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ORIGINAL RESEARCH - CLINICAL

MANP in Hypertension With Metabolic Syndrome

Proof-of-Concept Study of Natriuretic Peptide-Based Therapy for Cardiometabolic Disease

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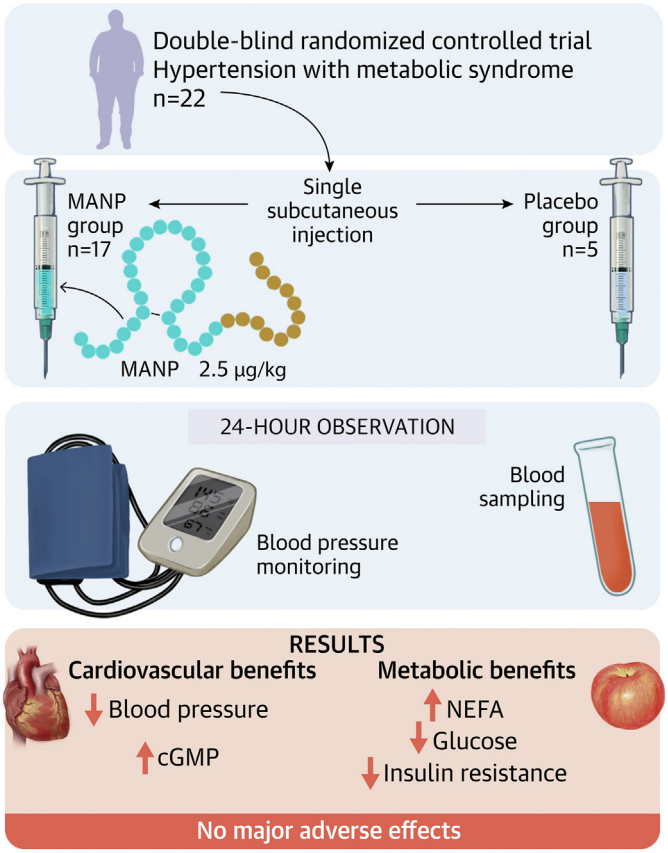
VISUAL ABSTRACT



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HIGHLIGHTS

- **MANP is an innovative ANP analog that targets the GC-A receptor and triggers the production of therapeutic cGMP.**
- **In subjects with HTN and MetS, a single subcutaneous administration of MANP is safe, well-tolerated, and increases plasma levels of cGMP.**
- **Subcutaneous administration of MANP in subjects with HTN and MetS can exert pleiotropic protections including blood pressure-lowering, lipolytic, and insulin-sensitizing effects.**

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SUMMARY

Hypertension and metabolic syndrome frequently coexist to increase the risk for adverse cardiometabolic outcomes. To date, no drug has been proven to be effective in treating hypertension with metabolic syndrome. M-atrial natriuretic peptide is a novel atrial natriuretic peptide analog that activates the particulate guanylyl cyclase A receptor. This study conducted a double-blind, placebo-controlled trial in 22 patients and demonstrated that a single subcutaneous injection of M-atrial natriuretic peptide was safe, well-tolerated, and exerted pleiotropic properties including blood pressure–lowering, lipolytic, and insulin resistance–improving effects. (MANP in Hypertension and Metabolic Syndrome [MANP-HTN-MS]; NCT03781739) (J Am Coll Cardiol Basic Trans Science 2024;9:18–29) © 2024 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

ANP = atrial natriuretic peptide

BMI = body mass index

BP = blood pressure

cGMP = 3′, 5′-cyclic guanylyl monophosphate

GC-A = particulate guanosine cyclase A

HOMA2 = updated Homeostatic Model Assessment

HTN = hypertension

MANP = M-atrial natriuretic peptide

MetS = metabolic syndrome

NEFA = nonesterified fatty acids

Hypertension (HTN) represents a highly prevalent disease affecting approximately 30% of the U.S. general population, based on data from 2015-2016 NHANES (National Health and Nutrition Examination Survey).[1] If the 2017 American College of Cardiology/American Heart Association multisociety definition of HTN is applied, prevalence rises to almost 45%.[2]

Metabolic syndrome (MetS) is a constellation of cardiovascular and metabolic risk factors that predispose patients to major cardiovascular disease. Importantly, HTN represents a central clinical characteristic of MetS. Indeed, HTN is present in 77% of patients affected by MetS, and conversely, visceral obesity is a key risk factor for the development of HTN.[3,4] Individually, HTN and MetS both represent significant risk factors for cardiovascular disease;[5,6] when they coexist, the risk is doubled.[7] Despite being 2 pathological conditions that are highly interrelated, none of the currently available antihypertensive medications have been reported to exert definitive favorable metabolic effects and no therapy has ever been specifically approved for treating HTN associated with MetS.

Atrial natriuretic peptide (ANP) is a cardiac hormone with pleiotropic actions and is secreted by cardiomyocytes in response to volume overload and myocardial stretch.[8] ANP binds to the particulate guanosine cyclase A (GC-A) receptor and activates the second messenger 3′, 5′-cyclic guanosine monophosphate (cGMP), which mediates biological actions. Preclinical and clinical studies have established that ANP is a key regulator of blood pressure (BP) homeostasis via vasodilation, natriuresis, and renin-angiotensin-aldosterone system suppression.[9-11] Importantly, ANP also acts as a modulator of metabolism by enhancing lipid mobilization, oxidation, browning of white adipocytes, and insulin sensitivity.[12-16] In healthy subjects, infusion of ANP mediated a lipolytic effect, which resulted in increasing plasma levels of glycerol and nonesterified fatty acid (NEFA), and enhanced energy expenditure.[17,18] Importantly, the metabolic effects of ANP are not affected by the presence of obesity, because, as reported by Galitzky et al,[19] ANP induces a similar rise in circulating levels of glycerol and NEFA in both lean and obese subjects. There is also a strong association between high levels of NPs and a favorable lipid profile.[20] In addition, a recent study reported by Ichiki et al[21] also underscores the potential gender-specific effect in relationship to the cardiovascular and metabolic protection of ANP/GC-A–based therapeutics.

Interestingly, both HTN and MetS can be considered states of ANP deficiency. Macheret et al[22] previously reported that human HTN is characterized by a lack of ANP increases. In patients with MetS, low circulating levels of ANP are present and inversely correlated with several key metabolic risk factors.[23] Conversely, Cannone et al[24,25] previously reported

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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 Author Center.

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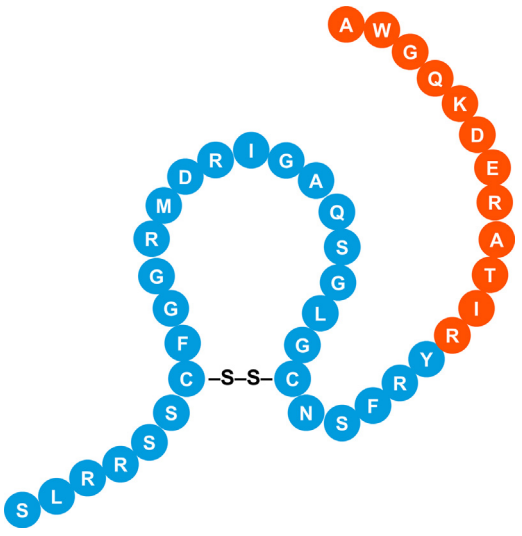
that the ANP gene variant rs5068, which is associated with higher circulating levels of ANP, is also related with lower prevalence of HTN and MetS. Altogether these studies imply that strategies aimed to augment the "ANP system" may be promising therapeutics to treat patients with HTN and MetS. Nonetheless, the short half-life of native ANP remains a primary challenge for its therapeutic implementation.

We previously engineered M-atrial natriuretic peptide (MANP), a novel ANP analog, to be a more potent and long-lasting peptide ligand for GC-A through enhanced production of its effector molecule cGMP.[26] MANP is a 40-amino acid peptide consisting of the 28 amino acids of native ANP with a unique 12-amino acid C-terminus extension (**Figure 1**). When compared to native ANP, MANP has been shown to be more resistant to enzymatic degradation by neprilysin and insulin-degrading enzyme.[27,28] In normal canines, intravenous infusion of MANP exhibited markedly greater and more sustained BP-lowering, natriuretic, glomerular filtration-enhancing, and aldosterone-suppressing actions compared with those of native ANP.[26] In a canine model of HTN induced by angiotensin II, intravenous administration of MANP potently reduced BP, enhanced diuresis and natriuresis, and inhibited angiotensin II-induced aldosterone production.[29] Most importantly, a first-in-human study involving subcutaneous administration of MANP was recently conducted in subjects with essential HTN.[30] The study showed that 3 single ascending doses of MANP were safe, well-tolerated, and increased plasma levels of cGMP. Moreover, MANP lowered BP values and aldosterone circulating levels. Nonetheless, the cardiovascular and metabolic properties of MANP along with its safety and tolerability remain to be investigated in subjects with cardiometabolic disease.

Here, for the first time, we designed and conducted a proof-of-concept study of MANP in patients with HTN and MetS. All subjects were on at least 1 antihypertensive medication and satisfied the criteria for MetS as defined by the National Cholesterol Education Program Adult Treatment Panel III. Our study was a double-blind, placebo-controlled phase I clinical trial in 22 subjects (17 receiving MANP) with HTN and MetS involving a single subcutaneous injection of MANP (2.5 μg/kg) or placebo. The primary goal was to establish safety, tolerability, and cGMP activation. Secondary objectives included BP-lowering effect and defining metabolic properties of MANP. In addition, we assessed whether baseline clinical characteristics, in particular levels of ANP and/or cGMP, could predict responsiveness to MANP.

FIGURE 1 Structure and Amino Acid Sequence of MANP



Amino acid sequence in red highlights the unique C-terminal extension of M-atrial natriuretic peptide (MANP) compared to native ANP.

METHODS

For an expanded Methods section, please see the Supplemental Appendix.

ETHICAL APPROVAL. Our study was a double-blind, placebo-controlled, proof-of-concept, clinical trial in subjects with HTN and MetS. The entire study was conducted at the Clinical Research and Trial Unit of the Mayo Clinic Center for Translational Science Activities under the Investigational New Drug no. 132148, and registered at ClinicalTrials.gov (NCT03781739). All procedures were performed following the ethical principles of the Declaration of Helsinki and its amendments, the US Food and Drug Administration Principles of Good Clinical Practice and International Conference on Harmonization Guidelines. All subjects who participated in this trial provided written, informed consent prior to enrollment. All documents related to this study have been reviewed and approved by the Ethics Committee of the Mayo Clinic.

STATISTICAL METHODS. In our prespecified data analysis plan, the primary cardiovascular and metabolic hypotheses were the following: 1) greater decrease in systolic BP compared to placebo within the 24 hours following MANP administration; 2) greater increase in plasma cGMP compared to placebo within the 24 hours following MANP administration;

and 3) greater increase in plasma NEFA compared to placebo within 4 hours of MANP administration. The secondary cardiovascular and metabolic hypotheses were the following: 1) decrease in systolic BP compared to baseline within the MANP group; 2) increase in plasma cGMP compared to baseline within the MANP group; and 3) increase in plasma NEFA compared to baseline within the MANP group. In the post hoc phase, changes in plasma glucose, plasma insulin, and the updated Homeostatic Model Assessment (HOMA2) indices within 4 hours of MANP administration were investigated. The responses to MANP at the individual level were also evaluated post hoc as the exploratory efforts. Our initial sample size and power calculations were conducted based on the primary outcomes and using previously published data,[16,26,29] which led to an estimation of 80% power to detect an effect size of 0.9.

Data for patients were summarized at baseline and after MANP or placebo injection. Distributions of data were examined for normality with Shapiro-Wilk test, and no data were excluded for analyses. All adverse events were summarized as count (percentage). Continuous variables were presented as mean ± SD or median (Q1-Q3), unless otherwise specified. For pharmacokinetics and pharmacodynamics data, repeated measures analysis of variance with an unstructured covariance matrix was used to compare the main group effects between placebo and MANP, and group differences at specific time points were evaluated. Comparison to baseline levels within single group were also conducted. Scheffe post hoc test for multiple pairwise comparisons was used to control type 1 error. Calculation of specific pharmacokinetic and pharmacodynamic indices were conducted using PKNCA package with R (version 4.1.1, CRAN Project).

For analyses on individual response to MANP, the C_{max} of plasma ANP-like peptides and plasma cGMP were determined by the greatest absolute values observed among 0.5, 1, 2, 4, 6, 12, and 24 hours post MANP administration. The mean change in systolic BP and diastolic BP were determined by the average value in systolic BP and diastolic BP changes occurring among 0.5, 1, 2, 4, 6, 12, and 24 hours post MANP administration in comparison with baseline values. Similarly, the median systolic BP change and maximal systolic BP change were defined as the median value or the maximal value in systolic BP change among 0.5, 1, 2, 4, 6, 12, and 24 hours post MANP administration. Gender-specific differences of these MANP-responsive indices were conducted using unpaired Student's *t*-tests assuming unequal variance. Spearman rank correlation coefficient (*r*) was leveraged to evaluate the associations between these MANP-responsive indices and continuous baseline variables. All statistical analyses were performed with SAS (version 9.4, SAS Institute Inc) and GraphPad Prism (version 9, GraphPad Software), and a 2-sided $P < 0.05$ was considered statistically significant.

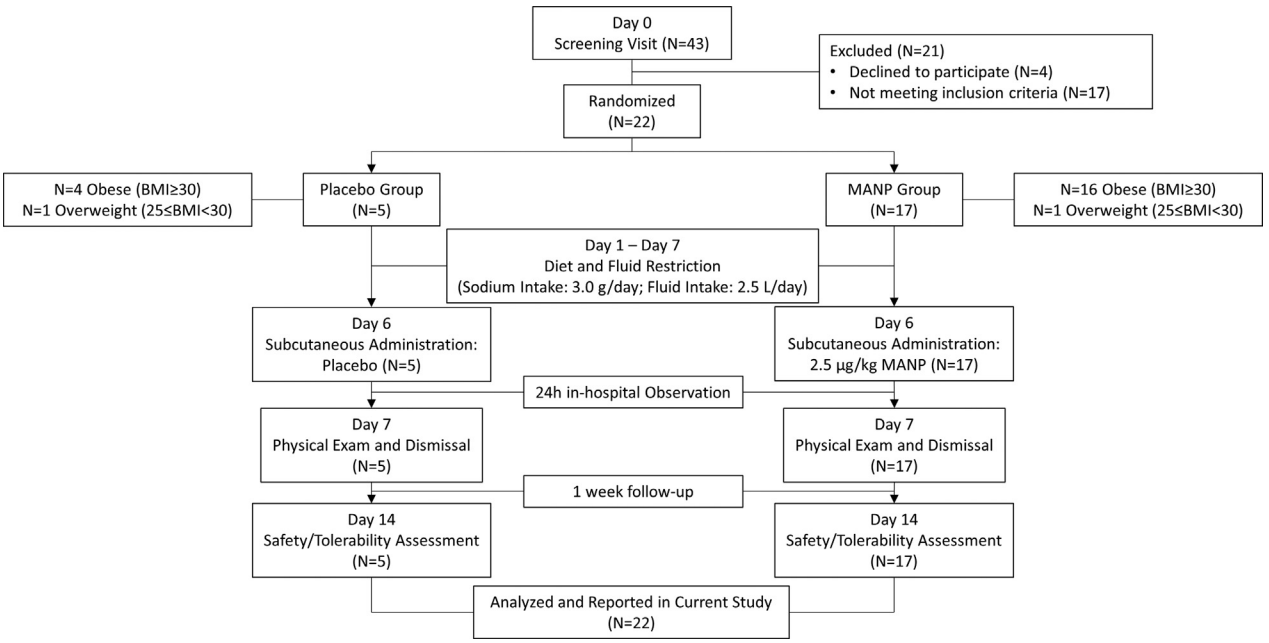
RESULTS

BASELINE CHARACTERISTICS. Our study consisted of a screening visit (defined as first visit on day 0), a 7-day period of diet and fluid restriction, MANP or placebo administration, and 24-hour observation (**Figure 2**). The detailed inclusion and exclusion criteria are listed in Supplemental Tables 1 and 2. A total of 22 subjects with HTN and MetS were enrolled and participated in this study. Among them, 17 were randomized to MANP and 5 to placebo. The clinical characteristics, overall and by treatment group, are summarized in **Table 1**. Overall, mean systolic and diastolic BP levels at baseline were 149 ± 8 mm Hg and 83 ± 11 mm Hg, respectively, though all patients were on at least 1 HTN medication. Mean values of body mass index (BMI) and waist circumference were 36 ± 5 kg/m^2 and 113 ± 9 cm, respectively, showing that our cohort was characterized by both general and abdominal obesity.

SAFETY. All 22 patients completed the treatment, and none of the stopping criteria were met. During the 24-hour period following MANP/placebo subcutaneous injection, no significant changes in electrocardiographic findings were observed, nor did drug-related, clinically relevant changes in safety laboratory parameters occur (Supplemental Table 3). **Table 2** summarizes all adverse events associated with the treatment during the 1-week follow-up period following MANP/placebo injection. No serious adverse events were observed, and orthostatic hypotension and vasovagal syncope were observed in only 2 patients receiving MANP.

PLASMA ANP-LIKE PEPTIDES AND cGMP. The C_{max} and T_{max} for plasma ANP-like peptides (MANP and endogenous ANP) and cGMP are reported in Supplemental Table 4. The changes (from baseline values) of plasma ANP-like peptides and cGMP levels are illustrated in **Figure 3**. In patients receiving MANP, a rapid and significant increase from baseline in plasma ANP-like peptides occurred 30 minutes after injection (48.9 ± 19.5 pg/mL, $P = 0.026$), and elevated levels of plasma ANP-like peptides persisted at 1-hour post injection (26.4 ± 12.8 pg/mL, $P = 0.059$). Meanwhile, a concurrent and significant elevation of plasma cGMP was also observed in the MANP group at 30 minutes (4.8 ± 2.0 pmol/mL, $P = 0.024$) and 1 hour

FIGURE 2 Consort Flow Diagram of the Study Design



A schematic summary of the study protocol. BMI = body mass index; MANP = M-atrial natriuretic peptide.

(2.9 ± 1.3 pmol/mL, $P = 0.035$) after administration; an increase, though not statistically significant, persisted until almost 4 hours post injection. No significant changes in plasma ANP-like peptides or cGMP levels occurred in the placebo group when compared to baseline.

SITTING BP. Sitting BP and heart rate values for both treatment groups are illustrated in Figure 4. In the MANP group, sitting systolic BP was reduced compared to baseline, though not statistically significant, with the greatest reduction occurring at 6 hours post injection (−5.7 ± 2.9 mm Hg, $P = 0.063$). At 12 hours post MANP dosing, systolic BP remained lower than baseline. In contrast, no reduction of systolic BP was observed in the placebo group. Sitting diastolic BP was lower than baseline at 6 hours after MANP administration (−2.2 ± 1.2 mm Hg, $P = 0.089$), though not statistically different. A slight and not statistically significant increase in heart rate was observed in both groups at 6 hours post injection and thereafter. There was no difference in heart rate between the MANP group and the placebo group at any time points.

INDIVIDUAL RESPONSE TO MANP. We further determined whether baseline features may influence the response to MANP at the individual level among the 17 patients who received MANP administration. Specifically, we evaluated the MANP response by: 1) C_{max} of plasma ANP-like peptides; 2) C_{max} of plasma cGMP; 3) mean change in systolic BP; and 4) mean change in diastolic BP, during the 24-hour follow-up period post MANP injection.

Whereas an increase in plasma ANP-like peptides was observed in all 17 patients, baseline ANP levels were positively associated with C_{max} of plasma ANP-like peptides ($r = 0.51$; $P = 0.039$) after MANP administration (Supplemental Figure 1A). Furthermore, C_{max} of plasma ANP-like peptides were positively associated with waist circumference ($r = 0.53$; $P = 0.030$), but not BMI ($r = 0.28$; $P = 0.277$). Age, gender, and baseline cGMP were not associated with C_{max} of plasma ANP-like peptides (Supplemental Figures 1A and 2A). Meanwhile, among these 17 patients, only 2 patients were found not to have enhancement in circulating levels of cGMP at any measured time points. C_{max} of plasma cGMP had no association with age, gender, BMI, waist circumference, baseline plasma ANP, or baseline plasma cGMP (Supplemental Figures 1B and 2B).

The reduction in systolic BP (at ≥1 time points post MANP administration) was observed in all patients, and the reduction in diastolic BP (at ≥1 time points post MANP administration) was observed in 15 of 17 patients who received MANP. We further calculated

the mean changes (relative to the baseline values) in both systolic BP and diastolic BP over the 24-hour post MANP administration period for each subject. Accordingly, the median of the mean change in systolic BP was found to be −3.4 mm Hg (Q1-Q3: −8.0 to −3.0 mm Hg), and the median of the mean change in diastolic BP was found to be −2.4 mm Hg (Q1-Q3: −3.3 to 0.7 mm Hg). The mean change in systolic BP was more prominent in male than female patients ($P = 0.008$) (Supplemental Figure 2C), whereas the mean change in diastolic BP was similar between the 2 genders ($P = 0.248$) (Supplemental Figure 2D). **Figures 5A and 5B** illustrate the correlation between the mean BP change with other baseline features. Interestingly, we observed a significantly positive association between the mean systolic BP change and baseline plasma cGMP, according to which of those patients with lower baseline plasma cGMP levels had greater reduction in mean systolic BP over a 24-hour period after MANP injection ($r = 0.66$; $P = 0.005$). This significant association was further validated by repeating the same analysis using median systolic BP change (baseline cGMP vs median systolic BP change: $r = 0.73$; $P < 0.001$) or maximal systolic BP change (baseline cGMP vs maximal systolic BP change: $r = 0.59$; $P = 0.012$) during the 24-hour period post MANP injection. The mean diastolic BP change appeared to not be affected by baseline plasma ANP, baseline plasma cGMP, age, or waist circumference and had a weak negative association with BMI ($r = −0.44$; $P = 0.077$).

METABOLIC EFFECTS OF MANP. Following overnight fasting, plasma metabolic parameters were measured before and at different time points during the 4 hours after MANP/placebo injection. The absolute values of different metabolic parameters after MANP/placebo injection are presented in Supplemental Table 5. The relative changes (compared to baseline) of different metabolic parameters are presented in **Table 3**. Plasma glucose levels did not alter significantly in the placebo group, whereas we observed a reduction in plasma glucose levels at 1 hour after MANP administration compared to baseline (−3.1 ± 1.2 mg/mL; $P = 0.056$) and, importantly, plasma glucose levels significantly decreased at both 2 hours (−4.7 ± 1.6 mg/mL; $P = 0.041$) and 4 hours (−13.1 ± 4.0 mg/mL; $P = 0.003$) post MANP administration. In the placebo group, plasma insulin increased at 1 and 2 hours post injection, whereas levels of plasma insulin remained stable in the MANP group. The variation in insulin levels of the MANP group vs placebo group reached statistical significance at both 1 ($P = 0.016$) and 2 hours ($P = 0.027$) post injection. We also observed an increase in plasma NEFA in the MANP group, reaching statistical significance at 1 hour post MANP administration ($P = 0.009$) (**Table 3**). The levels of plasma glycerol and triglycerides had no significant changes during the first 4 hours post MANP injection (**Table 3**).

TABLE 1 Baseline Characteristics

	All (N = 22)	Placebo (n = 5)	MANP (n = 17)
Age, y	65 ± 6	64 ± 4	65 ± 6
Male	6 (27)	1 (20)	5 (29)
BMI, kg/m^2	36 ± 5	37 ± 9	36 ± 4
Waist circumference, cm	113 ± 9	112 ± 8	114 ± 9
Baseline blood pressure, mm Hg			
Systolic	149 ± 8	148 ± 6	149 ± 9
Diastolic	83 ± 11	81 ± 12	83 ± 12
Baseline heart rate, beats/min	71 ± 13	67 ± 12	72 ± 13
eGFR, mL/min/1.73 m^2	78 ± 13	77 ± 10	78 ± 14
Laboratory values			
Serum creatinine, mg/dL	0.9 (0.8-1.0)	0.8 (0.8-1.0)	0.8 (0.8- 1.0)
BUN, mg/dL	16 (14-18)	15 (14-20)	17 (15- 19)
Fasting HDL cholesterol, mg/dL	49 (41-55)	51 (41-58)	48 (41-53)
Fasting triglycerides, mg/dL	135 (104-160)	148 (90-164)	133 (101-162)
Fasting plasma glucose, mg/dL	115 (100-144)	101 (100-182)	117 (102-140)
Serum sodium, mmol/L	141 (140-142)	142 (140-142)	141 (140-142)
Serum potassium, mmol/L	4.5 (4.1-4.8)	4.6 (4.2-4.8)	4.5 (4.1-4.8)
NT-proBNP, pg/mL	39 (24-69)	40 (20-76)	37 (23-64)
ANP, pg/mL	14.4 (7.6-37.0)	12.0 (5.3-23.0)	17.3 (9.0-48.7)
cGMP, pmol/mL	8.0 (5.0-10.0)	8.0 (4.4-11.2)	8.0 (4.8-10.1)
Antihypertension medications			
Alpha blockers	1 (5)	0 (0)	1 (6)
Beta blockers	4 (18)	1 (20)	3 (18)
ACE inhibitor	7 (32)	1 (20)	6 (35)
ARB	10 (45)	2 (40)	8 (47)
Diuretics	22 (100)	5 (100)	17 (100)
CCB	5 (23)	2 (40)	3 (18)
Total no. of antihypertension medications			
3 or more	6 (27)	1 (20)	5 (29)
2	14 (64)	3 (60)	11 (65)
1	2 (9)	1 (20)	1 (6)
0	0 (0)	0 (0)	0 (0)
Other medications			
Statins	19 (86)	5 (100)	14 (82)
Oral antidiabetic medications	9 (41)	2 (40)	7 (41)
Insulin	2 (9)	1 (20)	1 (6)
Comorbidities			
Diabetes mellitus type 1	1 (5)	0 (0)	1 (6)
Diabetes mellitus type 2	9 (41)	1 (20)	8 (47)
Class III obesity	5 (23)	1 (20)	4 (24)
Hyperlipidemia	18 (82)	5 (100)	13 (76)
Obstructive sleep apnea	9 (41)	2 (40)	7 (41)
Chronic kidney diseases	1 (5)	0 (0)	1 (6)

Values are mean ± SD, n (%), or median (Q1-Q3).

ACE = angiotensin-converting enzyme; ANP = atrial natriuretic peptide; ARB = angiotensin receptor blocker; BMI = body mass index; BUN = blood urea nitrogen; CCB = calcium channel blocker; cGMP = 3′, 5′-cyclic guanylyl monophosphate; eGFR = estimated glomerular filtration rate; HDL = high-density lipoprotein; MANP = M-atrial natriuretic peptide; NT-proBNP = N-terminal pro-B-type natriuretic peptide.

TABLE 2 Adverse Events

	Placebo (n = 5)	MANP (n = 17)
Orthostatic hypotension	0 (0)	1 (6)
Vasovagal syncope	0 (0)	1 (6)
Light or mild headache	0 (0)	0 (0)
Arrhythmia	0 (0)	0 (0)
Second- or third-degree AV block	0 (0)	0 (0)
Ventricular tachycardia >5 beats	0 (0)	0 (0)
Ventricular fibrillation or asystole	0 (0)	0 (0)
Tachycardia	0 (0)	0 (0)
Paresthesia	0 (0)	0 (0)
Dyspnea	0 (0)	0 (0)
Gastrointestinal symptoms	0 (0)	0 (0)

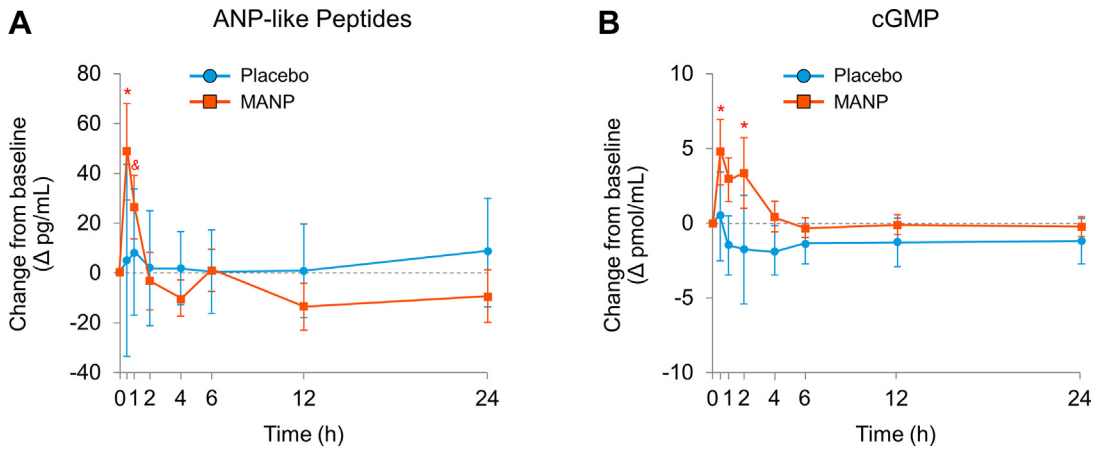
Values are n (%).
AV = atrioventricular; MANP = M-atrial natriuretic peptide.

To gain additional insights into the acute effect of MANP on insulin sensitivity, HOMA2 values were calculated, as indirect and surrogate indices, for the first 4 hours post injection (Table 4). Before treatment, there was no difference in HOMA2 insulin sensitivity values (P = 0.992) and HOMA2 insulin resistance values (P = 0.357) between the placebo and MANP groups. From baseline to 4 hours post injection, there was an overall increase in median HOMA2 insulin sensitivity values in the MANP group compared to baseline, but not in the placebo group (13.7% [Q1-Q3: 4.2% to 32.7%] vs −12.4% [Q1-Q3: −30.6% to 2.0%]) (Supplemental Figure 3A). In line with this, there was also a reduction in the median HOMA2 insulin resistance values in the MANP group compared to baseline, but not in the placebo group (−0.485 [Q1-Q3: −0.682 to −0.148] vs 0.485 [Q1-Q3: −0.148 to 1.022]) (Supplemental Figure 3B).

DISCUSSION

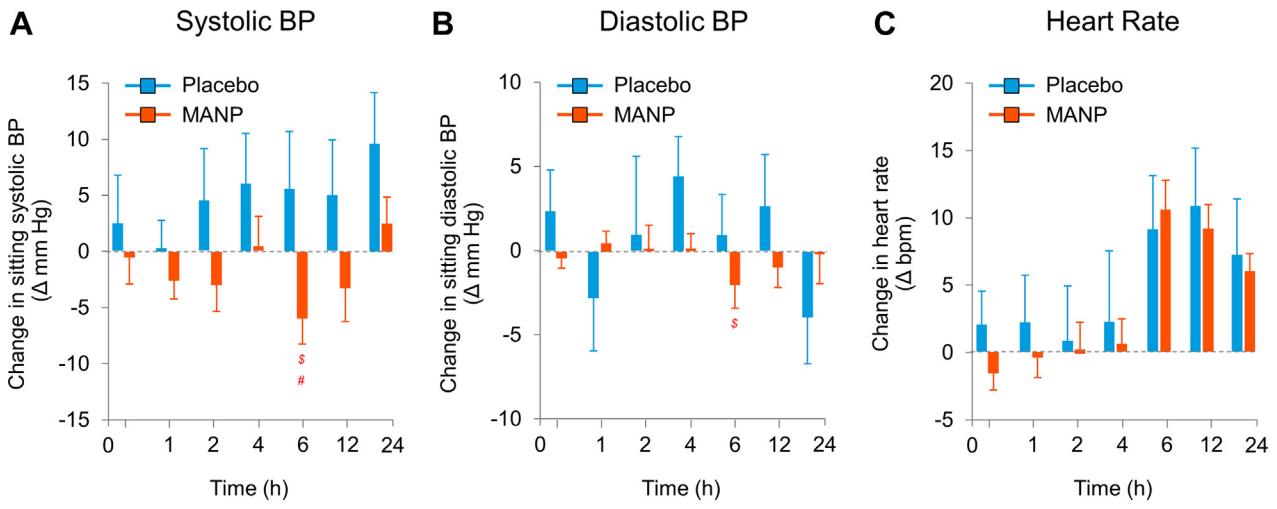
In this proof-of-concept human study, we investigated the safety, tolerability, pharmacokinetics, pharmacodynamics, and cardiovascular and metabolic properties of subcutaneous administration of MANP, a novel designer GC-A activating peptide, in subjects with HTN and MetS. We found that a single subcutaneous injection of MANP was safe and well-tolerated with increases in plasma cGMP and reductions in BP. Importantly, our data demonstrate a beneficial effect of MANP on reducing circulating levels of fasting glucose whereas insulin remained stable compared to baseline. Furthermore, the favorable HOMA2 profile associated with MANP treatment indirectly supports an improvement in insulin sensitivity. In addition, MANP increased NEFA circulating levels, suggesting a possible lipolytic effect. Therefore, the cardiovascular protective actions of MANP in patients with HTN and MetS may go beyond BP reduction and mediate favorable metabolic properties.

FIGURE 3 Effects of Subcutaneous Injection of MANP on Plasma ANP-Like Peptides and cGMP



Changes in circulating levels of atrial natriuretic peptide (ANP)-like peptides (M-atrial natriuretic peptide [MANP] and endogenous ANP) (A) and 3′, 5′-cyclic guanylyl monophosphate (cGMP) (B) during 24-hour post subcutaneous administration of MANP or placebo. Shown P values represent the comparison to baseline. *P < 0.05. &P = 0.059 at 1-hour post MANP administration. Data are mean ± SEM. Data compared using repeated measures analysis of variance with Scheffe post hoc test.

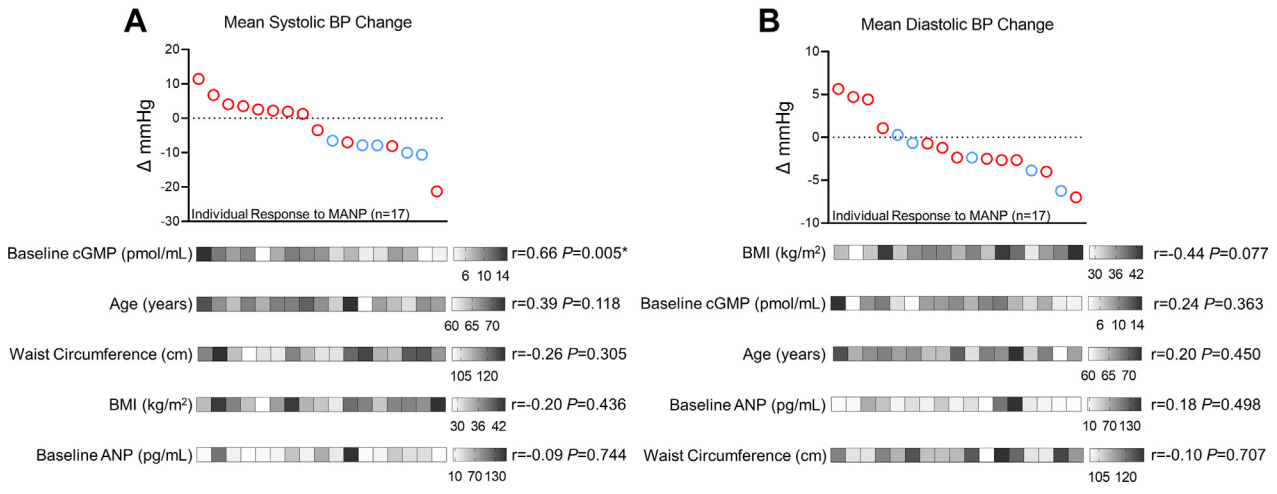
FIGURE 4 **Effects of Subcutaneous Injection of MANP on Sitting BP**



Changes in sitting systolic blood pressure (BP) (A), sitting diastolic BP (B), and heart rate (C) during 24-hour post subcutaneous administration of M-atrial natriuretic peptide (MANP) or placebo. $^{\$}P < 0.10$ compared to baseline; $^{\#}P < 0.07$ compared to placebo group. Data are mean ± SEM. Data compared using repeated measures analysis of variance with Scheffe post hoc test.

Failure to reach satisfactory control of BP in the general population has originated a continuous search for more efficacious pharmacological strategies targeting the pathophysiological pathways underlying HTN.[31] We recently reported the first-in-human study of MANP in patients with essential HTN.[30] Three ascending doses of MANP were investigated and proven to be safe, well-tolerated, and with beneficial BP lowering and neurohumoral effects. Importantly, this first-in-human study

FIGURE 5 **Correlations Between Baseline Features and MANP Response in BP Reduction**



Spearman correlation analysis on baseline characteristics and response to MANP. The responses to MANP are represented by the mean systolic BP change (A) and the mean diastolic BP change (B). For each subject, mean BP (systolic or diastolic) change is defined as the mean value of BP change among 0.5, 1, 2, 4, 6, 12, and 24 hours post MANP administration. (A, B) Each circle represents 1 subject with blue indicating male gender and red indicating female gender. $^{*}P < 0.05$. Abbreviations as in Figures 1 to 4.

TABLE 3 Changes in Plasma Levels of Metabolic Parameters After MANP/Placebo Administration

	0.5 h		1 h		2 h		4 h	
	Placebo	MANP	Placebo	MANP	Placebo	MANP	Placebo	MANP
Glucose, Δmg/dL	2.0 ± 3.0	−1.6 ± 1.2	1.2 ± 4.5	−3.1 ± 1.2	−4.2 ± 6.5	−4.7 ± 1.6[a]	−10.0 ± 6.3	−13.1 ± 4.0[a]
Insulin, ΔμIU/mL	3.8 ± 2.3	−1.0 ± 1.7	6.9 ± 6.6[a]	−1.6 ± 1.1[b]	4.2 ± 2.7[a]	0.4 ± 1.3[b]	3.1 ± 2.9	0.4 ± 3.7
NEFA, Δμmol/L	43.4 ± 40.6	72.8 ± 45.5	79.0 ± 28.0	108.5 ± 41.2[a]	81.5 ± 55.4	79.2 ± 49.2	129.7 ± 86.7	32.8 ± 34.2
Glycerol, Δμmol/L	1.6 ± 30.0	9.5 ± 14.0	7.6 ± 27.0	15.7 ± 10.1	8.0 ± 15.1	13.4 ± 21.8	21.1 ± 27.0	−15.7 ± 12.3
Triglycerides, Δmg/dL	−14.0 ± 7.1	−6.5 ± 2.1	−17.0 ± 8.3	−7.7 ± 2.8	−19.2 ± 9.3	−8.6 ± 2.8	−18.8 ± 11.4	−6.5 ± 3.7

Values are mean ± SEM. Shown values are relative changes compared to baseline is 0. [a]$P < 0.05$ compared to 0. [b]$P < 0.05$ compared to placebo group.
MANP = M-atrial natriuretic peptide; NEFA = nonesterified fatty acid.

demonstrates the efficacy of delivering a natriuretic peptide via subcutaneous administration, which is a route widely used for insulin and glucagon-like peptide-1 analogs that overcomes the need for a continuous intravenous infusion. Herein, we further extended the subcutaneous administration of MANP to subjects with HTN and MetS, a special population whose treatment remains an unmet need. A larger cohort of 22 subjects participated in the current trial. Moreover, and for the first time, administration of MANP was compared to placebo and was also investigated on top of current antihypertensive medications.

In the initial first-in-human study, 3 doses of MANP were investigated and 5 μg/kg was found to be the maximal tolerated dose as 2 of the 4 subjects experienced reduction of systolic BP > 30 mm Hg. Thus, here we administered MANP at the dose of 2.5 μg/kg, which was the medium dose of the initial study. In the current study, treatment of MANP at 2.5 μg/kg in patients with HTN and MetS was proven to be safe, and no major adverse events were observed. Of the 17 subjects receiving MANP, 1 subject developed orthostatic hypotension and 1 subject developed vasovagal syncope, both of which resolved without intervention or clinical consequence. Meanwhile, no electrocardiographic changes were observed in any of the subjects during the 24 hours of observation, and there were no local reactions at the injection sites. Thus, similarly to essential primary HTN, the safety and tolerability of subcutaneous administration of MANP is also proven in subjects with HTN and MetS.

The central role of ANP and its molecular target GC-A receptor in BP homeostasis has been well established. Activation of ANP/GC-A signaling lowers BP via its second messenger cGMP. Indeed, deletion of either ANP or GC-A gene in murine model leads to hypertensive phenotype.[32,33] Moreover, a relative deficiency in ANP has been separately reported in both HTN and MetS that may be of greater magnitude in Blacks,[34] thus strongly supporting the rationale of developing MANP, which is the most potent and long-lasting ANP analog proven in preclinical studies, as therapeutics for treating patients with HTN and MetS. In the current study, subcutaneous injection of a single dose of MANP increased circulating levels of ANP-like peptides in all 17 subjects with the peak in the first 2 hours. Importantly, the increase of ANP-like peptides was also followed by an increase in plasma cGMP that peaked in the first 6 hours. By contrast, an elevation in neither plasma ANP-like peptides nor cGMP was observed in the placebo group at any time points. Together our data demonstrate that subcutaneous injection of MANP can engage the molecular target and effectively trigger cGMP production to correct, at least partially, the state of ANP deficiency associated with HTN with MetS.

It is worth noting that among the 17 patients who received MANP, 15 were obese and 2 were overweight. Indeed, BMI above normal values is highly prevalent in patients with HTN and MetS. Importantly, obesity is also characterized by a relative ANP deficiency, which is mostly due to reduced expression of GC-A and increased expression of the natriuretic peptide clearance receptor on adipose tissue.[8,35,36] Given that HTN with MetS and obesity confer high risks of heart disease, diabetes, and stroke, the pleiotropic effects of cGMP may benefit these patients by preventing the development of cardiovascular events as well as providing long-term organ protection. Importantly, the increase in cGMP levels associated with MANP treatment was observed in both male and female subjects and was not affected by age, BMI, waist circumference, baseline ANP, and baseline cGMP. Therefore, MANP represents a promising therapy to rescue the status of ANP/cGMP deficiency of subjects with cardiometabolic disease.

Reduction in BP was observed over the 24-hour period of observation and was similar to the previous study conducted from our group in subjects with essential HTN,[30] the BP effect peaked within 12 hours. Importantly, during the same period. BP showed a

TABLE 4 HOMA2 Indices Before and After MANP/Placebo Administration

	Baseline		0.5 h		1 h		2 h		4 h	
	Placebo	MANP	Placebo	MANP	Placebo	MANP	Placebo	MANP	Placebo	MANP
HOMA2-S, %	63.4 (37.8- 89.8)	51.9 (39.4- 79.7)	41.7 (39.9- 42.7)	48.4 (34.2- 89.1)	39.3 (34.1- 50.4)	48.7 (35.6- 94.1)	41.6 (37.5- 50.1)	41.0 (27.9- 101.5)	44.5 (42.7- 56.2)	64.0 (34.8- 127.0)
HOMA2-IR	1.58 (1.11- 2.65)	1.93 (1.26- 2.62)	2.40 (2.34- 2.51)	2.10 (1.13- 2.96)	2.54 (1.98- 2.93)	2.05 (1.07- 2.81)	2.40 (2.00- 2.67)	2.44 (0.99- 3.58)	2.25 (1.94- 2.35)	1.57 (0.79- 2.90)

Values are median (Q1-Q3).
HOMA2 = updated Homeostatic Model Assessment; HOMA2-S = HOMA2 index for insulin sensitivity; HOMA2-IR = HOMA2 index for insulin resistance; MANP = M-atrial natriuretic peptide.

nonsignificant increase in the placebo group reaching highest BP values at 24 hours post injection. This directly ensures that the BP reduction observed in the MANP group are truly related to MANP administration and not just a time effect. Moreover, in both studies, we consistently observed greater reduction in systolic BP than diastolic BP with MANP treatment, which was probably due to the inhibitory effect of natriuretic peptides on sympathetic nervous system. Though similar levels of increase in plasma cGMP were observed in our study and the previous study in subjects with essential HTN,[30] in the current study the dose of 2.5 μg/kg MANP resulted in a lower BP reduction of approximately 5-10 mm Hg. The difference may be attributed, at least in part, to 2 reasons. First, MetS, obesity, and related presence of adipose tissue, which is rich in the natriuretic peptide clearance receptor, may have affected the ANP/cGMP pathway and resulted in milder biological effects including vasodilation. Indeed, whereas MANP has been proven to be resistant to degradation driven by neprilysin and insulin-degrading enzyme,[27] its relationship to natriuretic peptide clearance receptor-mediated clearance remains to be characterized. Second, conversely to the study by Chen et al,[30] in which hypertensive subjects were withdrawn from antihypertensive medications for 2 weeks, the design of the current trial evaluated MANP effect on top of other antihypertensive medications because those were withheld only on the day in which MANP was administered. This design closely resembled the clinical setting in real practice. Based on the observed BP effect, it is reasonable to conclude that a dose of 2.5 μg/kg may not represent the maximal tolerated dose of MANP in patients with HTN and MetS. Future studies are warranted to further investigate higher doses of MANP and longer duration of treatment. The association of baseline features and BP response to MANP were also explored in the current study, showing that greater BP effect was related to lower baseline cGMP levels and male gender. The mechanisms underlying how baseline cGMP and gender may influence MANP response is worth being further investigated. In addition, our analysis also showed associations among baseline ANP levels, waist circumference, and C_{max} of ANP-like peptides after MANP administration. Such relations require further studies in larger cohorts to be confirmed.

Natriuretic peptides also exert metabolic actions, and a crosstalk exists between the natriuretic peptide system and metabolism.[37] We have reported that the ANP genetic variant rs5068, which is associated with higher ANP plasma levels, is also associated with lower prevalence of obesity and MetS.[24,25,38] Thus, for the first time, our study investigated metabolic actions of MANP in humans with cardiometabolic disease. In our study, we observed an acute effect of MANP on lipolysis that is indicated by a significant increase of circulating NEFA at 1 hour post MANP administration. This observation aligns with previous reports of increased NEFA plasma levels following ANP infusion in both lean and obese individuals,[17,19] further ensuring an important link between GC-A/cGMP function and lipid mobilization in humans.

Our findings on glucose and insulin levels after MANP administration may support an improvement on insulin sensitivity. In subjects receiving MANP, we observed a reduction in glucose levels when compared to baseline, whereas insulin levels remained unchanged. Despite that the study may be statistically underpowered, we found an increase in HOMA2 insulin sensitivity index and a decrease in HOMA2 insulin resistance index at 4 hours in the MANP group compared with in the placebo group. Indeed, activation of GC-A receptor has been extensively linked to enhanced insulin sensitivity in animal models. Transgenic mice overexpressing B-type natriuretic peptide, another ligand of GC-A along with ANP, were reported to be protected against insulin resistance induced by high fat diet.[39] At the level of downstream signaling, transgenic mice overexpressing cGMP-dependent protein kinase had higher insulin sensitivity even on standard diet.[39] Mechanistically, Coue et al[40] provided insights into

the metabolic actions of GC-A activation through ANP and reported that ANP might favor insulin sensitivity by increasing glucose uptake in adipocytes. Our data further support the benefits of the natriuretic peptide/GC-A/cGMP signaling pathway on insulin sensitivity, and for the first time we showed that MANP may represent a promising GC-A–specific agonist with dual cardiovascular and metabolic actions in subjects with cardiometabolic disease. Along the line, Jordan et al[41] and Murphy et al[42] have also reported improved insulin sensitivity in subjects with obesity and hypertension treated with sacubitril/valsartan, further confirming the benefit of targeting the natriuretic peptide system as sacubitril blocks the degradation of ANP and B-type natriuretic peptide. A longer MANP effect on circulating NEFA, glucose, and insulin in HTN with MetS, especially those subjects with obesity or type 2 diabetes mellites, is of particular interest to be validated in future studies. Importantly, studies are also warranted to further investigate the mechanism through which MANPs induce lipolytic effect and improve insulin sensitivity.

STUDY LIMITATIONS. First, our study included a small cohort of 22 patients and our statistics are underpowered in some cases, thus a trial involving a larger cohort will be needed to confirm and extend current findings. Second, although our study established the safety and tolerability of MANP in subjects with HTN and MetS with a single injection over a 24-hour period, it remains to be determined whether daily MANP injections would be safe and tolerable over a longer period of time. Third, we only observed mild metabolic properties possibly due to a single administration and, consequently, a short period of observation. Future studies are certainly warranted to investigate metabolic actions of MANP during longer treatment with higher doses including a diverse cohort.

CONCLUSIONS

This proof-of-concept phase 1 clinical trial demonstrated safety and tolerability of subcutaneous injection of MANP, the best-in-class ANP analog, in subjects with HTN with MetS. Importantly, a single subcutaneous injection of MANP effectively boosted plasma levels of both ANP-like peptides and cGMP as well as reduced blood pressure. Our data also suggest an acute effect on improving insulin sensitivity and inducing lipolysis. Based on its unique combination of cardiovascular and metabolic actions, MANP may represent a future and comprehensive therapy for cardiometabolic disease. Future studies in larger cohorts are warranted to evaluate the long-term cardiometabolic effects of higher doses of MANP.

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PERSPECTIVES

COMPETENCY IN PATIENT CARE AND MEDICAL KNOWLEDGE: In a double-blind, placebo-controlled trial, patients with HTN and MetS benefited from a single subcutaneous administration of MANP, an innovative ANP analog, with an increase in the protective molecule cGMP, reduction in blood pressure, and favorable profile on glucose, insulin, and NEFA levels.

TRANSLATIONAL OUTLOOK: Given the safety, tolerability, and cardiovascular and metabolic properties of MANP, future trials are warranted to investigate the long-term cardiometabolic benefits of MANP in patients with HTN and MetS in larger and more diverse subjects.

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KEY WORDS cGMP, cyclic guanosine monophosphate, hypertension, insulin resistance, MANP, M-atrial natriuretic peptide, metabolic syndrome, MetS, NEFA, nonesterified fatty acids

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