Low Calorie Diet Enhances Renal, Hemodynamic, and Humoral Effects of Exogenous Atrial Natriuretic Peptide in Obese Hypertensives

Paolo Dessì-Fulgheri, Riccardo Sarzani, Massimiliano Serenelli, Paola Tamburrini, Diego Spagnolo, Laura Giantomassi, Emma Espinosa, Alessandro Rappelli

Abstract—The expression of the natriuretic peptide clearance receptor is abundant in human and rat adipose tissue, where it is specifically inhibited by fasting. In obese hypertensives, plasma atrial natriuretic peptide (ANP) levels were found to be lower than in obese normotensives. Therefore, the increased adipose mass might influence ANP levels and/or its biological activity. The aim of the present study was to evaluate whether the humoral, hemodynamic, and renal effects of exogenous ANP in obese hypertensives might be enhanced by a very low calorie diet. Eight obese hypertensives received a bolus injection of ANP (0.6 mg/kg) after 2 weeks of a normal calorie/normal sodium diet, and blood pressure (BP), heart rate, ANP, cGMP, plasma renin activity, and aldosterone were evaluated for 2 hours before and after the injection. Diuresis and natriuresis were measured every 30 minutes. The patients then started a low calorie/normal sodium diet (510 kcal/150 mmol/d) for 4 days, and then the ANP injection protocol was repeated. The low calorie diet induced a slight weight loss (from 90.6 ± 1.1 to 87.7 ± 1.2 kg; P < 0.01), which was accompanied by increase of cGMP excretion (from 146.0 ± 10.1 to 154.5 ± 9.5 nmol/24 h; P < 0.05) together with a reduction of BP (P < 0.01 versus basal levels). ANP injection after diet was followed by an increase of ANP levels similar to that observed before diet, but plasma cGMP, diuresis, and natriuresis increased significantly only after diet. Similarly, the decrease of BP after ANP administration was significantly higher after diet (change in mean arterial pressure, −6.4 ± 0.7 versus −4.0 ± 0.6 mm Hg; P < 0.05) as well as that of aldosterone (P < 0.01). These data show that a low calorie diet enhances the humoral, renal, and hemodynamic effects of ANP in obese hypertensives and confirm the importance of caloric intake in modulating the biological activity of ANP, suggesting that the natriuretic peptide system can play a role in the acute changes of natriuresis and diuresis associated with caloric restriction. (Hypertension. 1999;33:658-662.)

Key Words: obesity • hypertension • natriuretic peptides, atrial • cyclic GMP • diuresis • natriuresis

Several epidemiological and clinical studies have shown that excess weight and weight gain contribute to increase blood pressure (BP) in a large proportion of hypertensive patients. Such studies have also demonstrated that BP in the adult is highly correlated to body mass index (BMI). Although the association between obesity and hypertension is widely recognized, the pathophysiology of weight-related change in BP is a matter of debate. Several mechanisms have been suggested to play a role in the pathogenesis of obesity-related hypertension, such as increased plasma volume and cardiac output, enhanced sympathetic nervous system activity, hyperinsulinemia and insulin resistance, and nutritional factors such as high sodium intake and/or sodium retention. Moreover, the importance of weight gain per se in causing hypertension is reinforced by experimental studies showing that weight gain raises BP and weight loss reduces BP in normotensives and hypertensives, even when sodium intake was maintained constant.

In addition to a reduction in BP, the early phase of weight loss induced by severe caloric restriction or fasting is accompanied by a considerable increase in diuresis and natriuresis. Although the mechanisms of the natriuresis of fasting have been studied in the past, little attention has been given to a possible pathophysiological role of the natriuretic peptide (NP) system. The NP system is a major regulator of body fluid volumes and arterial pressure through several actions, including enhancement of natriuresis, inhibition of the renin-angiotensin-aldosterone system, direct vasodilation, and possible inhibitory effects on arginine vasopressin and on the sympathetic nervous system. This peptide family, and in particular atrial natriuretic peptide (ANP), acts by binding to specific natriuretic peptide receptors (NPr), either coupled (NPr-A and NPr-B) or uncoupled (NPr-C) with guanylyl cyclase activity. NPr-A mediates most of the known activities of ANP, and binding of ANP to this receptor is followed by an increase in plasma levels and urinary excretion of...
cGMP. NPR-C appears to mediate the clearance and the “buffering” of circulating natriuretic peptides and therefore is also known as the “clearance” receptor for NP.

As mentioned above, few studies have been performed in selected populations of obese hypertensive patients to analyze a possible role of the NP system either in the natriuresis of fasting or in impaired sodium handling in obesity-related hypertension. A blunted response of plasma ANP levels to saline load has been demonstrated in normotensive obese subjects, whereas Maoz et al. showed that caloric restriction induced an early increase of circulating ANP. ANP binding sites have been found in rat brown adipose tissue, and we have recently shown that human and rat adipose tissue contains very high levels of NPR-C mRNA. Other authors have shown the presence of abundant clearance receptor binding and protein in adipocytes. Moreover, in adipose tissue NPR-C expression is dramatically inhibited by fasting, indicating a nutritional regulation of this receptor. Plasma levels of ANP lower than that found in obese normotensive individuals have been shown in obese hypertensives, together with higher gene expression of the clearance receptor for NP in the adipose tissue. Finally, in experimental obesity, weight reduction might facilitate ANP activity, inducing diuresis and natriuresis. These findings are consistent with the hypothesis that the abundance of clearance receptors in the total adipose mass of obese hypertensives might play some role in obesity-related hypertension by reducing the biological activity of circulating ANP.

In an attempt to further investigate the role of increased adipose mass in modulating the biological activity of NP, we evaluated whether a short period of a low calorie diet in obese hypertensives is able to enhance the renal, hemodynamic, and humoral effects of a low dose of exogenous ANP injection.

**Methods**

**Subjects**

The patients studied consisted of 8 obese hypertensives (mean age, 51±4 years). Both male (n=3) and female (n=5) patients were included in the study, but only postmenopausal women not using hormone replacement therapy were selected. Obesity was defined as a BMI≥30 kg/m². All patients were affected by untreated mild to moderate uncomplicated essential hypertension, according to the World Health Organization classification. Patients affected by a secondary form of hypertension were excluded on the basis of clinical and laboratory data. Patients with overt insulin-dependent or -independent diabetes mellitus were also excluded from the study, and an oral glucose tolerance test was performed in all patients to also exclude those with impaired glucose tolerance according to the American Diabetes Association criteria. All patients had no history or clinical evidence of cardiovascular (except hypertension), renal (serum creatinine ≤1.4 mg/dL), or hepatic diseases.

**Protocol**

The protocol was approved by the Ethics Committee of the University of Ancona, and all patients gave their written informed consent to participate in the study. The patients entered a 2-week run-in period, during which they followed a normal calorie/normal sodium (150 mmol) diet given by the hospital dietitian. Thereafter, the patients were admitted to our department, where body weight, BP, heart rate, diuresis, natriuresis, plasma and urinary cGMP, ANP (after 30 minutes of recumbency), plasma renin activity (PRA), plasma aldosterone, and plasma catecholamines (after 2 hours of upright position) were evaluated daily for 1 week during hospitalization.

Body weight and height were measured with patients wearing undergarments (light clothing without shoes or slippers). BMI was then calculated as weight in kilograms divided by height in meters squared. BP was measured with a mercury sphygmomanometer using a cuff of proper size. On the second day of hospitalization, after overnight fasting and with the patients in the supine position, an antecubital vein in each arm was cannulated for blood sampling and ANP injection with a 20-gauge needle (Ethicon SpA). The cuff of an automatic oscillometric monitor (SpaceLab 90207, SpaceLab Inc), validated against a standard sphygmomanometer before each experiment, was then applied in the nondominant arm for BP and heart rate recording every 10 minutes for 2 hours before (7 to 9 AM) and after (9 to 11 AM) ANP injection. Mean arterial pressure (MAP) was calculated as diastolic BP plus one third of the pulse height.

To maintain an adequate urinary flow rate, all patients drank a fixed amount of water (200 mL/h) throughout the study period. Urine was collected every 30 minutes by spontaneous voiding during the 4 hours of the acute phase of the study, with the patients avoiding any postural change. Urinary volume, natriuresis, and creatinine excretion were measured in each urine sample, and plasma creatinine was measured every 30 minutes. The hourly means of diuresis, natriuresis, and creatinine clearance were then calculated. At time 0 (9 AM), a bolus of 0.6 μg/kg of synthetic human ANP (Cininalfa AG) dissolved in 10 mL of sterile saline was injected intravenously. Blood samples for ANP, cGMP, PRA, and plasma aldosterone were withdrawn 30 minutes before ANP injection, at the time of ANP bolus (time 0), and 15, 30, 60, 90, and 120 minutes after ANP injection. On the following day, the patients started a low calorie diet (510 kcal=121.4 J). The diet composition was as follows: 42% protein, 40% fat, 18% carbohydrate, and 35 mmol Na⁺.

To maintain sodium intake unchanged, 7 g NaCl supplement was added to the diet, resulting in a daily sodium intake of 154 mmol/d. The low calorie diet was maintained for 4 days, and on the morning of day 5 the ANP injection protocol was repeated, following exactly the same procedure described above.

**Hormonal Measurements**

Blood samples for ANP were collected in ice-chilled tubes containing 1 mg/mL EDTA (Sigma Chemical Co) and 500 IU/mL aprotinin (Trasylol, Bayer AG). After centrifugation, plasma was stored at −80°C until further processing. ANP was measured by radioimmunoassay after extraction from acidified plasma with C-18 cartridges (Sep-Pak, Waters Associated) with the use of a commercial kit (Eiken Chemical Co, PRA, plasma aldosterone, and plasma and urinary cGMP were measured by radioimmunoassay with the use of commercial kits (PRA and plasma aldosterone: Sorin Biomedica; cGMP: Advanced Magnetics Inc). Plasma catecholamines were measured by high-performance liquid chromatography with electrochemical detection (HLC-725CA, Tohso). Urinary sodium was measured by flame photometry, and plasma and urinary creatinine were measured with an automatic multianalyzer instrument.

**Statistical Analysis**

Data in the text, table, and figures are reported as mean±SEM. Comparisons of data before and after diet were performed by Student’s t test for paired data. Statistical analysis of data obtained after ANP injection, before and after diet, was performed by ANOVA for repeated measures, followed by Student’s t test for paired data with Bonferroni correction for multiple comparisons. For statistical analysis of MAP values after ANP administration, the mean of the BP recordings taken during the 2-hour period preceding the administration of ANP was taken as basal value of MAP. A P value <0.05 was considered statistically significant.

**Results**

**Effects of Diet on Basal Parameters**

The Table reports the mean±SEM levels of body weight, BP, heart rate, diuresis, natriuresis, plasma and urinary cGMP,
ANP, PRA, plasma and urinary aldosterone, and plasma catecholamines evaluated at the end of the run-in normal calorie diet and at the end of the low calorie diet period. As expected, a significant body weight loss was observed after 4 days of diet (from 90.6±1.1 to 87.7±1.2 kg; P<0.01).

A significant fall of both systolic and diastolic BP was also observed (from 161.2±2.7/100.5±0.9 mm Hg at the end of the run-in period to 150.3±2.0/94.9±1.0 mm Hg after 4 days of low calorie diet; P<0.01 for both systolic and diastolic BP). No significant difference of 24-hour urinary sodium excretion was observed during the study. Of the other parameters measured, only urinary cGMP increased significantly after the low calorie diet (from 146.0±10.1 to 154.5±9.5 pmol/L; P<0.05). Administration performed at baseline. When ANP injection was repeated after the low calorie diet, the trend of fall of MAP was initially similar, but at 30 minutes the fall (−6.4±0.7 mm Hg; P<0.01 versus basal value) was significantly greater than that observed after the first administration of the peptide (P<0.05), and 90 minutes after ANP injection the mean MAP (−5.1±1.0 mm Hg) was still significantly lower than basal values (P<0.01) and with the values measured when ANP injection was performed before the low calorie diet (−0.2±0.8 mm Hg; P<0.05).

The effects of ANP, before and after the low calorie diet, on plasma levels of cGMP are reported in Figure 2. When ANP was injected before the low calorie diet, no significant changes of circulating cGMP levels were observed. On the contrary, when the experiment was repeated after the low calorie diet, a significant increase of circulating cGMP was observed 30 minutes after ANP injection (from a basal value of 2.1±0.4 to 4.0±0.2 pmol/mL; P<0.05).

Figure 3 depicts the comparison of basal values of PRA and plasma aldosterone with the lowest values of these variables measured after ANP injection before and after the low calorie diet. PRA did not change significantly (before low calorie diet: from 11.5±2.6 to 10.9±3.8 pmol·min⁻¹·L⁻¹; P=NS; after low calorie diet: from 11.5±2.6 to 10.8±3.8 pmol·min⁻¹·L⁻¹; P=NS). A significant reduction of plasma aldosterone was observed when ANP injection was performed after the low calorie diet (from 333±50 to 327±58 pmol/L; P<0.05).

Effects of ANP Infusion Before and After Low Calorie Diet

The injection of 0.6 μg/kg of exogenous ANP was followed by a significant increase of circulating ANP, reaching a peak level 30 minutes after peptide administration. The values were within the pathophysiological range. Similar peak levels were obtained after ANP injection before and after the low calorie diet (15.2±2.6 and 15.4±1.9 pmol/mL, respectively; P=NS) because the dose administered was adjusted for body weight.

Figure 1 shows the changes of MAP values observed when ANP was injected in our obese patients before and after a low calorie diet. A significant decrease of MAP was observed after the first ANP injection, reaching statistical significance 30 minutes after ANP bolus (−4.0±0.6 mm Hg; P<0.05 versus basal value). However, the effect was evanescent; 60 minutes after ANP injection, MAP levels were not statistically different from basal values, and at 90 minutes the values were actually superimposable to those measured before ANP

### Table: Main Clinical and Laboratory Parameters Evaluated Before and After Low Calorie Diet

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Start of Low Calorie Diet (End of Run-In)</th>
<th>End of Low Calorie Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>90.6±1.1</td>
<td>87.7±1.2*</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>161.2±2.7</td>
<td>150.3±2.0*</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>100.5±0.9</td>
<td>94.9±1.0*</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>72±4</td>
<td>71±6</td>
</tr>
<tr>
<td>Diuresis, mL/24 h</td>
<td>1290±156</td>
<td>1325±145</td>
</tr>
<tr>
<td>Urinary Na⁺ excretion, mmol/24 h</td>
<td>139±18</td>
<td>135±20</td>
</tr>
<tr>
<td>ANP, fmol/mL</td>
<td>10.8±1.1</td>
<td>11.9±1.3</td>
</tr>
<tr>
<td>Plasma cGMP, pmol/mL</td>
<td>2.8±0.9</td>
<td>2.9±1.1</td>
</tr>
<tr>
<td>Urinary cGMP, nmol/24 h</td>
<td>146.0±10.1</td>
<td>154.5±9.5†</td>
</tr>
<tr>
<td>PRA, pmol·min⁻¹·L⁻¹</td>
<td>9.7±2.6</td>
<td>10.4±3.8</td>
</tr>
<tr>
<td>Plasma aldosterone, pmol/L</td>
<td>333±50</td>
<td>327±58</td>
</tr>
<tr>
<td>Urinary aldosterone, nmol/24 h</td>
<td>23.9±3.0</td>
<td>25.5±2.5</td>
</tr>
<tr>
<td>Adrenaline, nmol/L</td>
<td>0.53±0.06</td>
<td>0.51±0.08</td>
</tr>
<tr>
<td>Noradrenaline, nmol/L</td>
<td>1.47±0.13</td>
<td>1.43±0.15</td>
</tr>
<tr>
<td>Creatinine clearance, mL·min⁻¹·BSA</td>
<td>112±8</td>
<td>114±7</td>
</tr>
</tbody>
</table>

*P<0.01, †P<0.05 vs basal values.

### Figure 1. MAP (mean±SEM) after ANP injection (arrow) before (continuous line) and after (dotted line) low calorie diet. *P<0.05 vs time −10 minutes; †P<0.01 vs time −10 minutes; ‡P<0.05 before vs after low calorie diet.

### Figure 2. Plasma cGMP concentration (mean±SEM) after ANP injection (arrow) before (continuous line) and after (dotted line) low calorie diet. *P<0.05 vs time 0.
The biological effects of exogenous ANP administration in humans have been investigated extensively in the past. However, the renal and humoral effects of the peptide reported in the literature are strongly influenced by the dose of ANP used and whether it is injected as a single bolus or administered in continuous infusion. When low doses are used (as in our study), inducing changes in circulating ANP confined within the normal range, the humoral effects and those on diuresis, natriuresis, and BP showed a transient decrease, but these changes did not reach statistical significance (diuresis: from 225±30 to 270±40 mL/h; P=NS; natriuresis: from 9.1±0.8 to 10.1±2.0 mmol/h; P=NS). On the contrary, when ANP bolus injection was performed after the low calorie diet, the diuretic and natriuretic effect was greater and was statistically significant (diuresis: from 230±25 to 337±30 mL/h; P<0.01; natriuresis: from 7.8±1.1 to 12.2±2.1 mmol/h; P<0.01).

Glomerular filtration rate, evaluated by means of creatinine clearance, remained unchanged after the injection of ANP performed either before or after a low calorie diet (data not shown).

**Discussion**

The biological effects of exogenous ANP administration in humans have been investigated extensively in the past. However, the renal and humoral effects of the peptide reported in the literature are strongly influenced by the dose of ANP used and whether it is injected as a single bolus or administered in continuous infusion. When low doses are used (as in our study), inducing changes in circulating ANP confined within the normal range, the humoral effects and those on diuresis, natriuresis, and BP showed a transient decrease, but these changes did not reach statistical significance (diuresis: from 225±30 to 270±40 mL/h; P=NS; natriuresis: from 9.1±0.8 to 10.1±2.0 mmol/h; P=NS). On the contrary, when ANP bolus injection was performed after the low calorie diet, the diuretic and natriuretic effect was greater and was statistically significant (diuresis: from 230±25 to 337±30 mL/h; P<0.01; natriuresis: from 7.8±1.1 to 12.2±2.1 mmol/h; P<0.01).

Glomerular filtration rate, evaluated by means of creatinine clearance, remained unchanged after the injection of ANP performed either before or after a low calorie diet (data not shown).

**Figure 3.** PRA and aldosterone (mean±SEM) before (open columns) and after (closed columns) ANP injection performed under basal conditions or after a low calorie diet. The values after ANP injection are the mean±SEM of the lowest values measured during the 2 hours after peptide administration. **P<0.01 before vs after ANP injection.

**Figure 4.** Hourly mean of diuresis (mean±SEM) in the 2 hours before (open columns) and after (closed columns) ANP injection performed under basal conditions or after a low calorie diet. **P<0.01 before vs after ANP injection.

ANP in obese hypertensives did not significantly affect renal and systemic hemodynamics as well as PRA, plasma aldosterone, or cGMP. Only BP showed a transient decrease 30 minutes after ANP injection. However, in none of the previous studies concerning exogenous ANP administration was the possible influence of body weight and caloric intake in modulating the biological activity of ANP taken into account. Therefore, in the present study the injection of ANP was repeated after 4 days of low calorie/normal sodium diet (510 kcal/d=2114 J). Such caloric restriction was followed by a significant although slight body weight reduction, but the dose of the peptide, adjusted for body weight, induced changes in circulating ANP almost identical to those observed when peptide administration was performed before the low calorie diet. Nevertheless, when the ANP injection was repeated after the low calorie diet, all the well-known systemic and renal effects of ANP were significantly enhanced in comparison with the data obtained after the injection of the peptide in the basal state. Indeed, after 4 days of the low calorie diet, the basal urinary excretion of cGMP, the second messenger of the NP system, was significantly increased, suggesting an improvement of the biological activity of the NP system itself. Therefore, for the first time, we demonstrated that, in humans, caloric intake and body weight are important modulators of the humoral, renal, and hemodynamic effects not only of exogenous ANP but also of the endogenous NP system. Our findings are in agreement with those of Crandall et al., showing that, in experimental obesity, diuresis and natriuresis accompanying weight reduction may be due to increased activity of ANP.

Previous studies in animals and humans of our group and by others help to explain the findings of the present study. Rat and human adipose tissue contain very high levels of NPR-C mRNA, and the expression of this receptor is dramatically inhibited by fasting, indicating a nutritional regulation of its gene expression. Other authors have shown the presence of abundant clearance receptor binding and protein in adipocytes. Moreover, plasma levels of ANP in obese hypertensives were found to be lower than those measured in obese normotensive individuals, together with higher gene expression of the clearance receptor for NP in adipose tissue. These findings are consistent with the hypothesis that the increased total adipose mass of obese hypertensives, through the enhanced expression of the biologically inactive NPR-C, might influence the activity of NP. In other words, a larger number of NPR-C may capture more molecules of circulating ANP, preventing them from binding to the biologically active receptors, mainly at the renal level. Accordingly, a downregulation of NPR-C expression occurs after a low calorie diet in humans, the bioavailability of NP should be increased through a reduction of the peripheral clearance at the adipose tissue level, as we have shown in the rat. Unfortunately, for ethical reasons, we could not perform 2 adipose tissue biopsies before and after such a short period of time. This would have certainly made a valid contribution to the interpretation of our results. However, taken together these findings support the hypothesis that the NP system may play a role in the natriuresis of fasting.
Decrease of body weight after low calorie intake is associated with a decrease in the overactivity of the adrenergic nervous system usually found in obese hypertensives.\textsuperscript{29,30} In fact, most of the effects observed after weight loss in obese hypertensives (eg, BP reduction, enhanced sodium excretion, decrease PRA) might be due, at least in part, to normalization of the activity of the sympathetic nervous system. However, these findings are usually observed after prolonged low calorie intake and significant weight reduction. This does not seem to be the case in our study, since these findings were observed after only 4 days of a low calorie diet, which induced only a modest reduction of body weight (\( \pm 2.9 \pm 0.9 \) kg, ie, no more than 3\% of the basal weight) and was not accompanied by significant changes in the activity of the sympathetic nervous system, as judged by HR and plasma catecholamines, which remained unchanged during the study.

As demonstrated in Figure 3, the decrease of plasma aldosterone in response to ANP injection performed after the low calorie diet was greater than that of PRA. This might be due to the fact that ANP is a potent inhibitor of aldosterone secretion both in vitro and in vivo, independently of angiotensin II.\textsuperscript{31,32} This finding further demonstrates the enhanced biological activity of ANP after a low calorie diet.

In conclusion, the data of the present study suggest that a possible interaction between the NP-NP\(_{r}\) system and adipose tissue can play a role in explaining the acute changes of natriuresis, diuresis, and BP associated with short-term caloric restriction. Further studies on the NP-NP\(_{r}\) system and adipose tissue in obese hypertensive patients after prolonged caloric restriction might provide new insights into the multifactorial pathophysiology of obesity-related hypertension.

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References

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