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# Salt-Regulating Hormones in Young Normotensive Obese Subjects

## Effects of Saline Load

Giuseppe Licata, Massimo Volpe, Rosario Scaglione, Speranza Rubattu

**Abstract** To investigate whether the response of salt-regulating hormones to volume expansion is impaired in obese subjects, we assessed the effects of saline load (0.25 mL/kg · min · 120 min) in 9 young, healthy, normotensive obese subjects (body mass index, >30 kg/m<sup>2</sup>) and in 10 lean control subjects (body mass index, <25 kg/m<sup>2</sup>) matched for age, gender, height, and mean blood pressure. Hematocrit, plasma renin activity (PRA), plasma aldosterone (PA), atrial natriuretic factor (ANF), and urinary sodium excretion (U<sub>Na</sub>V) were evaluated. Saline load increased ANF levels significantly ( $P < .001$ ) in lean subjects at both 60 and 120 minutes, whereas they decreased in obese subjects. Such decreases became significant ( $P < .01$ ) at 120 minutes. Suppression of PRA and PA by saline load were more marked in lean than obese

subjects. Hematocrit decreased in both groups, and U<sub>Na</sub>V increased more in lean than obese subjects during saline load. Comparisons of percent changes in ANF, PRA, and PA after saline load showed that the responses of lean and obese subjects were significantly different ( $P < .001$  for ANF at both 60 and 120 minutes;  $P < .05$  for PRA and PA at both 60 and 120 minutes). In conclusion, the lack of ANF response and the reduced suppression of PRA and PA to saline load indicate a dysfunction of these systems in obese subjects. This alteration may be involved in the higher susceptibility of obese subjects to developing hypertension. (*Hypertension*. 1994;23[suppl 1]:I-20-I-24.)

**Key Words** • renin-angiotensin system • obesity • atrial natriuretic factor • hypertension, sodium-dependent

**A**lthough a strong association between obesity and high blood pressure has been well characterized, the physiological basis of obesity-induced hypertension is still unclear.<sup>1,2</sup> Several abnormalities recently have been associated with the mechanisms involved in obesity-related hypertension. They include sodium retention,<sup>3</sup> increased plasma volume and cardiac output,<sup>4</sup> hyperinsulinemia and insulin resistance,<sup>5</sup> and enhanced sympathetic nervous system activity.<sup>6</sup> Conflicting findings have been reported on plasma renin activity (PRA) and plasma aldosterone (PA) behavior in normotensive and hypertensive obese subjects.<sup>7-9</sup> In addition, few studies have addressed the significance of atrial natriuretic factor (ANF) in obesity-related hypertension.<sup>10</sup>

The relations among these factors in obese subjects might be of great importance because the renin-angiotensin-aldosterone system (RAAS) has been recognized as one of the fundamental control systems of salt, water, and blood pressure homeostasis.<sup>11</sup> In addition, RAAS dysregulation has been reported in subsets of hypertensive patients, such as "salt-sensitive non-modulators,"<sup>12</sup> and it may be involved in obesity-related hypertension. In fact, previous studies have indicated that obese adolescents<sup>3</sup> and fat-fed dogs<sup>13</sup> exhibit an enhanced blood pressure sensitivity to salt intake. Moreover, recent experimental data suggest that the regulatory mechanisms controlling sodium excretion and extracellular fluid volume are al-

tered in dogs with obesity-induced hypertension.<sup>2,14</sup> The ANF response to volume expansion is also a sensitive marker of the adaptive ability of the cardiovascular system to increased preload.<sup>15</sup>

In this study, the responses of PRA, ANF, and PA to volume expansion by saline load were investigated in young normotensive lean and obese subjects. Our final goal was to recognize early alterations of factors controlling sodium balance that could explain the higher susceptibility of obese subjects to developing hypertension. Because obesity is often associated with metabolic or cardiovascular abnormalities, we selected obese subjects without hypertension, lipid abnormalities, or insulin-dependent or -independent diabetes mellitus.

## Methods

### Subjects

A total of 19 subjects, 9 obese and 10 lean healthy subjects, were included in the study. Obese subjects were recruited from the obese population younger than 45 years attending the obesity center of the Internal Medicine Department at the University of Palermo (Italy). According to body mass index (BMI) values and the criteria of Garrow and Webster,<sup>16</sup> subjects were divided into two groups: The lean group consisted of 10 subjects (5 men and 5 women), aged 28 to 44 years (mean, 40.5 ± 3.5 years) with a BMI less than 25 kg/m<sup>2</sup> (mean, 24.2 ± 1.5); the obese group consisted of 9 subjects (4 men and 5 women), aged 27 to 42 years (mean, 35.3 ± 4.5 years) with a BMI greater than or equal to 30 kg/m<sup>2</sup> (mean, 39 ± 2).

Exclusion criteria were insulin-dependent or -independent diabetes mellitus, hyperlipoproteinemia, endocrine and cardiovascular diseases, hypertension, positive family history of hypertension or cardiovascular accidents, alcoholism, drug addition, and psychiatric problems.

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All obese and lean subjects were normotensive and matched as closely as possible with regard to age, gender, body height, systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean blood pressure (Table 1). SBP and DBP were obtained from the average of three measurements made with a mercury sphygmomanometer after subjects had been in a comfortable supine position for 5 minutes. DBP refers to Korotkoff phase V. Mean blood pressure was calculated from the sum of DBP plus one third of the arterial pulse pressure. Arterial pressure was measured with an appropriately sized cuff in obese subjects.<sup>17</sup>

Resting heart rate was obtained from electrocardiogram. Cardiovascular diseases were excluded on the basis of absence of chest pain or previous myocardial infarction and of appropriate investigations including chest radiograph, basal and 24-hour electrocardiograph monitoring, and M- and B-mode echocardiography. Echocardiographic left ventricular mass and left ventricular mass index were calculated according to the criteria of Devereux et al.<sup>18</sup> All subjects with unsatisfactory echocardiographic findings or with left ventricular hypertrophy were excluded. All obese subjects were untreated for at least 4 weeks before the study, and they maintained a normal diet.

The present study was approved by the Ethics Committee of our institution, and each patient gave informed consent after a detailed description of the study procedure.

### Experimental Protocol

All drug therapy was discontinued at least 4 weeks before the study. Alcohol, caffeine, cigarettes, and physical exercise were all prohibited within 24 hours of the study. After admission to the clinical ward, all subjects were maintained on a daily diet containing 100 mEq sodium, 50 mEq potassium, and 1500 mL water. Daily 24-hour urine collections were analyzed for sodium, potassium, and creatinine excretions. When subjects had achieved metabolic balance, as assessed by the stability of urinary electrolyte excretion, the studies were undertaken.

The study was begun at 7:30 AM with the subject in a comfortable seated position after voiding. The temperature (22°C) and lights of the study room were maintained constant. The patient was asked to drink 500 mL of water to ensure sufficient urinary flow.

Thirty minutes after insertion of two intravenous lines into superficial forearm veins, 20-minute baseline clearance periods were performed. Throughout the study, at the end of each period the patients drank an amount of water equivalent to the sum of blood loss and urinary flow. As soon as balance was achieved (consistent urinary volumes in two consecutive clearance periods), two 30-minute baseline periods were observed, and the average of the urinary and hormonal measurements obtained during the two baseline periods was used for analysis.

Subsequently, an intravenous isotonic saline load (0.9% NaCl, 0.25 mL/kg per minute) was started and maintained at a constant rate for 2 hours. Arterial blood pressure was measured at 10-minute intervals, and heart rate was continuously monitored by electrocardiogram. Urinary samples for determination of volume, sodium, and potassium were obtained at the end of each clearance period. Venous blood samples for determination of hematocrit, PRA, PA, and immunoreactive ANF levels were obtained at 30-minute intervals.

### Laboratory Methods

All blood samples were collected on ice; plasma was then separated and frozen until the time of the assay, which did not exceed 5 weeks. PRA was measured by radioimmunoassay according to the method described by Menard and Catt.<sup>19</sup> Plasma ANF levels were determined by radioimmunoassay as previously described by Volpe et al<sup>20</sup> using rabbit antiserum (RAS 8798, Peninsula Laboratories Europe), iodinated human

ANF (2000 Ci/mmol, Amersham, Berks, UK), and  $\alpha$ -human ANF (Bissendorf GmbH Peptide) as a standard. ANF was extracted from plasma with Sep-Pak C18 cartridges. Recoveries were determined on each plasma sample by adding to it a minimal amount of radiolabeled ANF, ranging from 74% to 90%. Intra-assay and interassay coefficients of variation were 6.5% and 10.5%, respectively. The radioimmunoassay sensitivity was 1 fmol per tube. PA concentrations were estimated by a radioimmunoassay method using a commercial kit (Sorin, Saluggia, Italy). Plasma insulin levels were also determined at baseline by a radioimmunoassay double-antibody method using a commercial kit (Sorin). Intra-assay variation was 7.5%, and interassay variation was 8%; sensitivity for detection of insulin was 2.5  $\mu$ U/mL. Urinary potassium and sodium levels were measured by a current ion-selective electrode method (Beckman).

### Statistical Analysis

Data are presented as mean  $\pm$  SD. Comparisons of the basal data of lean and obese subjects were performed by unpaired *t* test. Analysis of variance with Dunnett's correction was used to determine significant differences within the same group. Between-group comparisons of the responses to saline load were tested by two-factor analysis of variance (factoring for group and time) for repeated measures.

## Results

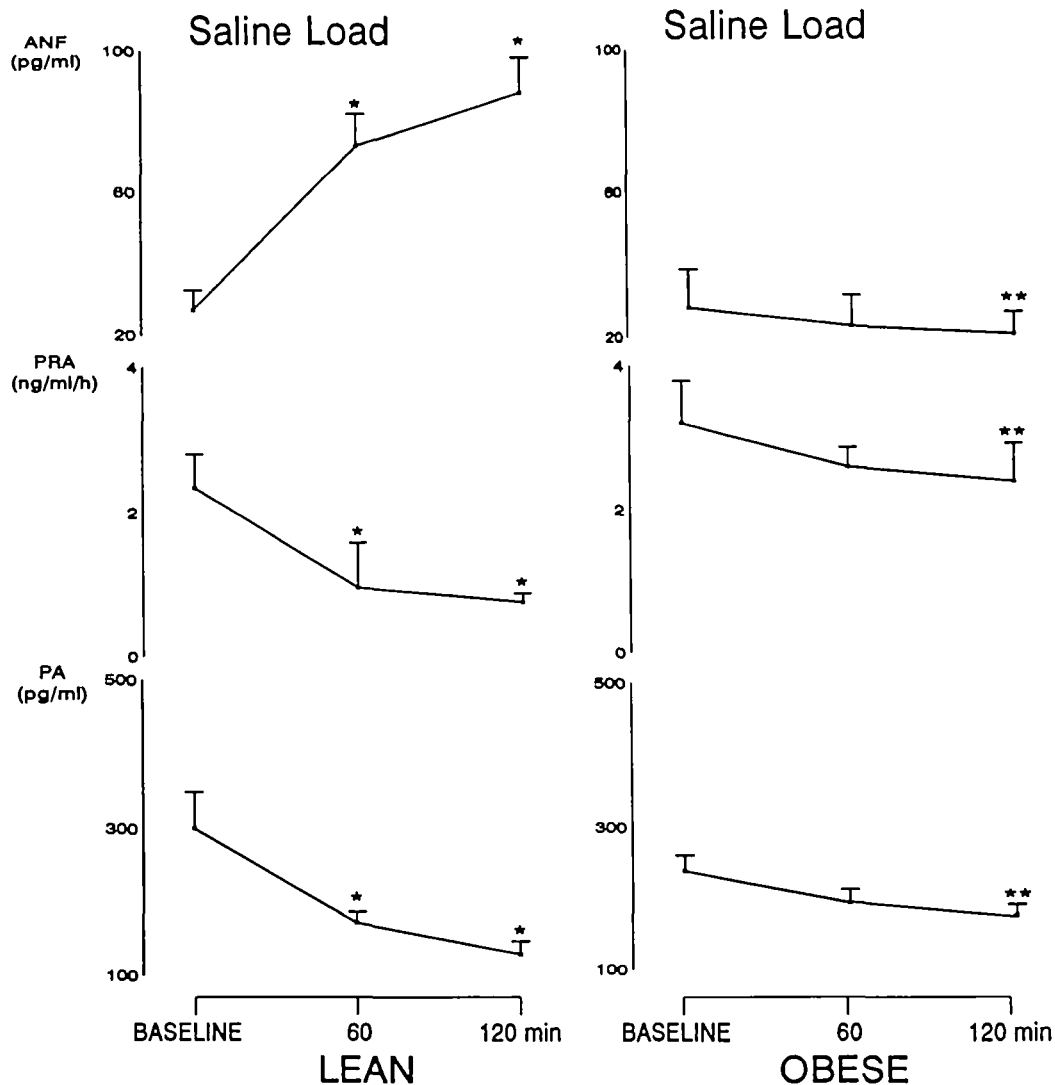
### Characteristics of Lean and Obese Subjects

The two groups of subjects were comparable with regard to clinical and metabolic baseline characteristics. In fact, no significant differences in age, height, blood pressure, heart rate, left ventricular mass index, serum

TABLE 1. Characteristics of the Study Groups

Characteristic	Lean (n=10)	Obese (n=9)	P
Age, y	37.5 $\pm$ 3.5	35.3 $\pm$ 4.5	NS
Sex, M/F	5/5	4/5	
Weight, kg	67.8 $\pm$ 3	104 $\pm$ 7	<.0001
Height, cm	165 $\pm$ 4	163 $\pm$ 5	NS
BMI, kg/m <sup>2</sup>	24.2 $\pm$ 1.5	39 $\pm$ 2	<.0001
SBP, mm Hg	127 $\pm$ 4	125 $\pm$ 5	NS
DBP, mm Hg	82 $\pm$ 3	84 $\pm$ 6	NS
HR, bpm	76.2 $\pm$ 3.1	78.2 $\pm$ 7	NS
LVMI, g/m <sup>2</sup>	106 $\pm$ 12	96 $\pm$ 10	NS
Glucose, mmol/L	5.0 $\pm$ 0.02	5.1 $\pm$ 0.02	NS
Serum creatinine, $\mu$ mol/L	76 $\pm$ 8.8	79.6 $\pm$ 8.8	NS
Total cholesterol, mmol/L	4.9 $\pm$ 0.3	5 $\pm$ 0.3	NS
U <sub>Na</sub> V, mmol/24 h	127 $\pm$ 16	130 $\pm$ 10	NS
PRA, ng/mL/h	2.3 $\pm$ 0.5	3.1 $\pm$ 0.6	NS
PA, pg/mL	296 $\pm$ 50	276 $\pm$ 24	NS
ANF, pg/mL	26 $\pm$ 7	29 $\pm$ 11	NS
IRI, $\mu$ U/mL	9.1 $\pm$ 3.2	14.6 $\pm$ 3.7	<.05

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; bpm, beats per minute; LVMI, echocardiographic left ventricular mass index; U<sub>Na</sub>V, urinary sodium excretion rate; PRA, plasma renin activity; PA, plasma aldosterone; ANF, atrial natriuretic factor; and IRI, serum immunoreactive insulin.



Line graphs show responses of atrial natriuretic factor (ANF), plasma renin activity (PRA), and plasma aldosterone (PA) to saline load in young normotensive lean and obese subjects. \* $P < .001$ , \*\* $P < .01$  vs baseline.

glucose, total cholesterol, and creatinine were observed. Baseline PRA, PA, ANF, and urinary sodium excretion rate were similar in the two groups. Insulin levels were significantly ( $P < .05$ ) higher in obese compared with lean subjects (Table 1).

#### Hormonal Responses to Saline Load

In lean subjects saline load was associated with a progressive increase in plasma ANF levels (from  $26 \pm 7$  to  $72 \pm 10$  and  $87 \pm 11$  pg/mL at 60 and 120 minutes, respectively), which became significant ( $P < .001$ ) at 60 minutes, whereas PRA ( $P < .001$ ) and PA ( $P < .001$ ) were progressively reduced (PRA, from  $2.3 \pm 0.5$  to  $0.9 \pm 0.2$  and  $0.7 \pm 0.1$  ng/mL per hour; PA, from  $296 \pm 50$  to  $170 \pm 16$  and  $126 \pm 18$  pg/mL) (Figure, left panel). Urinary sodium excretion rate ( $P < .001$ ) increased significantly after 60 and 120 minutes (Table 2).

As shown in the Figure (right panel), in the obese subjects ANF levels did not increase and were slightly reduced in response to saline loading (from  $29 \pm 11$  to  $24 \pm 9$  and  $22 \pm 6$  pg/mL); this reduction was significant

( $P < .01$ ) at 120 minutes. PRA and PA values were suppressed by a saline load (PRA, from  $3.1 \pm 0.6$  to  $2.6 \pm 0.3$  and  $2.4 \pm 0.6$  ng/mL per hour; PA, from  $276 \pm 24$  to  $244 \pm 18$  and  $222 \pm 13$  pg/mL) in the obese subjects to a lesser extent than in the lean subjects; these values became significant (both  $P < .01$ ) only at 120 minutes. Saline load also increased urinary sodium excretion rate in obese subjects but was significant ( $P < .01$ ) only at 120 minutes (Table 2).

A mild but not significant increase in SBP and DBP was observed in both groups at both experimental times. This increase was similar in lean and obese subjects (Table 2). Comparisons of percent changes in ANF, PRA, and PA after saline load show that the responses of lean and obese subjects were significantly different ( $P < .001$  for ANF at both 60 and 120 minutes;  $P < .05$  for PRA and PA at both 60 and 120 minutes). Finally, volume expansion induced comparable reductions of hematocrit in the two groups from  $0.42 \pm 0.2$  to  $0.40 \pm 0.2$  and  $0.39 \pm 0.2$  in lean subjects, and from  $0.45 \pm 0.4$  to  $0.43 \pm 0.5$  and  $0.41 \pm 0.5$  in obese subjects.

**TABLE 2. Effects of Saline Load on Urinary Sodium Excretion and Systolic and Diastolic Blood Pressures in Lean and Obese Subjects**

	Baseline	60 Minutes	120 Minutes
<b>Lean subjects (n=10)</b>			
U <sub>Na</sub> V, mmol/min	112±8	504±51*	816±64*
SBP, mm Hg	127±4	128±3	129±2
DBP, mm Hg	82±3	84±2	84±3
<b>Obese subjects (n=9)</b>			
U <sub>Na</sub> V, mmol/min	148±56	265±105	680±210†
SBP, mm Hg	125±5	128±4	129±2
DBP, mm Hg	84±6	86±3	86±3

U<sub>Na</sub>V indicates urinary sodium excretion rate; SBP, systolic blood pressure; and DBP, diastolic blood pressure.

\* $P < .001$ , † $P < .01$  vs baseline.

### Discussion

Our study demonstrates remarkable differences in the hormonal responses to an acute saline load between young normotensive lean and obese subjects. More specifically, in the obese subjects the major physiological adjustments occurring in response to an acute saline load, ie, suppression of PRA and PA levels and increased urinary sodium excretion values, were significantly smaller or delayed compared with those reported in lean subjects. The physiological secretory response of ANF promoted by saline load in lean subjects was not detectable in obese subjects. In fact, ANF levels were slightly reduced at both 60 and 120 minutes of saline load in young normotensive obese subjects.

It seems unlikely that these differences may have been accounted for by a different baseline degree of volume depletion because both groups were maintained on the same intake of fluids and sodium. In addition, volume expansion was similar in both groups, as documented by the induced changes in hematocrit values. Similarly, the abnormal responses observed in the obese subjects cannot be related to concomitant alterations often associated with obesity, ie, hypertension, diabetes, or lipid abnormalities because the obese subjects selected for this study were not affected by these concomitant diseases. The abnormal hormonal responses to saline load may be related to the higher susceptibility of obese subjects to developing hypertension. In fact, hyposecretion of ANF in response to a saline load has been reported in different rat models of genetic hypertension, including stroke-prone spontaneously hypertensive rats.<sup>21</sup> In addition, a smaller suppression in renin and PA levels after saline load has been recently reported by West et al<sup>13</sup> in hypertensive obese dogs compared with lean dogs.

Several experimental data suggest that activation of sodium-retaining systems during obesity, such as the RAAS and plasma insulin levels, could be involved in obesity-induced hypertension.<sup>13,15</sup> These parameters may be altered in obese animals and humans, but only some indirect information is available on ANF levels in obesity.<sup>10,22</sup>

In particular, increased insulin levels and insulin resistance have been recognized in both normotensive and hypertensive obese subjects<sup>5,6,14,22</sup> and have been associated with obesity-induced hypertension in view of the effects of insulin on the kidney, the cardiovascular system, and the cellular mechanisms of sodium transport. In fact, insulin can promote sodium retention through an increased sodium reabsorption by the proximal tubule.<sup>23,24</sup> This action, if prolonged, could determine extracellular volume expansion involving salt-regulating hormonal systems. On the other hand, insulin could promote sodium retention through increased angiotensin-induced aldosterone production,<sup>25</sup> enhanced sympathoadrenergic activity,<sup>5,6</sup> or reduction in ANF release.<sup>10</sup>

The hormonal responses reported in our obese subjects were reduced and delayed compared with those observed in lean subjects. These abnormal hormonal responses may be involved in the sodium sensitivity described in obese individuals.<sup>3,7,22</sup> Further studies will be required to test whether the dysregulation in ANF and the RAAS may be involved in the higher susceptibility of obese subjects to hypertension and cardiovascular risk.

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