# B-type natriuretic peptide modulates ghrelin, hunger and satiety in healthy men

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# B-type natriuretic peptide modulates ghrelin, hunger and satiety in healthy men

*Vila et al. Anorexigenic effects of BNP in men*

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## ABSTRACT

Chronic heart failure is accompanied by anorexia and increased release of B-type natriuretic peptide (BNP) from ventricular cardiomyocytes. The pathophysiological mechanisms linking heart failure and appetite regulation remain unknown. Here we investigated the impact of intravenous BNP administration on appetite-regulating hormones and subjective ratings of hunger and satiety in ten healthy volunteers. Participants received in a randomized, placebo-controlled, cross-over, single-blinded study (subject) once placebo and once 3.0 pmol/kg/min human BNP-32 administered as continuous infusion during four hours. Circulating concentrations of appetite-regulating peptides were measured hourly. Subjective ratings of hunger and satiety were evaluated by visual analog scales. BNP inhibited the fasting-induced increase in total and acylated ghrelin concentrations over time (P=0.043 and P=0.038, respectively). In addition, BNP decreased the subjective rating of hunger (P=0.009) and increased the feeling of satiety (P=0.012) when compared to placebo. There were no significant changes in circulating peptide YY, glucagon-like peptide 1, oxyntomodulin, pancreatic polypeptide, leptin and adiponectin concentrations. In summary, our results demonstrate that BNP exerts anorectic effects and reduces ghrelin concentrations in men. These data, taken together with the known cardiovascular properties of ghrelin, support the existence of a heart-gut-brain axis, which could be therapeutically targeted in patients with heart failure, obesity.




**Clinical Trial Registration —** http:www.clinicaltrials.gov. Unique identifier: NCT01375153.

**KEYWORDS —** natriuretic peptides, physiology, appetite regulation, ghrelin, adiponectin

## INTRODUCTION

The control of appetite and energy balance is tightly regulated by a complex neuronal network in the brainstem and hypothalamus that receives information from the periphery via nutrients, hormones and afferent nervous fibres (1). Important members of this network are the gut-derived hormones ghrelin and peptide YY (PYY), which modulate not only appetite in the short-term, but also energy balance in the long-term (2-4).

Appetite and body mass index (BMI) decrease progressively in patients with heart failure, and cachexia accompanies end-stage disease (5). The changes in body composition have prognostic importance, as lower waist circumference and BMI are associated with a worse outcome in patients with heart failure (6).

The biochemical diagnosis and prognosis of heart failure is strongly dependent on the circulating concentrations of B-type natriuretic peptide (BNP), which is secreted from ventricular myocytes in response to cell stretching and stress, and reflects the degree of cardiac dysfunction (7, 8, 9). BNP has hormone properties, binds to transmembrane receptors and induces natriuresis, diuresis and vasodilatation, thereby reducing the preload (8). Recent studies corroborate an association between BNP concentrations and appetite regulation. BNP transgenic mice are protected against diet-induced obesity and insulin resistance (10). Cross-sectional studies reveal an inverse correlation between circulating BNP and BMI not only in patients with heart failure, but also in healthy people (11). Heart failure is associated with resistance to the orexigenic hormone ghrelin (12) and ghrelin administration improves both left ventricular function and muscle wasting (13, 14).

Here we investigated the acute effect of intravenous BNP administration on appetite-regulating hormones and on subjective ratings of hunger and satiety in a placebo-controlled cross-over study conducted in healthy men.

METHODS

*Study subjects*

The protocol was approved by the Institutional Review Board, registered at www.ClinicalTrial.gov (NCT01375153) and performed according to the Declaration of Helsinki. Ten healthy male subjects were recruited in the study after prior oral and written informed consent. At the screening day, a careful medical history was taken. Inclusion criteria were: normal BMI, no history of concomitant or chronic disease, no smoking, no drug- and alcohol-abuse, and no medication, including non-prescription drugs. A thorough medical examination and biochemical tests for glucose, HbA1c, BNP, electrolytes, CRP, lipid values, renal, liver and thyroid function were performed before enrollment in the study. Blood pressure was measured in supine and sitting positions. An electrocardiogram was recorded. Only subjects having all values within the normal range were included in the study. They were aged between 21 and 29 years and had BMIs ranging from 22 to 24.6 $kg/m^2$.

*Study protocol*

This was a randomized placebo-controlled single-blind (subject) cross-over study. All subjects were examined on two sessions, scheduled at least three weeks apart, where they received once placebo and once BNP. At both sessions, participants came to the clinical trials center at 8:00 AM after having fasted overnight and remained fasted till the end of the study. In order to avoid BNP effects on blood pressure, all participants were confined to bed rest and remained in laying position throughout the study period (15).

Cannulas were placed in the antecubital veins of the right and left arm for infusions and blood sampling, respectively. After venous cannulation, the subjects relaxed about 20 min. Then, 3 pmol/kg/min active BNP (BNP-32, containing aminoacids 77-108 of the preproBNP, obtained from American Peptide Company, Sunnyvale, CA) or placebo (0.9% NaCl) were administered as a continuous intravenous infusion during four hours (time point 0 till 4

hours). Blood samples were obtained at time points -5 min, 0, 1, 2, 3 and 4 hours. The subjective ratings of hunger and satiety were marked on a 10-cm visual analog scale (VAS) half-hourly during the four study hours.

Primary outcome measurements were circulating ghrelin, acylated ghrelin, PYY and adiponectin concentrations. Secondary outcome measurements were the ratings of hunger and satiety in the VAS, and plasma glucagon-like peptide 1 (GLP-1), oxyntomodulin, pancreatic polypeptide (PP), and leptin concentrations.

*Blood sampling and assays*

Blood samples for the measurement of ghrelin, PYY, oxyntomodulin, PP, leptin and adiponectin were obtained in collection tubes containing EDTA. Blood samples for the measurement of BNP were obtained in collection tubes containing heparin. Blood samples for the measurement of GLP-1 were obtained in collection tubes containing EDTA, and were immediately supplemented with DPP IV inhibitor. All these samples were immediately cooled on ice, centrifuged at 3000 rpm for 10 min and then frozen at -20°C. Samples for the measurement of acylated ghrelin were withdrawn in EDTA supplemented with the protease inhibitor Trasylol (SIGMA), centrifuged at 3000 rpm for 10 min, acidified with 1N HCl (1/10) and frozen at -20°C. Assays were performed at the very end of the study in duplicates, with samples from both study days of each individual analysed within one assay.

Total ghrelin concentrations were measured using a commercially available RIA kit with inter- and intra-assay coefficients of variation being 5% and 8%, respectively (Bachem-Peninsula Laboratories, San Carlos, CA). Acylated ghrelin was determined using a commercially available sandwich ELISA kit with no cross-reactivity with des-octanoyl-ghrelin (Millipore, Billerica, MA, USA). The inter-assay and intra-assay coefficients were 7.5–12% and 0.9–7.5%, respectively. Plasma $PYY_{3-36}$ concentrations were quantified using a RIA kit (Millipore) with inter- and intra-assay variations of 8.2 and 9%, respectively. Active

GLP-1 was determined using an ELISA kit (Millipore) with inter- and intra-assay variations being 6-10%. Oxyntomodulin concentrations were quantified with a RIA kit (Phoenix Pharmaceuticals, Burlingame, CA). PP was quantified using a sandwich ELISA (Millipore) with intra-assay CVs being 3.3–5% and inter-assay CVs being 5–9.8%. Leptin and adiponectin were measured with RIA kits (Millipore), both having inter- and intra-assay variations of 5-8%. BNP concentrations were measured using the commercially available ARCHITECT BNP immunoassay on the ARCHITECT System (Abbott Laboratories, Vienna, Austria).

*Statistical evaluation*

Data were analyzed using the statistical software SPSS release 12.0.1 (SPSS, Inc., Chicago, IL). Subjects' characteristics are expressed as mean and range, or mean ± SE. The values of primary and secondary outcome parameters are expressed as mean ± SE. The significance of differences between the two study days was assessed by repeated measurements analysis of variance (ANOVA) with the interaction between time and treatment (time*treatment) being the term of interest. $P < 0.05$ was considered statistically significant. Post hoc paired t-tests for comparing the intervention-induced changes at each time point were performed only when repeated measurements ANOVA revealed significant results.

RESULTS

The intravenous infusion of 3 pmol/kg/min BNP significantly increased plasma BNP concentrations, which remained constant between 400 and 500 pg/ml during the last two hours of the study (Figure 1). In accordance with previous reports, BNP did not modulate blood pressure in the supine position (15).

BNP infusion decreased total ghrelin concentrations (P=0.043 RM-ANOVA), reaching 778 ± 113 pg/ml in BNP sessions as compared to 988 ± 122 pg/ml during placebo sessions at time

point 4 hours (P=0.012, post hoc t-tests) (Figure 2A-B). In parallel, BNP reduced the increase in acylated ghrelin concentrations during fasting (P=0.038 RM-ANOVA), with significant differences observed at time points 2, 3 and 4 hours (P=0.014, P=0.002 and P=0.029 respectively, post hoc t-tests) (Figure 2C-D). However, BNP did not significantly modify plasma $PYY_{3-36}$ (P=0.716), active GLP-1 (P=0.265), oxyntomodulin (P=0.419), pancreatic polypeptide (P=0.577), leptin (P=0.533), and adiponectin (P=0.180), concentrations (Figure 3A-F).

The increase in the subjective rating of hunger with time during fasting was reduced during the BNP infusion (P=0.009 RM-ANOVA) (Figure 4A). At time point 4 hours, the changes in scores of Hunger-VAS versus baseline were 36.2 ± 4.9 mm in placebo sessions and 13.4 ± 6.1 mm in BNP sessions (P=0.024) (Figure 4B). In parallel, BNP infusion abolished the decrease in the subjective rating of satiety during fasting (P=0.012 RM-ANOVA), achieving a decrease of 21.7 ± 5.9 mm in placebo sessions versus 0.3 ± 3.6 mm in BNP sessions (P=0.018, Figure 4C-D).

## DISCUSSION

Here we report that the cardiac hormone BNP directly impacts appetite via reducing the circulating concentrations of the appetite-stimulating hormone ghrelin, thereby decreasing hunger and inducing the feeling of satiety. These anorexigenic properties of BNP suggest the existence of a pathway originating from the heart, linking heart dysfunction and appetite regulation.

The gut-brain axis with its main hormone ghrelin is one of the key players controlling appetite regulation, as it not only transmits information on the feeding status, but also coordinates information coming from other hormones and organs, adapting appetite in response to stressors (3, 16, 17). Ghrelin is a potent orexigenic peptide and the BNP-induced

reduction in the ratings of hunger, and increase in the feeling of satiety might therefore be, at least in part, mediated by the decrease in circulating ghrelin concentrations (2). Nevertheless, BNP receptors are widely spread throughout the brain, and potential direct effects of BNP on appetite regulating centres cannot be excluded (18).

Interestingly, BNP administration did not change the concentrations of the anorexigenic gut hormone PYY. The secretion of ghrelin and PYY in response to food intake is mediated via the cholinergic system. Pharmacological modulation of the cholinergic system alters both ghrelin and PYY secretion during fasting (19). The fact that BNP administration impacts only ghrelin and not PYY speaks against a possible mediation of BNP effects via the cholinergic system. This hypothesis is supported by a physiological study in eels, revealing that BNP effects are dependent on beta-adrenergic blockade, but not on cholinergic blockade (20). The fact that BNP does not influence GLP-1, oxyntomodulin and PP is somewhat expected in this fasting study, as all these peptides are mainly modulated in response to food intake.

It is important to emphasize that this trial investigated only the acute effects of BNP administration in healthy men. The continuous infusion of BNP led to plasma BNP concentrations between 400 and 500 pg/ml. These concentrations are in agreement with other studies using similar infusion protocols, and similar to the values found in patients with heart failure (21). Patients with heart failure associated anorexia have increased ghrelin concentrations when compared to healthy people, as expected from the strong negative correlation between ghrelin and BMI in all studied populations (2, 22). We suggest that the continuous BNP oversecretion in patients with heart failure could thus impede a further increase in ghrelin. This hypothesis is supported by the fact that patients with cardiac cachexia, despite having increased ghrelin concentrations, significantly benefit from the pharmacological administration of ghrelin (23). In addition to increasing appetite, ghrelin

displays also positive cardiovascular properties, such as the stimulation of left ventricular function, vasodilatation and anti-inflammatory effects (13, 14, 17).

Several studies have addressed the positive correlation between circulating concentrations of BNP and adiponectin not only in patients with heart failure, but also in healthy people (24). BNP enhances adiponectin mRNA expression in human adipocytes in primary culture (25). In contrast to these in vitro effects, we show that systemic BNP administration does not significantly change adiponectin concentrations. Taken together, these results demonstrate that the relationship between BNP and adiponectin is not just a direct one.

Strength of the study is the use of an adequate randomised cross-over protocol for investigating novel effects of BNP on appetite-regulating hormones, hunger and satiety, independently from effects on blood pressure. Study weaknesses are the single blind design and the lack of a meal intake, which was conditioned by the fact that subjects had to remain in supine position. Therefore, we cannot exclude BNP effects on the meal-induced changes in GLP-1, oxyntomodulin and PP.

In summary, here we demonstrate that intravenous administration of BNP reduces circulating ghrelin concentrations, decreases hunger and increases the feeling of satiety in healthy people. These data, taken together with the known cardioprotective effects of ghrelin, support the existence of a bidirectional heart-gut cross talk, which might play a central role in adapting appetite and energy homeostasis to the degree of heart dysfunction. Heart-gut signaling links two vital human functions and might be further investigated as a potential therapeutical target not only in heart failure, but also in obesity and eating disorders.

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Author contributions: G.V., B.H., A.L and M.C. designed the study. G.V., G.G., M.R., E.E., H.E., B.D. and T.M. performed research. G.V., A.L and M.C analysed the data. G.V. wrote the manuscript. All authors approved the final version of the manuscript.

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LITERATURE

1. Coll AP, Farooqi IS, O'Rahilly S. The hormonal control of food intake. Cell. 2007;129:251-262.
2. Murphy KG, Dhillo WS, Bloom SR. Gut peptides in the regulation of food intake and energy homeostasis. Endocr Rev. 2006;27:719-727.
3. Castañeda TR, Tong J, Datta R, Culler M, Tschöp MH. Ghrelin in the regulation of body weight and metabolism. Front Neuroendocrinol. 2010;31:44-60.
4. Batterham RL, ffytche DH, Rosenthal JM, Zelaya FO, Barker GJ, Withers DJ, Williams SC. PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans. Nature. 2007;450:106-109.
5. von Haehling S, Lainscak M, Springer J, Anker SD. Cardiac cachexia: a systematic overview. Pharmacol Ther. 2009;121:227-252.
6. Curtis JP, Selter JG, Wang Y, Rathore SS, Jovin IS, Jadbabaie F, Kosiborod M, Portnay EL, Sokol SI, Bader F, Krumholz HM. The obesity paradox: body mass index and outcomes in patients with heart failure. Arch Intern Med. 2005;165:55-61.
7. Clerico A, Giannoni A, Vittorini S, Passino C. Thirty years of the heart as an endocrine organ: physiological role and clinical utility of cardiac natriuretic hormones. Am J Physiol Heart Circ Physiol. 2011;301:H12-20.
8. Braunwald E. Biomarkers in heart failure. N Engl J Med. 2008;358:2148-2159.
9. Vila G, Resl M, Stelzeneder D, Struck J, Maier C, Riedl R, Hülsmann M, Pacher R, Luger A, Clodi M. Plasma NT-proBNP increases in response to LPS administration in healthy men. J Appl Physiol 2008;105:1741-1745.
10. Miyashita K, Itoh H, Tsujimoto H, Tamura N, Fukunaga Y, Sone M, Yamahara K, Taura D, Inuzuka M, Sonoyama T, Nakao K. Natriuretic peptides/cGMP/cGMP-

dependent protein kinase cascades promote muscle mitochondrial biogenesis and prevent obesity. Diabetes. 2009;58:2880-2892.

11. Das SR, Drazner MH, Dries DL, Vega GL, Stanek HG, Abdullah SM, Canham RM, Chung AK, Leonard D, Wians FH Jr, de Lemos JA. Impact of body mass and body composition on circulating levels of natriuretic peptides: results from the Dallas Heart Study. Circulation. 2005;112:2163-2168.
12. Lund LH, Williams JJ, Freda P, LaManca JJ, LeJemtel TH, Mancini DM. Ghrelin resistance occurs in severe heart failure and resolves after heart transplantation. Eur J Heart Fail. 2009;11:789-794.
13. Vestergaard ET, Andersen NH, Hansen TK, Rasmussen LM, Moller N, Sorensen KE, Sloth E, Jorgensen JO. Cardiovascular effects of intravenous ghrelin infusion in healthy young men. Am J Physiol Heart Circ Physiol. 2007;293:H3020-3026.
14. Nagaya N, Moriya J, Yasumura Y, Uematsu M, Ono F, Shimizu W, Ueno K, Kitakaze M, Miyatake K, Kangawa K. Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure. Circulation. 2004;110:3674-3679.
15. van der Zander K, Houben AJ, Hofstra L, Kroon AA, de Leeuw PW. Hemodynamic and renal effects of low-dose brain natriuretic peptide infusion in humans: a randomized, placebo-controlled crossover study. Am J Physiol Heart Circ Physiol. 2008;285:H1206-1212.
16. Seoane LM, Al-Massadi O, Caminos JE, Tovar SA, Dieguez C, Casanueva FF. Sensory stimuli directly acting at the central nervous system regulate gastric ghrelin secretion: an ex-vivo organ culture study. Endocrinology 2007;148:3998-4006.

17. Vila G, Maier C, Riedl M, Nowotny P, Ludvik B, Luger A, Clodi M. Bacterial endotoxin induces biphasic changes in plasma ghrelin in healthy humans. J Clin Endocrinol Metab. 2007;92:3930-3934.
18. Cao LH, Yang XL. Natriuretic peptides and their receptors in the central nervous system. Prog Neurobiol. 2008;84:234-248.
19. Maier C, Riedl M, Vila G, Nowotny P, Wolzt M, Clodi M, Ludvik B, Luger A. Cholinergic regulation of ghrelin and peptide YY release may be impaired in obesity. Diabetes. 2008;57:2332-2340.
20. Nobata S, Ventura A, Kaiya H, Takei Y. Diversified cardiovascular actions of six homologous natriuretic peptides (ANP, BNP, VNP, CNP1, CNP3 and CNP4) in conscious eels. Am J Physiol Regul Integr Comp Physiol. 2010;298:R1549-1559.
21. Lainchbury JG, Richards AM, Nicholls MG, Espiner EA, Yandle TG. Brain natriuretic peptide and neutral endopeptidase inhibition in left ventricular impairment. J Clin Endocrinol Metab. 1999;84:723-729.
22. Nagaya N, Uematsu M, Kojima M, Date Y, Nakazato M, Okumura H, Hosoda H, Shimizu W, Yamagishi M, Oya H, Koh H, Yutani C, Kangawa K. Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. Circulation. 2001;104:2034-2038.
23. Akamizu T, Kangawa K. Therapeutical applications of ghrelin to cachexia utilizing its appetite-stimulatory effect. Peptides. 2011;11:2295-2300.
24. Fujimatsu D, Kotooka N, Inoue T, Nishiyama M, Node K Association between high molecular weight adiponectin and metabolic parameters. J Atheroscler Thromb. 2009;16:553-559.
25. Tsukamoto O, Fujita M, Kato M, Yamazaki S, Asano Y, Ogai A, Okazaki H, Asai M, Nagamachi Y, Maeda N, Shintani Y, Minamino T, Asakura M, Kishimoto I,

Funahashi T, Tomoike H, Kitakaze M. Natriuretic peptides enhance the production of adiponectin in human adipocytes and in patients with chronic heart failure. J Am Coll Cardiol. 2009;53:2070-2077.

**Figure legends**

**Figure 1**. Changes in circulating BNP concentrations.

BNP administered as a continuous intravenous infusion of 3pmol/kg/min. Horizontal black bar shows infusion duration. Data are presented as mean ± SEM. ●, 3pmol/kg/min BNP; ○ placebo.

**Figure 2**. Effects of BNP on acylated and total ghrelin concentrations

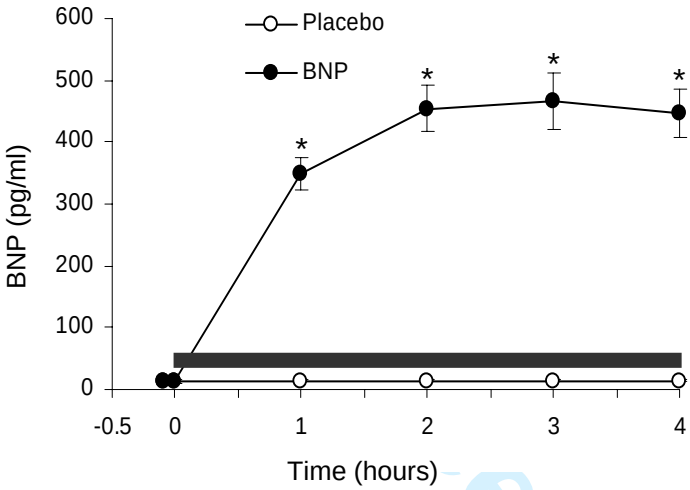
(A) Profile of changes in plasma ghrelin concentrations, (B) Differences between ghrelin concentrations at baseline and at time-point 4 hours, (C) Profile of changes in acylated ghrelin concentrations, (D) Differences between acylated ghrelin concentrations at baseline and at time-point 4 hours. Data are presented as mean ± SEM. ●, 3pmol/kg/min BNP; ○ placebo. Horizontal black bar shows infusion duration.

**Figure 3**. Effects of BNP on gut peptides and adipokines

BNP-induced changes in circulating (A) $PYY_{3-36}$ concentrations, (B) GLP-1 concentrations, (C) oxyntomodulin concentrations (D) PP concentrations, (E) leptin concentrations and (F) adiponectin concentrations. Data are presented as mean ± SEM. ●, 3pmol/kg/min BNP; ○ placebo. Horizontal black bar shows infusion duration.

**Figure 4**. Effects of BNP on subjective ratings of hunger and satiety, as measured by VAS. Scores are depicted as change from the 0 value (mm). (A) Profile of hunger-VAS values, (B) Differences between hunger-VAS values at baseline and at time-point 4 hours, (C) Profile of satiety-VAS values, (D) Differences between satiety-VAS values at baseline and at time-point 4 hours. Data are presented as mean ± SEM. ●, 3pmol/kg/min BNP; ○ placebo. Horizontal black bar shows infusion duration.

Figure 1



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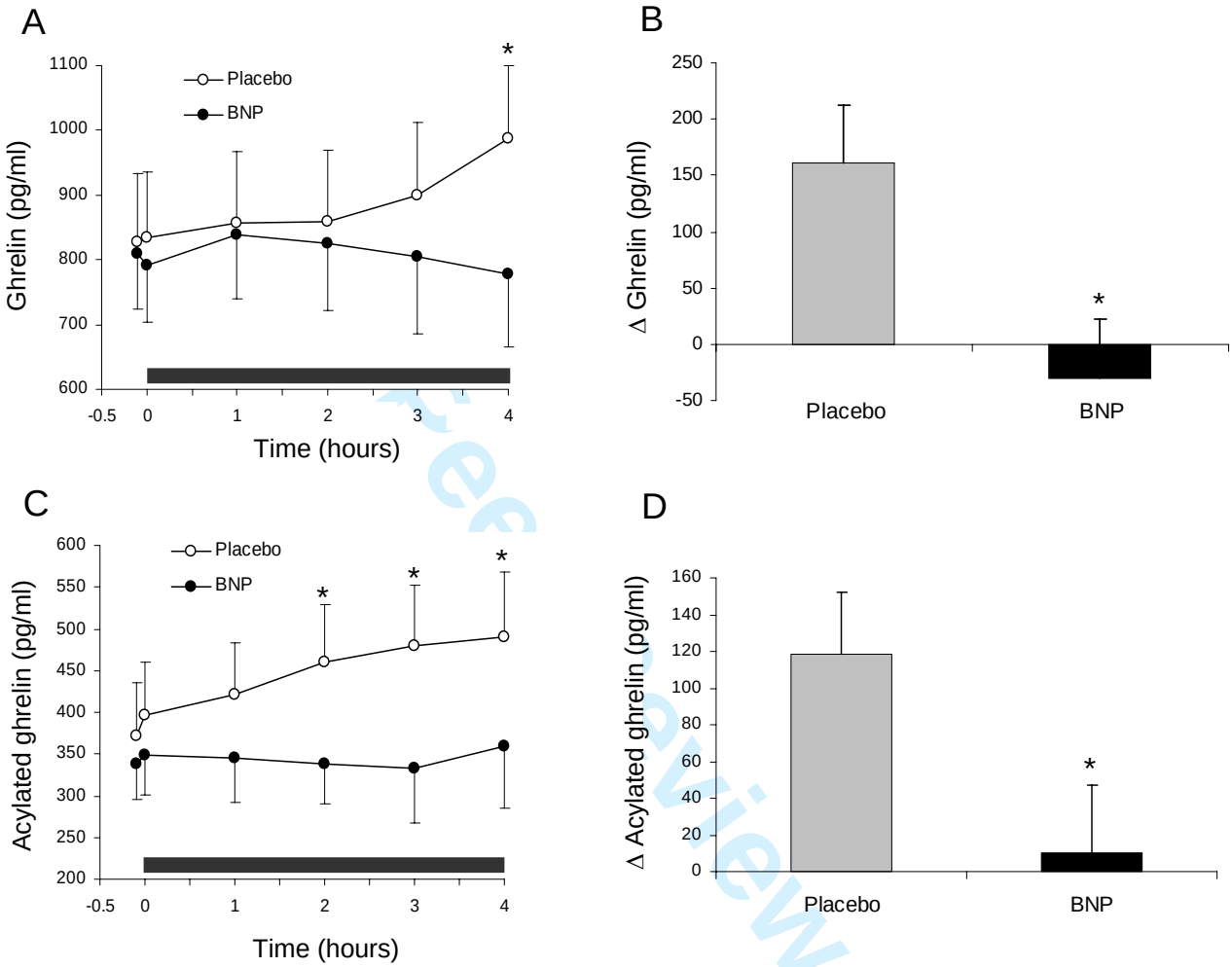
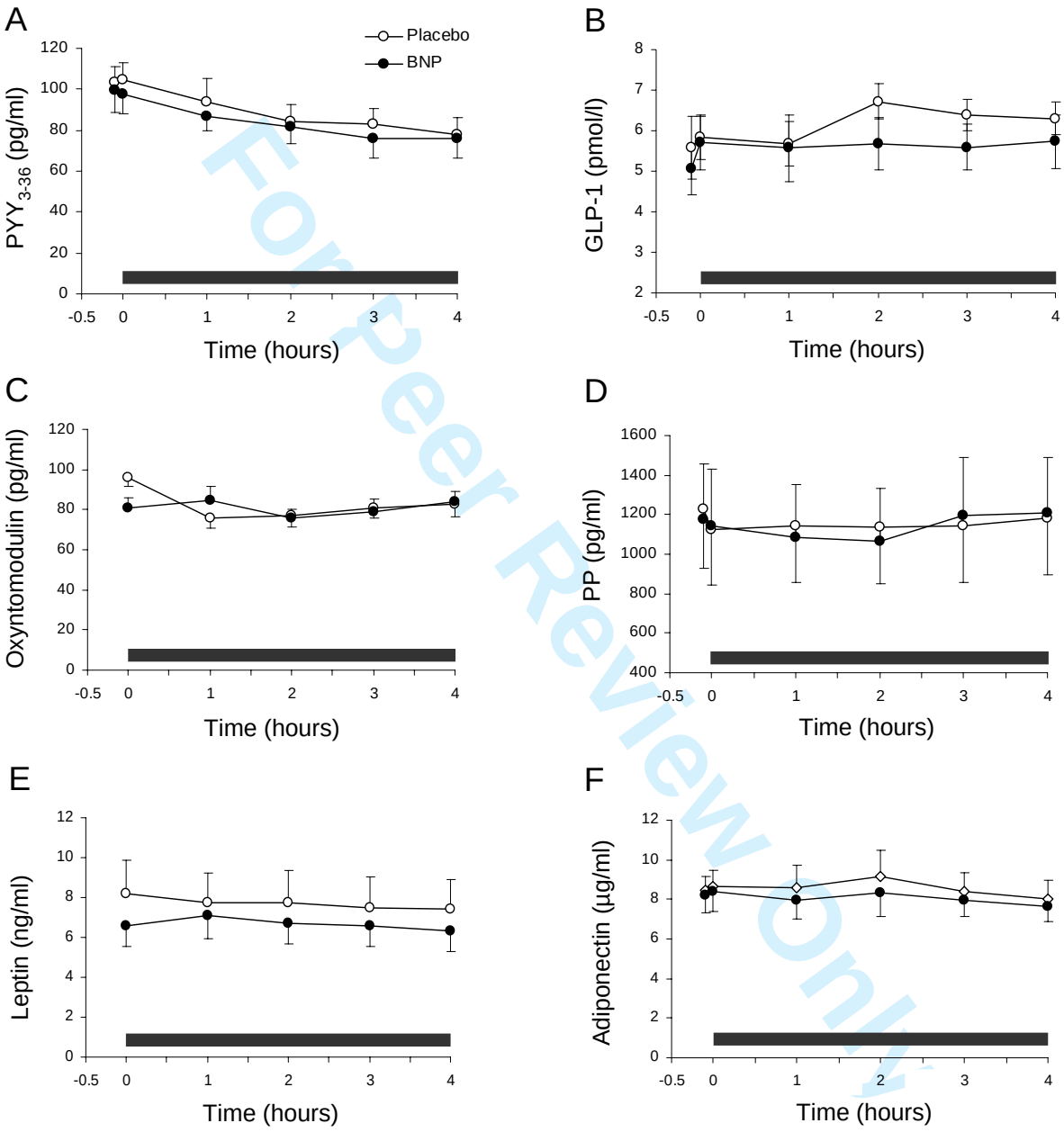
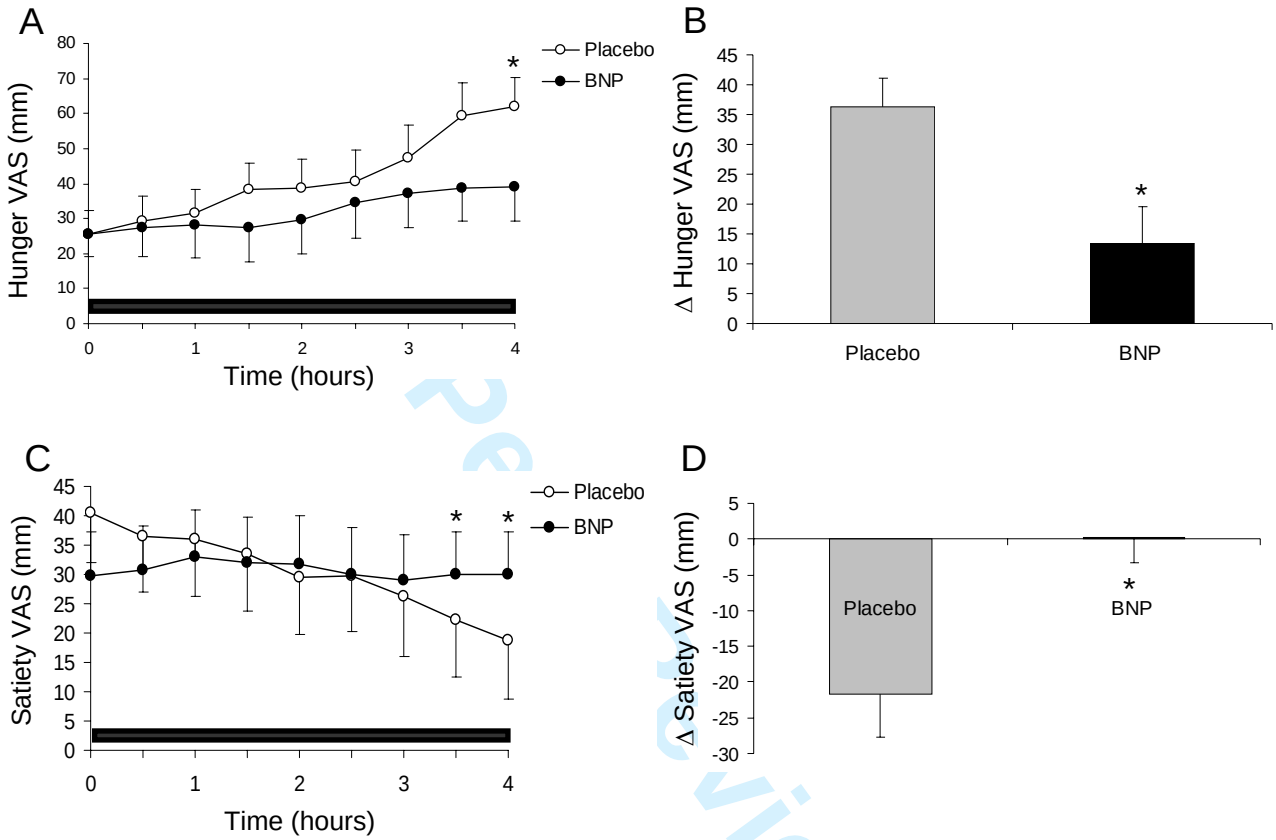


# Figure 3



# Figure 4



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## ABSTRACT

Chronic heart failure is accompanied by anorexia and increased release of B-type natriuretic peptide (BNP) from ventricular cardiomyocytes. The pathophysiological mechanisms linking heart failure and appetite regulation remain unknown. Here we investigated the impact of intravenous BNP administration on appetite-regulating hormones and subjective ratings of hunger and satiety in ten healthy volunteers. Participants received in a randomized, placebo-controlled, cross-over, single-blinded study (subject) once placebo and once 3.0 pmol/kg/min human BNP-32 administered as continuous infusion during four hours. Circulating concentrations of appetite-regulating peptides were measured hourly. Subjective ratings of hunger and satiety were evaluated by visual analog scales. BNP inhibited the fasting-induced increase in total and acylated ghrelin concentrations over time (P=0.043 and P=0.038, respectively). In addition, BNP decreased the subjective rating of hunger (P=0.009) and increased the feeling of satiety (P=0.012) when compared to placebo. There were no significant changes in circulating peptide YY, glucagon-like peptide 1, oxyntomodulin, pancreatic polypeptide, leptin and adiponectin concentrations. In summary, our results demonstrate that BNP exerts anorectic effects and reduces ghrelin concentrations in men. These data, taken together with the known cardiovascular properties of ghrelin, support the existence of a heart-gut-brain axis, which could be therapeutically targeted in patients with heart failure, obesity.



**Clinical Trial Registration —** http:www.clinicaltrials.gov. Unique identifier: NCT01375153.

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## INTRODUCTION

The control of appetite and energy balance is tightly regulated by a complex neuronal network in the brainstem and hypothalamus that receives information from the periphery via nutrients, hormones and afferent nervous fibres (1). Important members of this network are the gut-derived hormones ghrelin and peptide YY (PYY), which modulate not only appetite in the short-term, but also energy balance in the long-term (2-4).

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METHODS

*Study subjects*

The protocol was approved by the Institutional Review Board, registered at www.ClinicalTrial.gov (NCT01375153) and performed according to the Declaration of Helsinki. Ten healthy male subjects were recruited in the study after prior oral and written informed consent. At the screening day, a careful medical history was taken. Inclusion criteria were: normal BMI, no history of concomitant or chronic disease, no smoking, no drug- and alcohol-abuse, and no medication, including non-prescription drugs. A thorough medical examination and biochemical tests for glucose, HbA1c, BNP, electrolytes, CRP, lipid values, renal, liver and thyroid function were performed before enrollment in the study. Blood pressure was measured in supine and sitting positions. An electrocardiogram was recorded. Only subjects having all values within the normal range were included in the study. They were aged between 21 and 29 years and had BMIs ranging from 22 to 24.6 kg/m$^2$.

*Study protocol*

This was a randomized placebo-controlled single-blind (subject) cross-over study. All subjects were examined on two sessions, scheduled at least three weeks apart, where they received once placebo and once BNP. At both sessions, participants came to the clinical trials center at 8:00 AM after having fasted overnight and remained fasted till the end of the study. In order to avoid BNP effects on blood pressure, all participants were confined to bed rest and remained in laying position throughout the study period (15).

Cannulas were placed in the antecubital veins of the right and left arm for infusions and blood sampling, respectively. After venous cannulation, the subjects relaxed about 20 min. Then, 3 pmol/kg/min active BNP (BNP-32, containing aminoacids 77-108 of the preproBNP, obtained from American Peptide Company, Sunnyvale, CA) or placebo (0.9% NaCl) were administered as a continuous intravenous infusion during four hours (time point 0 till 4

hours). Blood samples were obtained at time points -5 min, 0, 1, 2, 3 and 4 hours. The subjective ratings of hunger and satiety were marked on a 10-cm visual analog scale (VAS) half-hourly during the four study hours.

Primary outcome measurements were circulating ghrelin, acylated ghrelin, PYY and adiponectin concentrations. Secondary outcome measurements were the ratings of hunger and satiety in the VAS, and plasma glucagon-like peptide 1 (GLP-1), oxyntomodulin, pancreatic polypeptide (PP), and leptin concentrations.

*Blood sampling and assays*

Blood samples for the measurement of ghrelin, PYY, oxyntomodulin, PP, leptin and adiponectin were obtained in collection tubes containing EDTA. Blood samples for the measurement of BNP were obtained in collection tubes containing heparin. Blood samples for the measurement of GLP-1 were obtained in collection tubes containing EDTA, and were immediately supplemented with DPP IV inhibitor. All these samples were immediately cooled on ice, centrifuged at 3000 rpm for 10 min and then frozen at -20°C. Samples for the measurement of acylated ghrelin were withdrawn in EDTA supplemented with the protease inhibitor Trasylol (SIGMA), centrifuged at 3000 rpm for 10 min, acidified with 1N HCl (1/10) and frozen at -20°C. Assays were performed at the very end of the study in duplicates, with samples from both study days of each individual analysed within one assay.

Total ghrelin concentrations were measured using a commercially available RIA kit with inter- and intra-assay coefficients of variation being 5% and 8%, respectively (Bachem-Peninsula Laboratories, San Carlos, CA). Acylated ghrelin was determined using a commercially available sandwich ELISA kit with no cross-reactivity with des-octanoyl-ghrelin (Millipore, Billerica, MA, USA). The inter-assay and intra-assay coefficients were 7.5–12% and 0.9–7.5%, respectively. Plasma $PYY_{3-36}$ concentrations were quantified using a RIA kit (Millipore) with inter- and intra-assay variations of 8.2 and 9%, respectively. Active

GLP-1 was determined using an ELISA kit (Millipore) with inter- and intra-assay variations being 6-10%. Oxyntomodulin concentrations were quantified with a RIA kit (Phoenix Pharmaceuticals, Burlingame, CA). PP was quantified using a sandwich ELISA (Millipore) with intra-assay CVs being 3.3–5% and inter-assay CVs being 5–9.8%. Leptin and adiponectin were measured with RIA kits (Millipore), both having inter- and intra-assay variations of 5-8%. BNP concentrations were measured using the commercially available ARCHITECT BNP immunoassay on the ARCHITECT System (Abbott Laboratories, Vienna, Austria).

*Statistical evaluation*

Data were analyzed using the statistical software SPSS release 12.0.1 (SPSS, Inc., Chicago, IL). Subjects' characteristics are expressed as mean and range, or mean ± SE. The values of primary and secondary outcome parameters are expressed as mean ± SE. The significance of differences between the two study days was assessed by repeated measurements analysis of variance (ANOVA) with the interaction between time and treatment (time*treatment) being the term of interest. $P < 0.05$ was considered statistically significant. Post hoc paired t-tests for comparing the intervention-induced changes at each time point were performed only when repeated measurements ANOVA revealed significant results.

RESULTS

The intravenous infusion of 3 pmol/kg/min BNP significantly increased plasma BNP concentrations, which remained constant between 400 and 500 pg/ml during the last two hours of the study (Figure 1). In accordance with previous reports, BNP did not modulate blood pressure in the supine position (15).

BNP infusion decreased total ghrelin concentrations (P=0.043 RM-ANOVA), reaching 778 ± 113 pg/ml in BNP sessions as compared to 988 ± 122 pg/ml during placebo sessions at time

point 4 hours (P=0.012, post hoc t-tests) (Figure 2A-B). In parallel, BNP reduced the increase in acylated ghrelin concentrations during fasting (P=0.038 RM-ANOVA), with significant differences observed at time points 2, 3 and 4 hours (P=0.014, P=0.002 and P=0.029 respectively, post hoc t-tests) (Figure 2C-D). However, BNP did not significantly modify plasma $PYY_{3-36}$ (P=0.716), active GLP-1 (P=0.265), oxyntomodulin (P=0.419), pancreatic polypeptide (P=0.577), leptin (P=0.533), and adiponectin (P=0.180), concentrations (Figure 3A-F).

The increase in the subjective rating of hunger with time during fasting was reduced during the BNP infusion (P=0.009 RM-ANOVA) (Figure 4A). At time point 4 hours, the changes in scores of Hunger-VAS versus baseline were 36.2 ± 4.9 mm in placebo sessions and 13.4 ± 6.1 mm in BNP sessions (P=0.024) (Figure 4B). In parallel, BNP infusion abolished the decrease in the subjective rating of satiety during fasting (P=0.012 RM-ANOVA), achieving a decrease of 21.7 ± 5.9 mm in placebo sessions versus 0.3 ± 3.6 mm in BNP sessions (P=0.018, Figure 4C-D).

## DISCUSSION

Here we report that the cardiac hormone BNP directly impacts appetite via reducing the circulating concentrations of the appetite-stimulating hormone ghrelin, thereby decreasing hunger and inducing the feeling of satiety. These anorexigenic properties of BNP suggest the existence of a pathway originating from the heart, linking heart dysfunction and appetite regulation.

The gut-brain axis with its main hormone ghrelin is one of the key players controlling appetite regulation, as it not only transmits information on the feeding status, but also coordinates information coming from other hormones and organs, adapting appetite in response to stressors (3, 16, 17). Ghrelin is a potent orexigenic peptide and the BNP-induced

reduction in the ratings of hunger, and increase in the feeling of satiety might therefore be, at least in part, mediated by the decrease in circulating ghrelin concentrations (2). Nevertheless, BNP receptors are widely spread throughout the brain, and potential direct effects of BNP on appetite regulating centres cannot be excluded (18).

Interestingly, BNP administration did not change the concentrations of the anorexigenic gut hormone PYY. The secretion of ghrelin and PYY in response to food intake is mediated via the cholinergic system. Pharmacological modulation of the cholinergic system alters both ghrelin and PYY secretion during fasting (19). The fact that BNP administration impacts only ghrelin and not PYY speaks against a possible mediation of BNP effects via the cholinergic system. This hypothesis is supported by a physiological study in eels, revealing that BNP effects are dependent on beta-adrenergic blockade, but not on cholinergic blockade (20). The fact that BNP does not influence GLP-1, oxyntomodulin and PP is somewhat expected in this fasting study, as all these peptides are mainly modulated in response to food intake.

It is important to emphasize that this trial investigated only the acute effects of BNP administration in healthy men. The continuous infusion of BNP led to plasma BNP concentrations between 400 and 500 pg/ml. These concentrations are in agreement with other studies using similar infusion protocols, and similar to the values found in patients with heart failure (21). Patients with heart failure associated anorexia have increased ghrelin concentrations when compared to healthy people, as expected from the strong negative correlation between ghrelin and BMI in all studied populations (2, 22). We suggest that the continuous BNP oversecretion in patients with heart failure could thus impede a further increase in ghrelin. This hypothesis is supported by the fact that patients with cardiac cachexia, despite having increased ghrelin concentrations, significantly benefit from the pharmacological administration of ghrelin (23). In addition to increasing appetite, ghrelin

displays also positive cardiovascular properties, such as the stimulation of left ventricular function, vasodilatation and anti-inflammatory effects (13, 14, 17).

Several studies have addressed the positive correlation between circulating concentrations of BNP and adiponectin not only in patients with heart failure, but also in healthy people (24). BNP enhances adiponectin mRNA expression in human adipocytes in primary culture (25). In contrast to these in vitro effects, we show that systemic BNP administration does not significantly change adiponectin concentrations. Taken together, these results demonstrate that the relationship between BNP and adiponectin is not just a direct one.

Strength of the study is the use of an adequate randomised cross-over protocol for investigating novel effects of BNP on appetite-regulating hormones, hunger and satiety, independently from effects on blood pressure. Study weaknesses are the single blind design and the lack of a meal intake, which was conditioned by the fact that subjects had to remain in supine position. Therefore, we cannot exclude BNP effects on the meal-induced changes in GLP-1, oxyntomodulin and PP.

In summary, here we demonstrate that intravenous administration of BNP reduces circulating ghrelin concentrations, decreases hunger and increases the feeling of satiety in healthy people. These data, taken together with the known cardioprotective effects of ghrelin, support the existence of a bidirectional heart-gut cross talk, which might play a central role in adapting appetite and energy homeostasis to the degree of heart dysfunction. Heart-gut signaling links two vital human functions and might be further investigated as a potential therapeutical target not only in heart failure, but also in obesity and eating disorders.

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LITERATURE

1. Coll AP, Farooqi IS, O'Rahilly S. The hormonal control of food intake. Cell. 2007;129:251-262.
2. Murphy KG, Dhillo WS, Bloom SR. Gut peptides in the regulation of food intake and energy homeostasis. Endocr Rev. 2006;27:719-727.
3. Castañeda TR, Tong J, Datta R, Culler M, Tschöp MH. Ghrelin in the regulation of body weight and metabolism. Front Neuroendocrinol. 2010;31:44-60.
4. Batterham RL, ffytche DH, Rosenthal JM, Zelaya FO, Barker GJ, Withers DJ, Williams SC. PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans. Nature. 2007;450:106-109.
5. von Haehling S, Lainscak M, Springer J, Anker SD. Cardiac cachexia: a systematic overview. Pharmacol Ther. 2009;121:227-252.
6. Curtis JP, Selter JG, Wang Y, Rathore SS, Jovin IS, Jadbabaie F, Kosiborod M, Portnay EL, Sokol SI, Bader F, Krumholz HM. The obesity paradox: body mass index and outcomes in patients with heart failure. Arch Intern Med. 2005;165:55-61.
7. Clerico A, Giannoni A, Vittorini S, Passino C. Thirty years of the heart as an endocrine organ: physiological role and clinical utility of cardiac natriuretic hormones. Am J Physiol Heart Circ Physiol. 2011;301:H12-20.
8. Braunwald E. Biomarkers in heart failure. N Engl J Med. 2008;358:2148-2159.
9. Vila G, Resl M, Stelzeneder D, Struck J, Maier C, Riedl R, Hülsmann M, Pacher R, Luger A, Clodi M. Plasma NT-proBNP increases in response to LPS administration in healthy men. J Appl Physiol 2008;105:1741-1745.
10. Miyashita K, Itoh H, Tsujimoto H, Tamura N, Fukunaga Y, Sone M, Yamahara K, Taura D, Inuzuka M, Sonoyama T, Nakao K. Natriuretic peptides/cGMP/cGMP-

dependent protein kinase cascades promote muscle mitochondrial biogenesis and prevent obesity. Diabetes. 2009;58:2880-2892.

11. Das SR, Drazner MH, Dries DL, Vega GL, Stanek HG, Abdullah SM, Canham RM, Chung AK, Leonard D, Wians FH Jr, de Lemos JA. Impact of body mass and body composition on circulating levels of natriuretic peptides: results from the Dallas Heart Study. Circulation. 2005;112:2163-2168.
12. Lund LH, Williams JJ, Freda P, LaManca JJ, LeJemtel TH, Mancini DM. Ghrelin resistance occurs in severe heart failure and resolves after heart transplantation. Eur J Heart Fail. 2009;11:789-794.
13. Vestergaard ET, Andersen NH, Hansen TK, Rasmussen LM, Moller N, Sorensen KE, Sloth E, Jorgensen JO. Cardiovascular effects of intravenous ghrelin infusion in healthy young men. Am J Physiol Heart Circ Physiol. 2007;293:H3020-3026.
14. Nagaya N, Moriya J, Yasumura Y, Uematsu M, Ono F, Shimizu W, Ueno K, Kitakaze M, Miyatake K, Kangawa K. Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure. Circulation. 2004;110:3674-3679.
15. van der Zander K, Houben AJ, Hofstra L, Kroon AA, de Leeuw PW. Hemodynamic and renal effects of low-dose brain natriuretic peptide infusion in humans: a randomized, placebo-controlled crossover study. Am J Physiol Heart Circ Physiol. 2008;285:H1206-1212.
16. Seoane LM, Al-Massadi O, Caminos JE, Tovar SA, Dieguez C, Casanueva FF. Sensory stimuli directly acting at the central nervous system regulate gastric ghrelin secretion: an ex-vivo organ culture study. Endocrinology 2007;148:3998-4006.

17. Vila G, Maier C, Riedl M, Nowotny P, Ludvik B, Luger A, Clodi M. Bacterial endotoxin induces biphasic changes in plasma ghrelin in healthy humans. J Clin Endocrinol Metab. 2007;92:3930-3934.
18. Cao LH, Yang XL. Natriuretic peptides and their receptors in the central nervous system. Prog Neurobiol. 2008;84:234-248.
19. Maier C, Riedl M, Vila G, Nowotny P, Wolzt M, Clodi M, Ludvik B, Luger A. Cholinergic regulation of ghrelin and peptide YY release may be impaired in obesity. Diabetes. 2008;57:2332-2340.
20. Nobata S, Ventura A, Kaiya H, Takei Y. Diversified cardiovascular actions of six homologous natriuretic peptides (ANP, BNP, VNP, CNP1, CNP3 and CNP4) in conscious eels. Am J Physiol Regul Integr Comp Physiol. 2010;298:R1549-1559.
21. Lainchbury JG, Richards AM, Nicholls MG, Espiner EA, Yandle TG. Brain natriuretic peptide and neutral endopeptidase inhibition in left ventricular impairment. J Clin Endocrinol Metab. 1999;84:723-729.
22. Nagaya N, Uematsu M, Kojima M, Date Y, Nakazato M, Okumura H, Hosoda H, Shimizu W, Yamagishi M, Oya H, Koh H, Yutani C, Kangawa K. Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. Circulation. 2001;104:2034-2038.
23. Akamizu T, Kangawa K. Therapeutical applications of ghrelin to cachexia utilizing its appetite-stimulatory effect. Peptides. 2011;11:2295-2300.
24. Fujimatsu D, Kotooka N, Inoue T, Nishiyama M, Node K Association between high molecular weight adiponectin and metabolic parameters. J Atheroscler Thromb. 2009;16:553-559.
25. Tsukamoto O, Fujita M, Kato M, Yamazaki S, Asano Y, Ogai A, Okazaki H, Asai M, Nagamachi Y, Maeda N, Shintani Y, Minamino T, Asakura M, Kishimoto I,

Funahashi T, Tomoike H, Kitakaze M. Natriuretic peptides enhance the production of adiponectin in human adipocytes and in patients with chronic heart failure. J Am Coll Cardiol. 2009;53:2070-2077.

**Figure legends**

**Figure 1**. Changes in circulating BNP concentrations.

BNP administered as a continuous intravenous infusion of 3pmol/kg/min. Horizontal black bar shows infusion duration. Data are presented as mean ± SEM. ●, 3pmol/kg/min BNP; ○ placebo.

**Figure 2**. Effects of BNP on acylated and total ghrelin concentrations

(A) Profile of changes in plasma ghrelin concentrations, (B) Differences between ghrelin concentrations at baseline and at time-point 4 hours, (C) Profile of changes in acylated ghrelin concentrations, (D) Differences between acylated ghrelin concentrations at baseline and at time-point 4 hours. Data are presented as mean ± SEM. ●, 3pmol/kg/min BNP; ○ placebo. Horizontal black bar shows infusion duration.

**Figure 3**. Effects of BNP on gut peptides and adipokines

BNP-induced changes in circulating (A) $PYY_{3-36}$ concentrations, (B) GLP-1 concentrations, (C) oxyntomodulin concentrations (D) PP concentrations, (E) leptin concentrations and (F) adiponectin concentrations. Data are presented as mean ± SEM. ●, 3pmol/kg/min BNP; ○ placebo. Horizontal black bar shows infusion duration.

**Figure 4**. Effects of BNP on subjective ratings of hunger and satiety, as measured by VAS. Scores are depicted as change from the 0 value (mm). (A) Profile of hunger-VAS values, (B) Differences between hunger-VAS values at baseline and at time-point 4 hours, (C) Profile of satiety-VAS values, (D) Differences between satiety-VAS values at baseline and at time-point 4 hours. Data are presented as mean ± SEM. ●, 3pmol/kg/min BNP; ○ placebo. Horizontal black bar shows infusion duration.