

# Novel Protein Therapeutics for Systolic Heart Failure

## Chronic Subcutaneous B-Type Natriuretic Peptide

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### Objectives

The purpose of the present study was to translate our laboratory investigations to establish safety and efficacy of 8 weeks of chronic SC B-type natriuretic peptide (BNP) administration in human Stage C heart failure (HF).

### Background

B-Type natriuretic peptide is a cardiac hormone with vasodilating, natriuretic, renin-angiotensin inhibiting, and lusitropic properties. We have previously demonstrated that chronic cardiac hormone replacement with subcutaneous (SC) administration of BNP in experimental HF resulted in improved cardiovascular function.

### Methods

We performed a randomized double-blind placebo-controlled proof of concept study comparing 8 weeks of SC BNP (10  $\mu\text{g}/\text{kg}$  bid) ( $n = 20$ ) with placebo ( $n = 20$ ) in patients with ejection fraction  $<35\%$  and New York Heart Association functional class II to III HF. Primary outcomes were left ventricular (LV) volumes and LV mass determined by cardiac magnetic resonance imaging. Secondary outcomes include LV filling pressure by Doppler echo, humoral function, and renal function.

### Results

Eight weeks of chronic SC BNP resulted in a greater reduction of LV systolic and diastolic volume index and LV mass index as compared with placebo. There was a significantly greater improvement of Minnesota Living with Heart Failure score, LV filling pressure as demonstrated by the reductions of E/e' ratio, and decrease in left atrial volume index as compared with placebo. Glomerular filtration rate was preserved with SC BNP, as was the ability to activate plasma 3',5'-cyclic guanosine monophosphate ( $p < 0.05$  vs. placebo).

### Conclusions

In this pilot proof of concept study, chronic protein therapy with SC BNP improved LV remodeling, LV filling pressure, and Minnesota Living with Heart Failure score in patients with stable systolic HF on optimal therapy. Renin-angiotensin was suppressed, and glomerular filtration rate was preserved. Subcutaneous BNP represents a novel, safe, and efficacious protein therapeutic strategy in human HF. Further studies are warranted to determine whether these physiologic observations can be translated into improved clinical outcomes and ultimately delay the progression of HF. (Cardiac Hormone Replacement With BNP in Heart Failure: A Novel Therapeutic Strategy; NCT00252187) (J Am Coll Cardiol 2012;60:2305-12) © 2012 by the American College of Cardiology Foundation

The American Heart Association/American College of Cardiology classification of heart failure (HF) Stage A to D emphasizes the need to develop therapeutic strategies to prevent the progression of HF (1). B-type natriuretic peptide (BNP) is a 32-amino acid peptide produced mainly

by cardiomyocytes and plays an important role in cardiorenal homeostasis. Specifically, BNP binds to the natriuretic peptide-A receptor (NPR-A), which via 3',5'-cyclic guanosine monophosphate (cGMP) mediates natriuresis; vasodilation; renin-angiotensin-aldosterone inhibition; and anti-hypertrophic, anti-fibrotic, and positive lusitropic properties (2). Despite activation of both plasma and cardiac BNP in HF, exogenous administration of BNP resulted in activation of its second messenger cGMP with favorable cardiac unloading actions (3). In both experimental and

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human HF, investigations have supported the hypothesis that the synthetic capacity of the cardiomyocytes might be overwhelmed in severe HF relative to the demands of the system, leading to a state of relative deficiency of BNP (4).

# Abbreviations and Acronyms

<b>BNP</b>	= B-type natriuretic peptide
<b>cGMP</b>	= 3',5'-cyclic guanosine monophosphate
<b>CRU</b>	= Clinic Research Unit
<b>GFR</b>	= glomerular filtration rate
<b>LA</b>	= left atrium/atrial
<b>LV</b>	= left ventricle/ventricular
<b>LVEF</b>	= left ventricular ejection fraction
<b>MI</b>	= myocardial infarction
<b>MLHF</b>	= Minnesota Living with Heart Failure score
<b>MRI</b>	= magnetic resonance imaging
<b>NYHA</b>	= New York Heart Association
<b>SC</b>	= subcutaneous

Recent investigations have demonstrated that, in severe HF, despite high levels of immunoreactive BNP detected in the plasma, there was very little biologically active mature BNP<sub>1–32</sub>—suggesting the presence of abnormal molecular forms with reduced biological actions (5–7).

Intravenous infusion of human BNP (Nesiritide) is approved by the U.S. Food and Drug Administration for the inpatient management of acute decompensated congestive heart failure (3). The FUSION I and II studies (Follow-Up Serial Infusions of Nesiritide in Advanced Heart Failure) evaluated the use of outpatient serial intravenous infusions of BNP in patients with Stage D HF (8,9). Most recently, findings of the 7,000-patient ASCEND Trial (Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure) reported that 1- to 7-day infusion of BNP

resting left ventricular ejection fraction (LVEF) of 35% or less and stable mild symptoms of HF (NYHA functional class II and III). Subjects were admitted to the Clinic Research Unit (CRU) at St. Mary's Hospital, Mayo Clinic CTSA, Rochester, Minnesota, the afternoon before the scheduled active renal clearance study day. Cardiac MRI was performed, and the 6-min walk and Minnesota Living with Heart Failure (MLHF) Questionnaire was assessed. On the following morning, the patients underwent a short renal clearance test with Lothalamate Meglumine (Conray 60%) given subcutaneously to measure the glomerular filtration rate (GFR). Echocardiography was performed for determination of baseline LV filling pressure. Venous blood samples were also collected for determination of BNP, cGMP, and plasma renin activity. At the completion of the short renal clearance test, the patients were randomized to the SC BNP or placebo group. The patients were then instructed about the proper technique for diluting the BNP or placebo, and the SC injection was given in the anterior abdominal wall. Thirty minutes after the first dose, a venous blood sample for determination of BNP and cGMP was drawn. The patients then self-administered the second dose of SC BNP or placebo 12 h after the first dose. Subsequently, the third dose was self-administered under supervision 12 h later. The patients were then dismissed with instructions and enough supplies for 8 weeks of SC administration twice/day.

Participants who experienced symptomatic hypotension as defined by systolic BP <85 mm Hg or had symptoms of lightheadedness or visual disturbances during the baseline visit in the CRU were discontinued from the study.

At the end of the 8-week study period,  $\pm 3$  days, the patients were admitted to the CRU in the afternoon before the final renal clearance study. In our previous pilot dose finding study (12), we demonstrated that the blood pressure returned to baseline 2 h after the administration of SC BNP. Hence, in the current study, patients received their self-administered SC injection at least 2 h before the cardiac MRI, 6 min walk, and MLHF Questionnaire assessment. The next morning, echocardiography was performed, after which, a short renal clearance test and humeral determination were carried out before and after a final dose of SC injection in the same manner as the baseline study. The randomization table was provided by the statistician, and it was created to allow for a final  $n = 20$  in each group with completed primary endpoint data.

**Exclusion criteria specification.** Exclusion criteria include: myocardial infarction (MI) within 3 months of screening; unstable angina within 14 days of screening or any evidence of myocardial ischemia; valvular stenosis, hypertrophic, restrictive, or obstructive cardiomyopathy, constrictive pericarditis, primary pulmonary hypertension, or biopsy proven active myocarditis; sustained ventricular tachycardia or ventricular fibrillation within 14 days of screening; persistent atrial fibrillation; second- or third-degree atrioventricular block without a permanent cardiac

was safe with no adverse renal or mortality properties and was associated with modest improvement in dyspnea (10).

We have previously reported that subcutaneous (SC) administration of BNP in experimental HF resulted in improved cardiac output with reduced systemic vascular resistance and cardiac filling pressures (11). Importantly, we translated these studies to humans and completed a dosing finding study that reported that acute SC administration of BNP at a dose of 10  $\mu\text{g}/\text{kg}$  in human Stage C HF resulted in increased plasma BNP and cGMP and urinary cGMP excretion, associated with favorable cardiorenal and humoral responses (12).

The objective of the present study was to further translate our laboratory investigations to establish safety and efficacy of 8 weeks of chronic SC BNP administration in human Stage C HF.

## Methods

We performed a randomized double-blind placebo-controlled proof of concept study comparing 8 weeks of SC BNP (10  $\mu\text{g}/\text{kg}$ ) with SC placebo twice/day, in patients with ejection fraction (EF) <35% and New York Heart Association (NYHA) functional class II to III stable HF. Primary outcomes were left ventricular (LV) volumes and LV mass determined by cardiac magnetic resonance imaging (MRI). Secondary outcomes include LV filling pressure by Doppler echo, humoral function, and renal function. The protocol was approved by the Mayo Clinic Institutional Review Board, and informed consent was obtained from all participants.

**Study protocol.** From May 2003 to May 2008, recruitment was limited to subjects 18 years of age and above with a

pacemaker; cardiovascular accident within 3 months of screening or other evidence of significantly compromised central nervous system perfusion; total bilirubin of  $>1.5$  mg/dl or an aspartate transaminase and alanine transaminase  $1.5\times$  the upper limit of normal; serum creatinine of  $>3.0$  mg/dl; serum sodium of  $<125$  mEq/dl or  $>160$  mEq/dl; serum potassium of  $<3.5$  mEq/dl or  $>5.0$  mEq/dl; serum digoxin level of  $>2.0$  ng/ml; systolic pressure of  $<85$  mm Hg; LVEF  $>35\%$  within 24 months of screening; unable to self-administer SC injection twice/day; diagnosed with acquired immunodeficiency syndrome or known positive human immunodeficiency virus titer; other acute or chronic medical conditions or laboratory abnormality that might increase the risks associated with study participation or might interfere with interpretation of the data; received an investigational drug within 1 month before dosing; unable to undergo cardiac MRI; in the opinion of the investigator, is unlikely to comply with the study protocol or is unsuitable for any reasons.

**Analytic methods.** The concentration of iothalamate in the plasma and urine collected during the short renal clearance test was determined with capillary electrophoresis in the Mayo Renal laboratory for the determination of GFR. Specific plasma radioimmunoassays, including BNP, cGMP, and renin, were performed on the basis of previously published methods by the co-investigator (12).

**Assessment of LV filling pressure and left atrium volume by echocardiography.** Pulsed wave Doppler examination of mitral (before and with Valsalva maneuver) and pulmonary venous inflow as well as Doppler tissue imaging of the mitral annulus were performed. Left atrial (LA) volume was calculated from 2-dimensional measurements and was indexed to body surface area as previously described (13).

**Cardiac MRI.** Patients underwent cardiac MRI for measurement of LVEF, LV end diastolic volume, LV end systolic volume, and LV mass. All examinations were performed with a 1.5-T MRI system (Signa Twin Speed Excite, GE Healthcare, Waukesha, Wisconsin). After initial localization scans, short-axis cine images were obtained at 8-mm intervals from base to apex with gated, balanced steady state electrocardiography. Two-chamber, 3-chamber, and 4-chamber cine balanced steady-state free precession acquisitions were then acquired with the short-axis images for prescription. Images were transferred to a workstation (GE Advantage Windows, GE Healthcare), and a software analysis package (Mass Analysis, version 6, Medis, Leiden, the Netherlands) was used to trace endocardial and epicardial contours around each end-diastolic slice, and endocardial contours around each end-systolic slice. The EF, end-systolic volume, end-diastolic volume, and LV mass were then computed by adding the contribution of the individual slices. All cardiac MR images were reviewed in a blinded fashion (J.F.G.).

**Data analysis.** Continuous measurements that are normally distributed are expressed as mean  $\pm$  SD, and those that are skewed are expressed as median and quartiles, whereas cate-

gorical variables are reported as a number (percentage). Chi-square tests or Fisher exact tests were used to compare categorical variables between BNP group and placebo group, whereas 2-sample *t* tests or rank sum tests were used to compare the continuous variables between the 2 groups. Comparisons from baseline to 8 weeks within groups were completed with paired *t* tests for normally distributed variables and signed-rank tests for non-normal differences. A *p* value  $<0.05$  was considered significant in this study.

## Results

**Participants.** We randomized 45 patients to receive SC BNP ( $n = 24$ ) or SC placebo ( $n = 21$ ) injections twice/day for 8 weeks. Three subjects in the BNP group developed symptomatic hypotension as defined by systolic BP  $<85$  mm Hg or had symptoms of lightheadedness or visual disturbances during the baseline visit in the CRU and were excluded from the study as per protocol. One subject in the BNP group withdrew from the study for personal reasons. One subject in the placebo group had worsening HF symptoms during the 8-week period and withdrew from the study. Hence, in the final analysis, we had  $n = 20$  in the BNP group and  $n = 20$  in the placebo group.

The baseline demographic and clinical characteristics between the study groups are similar, except for a higher prevalence of history of hypertension in the placebo group as compared with the BNP group (Table 1). However, despite this difference, the baseline systolic and diastolic blood pressure and heart rate were similar between the 2 groups. The mean EF, plasma BNP levels, and distribution of NYHA functional class II versus class III patients were similar between the placebo and BNP groups. Most (95%) patients were treated with an angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker or nitrates, in combination with a beta-blocker and a diuretic. Approximately 20% were also treated with spironolactone in each group. Eighty-five percent of the subjects in the BNP group and 80% of the subjects in the placebo group were receiving target doses of angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker/nitrates, whereas 80% of the subjects in the BNP group and 85% of the subjects in the placebo group were receiving target doses of beta blockers.

**Effects of SC BNP on LV structure and function.** Figure 1 illustrates the change from baseline to 8 weeks of LV structure and function as assessed by MRI. Eight weeks of chronic SC BNP administered twice daily resulted in a significantly greater reduction in LV end-systolic volume index as compared with placebo (BNP group:  $-5 \pm 13$  ml/m<sup>2</sup> vs. placebo group:  $+6 \pm 10$  ml/m<sup>2</sup>,  $p = 0.004$ ). Similarly, there was a greater reduction in LV end-diastolic volume ( $-10 \pm 15$  ml/m<sup>2</sup>) in the BNP group as compared with the placebo group ( $+6 \pm 12$  ml/m<sup>2</sup>) at 8 weeks compared with baseline ( $p = 0.001$ ). These reductions in LV volumes were associated with a greater decrease in LV mass index in the BNP group ( $-4 \pm 10$  mg/m<sup>2</sup>) as



**Table 1** Baseline Characteristics

Baseline Characteristics	Placebo (n = 20)	BNP (n = 20)	p Value
Female	8 (40)	4 (20)	0.17
NYHA functional class II	10 (50)	12 (60)	0.53
NYHA functional class III	10 (50)	8 (40)	0.55
LVEF (%)	31 ± 7	32 ± 9	0.79
Systolic blood pressure (mm Hg)	125 ± 16	133 ± 17	0.14
Diastolic blood pressure (mm Hg)	70 ± 11	73 ± 11	0.34
Heart rate (beats/min)	69 ± 12	66 ± 13	0.59
Plasma BNP (pg/ml)	57 (20,250)	51 (15,121)	0.67
GFR (ml/1.73 m <sup>2</sup> )	78 ± 21	74 ± 23	0.59
History of PVD	1 (5)	2 (10)	0.55
History of COPD	1 (5)	2 (10)	0.55
Ischemic cardiomyopathy	10 (50)	10 (50)	1.00
History of hypertension	16 (80)	8 (40)	0.010
History of arrhythmia	4 (20)	5 (25)	0.70
History of smoking	7 (35)	9 (45)	0.52
History of diabetes	7 (35)	9 (45)	0.52
History of high cholesterol	16 (80)	13 (65)	0.29
ACEI or Angio II or nitrates	19 (95)	20 (100)	0.31
Digoxin	8 (42)	7 (35)	0.65
Beta blocker	19 (95)	20 (100)	0.31
Diuretic (loop)	14 (70)	15 (75)	0.72
Coumadin	1 (6)	2 (13)	0.48
Spironolactone	4 (20)	4 (21)	0.94
Diuretic (thiazide)	3 (16)	4 (20)	0.73
Antiarrhythmics	0 (0)	5 (25)	0.017

Values are n (%), mean ± SD, or median (quartiles).

ACEI = angiotensin converting enzyme inhibitor; Angio II = angiotensin II receptor blocker; BNP = B-type natriuretic peptide; COPD = chronic obstructive pulmonary disease; GFR = glomerular filtration rate; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; PVD = peripheral vascular diseases.

compared with the placebo group ( $+6 \pm 13$  mg/m<sup>2</sup>) ( $p = 0.006$ ). The LVEF was unchanged in both the BNP and placebo groups. The aforementioned changes in LV structure occurred in the absence of significant changes from baseline to 8 weeks; in heart rate (BNP group:  $67 \pm 12$  beats/min to  $65 \pm 12$  beats/min; placebo group:  $74 \pm 12$  beats/min to  $73 \pm 14$  beats/min); systolic blood pressure (BNP group:  $140 \pm 20$  mm Hg to  $135 \pm 25$  mm Hg; placebo group:  $121 \pm 17$  mm Hg to  $126 \pm 15$  mm Hg) and diastolic blood pressure (BNP group:  $79 \pm 12$  mm Hg to  $77 \pm 13$  mm Hg; placebo group:  $74 \pm 12$  mm Hg to  $75 \pm 12$  mm Hg) measured when the MRI was performed.

Figure 2 illustrates echocardiographic assessment of LV filling pressure. The SC BNP improved LV filling pressure as demonstrated by a greater reduction of Doppler mitral inflow velocity (E) to mitral annulus tissue Doppler velocity (e') ratio E/e' and the greater decrease in LA volume index compared with placebo. The aforementioned changes occurred in the absence of significant changes from baseline to 8 weeks; in heart rate (BNP group:  $62 \pm 15$  beats/min to  $66$  beats/min  $\pm 13$  beats/min; placebo group:  $66 \pm 11$  beats/min to  $70 \pm 11$  beats/min); systolic blood pressure (BNP group:  $133 \pm 20$  mm Hg to  $129 \pm 36$  mm Hg; placebo group:  $123 \pm 16$  mm Hg to  $123 \pm 15$  mm Hg) and diastolic

blood pressure (BNP group:  $72 \pm 13$  mm Hg to  $74 \pm 12$  mm Hg; placebo group:  $69 \pm 11$  mm Hg to  $69 \pm 12$  mm Hg) measured at the time when the echo was performed.

**Effects of SC BNP on clinical well-being.** The MLHF score was assessed at baseline and at 8 weeks. Chronic SC BNP administration resulted in a greater improvement of MLHF as compared with the placebo group at 8 weeks. Specifically, there was a greater decrease of the MLHF score in the BNP group ( $-9 \pm 12$ ) as compared with the placebo group ( $+1 \pm 14$ ) (BNP vs. placebo  $p = 0.013$ ). There was a trend for a greater increase in 6-min walk distance in the BNP group versus placebo ( $2.2 \pm 70$  m vs.  $0.49 \pm 37$  m,  $p = 0.091$ ).

**Effects of SC BNP on neurohumoral parameters.** The SC BNP administration resulted in the increase of the second messenger, plasma cGMP levels both at time of first dosing and with the last dosing at 8 weeks (Fig. 3). Thus, 8 weeks of twice daily administration of SC BNP did not result in the development of tolerance to BNP as demonstrated by a similar activation of the second messenger cGMP, after 8 weeks as compared with baseline. There was a greater decrease in plasma renin activity with SC BNP compared with placebo at 8 weeks ( $-4 \pm 7$  ng/ml/h vs.  $+2 \pm 5$  ng/ml/h,  $p = 0.005$ ).

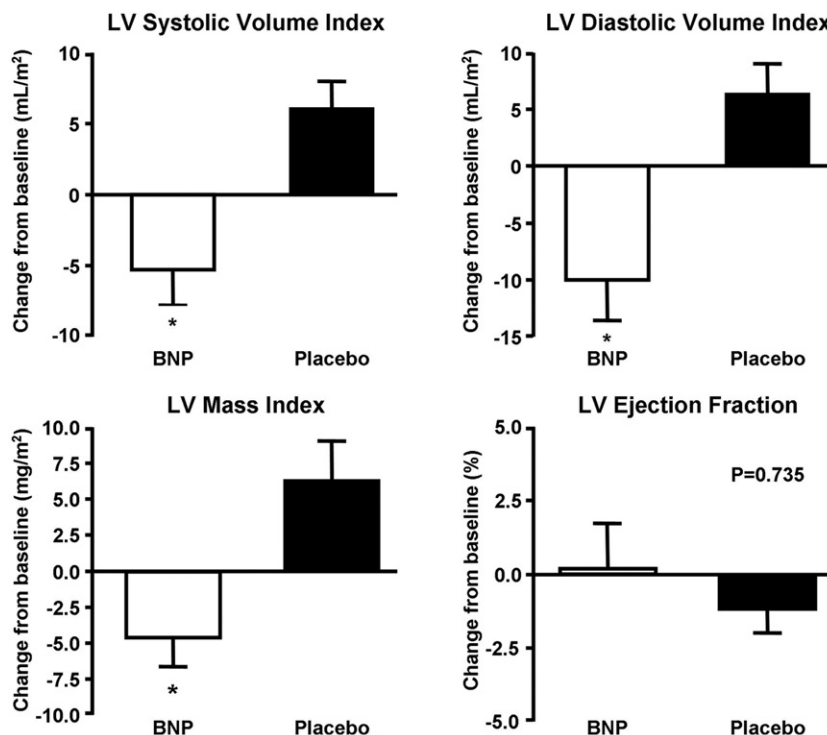
**Effects of SC BNP on renal function.** Glomerular filtration rate (GFR) as determined by iothalamate clearance was preserved with SC BNP ( $+6.9 \pm 14$  ml/1.73 m<sup>2</sup>) with a trend toward a greater increase at 8 weeks as compared with placebo ( $-2.8 \pm 25$  ml/1.73 m<sup>2</sup>) ( $p = 0.14$  BNP vs. placebo). In addition, plasma cystatin C trended to decrease in the BNP group ( $-0.04 \pm 0.2$  mg/dl,  $n = 16$ ) at 8 weeks, whereas it trended to increase in the placebo group ( $+0.09 \pm 0.2$  mg/dl,  $n = 18$ ) ( $p = 0.1$  BNP vs. placebo).

**Serious adverse events and adverse events.** SERIOUS ADVERSE EVENTS. Three subjects in the BNP group developed hypotension as defined by systolic BP  $<85$  mm Hg or had symptoms of lightheadedness or visual disturbances during the baseline visit in the CRU and were excluded from the study as per protocol. All subjects recovered with placement at Trendelenburg position and fluid administration without any clinical consequences. One subject in the placebo group had worsening HF symptoms during the 8-week period and withdrew from the study.

**ADVERSE EVENTS.** Adverse events are reported in Table 2. There was a strong trend for higher incidences of lightheadedness in the BNP group as compared with the placebo group. However, it must be noted that, in these patients, it did not result in the discontinuation of the study drug. In the placebo group, more patients developed fatigue and shortness of breath as compared with the BNP group. However, this did not achieve statistical significance.

## Discussion

In this proof of concept human study, 8 weeks of protein therapy with SC BNP administered twice daily improved LV remodeling, diastolic function, and clinical symptoms



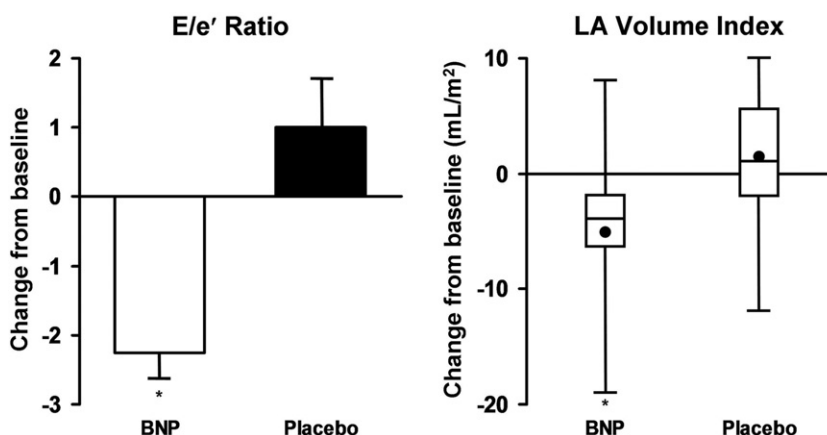
**Figure 1** Effects of SC BNP on LV Structure and Function

Changes in left ventricular (LV) end-systolic volume index (\**p* = 0.004), LV end-diastolic volume index (\**p* = 0.001), LV mass index (\**p* = 0.006), and LV ejection fraction from baseline to 8 weeks in B-type natriuretic peptide (BNP) and placebo groups. \**p* value BNP versus placebo. SC = subcutaneous.

(i.e., MLHF score) in patients with stage C systolic HF on optimal medical therapy. Furthermore, plasma renin activity was suppressed, and GFR was preserved. Importantly, there was no development of tolerance to chronic SC BNP

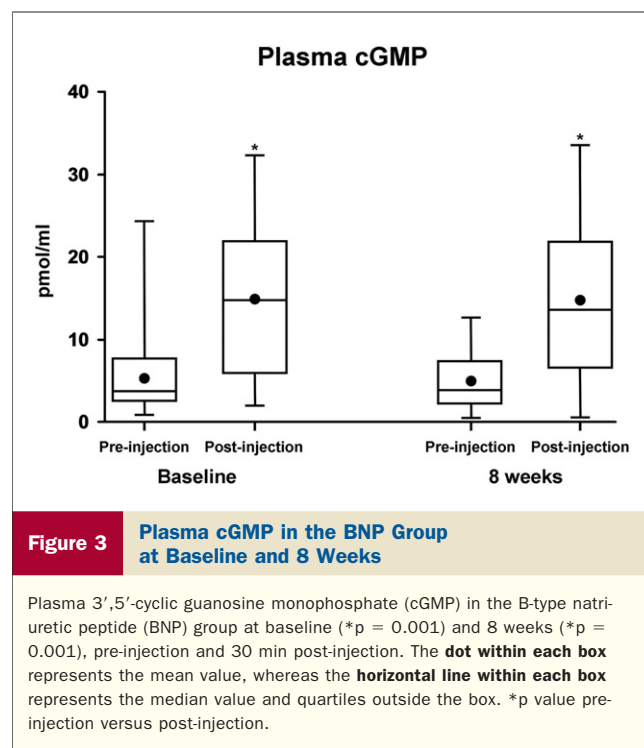
administration as evident by a preserved activation of cGMP, its second messenger after 8 weeks of therapy.

Despite recent advances in the treatment of overt symptomatic (Stage C/D) HF, mortality and morbidity among



**Figure 2** Change in LV Doppler E/e' Ratio and LA Volume Index

Change in left ventricular (LV) Doppler E/e' ratio (\**p* = 0.001) and left atrium (LA) volume index (\**p* = 0.003) from baseline to 8 weeks in B-type natriuretic peptide (BNP) and placebo groups. The dot within each box represents the mean value, whereas the horizontal line within each box represents the median value and quartiles outside the box. \**p* value BNP versus placebo.



patients with HF remains high. Our study addresses the concept of reverse LV remodeling with chronic administration of the cardiac peptide BNP as novel protein therapy for stage C systolic HF. The rationale for using BNP as a novel protein therapeutic for reducing LV remodeling was also strengthened by our pilot study, which reported that a 3-day infusion of BNP in humans with first-time anterior acute MI was associated with reduced LV systolic and diastolic volumes 1 month after acute MI (14).

The current study is the first to report the potential favorable LV remodeling actions of chronic BNP administration in human stage C systolic HF. The degree of LV remodeling seen in the current study is comparable to a previous MRI sub-study of the MERIT-HF (Metoprolol CR/XL Randomized Intervention Trial in Heart Failure). In that sub-study (n = 41), Groenning et al. (15) reported a reduction in LV end-systolic volume index of  $-9.7 \text{ ml/m}^2$  and LV end-diastolic volume index of  $-10 \text{ ml/m}^2$  at 5 to 7 weeks after therapy. In the current study, we report a reduction in left LV end-systolic volume index of  $-5 \pm 13 \text{ ml/m}^2$  and a reduction in LV end-diastolic volume ( $-10 \pm 15 \text{ ml/m}^2$ ) in the BNP group after 8 weeks of therapy. In a recent meta-analysis by Kramer et al. (16), they reported that a decrease of 10 ml in the mean change of end-diastolic volume was associated with a 1.9-fold (95% confidence interval: 1.2 to 3.2,  $p < 0.012$ ) increased odds that an intervention would have significantly favorable effects on mortality. Furthermore, a decrease of 10 ml in the mean end-systolic volume change corresponded to a relative odds ratio of 0.96 for mortality (95% confidence interval: 0.93 to 0.98,  $p < 0.01$ ). Plasma renin activity was suppressed to a

greater extent in the BNP group as compared with the placebo group. The effects of BNP on LV structure might be due to the indirect unloading and diuretic effects of BNP. In the current study, we did not have to reduce the dose of diuretics in any patients. However; we did not have to increase the dose of the diuretics as well. The diuretic and natriuretic actions of BNP might have resulted in decreased filling pressure in the LV contributing to the improved remodeling. Unfortunately, we did not measure the plasma volume of the patients. These findings confirm and extend previous preclinical studies that reported that BNP acting via the NPR-A has potent anti-hypertrophic and anti-fibrotic properties. Specifically, NPR-A is present on cardiomyocytes (17,18), and incubation with BNP suppresses the response to hypertrophic factors in isolated cardiomyocytes (19). Furthermore, we and others have demonstrated that cardiomyocyte specific disruption of NPR-A results in exaggerated hypertrophy and fibrosis in response to cardiac stress (20). These studies suggest that the cardiac hormone BNP regulates myocardial structure by direct anti-hypertrophic effects on the cardiomyocyte, which complement their effects to reduce load and suppress pro-hypertrophic humoral factors. Furthermore, transcripts for NPR-A are also present in isolated fibroblasts (18), and in vitro studies have demonstrated that BNP provides broad opposition to fibroblast activation and collagen production (21). Thus, direct actions of BNP on both the cardiomyocyte and the fibroblast might contribute to the anti-hypertrophic and anti-fibrotic effects of BNP. However, without a washout time for the SC BNP, we cannot exclude that the acute unloading effects of BNP might account for the changes in LV systolic and diastolic volumes. However, assessment of LV mass index by MRI is not affected by acute loading conditions. Hence, the greater decrease in LV mass index in the BNP group as compared with the placebo group might suggest that SC BNP has a favorable effect in the LV mass. There was no significant correlation between baseline systolic blood pressure and the changes in LV systolic and diastolic volumes and mass index at 8 weeks.

In the current study, 8 weeks of chronic BNP administration also resulted in an improvement of LV filling

**Table 2** Adverse Events

	BNP (n = 20)	Placebo (n = 20)	p Value
Lightheadedness	3 (15%)	0 (0%)	0.07
SOB and fatigue	3 (15%)	7 (35%)	0.14
Injection site sting/itchy	4 (20%)	3 (15%)	0.68
Flu-like symptoms	1 (5%)	2 (10%)	0.55
Flushing	3 (15%)	1 (5%)	0.29
Loose stools	2 (10%)	0 (0%)	0.15
Atrial fibrillation	0 (0%)	1 (5%)	0.31
Hyperkalemia	1 (5%)	1 (5%)	1.00
Edema	1 (5%)	0 (0%)	0.31
Other symptoms	2 (10%)	2 (10%)	1.00

SOB = shortness of breath.

pressure as measured by a greater decrease in Doppler E/e' ratios and LA volume index in the BNP group as compared with the placebo group. Studies in isolated cardiomyocytes preparations report improvement in the speed of myocyte/myocardial relaxation (22) and increases in resting cell length after BNP or cGMP exposure. In *in vivo* studies in experimental canine HF, we have described the pro-lusitropic effects of BNP (23). Additionally, a key integrated physiological study demonstrated that, in patients with isolated diastolic dysfunction, infusion of BNP significantly attenuated the increase in pulmonary capillary wedge pressure during exercise, without an effect on resting hemodynamic status (24). These findings suggest possible direct and indirect effects to improve operant diastolic function. Hence, the greater improvement of LV structure and filling pressure in the BNP group might account for the improvement in MLHF scores as compared with the placebo group. The findings of the current study are different from the ASCEND and FUSION studies (9,10). However, there are major differences in the patient population, route, dose, and duration of administration of BNP in the 3 studies. The current study recruited stable NYHA functional class II to III patients, whereas the patients in both the FUSION and ASCEND studies were NYHA functional class III to IV. In the FUSION study, the patients received once/week infusions, whereas in the ASCEND study, they received up to 6 days of infusion. In our current pilot study, the patients received twice/day SC administration for 8 weeks.

There was no development of tolerance to 8 weeks of twice daily SC BNP administration as evident by a preserved activation of cGMP, its second messenger. This is consistent with our previous findings in a canine model of HF where we demonstrated that 10 days of SC BNP administration did not result in the development of tolerance (25). In the current study, we demonstrated that 8 weeks of SC BNP preserved GFR with a strong trend for GFR to improve and plasma cystatin C to decrease as compared with placebo.

Three (12.5%) of the subjects did not tolerate 10  $\mu$ /kg bolus SC BNP injections and were excluded from the study as per study protocol. Another 3 (12.5%) subjects developed symptoms of lightheadedness during the 8 week period, which resolved without withdrawal of study drug. One subject in the placebo group withdrew from the study due to worsening HF symptoms, and there was a trend for more subjects in the placebo group developing symptoms of worsening shortness of breath or fatigue as compared with the BNP group. Indeed, in the ASCEND trial, there was significantly greater symptomatic and asymptomatic hypotension in the BNP-treated group compared with placebo. Therefore, a limiting factor of the use of BNP in our study and others is hypotension. Further studies are required to test lower doses of SC BNP.

**Study limitations.** This study was designed as a translational proof of concept investigation of the chronic use of twice daily SC BNP administration in patients with stage C

systolic HF and not as a definitive clinical trial, because the sample size is small. Furthermore, we only tested a single dose at 10  $\mu$ /kg. Further studies that are adequately powered to evaluate potential clinical efficacy in HF are warranted. Due to limited resources and funding, we were not able to include an active drug run-in period before randomization of the subjects to the treatment groups, and hence we were not able to do an intention to treat analysis, which is a limitation of the study design. Because of the use of MRI, none of our patients had a defibrillator or biventricular pacing. With the results from the recently published EMPHASIS (Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure) trial (26), our patient population should be treated with spironolactone antagonist; however, only 20% of them were taking a spironolactone antagonist.

## Conclusions

This proof of concept pilot study is the first to demonstrate that SC BNP administration represents a novel, safe, and efficacious protein therapeutic strategy in human stage C systolic HF with favorable actions of myocardial structure and function and patient well-being. Further studies are warranted to determine whether these functional responses can be translated into improved clinical outcomes contributing to delaying the progression of HF.

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