDEV upregulation might contribute to NP desensitization. Thus, there might be a role for combined treatment with PDEV inhibitors and BNP to maximize the benefits of endogenous and exogenous NPs.

The present study was the first-in-human study to define the acute biological effects of combination tadalafil and BNP on the cardiorenal response to VE in PSD. There was a significant improvement in cardiac function in response to acute VE with combination tadalafil and BNP administration in subjects with PSD and renal dysfunction. However, there was a worsening of renal function that might have been due to the decrease in blood pressure and RPF observed with combination tadalafil with BNP. With decreases in renal perfusion pressure, sodium delivery in the descending loop of Henle decreases, which results in greater sodium reabsorption and reduced sodium excretion (21). Although autoregulatory mechanisms in the kidney attempts to maintain a relatively constant GFR with higher renal artery blood pressures, GFR reduces when the renal vasodilatory and vasoconstrictive homeostatic mechanisms are overwhelmed (22).

Clinical and therapeutic implications include modulation of the NP system and its potential beneficial cardiac and renal effects. Sacubitril/valsartan, which has been approved for the treatment of HF with reduced EF, exerts its action in part via neprilysin inhibition and potentiation of the NP pathway (23). By decreasing NP degradation, there are important outcome benefits for patients with systolic dysfunction and HF. In addition, different patient populations may have different endogenous levels of NPs; therefore, therapeutic options should focus on both inhibition of NP degradation and exogenous administration of NPs (24,25).

Given the findings of this study regarding blood pressure reduction and effects on renal parameters, we believe further investigation is necessary with different doses of PDEV inhibitors and subcutaneous BNP to determine if there may be beneficial cardiac and renal effects at different doses. Furthermore, additional investigation is necessary in unique populations, such as the hypertensive population, to see if there may be a clinical benefit.

In summary, the results demonstrated that in human subjects with PSD and renal dysfunction, combination tadalafil and BNP compared with tadalafil alone resulted in cardiac function improvement but reduced renal function with acute VE.

STUDY LIMITATIONS. A limitation of this study was that only 1 dose of tadalafil and SC BNP was tested. Future studies are needed to further evaluate the effects of lower, non-hypotensive doses of tadalafil and SC BNP. The study also assessed acute physiological derangements, and hence, it might not reflect the long-term effects of tadalafil and SC BNP on cardiorenal function and the neurohormonal effects in PSD.

CONCLUSIONS

In this first-in-human study of subjects with PSD and renal dysfunction, pre-treatment with tadalafil and BNP compared with tadalafil alone resulted in improved cardiac function but worsened renal function with VE. Combination tadalafil and BNP demonstrated potentiation of the cGMP pathway, with increased plasma and urinary cGMP levels. However, combination tadalafil and BNP also led to decreased blood pressures and reduced renal perfusion, which might explain why there was worsened renal function. There was a differential cardiac versus renal response to VE with combination of tadalafil and BNP in subjects with PSD and renal dysfunction.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: In PSD, which is defined as systolic dysfunction in the absence of signs and symptoms of HF, inhibition of PDEV in combination with exogenous BNP administration resulted in improved cardiac function but worsened renal function in response to acute VE.

TRANSLATIONAL OUTLOOK: Understanding the cardiorenal effects and outcomes of combination PDEV with exogenous BNP may impact the clinical management of patients with PSD and renal dysfunction.

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APPENDIX For supplemental figures and tables, please see the online version of this paper.