Relative Atrial Natriuretic Peptide Deficiency and Inadequate Renin and Angiotensin II Suppression in Obese Hypertensive Men

Camilla L. Asfærg, Søren J. Nielsen, Ulrik B. Andersen, Allan Linneberg, Daniel V. Møller, Paula L. Hedley, Michael Christiansen, Jens P. Goetze, Murray Esler, Jørgen L. Jeppesen

Abstract—Obesity is a strong risk factor for hypertension, but the mechanisms by which obesity leads to hypertension are incompletely understood. On this background, we assessed dietary sodium intake, serum levels of natriuretic peptides (NPs), and the activity of the renin–angiotensin system in 63 obese hypertensive men (obeseHT: body mass index, ≥30.0 kg/m²; 24-hour ambulatory blood pressure, ≥130/80 mm Hg), in 40 obese normotensive men (obeseNT: body mass index, ≥30.0 kg/m²; 24-hour ambulatory blood pressure, <130/80 mm Hg), and in 27 lean normotensive men (leanNT: body mass index, 20.0–24.9 kg/m²; 24-hour ambulatory blood pressure, <130/80 mm Hg). All study subjects were medication free. As a surrogate estimate for dietary sodium intake, we measured sodium excretion in a 24-hour urine collection and we measured serum levels of midregional proatrial NP and plasma levels of renin and angiotensin II. The obese men had higher mean (±SD) urinary sodium excretion (obeseHT, 213.6±85.2 mmol; obeseNT, 233.0±70.0 mmol) than the lean normotensive men (leanNT, 155.5±51.7 mmol; P=0.003). ObeseHT had lower (median [interquartile range]) serum midregional proatrial NP levels (49.2 [37.3–64.7] pmol/L) than leanNT (69.3 [54.3–82.9] pmol/L; P=0.003), whereas obeseNT had midregional proatrial NP levels in between (54.1 [43.2–64.7] pmol/L); obeseNT had lower (median [interquartile range]) plasma levels of renin (5.0 [3.0–8.0] mIU/L versus 9.0 [4.0–18.0]) and angiotensin II (2.4 [1.5–3.5] pmol/L versus 4.2 [2.2–7.9]) than obeseHT (P<0.049), whereas obeseHT had similar plasma levels of renin and angiotensin II as leanNT (P=0.19). Thus, despite a high sodium intake and a high blood pressure, obese hypertensive men have a relative NP deficiency and an inadequate renin–angiotensin system suppression. (Hypertension. 2013;62:00-00.)

Key Words: aldosterone • angiotensin • hypertension • natriuretic peptides • renin • sympathetic nervous system

Obesity is a strong risk factor for hypertension, but the mechanisms by which obesity leads to hypertension are incompletely understood. Nevertheless, abnormalities in the natriuretic peptide (NP) system, the renin–angiotensin–aldosterone system (RAAS), and the sympathetic nervous system (SNS), together with disturbances of the normal glucose and insulin metabolism, have all been implicated in the pathogenesis of obesity-related hypertension. In particular, the finding that obese individuals have decreased circulating NP concentrations, described as natriuretic handicap, has recently attracted attention. So on this background, we initiated this study program hoping to shed some new light on the pathophysiology of obesity-related hypertension by assessing components belonging to all of the physiological systems mentioned above in a phenotypically well-characterized population of obese men, with or without hypertension, and normotensive lean control men. Specifically, we measured serum levels of midregional proatrial NP (MR-proANP), all RAAS components, urine and plasma noradrenaline levels, which have been shown to predict future blood pressure (BP) elevation, fasting and postglucose serum insulin levels, and sodium excretion in a 24-hour urine collection, an established surrogate estimate for dietary sodium intake. Furthermore, we measured BP by 24-hour ambulatory BP (AMBP) recordings, the gold standard to make the most accurate diagnosis of hypertension, and body composition by dual-energy X-ray absorptiometry scanning, which made it possible for us to control for any underlying differences in fat and lean body mass, an important issue when studying obesity-related disorders.
Methods
An expanded Methods section is provided in the online-only Data Supplement, which also includes information on the specific biochemical assays used.

Study Population
Study subjects were recruited by advertising and from 2 population-based studies from the Research Center for Prevention and Health, Copenhagen University Hospital Glostrup, Denmark. We aimed to study ≥100 obese men (body mass index [BMI], ≥30 kg/m²), with or without hypertension, and ≥25 lean men (BMI, ≤25 kg/m²) who were normotensive. To achieve this goal, we screened and examined 115 obese men and 33 lean men. Inclusion criteria for the obese men were BMI ≥ 30 kg/m²; age between 25 and 69 years; never treated for hypertension; no medication at all in the 3 weeks before study; no diagnosis of diabetes mellitus and a screening fasting plasma glucose < 7.0 mmol/L; no cardiovascular disease or other chronic diseases; no nighttime work; and a complete set of data. The criteria for the lean men were the same except for BMI between 20.0 and 24.9 kg/m² and mean 24-hour AMBP < 130/80 mm Hg. In total, 103 obese men and 27 lean normotensive men (leanNT) fulfilled all criteria and were included.

Study Program
The study was conducted over 3 visits. At the first visit, the participants were screened, filled out a questionnaire, had their office BP and an ECG recorded, and had their body composition assessed by dual-energy X-ray absorptiometry scanning. At the second visit, insulin and glucose metabolism was assessed by an oral glucose tolerance test. After the test, the participants were served lunch and the 24-hour AMBP measurement was started. The participants were instructed to perform a 24-hour urine collection, starting in the morning before the day of the third visit for measurement of urinary sodium, potassium, albumin, creatinine, and catecholamines. At the third visit, blood samples were drawn for the evaluation of SNS activity, RAAS activity, lipid status, kidney function including electrolytes, and the cardiac NP system. The participants had fasted for 12 hours before the second and third visit.

All subjects were on their regular weight maintenance diet. The participants were instructed to abstain from beverages containing caffeine and alcohol 24 hours before the second and third visit. Three days before the second and third visit, the participants were instructed to avoid strenuous physical exercise.

The study was conducted according to the principles of the Helsinki declaration, and all participants gave informed written consent before inclusion. The study was approved by the Danish Ethical Committee (HB-2007-040).

BP Measurements
BP was measured by automatic 24-hour AMBP recordings (Spacelabs ABP 90207, Redmont, CA). To fulfill the criteria for a complete data set, the 24-hour AMBP recording required ≥14 readings during daytime and ≥7 readings during nighttime in accordance with the European guidelines for AMBP measurements.

Body Composition
Body weight with subjects in underwear was measured to the nearest 0.1 kg and height to the nearest 0.1 cm. Waist and hip circumference was measured as illustrated in Figure 1. We used dual-energy X-ray absorptiometry scanning (GE Lunar, Madison, WI) to determine body composition accurately. Total body scans were autoanalyzed with software version 11.0. The android and gynoid regions were defined according to the default software readings, as shown in Figure 1.

Blood and Urine Samples
Blood samples were obtained from a brachial vein. Urine was collected for 24 hours in bottles containing 15 mL of 2 mol/L hydrochloric acid.

Cardiac NP System
By measuring circulating levels of MR-proANP and brain NP (BNP), the cardiac NP system was assessed. We chose to measure MR-proANP concentrations rather than ANP because ANP is a labile molecule with short plasma half-life. MR-proANP in serum was measured with a commercially available sandwich chemiluminescence immunoassay (BRAHMS AG, Hennigsdorf/Berlin, Germany). Serum concentrations of BNP were measured with a commercially available chemiluminescent microparticle immunoassay on an Architect System (Abbott Laboratories, IL).

RAAS Measurements
RAAS activity was assessed after 60 minutes rest in the supine position. The assays used for the determination of plasma levels of angiotensinogen and renin, and plasma levels of angiotensin I and II and aldosterone, and serum angiotensin-converting enzyme activity have been described in detail elsewhere.

SNS Measurements
SNS activity was assessed from measurement of plasma adrenaline and noradrenaline after 60-minute rest in the supine position, as described in detail elsewhere, and from measurement of adrenaline and noradrenaline in the 24-hour urine collections.

Insulin and Glucose Measurements
Blood samples were drawn for measurement of plasma glucose and serum insulin concentrations at time 0, 30, 60, 90, and 120 minutes after an oral glucose load of 75 g dissolved in 250 mL water.

Lipids, Sodium, Potassium, and Creatinine Clearance
Total blood cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, and triglycerides were measured using standard laboratory techniques. Serum and urinary concentrations of sodium, potassium, albumin, and creatinine were measured with a Vitros 700 Analyzer (Johnson & Johnson). Creatinine clearance was calculated from the 24-hour urinary collection with correction for body surface area calculated by the Mosteller formula.

Lifestyle Information
Information about smoking habits, alcohol consumption, physical activity, and parental history of hypertension and diabetes mellitus was obtained from the questionnaire filled out during the first visit. Participants, who were sedentary or only active < 4 hours per week, were defined as having a low level of physical activity. All participants were interviewed about sleep disorders using the Epworth Sleepiness Score.

Definition of Hypertension
Presence of hypertension and normotension was defined according to the recommendations from the International Database on Ambulatory BP monitoring in relation to Cardiovascular Outcomes (IDACO) as a mean 24-hour systolic BP ≥ 130 mm Hg or a mean 24-hour diastolic BP ≥ 80 mm Hg, and a mean 24-hour BP < 130/80 mm Hg, respectively.

Statistical Analysis
Data analyses were performed using SPSS (version 17.0, SPSS, Chicago, IL). Unless specified otherwise, continuous data are presented as mean ± SD or median and interquartile range for normal and skewed distributions, respectively. Group comparisons of continuous data were performed using ANOVA. Non-normally distributed data were log-transformed to fulfill the normal distribution criteria. Comparisons between groups were corrected with Tukey for multiple comparisons. χ² test was used for group comparisons for categorical data. Adjusted for age, partial Spearman correlation coefficients were calculated between selected variables of interest. All statistical tests were 2-sided, and a P value of < 0.05 was considered significant.

Results
Expanded results are provided in the online-only Data Supplement.
Demographic and Lifestyle Characteristics
Of the 103 obese men, 40 were normotensive (obeseNT) and 63 were diagnosed with hypertension for the first time (obeseHT). None of the obese participants qualified for a diagnosis of sleep apnea. There was no difference in age between the 3 groups (leanNT, 51.5±8.4 years; obeseNT, 47.9±9.8 years; obeseHT, 50.3±10.4 years; \( P=0.41 \)), and there were no differences in parental history of diabetes mellitus, smoking habit, and level of physical activity between the 3 groups (\( P \geq 0.15 \); Table S1 in the online-only Data Supplement). More obeseHT had a parental history of hypertension compared with leanNT (\( P=0.004 \); Table S1).

Body Composition Characteristics
Body weight was similar in the 2 obese groups (obeseNT, 106.0±10.6 kg; obeseHT, 106.1±11.1 kg; \( P=0.99 \)), and much higher than in the lean men (leanNT, 74.9±6.7 kg; \( P<0.001 \)). There were no differences between the 2 obese groups regarding BMI, waist circumference, waist:hip ratio, android fat mass, gynoid fat mass, and total fat mass (\( P \geq 0.19 \); Table S1), but all these factors were higher in the obese men compared with the lean men (\( P<0.001 \)).

Hemodynamic Characteristics
ObeseHT had higher BP compared with obeseNT and leanNT (Table). ObeseNT had slightly but significantly higher systolic BP than leanNT, whereas diastolic BP did not differ between the 2 normotensive groups (\( P=0.35 \)).

Renal Function and Electrolytes
The obese men had higher mean (±SD) urinary sodium excretion (obeseHT, 213.6±85.2 mmol; obeseNT, 233.0±70.0 mmol) than the lean normotensive men (leanNT, 155.5±51.7 mmol; \( P=0.003 \); Figure 2A). The obese men had also higher serum sodium and serum creatinine levels, and lower creatinine clearance compared with the lean men (\( P<0.001 \); Table S1). However, there were no differences in these variables between the 2 obese groups (\( P \geq 0.44 \)).

NPs and RAAS
Despite higher sodium excretion (Figure 2A) and higher BP levels (Table), obeseHT had lower serum levels of MR-proANP compared with leanNT (Table; Figure 2B). Despite higher BP levels, obeseHT had similar MR-proANP levels as obeseNT (Table; Figure 2B). Despite higher sodium excretion...
Table. Hemodynamic and Neuroendocrine Characteristics of the 3 Study Groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Lean Normotensive Men (n=27)</th>
<th>Obese Normotensive Men (n=40)</th>
<th>Obese Hypertensive Men (n=63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h mean SBP, mm Hg</td>
<td>112±7</td>
<td>117±6*</td>
<td>137±11†</td>
</tr>
<tr>
<td>24-h mean DBP, mm Hg</td>
<td>72±4</td>
<td>73±4</td>
<td>83±6‡</td>
</tr>
<tr>
<td>Natriuretic peptides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR-proANP pmol/L</td>
<td>69.3 (54.3–82.9)</td>
<td>54.1 (43.2–64.7)</td>
<td>49.2 (37.3–64.7)†</td>
</tr>
<tr>
<td>BNP pg/mL</td>
<td>9.40 (5.30–13.0)</td>
<td>6.85 (3.75–11.8)</td>
<td>5.30 (2.80–11.6)</td>
</tr>
<tr>
<td>RAAS activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renin, mIU/L</td>
<td>8.0 (3.0–17.0)</td>
<td>5.0 (3.0–8.0)</td>
<td>9.0 (4.0–18.0)§</td>
</tr>
<tr>
<td>Angiotensinogen, nmol/L</td>
<td>854.7±147.7</td>
<td>868.4±173.0</td>
<td>970.4±214.3§§</td>
</tr>
<tr>
<td>Aldosterone, pmol/L</td>
<td>94.0 (61.0–169.0)</td>
<td>98.1 (61.5–133.5)</td>
<td>118.0 (77.0–149.0)</td>
</tr>
<tr>
<td>Angiotensin I, pmol/L</td>
<td>7.0 (2.9–11.0)</td>
<td>3.8 (0.8–7.1)</td>
<td>8.0 (3.2–14.0)§</td>
</tr>
<tr>
<td>Angiotensin II, pmol/L</td>
<td>5.1 (3.2–9.8)</td>
<td>2.4 (1.5–3.5)†</td>
<td>4.2 (2.2–7.9)§§</td>
</tr>
<tr>
<td>ACE activity, Units</td>
<td>39.4±8.9</td>
<td>44.9±10.2</td>
<td>43.1±9.3</td>
</tr>
<tr>
<td>Aldosterone:renin ratio</td>
<td>16.9 (5.8–28.5)</td>
<td>16.3 (9.4–39.2)</td>
<td>12.5 (6.8–29.0)</td>
</tr>
<tr>
<td>SNS activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma noradrenaline, nmol/L</td>
<td>1.49 (1.11–1.82)</td>
<td>1.16 (0.83–1.47)*</td>
<td>1.47 (1.03–1.82)‡</td>
</tr>
<tr>
<td>Urine noradrenaline, pmol/24 h</td>
<td>0.37 (0.29–0.46)</td>
<td>0.37 (0.32–0.51)</td>
<td>0.45 (0.34–0.55)*</td>
</tr>
</tbody>
</table>

Data are presented as mean (±SD) or as median (interquartile range). ACE indicates angiotensin-converting enzyme; BNP, brain natriuretic peptide; DBP, diastolic blood pressure; MR-proANP, midregional proatrial natriuretic peptide; RAAS, renin–angiotensin–aldosterone system; SBP, systolic blood pressure; and SNS, sympathetic nervous system.

*P<0.05 vs lean normotensive men.
†P<0.01 vs lean normotensive men.
‡P<0.01 vs obese normotensive men.
§P<0.05 vs obese normotensive men.

Lipids, Glucose, and Insulin

The obese subjects had higher serum triglyceride levels and lower high-density lipoprotein cholesterol levels compared with the lean men (P<0.001; Table S3). The obese subjects had higher glucose and insulin values than the lean men (P<0.001; Table S3; Figure S1B and S1C), but between the 2 obese groups there were no significant differences (P≥0.26), although obeseHT had ~20% higher insulin levels.

Additional Analyses

If we confined our analyses to a single group comparison of obeseHT with obeseNT, that is, studying hypertension in obesity, obeseHT had also significantly higher urinary noradrenaline excretion (P=0.033) and higher levels of plasma aldosterone (P=0.043). Studying the 103 obese men with partial Spearman correlation analyses, adjusted for age, 24-hour urine noradrenaline was correlated with plasma renin levels (r=0.31; P=0.002), whereas angiotensinogen was not correlated with renin (r=−0.046; P=0.65) or any other components of the RAAS (r=−0.09–0.15; P=0.13). In the 103 obese men, adjusted for age, serum MR-proANP levels were negatively correlated with 24-hour mean systolic BP (r=−0.22; P=0.024), 24-hour mean diastolic BP (r=−0.29; P=0.0028) plasma renin levels (r=−0.27; P=0.0053), plasma angiotensin II levels (r=−0.29; P=0.0031), plasma aldosterone levels (r=−0.32; P=0.0010), and 24-hour urinary noradrenaline excretion (r=−0.29; P=0.0031), whereas no relationship was found between serum MR-proANP levels and 24-hour sodium excretion (r=−0.06; P=0.54). In the lean controls and adjusted

excretion (Figure 2A), obeseNT had lower MR-proANP levels compared with the leanNT, although the difference did not reach statistical significance (P=0.080; Table). Adjustments for lifestyle risk factors (smoking habit and physical activity) did not affect the results with respect to MR-proANP (data not shown). Regarding BNP, the relationships resembled those of MR-proANP (Table), although none of the differences reached statistical significance (P≥0.092). Nevertheless, obeseHT had the lowest BNP levels (Table).

Despite similar sodium excretion (Figure 2A), obeseNT had lower plasma levels of angiotensinogen, renin, angiotensin I, and angiotensin II compared with obeseHT (P<0.049; Table; Figure 2C and 2D). Compared with leanNT, obeseNT had lower plasma levels of angiotensin II (P<0.001; Table; Figure 2D). Comparing obeseHT with leanNT, angiotensinogen was the only variable that was significantly higher in obeseHT (P=0.025; Table). Among the 3 groups, there were no significant differences in angiotensin-converting enzyme activity, aldosterone, or aldosterone:renin ratio (P≥0.053; Table).

SNS

ObeseHT had higher levels of plasma noradrenaline compared with obeseNT (Table). Plasma noradrenaline was lower in obeseNT than in leanNT (Table). With respect to urine noradrenaline, obeseHT had higher values compared with leanNT (Table; Figure S1A), whereas in comparison with obeseNT, the difference did not reach statistical significance (P=0.074). There were no differences in adrenaline levels among the 3 groups (P≥0.25; Table S2).
for age, the serum MR-proANP levels were positively, albeit not significantly, correlated with 24-hour urinary sodium excretion ($r=0.26; P=0.21$).

### Discussion

The principal findings of this study were that, despite a high sodium intake and a high BP, obese hypertensive men have a relative NP deficiency and an inadequate suppression of their RAAS.

Although the cardiac NPs for a long time have been regarded as essential physiological regulators of BP, their pivotal role in the pathogenesis of human hypertension has recently been highlighted in genetic studies. Thus, common genetic variants in the loci encoding NP precursor A and B ($NPPA$ and $NPPB$, respectively) and the NP clearance receptor ($NPR3$), all related to lower circulating NP concentrations, have been shown to be associated with higher systolic and diastolic BP and an increased risk of hypertension. Consequently, if our obese hypertensive men had had higher circulating NP levels, it might be speculated that their BP would have been lower or may be even normal.

In the sections below, we discuss in detail the relative NP deficiency or natriuretic handicap seen in obese subjects that could be 1 mechanism by which obesity promotes hypertension, as we discuss signs of abnormal regulation of the RAAS and increased activity of the SNS as 2 other possible mechanisms by which obesity could lead to hypertension.

### Signs of Disrupted ANP Response to Sodium in obeseHT

In normal healthy volunteers, a sodium-enriched diet or a sodium load lead to higher circulating ANP levels, presumably through volume-induced atrial stretching/distention, the primary stimulus for ANP release. This normal physiological response to a high sodium intake seems somehow to be disrupted in our obese hypertensive men. Thus, despite a much higher sodium intake compared with leanNT, obeseHT had substantially lower serum levels of MR-proANP,
and BNP for that matter, although the difference only reached statistical significance for MR-proANP in our study.

**Signs of Disrupted NP Response to Higher BP in ObeseHT**

In normal healthy volunteers, infusion of vasopressors, such as angiotensin II, noradrenaline, or phenylephrine, leads to higher circulating ANP levels. This vasopressor-induced ANP release seems to be primarily mediated by hemodynamic changes, causing atrial stretching/distention, as additional infusions of a BP-lowering substance, such as sodium nitroprusside, will decrease ANP levels to basal values without affecting the circulating levels of the infused vasopressor. This normal physiological response to a higher BP also seems in some way to be disrupted in our obese hypertensive men. Thus, despite a much higher BP compared with the leanNT, obeseHT had substantially lower serum levels of MR-proANP, and BNP for that matter, although the difference only reach statistical significance for MR-proANP in our study.

**Signs of Disrupted RAAS and SNS Response to Sodium in obeseHT**

The normal response of the RAAS and the SNS to a high salt intake is decreased activity. This normal physiological RAAS response to a high salt intake also seems to be disrupted in our obese hypertensive men as it has been described in several other studies of obese normotensive and hypertensive subjects. Thus, despite a higher salt intake compared with leanNT, obeseHT had similar plasma renin concentrations, and similar plasma concentrations of angiotensin I, angiotensin II, and aldosterone, and even higher 24-hour urine excretion of noradrenaline compared with leanNT. In contrast to these abnormal responses, obeseNT overall seemed to have a more normal RAAS and SNS response to a high salt intake, as they, despite similar high salt intake, had lower plasma renin levels, and lower plasma concentrations of angiotensin I, angiotensin II, and aldosterone, and lower 24-hour urinary excretion of noradrenaline compared with obeseHT. The normal RAAS and SNS response to a high salt intake might have protected obeseHT from hypertension. This was despite the fact that they actually showed some degree of abnormal NP response to high salt intake, albeit not statistically significant, compared with leanNT. Finally, we also speculate that leanNT were normotensive because of their higher MR-proANP levels counterbalancing their RAAS activity.

**Limitations and Strengths**

The cross-sectional design of this study in addition to the use of a 24-hour urine excretion of sodium as a measure of daily salt intake instead of performing a controlled diet study may be perceived as limitations. Nevertheless, a 24-hour urine excretion of sodium is a well-established surrogate for sodium intake, and compared with figures from controlled diet studies reported in the medical literature, there is no doubt that our obese men must have had a high daily intake of sodium. Furthermore, our assessment of SNS activity may also be perceived as a limitation, but the fact that we could demonstrate a significantly higher 24-hour urinary noradrenalin excretion in the obese hypertension men, a finding in agreement with the prevailing view that SNS activation is important in the initiation and maintenance of obesity-related hypertension, increases the credibility of our results. Finally, we also have to acknowledge that the Epworth Sleepiness Score is not an optimal method to assess obstructive sleep apnea, so cases with this obesity-related sleep disorder may have been missed.

Perspectives

The present study indicates that obese hypertensive men have multiple disturbances related to salt and water homeostasis and BP control. Given their high salt intake and high 24-hour AMBP, obese hypertensive men have lower than expected circulating NP levels and higher than expected RAAS activity. The cross-sectional nature of our study does not allow us to draw conclusions about cause-effect relationships. Nevertheless, the positive relationship found between 24-hour urine excretion of noradrenaline and plasma renin levels in our study and findings in other studies suggest that an increase in the sympathetic outflows to the kidneys, stimulating renin release, may interfere with the normal suppression of the RAAS in our obese hypertensive men on their regular high-salt diet. With respect to the cause of the relative NP deficiency, this phenomenon may be related to hyperinsulinemia because higher fasting serum insulin levels have been shown to be negatively correlated with circulating NP levels, although this mechanism needs further exploration. Finally, it needs to be mentioned that, as is shown in the Table and Figure 2C and 2D, some obeseHT had in fact suppressed RAAS demonstrating that hypertension indeed is a very heterogeneous disease with different causes and presentations, also in obese subjects.

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**Disclosures**

None.

**References**


**Novelty and Significance**

What Is New?

- Given their high salt intake and high 24-hour ambulatory blood pressure, obese hypertensive men have the following:
  - Lower than expected circulating natriuretic peptide levels.
  - Higher than expected renin–angiotensin–aldosterone system activity.

What Is Relevant?

- Obese hypertensive men have multiple disturbances related to salt and water homeostasis and blood pressure control pointing to a multifactorial pathogenesis of obesity-related hypertension.
Relative Atrial Natriuretic Peptide Deficiency and Inadequate Renin and Angiotensin II Suppression in Obese Hypertensive Men

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Relative Atrial Natriuretic Peptide Deficiency and Inadequate Renin and Angiotensin II Suppression in Obese Hypertensive Men

Short title: ANP and RAS in Obese Hypertensive Men

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Extended Method Section Online-Only Data Supplement

Study Population
Study subjects were recruited by local advertising and from two population-based studies from the Research Centre for Prevention and Health, Copenhagen University Hospital Glostrup, Denmark. We aimed to study at least 100 obese men (body mass index (BMI) $\geq 30$ kg/m$^2$), with or without hypertension, and at least 25 lean men (BMI $\leq 25$ kg/m$^2$) who were normotensive. To achieve this goal, we screened and examined 115 obese men and 33 lean men. Inclusion criteria for the obese men were: BMI $\geq 30$ kg/m$^2$, age between 25 and 69 years, never treated for hypertension, no medication at all in the three weeks prior to study, no diagnosis of diabetes and a screening fasting plasma glucose $< 7.0$ mmol/L, no cardiovascular disease or other chronic diseases, no night time work, and a complete set of data. The criteria for the lean men were the same except for: BMI between 20.0 and 24.9 kg/m$^2$ and mean 24-hour ambulatory blood pressure (AMBP) $< 130/80$ mm Hg. In total 103 obese men and 27 lean normotensive men (LeanNT) fulfilled all criteria and were included.

Study Program
The study program was conducted over three visits, separated by at least two days and with a mean interval of seven days. The first visit took place between 08.00 and 16.00. The participants had fasted for 4 hours before the visit. At the first visit, the participants were screened, filled out a questionnaire, had their office BP and an electrocardiogram recorded, and had their body composition assessed by dual energy X-ray absorptiometry (DXA) scanning. At the second visit, insulin and glucose metabolism was assessed by an oral glucose tolerance test. The test was done between 07.30 and 11.00 after 12 hours fast. After the test, the participants were served lunch and the 24-hour AMBP measurement was started. The participants were instructed to perform a 24-hour urine collection, starting in the morning before the day of the third visit for measurement of urinary sodium, potassium, albumin, creatinine, and catecholamines. At the third visit, between 07:30 and 11.00, blood samples were drawn for the evaluation of sympathetic nervous system (SNS) activity, renin-angiotensin-aldosterone system (RAAS) activity, lipid status, kidney function including electrolytes, and the cardiac natriuretic peptide (NP) system. The participants had fasted for 12 hours prior to the visit.

All subjects were on their regular weight maintenance diet. They were instructed to consume the same food components in the time period before the various tests, and no attempt was made to influence their sodium intake. However, as Danish men according to the Danish Veterinary and Food Administration (www.foedevarestyrelsen.dk) consume 9 to 11 grams of salt a day, in contrast to the recommended amount of 1.5 to 2.3 grams of salt a day, we assumed that our research subjects would also be on a high salt diet in their everyday life. The participants were instructed to abstain from beverages containing caffeine and alcohol 24 hours before the second and third visits. Three days prior to the second and third visit, the participants were instructed to avoid strenuous physical exercise.

The study was conducted according to the principles of the Helsinki declaration, and all participants gave informed written consent before inclusion. The study was approved by the Danish ethical committee (HB-2007-040).

BP Measurements
BP was measured by automatic 24-hour AMBP recordings (Spacelabs ABP 90207, Redmont, CA). Day-time was set from 07.00 to 23.00, and BP was measured every 20 minute. Night-time was set from 23.00 to 07.00, unless the subject reported other sleeping habits, and BP was measured every 30 minute during night-time. A cuff size of 14 cm was chosen for 24-32 cm upper arm circumference, and of 16 cm for >32 cm upper arm circumference. To fulfill the criteria for a complete data set, the 24-hour AMBP recording required at least 14 readings during day-time and at least 7 readings during night-time in accordance with the European guidelines for AMBP measurements.

Body Composition
Body weight with subjects in underwear was measured to the nearest 0.1 kg and height to the nearest 0.1 cm. Waist and hip circumference was measured as illustrated in Figure 1. We used DXA scanning (GE Lunar, Madison, WI) to determine body composition accurately. Total body scans were auto-analyzed with software version 11.0. The android and gynoid regions were defined according to the default software readings, as shown in Figure 1. One investigator performed all the analyses.

Blood and Urine Samples
Blood samples, serum and EDTA plasma, were obtained from a brachial vein. Following separation, the samples were stored at minus 80 degree Celsius until analyses. Urine was collected for 24 hours in bottles containing 15 mL of 2 M hydrochloric acid.

Cardiac NP System
The activity of the cardiac NP system was assessed by measuring circulating levels of mid-regional pro-atrial natriuretic peptide (MR-proANP) and brain natriuretic peptide (BNP). We chose to measure MR-proANP concentrations rather than ANP, as ANP is a very labile molecule with short plasma half-life. MR-proANP in serum was measured with a commercially available sandwich chemiluminescence immunoassay (BRAHMS AG, Hennigsdorf/Berlin, Germany). From the literature, the MR-proANP assay has a limit of quantitation of 6.0 pmol/L. Intra-assay coefficient of variation (CV) is <10% and <20% for samples containing 23-3000 pmol/L and 18-22.8 pmol/L proANP respectively. Inter-assay CV is 20% for a MR-proANP concentration of 18 pmol/L and 10% for a proANP concentration of 65 pmol/L. The interassay imprecision profiles in our laboratory (as evaluated from 20 separate runs) were 2.8% at 100 pmol/L and 2.4% at 510 pmol/L, respectively. Serum concentrations of BNP were measured with a commercially available chemiluminescent microparticle immunoassay on an Architect System (Abbott Laboratories, IL, USA). The sensitivity of the assay is ≤10pg/mL, and the intraassay and interassay CV are <5% and <5%, respectively.

RAAS Measurements
RAAS activity was assessed after 60 minutes rest in the supine position. The assays used for the determination of plasma levels of angiotensinogen and renin, and plasma levels of angiotensin I and II and aldosterone, and serum angiotensin-converting enzyme (ACE)-activity have been described in detailed elsewhere. Blood samples for measurement of angiotensin I and II were obtained in tubes prepared with EDTA and phenanthroline. In our laboratory, the intraassay and interassay CV for plasma levels of renin are 2.9% and 8.3%, for angiotensin I 6.2% and 13.0%, for angiotensin II 4.0% and 13.0%, for
angiotensinogen 2.8% and 5.6%, for aldosterone.7% and 3.9%, and for ACE activity 2.2% and 4.3%, respectively.

**SNS Measurements**

SNS activity was assessed from measurement of plasma adrenaline and noradrenaline after 60 min rest in the supine position, as described in detail elsewhere, and from measurement of adrenaline and noradrenaline in the 24-hour urine collections. Plasma noradrenaline and adrenaline and urinary noradrenaline and adrenaline were analyzed by radioimmunoassay using a commercial kit (Labor Diagnostika Nord, Nordhorn, Germany). In our laboratory, the intraassay and interassay CV for catecholamines are 4.6% and 6.1%, respectively.

**Insulin and Glucose Measurements**

Blood samples were drawn at time 0, 30, 60, 90, and 120 minutes after an oral glucose load of 75 gram dissolved in 250 ml water. Plasma glucose concentrations were measured by an automated analyzer (Roche Diagnostics, Mannheim, Germany). Serum insulin concentrations were measured with a commercial ELISA kit (DAKoCytomation Ltd, Cambridgeshire, UK).

**Lipids, Sodium, Potassium, and Creatinine Clearance**

Total blood cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured using standard laboratory techniques. Serum and urinary concentrations of sodium, potassium, albumin, and creatinine were measured with a Vitros 700 Analyzer (Johnson & Johnson). Creatinine clearance was calculated from the 24-hours urinary collection as creatinine clearance = [urine creatinine (milligrams per deciliter) / serum creatinine (milligrams per deciliter)] X [urine volume (milliliters) / time (minutes)] with correction for body surface area calculated by the Mosteller formula.

**Lifestyle Information**

Information about smoking habits, alcohol consumption, physical activity, and parental history of hypertension and diabetes was obtained from the questionnaire filled out during the first visit. Participants, who were sedentary or only active less than 4 hours per week, were defined as having a low level of physical activity. All participants were interviewed about sleep disorders using the Epworth Sleepiness Score.

**Definition of Hypertension**

Presence of hypertension and normotension was defined according to the recommendations from the International Database on Ambulatory BP monitoring in relation to Cardiovascular Outcomes (IDACO) as a mean 24-hour systolic BP ≥130 mm Hg and/or a mean 24-hour diastolic BP ≥80 mmHg and a mean 24-hour BP <130/80 mm Hg, respectively.

**Statistical Analysis**

Data analyses were performed using SPSS (version 17.0, SPSS, Chicago IL). Unless specified otherwise, continuous data are presented as mean ± SD or median and interquartile range for normal and skewed distributions, respectively. Group comparisons of continuous data were performed using analysis of variance. Non-normally distributed
data were log-transformed in order to fulfill the normal distribution criteria. Comparisons between groups were corrected with Tukey for multiple comparisons. Chi-square was used for group comparisons for categorical data. Adjusted for age, partial Spearman correlation coefficients were calculated between selected variables of interest. All statistical tests were two-sided, and a p-value of <0.05 was considered significant.
References


Table S1. Demographic, Anthropometric, Hemodynamic, and Renal Characteristics of the Three Study Groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Lean normotensive</th>
<th>Obese normotensive</th>
<th>Obese hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>men (n=27)</td>
<td>men (n=40)</td>
<td>men (n=63)</td>
</tr>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>51.5±8.4</td>
<td>47.9±9.8</td>
<td>50.3±10.4</td>
</tr>
<tr>
<td>Parental hypertension, %</td>
<td>11.1</td>
<td>32.5</td>
<td>47.6*</td>
</tr>
<tr>
<td>Parental diabetes, %</td>
<td>22.2</td>
<td>30.0</td>
<td>25.4</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>29.6</td>
<td>22.5</td>
<td>17.5</td>
</tr>
<tr>
<td>High physical activity, %</td>
<td>63.0</td>
<td>52.5</td>
<td>41.3</td>
</tr>
<tr>
<td><strong>Anthropometric</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74.9±6.7</td>
<td>106.0±10.6*</td>
<td>106.1±11.1*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.8±1.5</td>
<td>32.9±2.0*</td>
<td>33.5±2.4*</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>84.4±4.8</td>
<td>111.9±7.3*</td>
<td>114.1±6.9*</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.89±0.03</td>
<td>1.00±0.04*</td>
<td>1.02±0.04*</td>
</tr>
<tr>
<td>Android fat mass, %</td>
<td>28.9±10.0</td>
<td>45.7±4.8*</td>
<td>46.5±5.7*</td>
</tr>
</tbody>
</table>

* p < 0.05
<table>
<thead>
<tr>
<th></th>
<th>Mean (±SD) Lean Normotensive Men</th>
<th>Mean (±SD) Obese Normotensive Men</th>
<th>Mean (±SD) Obese Hypertensive Men</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gynoid fat mass, %</strong></td>
<td>24.8±6.1</td>
<td>36.2±5.1*</td>
<td>36.6±5.3*</td>
</tr>
<tr>
<td><strong>Total fat mass, %</strong></td>
<td>20.9±6.2</td>
<td>34.0±4.7*</td>
<td>34.8±4.5*</td>
</tr>
</tbody>
</table>

**Haemodynamic**

<table>
<thead>
<tr>
<th></th>
<th>Mean (±SD) Lean Normotensive Men</th>
<th>Mean (±SD) Obese Normotensive Men</th>
<th>Mean (±SD) Obese Hypertensive Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hours mean SBP, mmHg</td>
<td>112±7</td>
<td>117±6†</td>
<td>137±11‡</td>
</tr>
<tr>
<td>24-hours mean DBP, mmHg</td>
<td>72±4</td>
<td>73±4</td>
<td>83±6‡</td>
</tr>
<tr>
<td>24-hours mean MAP, mmHg</td>
<td>85±4</td>
<td>88±4</td>
<td>101±6‡</td>
</tr>
<tr>
<td>24-hours mean heart rate, beat/min</td>
<td>66±7</td>
<td>69±8</td>
<td>70±9</td>
</tr>
</tbody>
</table>

**Renal**

<table>
<thead>
<tr>
<th></th>
<th>Mean (±SD) Lean Normotensive Men</th>
<th>Mean (±SD) Obese Normotensive Men</th>
<th>Mean (±SD) Obese Hypertensive Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium, mmol/L</td>
<td>140.2±1.2</td>
<td>141.3±1.4†</td>
<td>141.6±1.6*</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>4.1±0.3</td>
<td>3.9±0.7</td>
<td>4.0±0.3</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>80.7±9.6</td>
<td>93.1±11.8*</td>
<td>90.8±11.4*</td>
</tr>
<tr>
<td>24-hours urine sodium, mmol</td>
<td>155.5±51.7</td>
<td>233.0±70.0*</td>
<td>213.6±85.2*</td>
</tr>
<tr>
<td>24-hours urine creatinine, mmol</td>
<td>16.5±4.0</td>
<td>18.0±5.0</td>
<td>18.3±5.0</td>
</tr>
<tr>
<td>Creatinine clearance, ml•min⁻¹•1.73m⁻²</td>
<td>126.6±25.1</td>
<td>101.6±27.3*</td>
<td>106.2±26.8*</td>
</tr>
</tbody>
</table>

Data are presented as mean (±SD) or as frequency in percent. DBP = diastolic blood pressure, MAP = mean arterial blood pressure, SBP = systolic blood pressure. *P<0.01 vs. lean normotensive men, †P<0.05 vs. lean normotensive men, ‡P<0.01 vs. obese normotensive men.
Table S2. The Renin-Angiotensin-Aldosterone System, the Cardiac Natriurectic Peptide System, and the Sympathetic Nervous System of the Three Study Groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Lean normotensive men (n=27)</th>
<th>Obese normotensive men (n=40)</th>
<th>Obese hypertensive men (n=63)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RAAS Activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renin, mIU/L</td>
<td>8.0 (3.0-17.0)</td>
<td>5.0 (3.0-8.0)</td>
<td>9.0 (4.0-18.0)</td>
</tr>
<tr>
<td>Angiotensinogen, nmol/L</td>
<td>854.7±147.7</td>
<td>868.4±173.0</td>
<td>970.4±214.3†</td>
</tr>
<tr>
<td>Aldosterone, pmol/L</td>
<td>94.0 (61.0-169.0)</td>
<td>98.1 (61.5-133.5)</td>
<td>118.0 (77.0-149.0)</td>
</tr>
<tr>
<td>Angiotensin I, pmol/L</td>
<td>7.0 (2.9-11.0)</td>
<td>3.8 (0.8-7.1)</td>
<td>8.0 (3.2-14.0)‡</td>
</tr>
<tr>
<td>Angiotensin II, pmol/L</td>
<td>5.1 (3.2-9.8)</td>
<td>2.4 (1.5-3.5)†</td>
<td>4.2 (2.2-7.9)†</td>
</tr>
<tr>
<td>ACE activity, Units</td>
<td>39.4±8.9</td>
<td>44.9±10.2</td>
<td>43.1±9.3</td>
</tr>
<tr>
<td>Aldosterone to renin ratio</td>
<td>16.9 (5.8-28.5)</td>
<td>16.3 (9.4-39.2)</td>
<td>12.5 (6.8-29.0)</td>
</tr>
<tr>
<td><strong>Natriuretic peptides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR-proANP pmol/L</td>
<td>69.3 (54.3-82.9)</td>
<td>54.1 (43.2-64.7)</td>
<td>49.2 (37.3-64.7)†</td>
</tr>
<tr>
<td>BNP pg/mL</td>
<td>9.40 (5.30-13.0)</td>
<td>6.85 (3.75-11.8)</td>
<td>5.30 (2.80-11.6)</td>
</tr>
<tr>
<td><strong>SNS Activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Obese Hypertensive Men</td>
<td>Lean Hypertensive Men</td>
<td>Obese Normotensive Men</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------------</td>
<td>-----------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td><strong>Plasma adrenaline, nmol/L</strong></td>
<td>0.08 (0.03-0.14)</td>
<td>0.07 (0.04-0.12)</td>
<td>0.09 (0.05-0.12)</td>
</tr>
<tr>
<td><strong>Plasma noradrenaline, nmol/L</strong></td>
<td>1.49 (1.11-1.82)</td>
<td>1.16 (0.83-1.47)</td>
<td>1.47 (1.03-1.82)</td>
</tr>
<tr>
<td><strong>Urine adrenaline, µmol/24-hr</strong></td>
<td>0.04 (0.03-0.06)</td>
<td>0.04 (0.03-0.05)</td>
<td>0.04 (0.03-0.06)</td>
</tr>
<tr>
<td><strong>Urine noradrenaline, µmol/24-hr</strong></td>
<td>0.37 (0.29-0.46)</td>
<td>0.37 (0.32-0.51)</td>
<td>0.45 (0.34-0.55)</td>
</tr>
</tbody>
</table>

Data are presented as mean (±SD) or as median (interquartile range). ACE activity = angiotensin converting enzyme activity, MR-proANP = mid-regional pro-atrial natriuretic peptide, BNP = brain natriuretic peptide, RAAS = renin-angiotensin-aldosterone system, SNS = sympathetic nervous system. *P<0.05 vs. obese normotensive men. †P<0.01 vs. lean normotensive men, ‡P<0.01 vs. obese normotensive men, §P<0.05 vs. lean normotensive men,
Table S3. Lipid and Metabolic Characteristics of the Three Study Groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Lean normotensive men (n=27)</th>
<th>Obese normotensive men (n=40)</th>
<th>Obese hypertensive men (n=63)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.96 (0.65-1.27)</td>
<td>1.41 (1.03-1.79)$^*$</td>
<td>1.46 (0.97-2.52)$^+$</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.03±0.84</td>
<td>5.31±0.90</td>
<td>5.53±1.26</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.30±0.31</td>
<td>1.10±0.25$^+$</td>
<td>1.10±0.30$^+$</td>
</tr>
<tr>
<td><strong>Metabolic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose 0 hr, mmol/L</td>
<td>5.14±0.43</td>
<td>5.72±0.53$^+$</td>
<td>5.85±0.57$^+$</td>
</tr>
<tr>
<td>Glucose 2 hr, mmol/L</td>
<td>4.74±1.13</td>
<td>5.92±1.66$^+$</td>
<td>6.57±2.08$^+$</td>
</tr>
<tr>
<td>Glucose response, mmol/L/hr</td>
<td>12.7±2.38</td>
<td>15.7±3.01$^+$</td>
<td>16.7±3.44$^+$</td>
</tr>
<tr>
<td>Insulin 0 hr, pmol/L</td>
<td>26.0 (21.0-34.0)</td>
<td>54.0 (44.5-67.0)$^+$</td>
<td>66.0 (45.0-87.0)$^+$</td>
</tr>
<tr>
<td>Insulin 2 hr, pmol/L</td>
<td>153.0 (82.0-179.0)</td>
<td>197.0 (116.5-311.5)</td>
<td>237.0 (122.0-503.0)$^+$</td>
</tr>
<tr>
<td>Insulin response, pmol/L/hr</td>
<td>341.0 (211.3-466.0)</td>
<td>708.5 (493.6-832.6)$^+$</td>
<td>845.3 (489.3-1023.3)$^+$</td>
</tr>
</tbody>
</table>
Data are presented as mean (±SD) or as median (interquartile range). HDL = high-density lipoprotein. Glucose and insulin response refers to response to an oral glucose tolerance test. †P<0.01 vs. lean normotensive men; ‡P<0.05 vs. lean normotensive men
Figure S1. Box plots of 24-hour urine noradrenaline excretion (Figure S1A), plasma glucose concentrations (Figure S1B), serum insulin concentrations (Figure S1C) in the three groups of men. NT=normotensive, HT=hypertensive. P-values for group differences depicted over appropriate boxes after adjustment for multiple comparisons. Ends of the boxes define 25th and 75th percentiles, with line at the median, dotted line defines mean, and error bars define 10th and 90th percentile, and values outside the 10th and 90th percentile are shown in black circles.