Blood Pressure, Sodium Intake, Insulin Resistance, and Urinary Nitrate Excretion

Francesco S. Facchini, Carlos DoNascimento, Gerald M. Reaven, Jeannie W. Yip, Xi Ping Ni, Michael H. Humphreys

Abstract—The objective of this study was to investigate the relationships among various humoral factors thought to be involved in the regulation of blood pressure during high NaCl intake. Nineteen healthy subjects underwent sequential 5-day periods ingesting a low-sodium (25 mmol/d) or high-sodium (200 mmol/d) diet. Insulin resistance was assessed by the steady-state plasma glucose concentration at the end of a 3-hour insulin suppression test. Insulin resistance correlated inversely with natriuresis (P=0.04) and directly with increase in weight (P=0.03). The increase in mean arterial pressure associated with the high-sodium diet correlated directly with the gain in weight (P<0.05) and inversely with the increase in urinary nitrate excretion (P<0.0001). In a multiple regression model, more than 2/3 of the variance in mean arterial pressure was accounted for by the gain in weight and change in urinary nitrate excretion. The steady-state plasma glucose concentrations obtained with the 2 diets were similar, indicating that insulin resistance was unaffected by sodium intake. During high sodium intake, plasma renin activity and aldosterone decreased and plasma atrial natriuretic peptide increased; these changes did not correlate with the change in mean arterial pressure, insulin resistance, or change in urinary nitrate excretion. To the extent that urinary nitrate excretion reflects activity of the endogenous nitric oxide system, these results suggest that the salt sensitivity of mean arterial pressure may be related to blunted generation of endogenous nitric oxide. The results also demonstrate that insulin-resistant individuals have an impaired natriuretic response to high sodium intake. (Hypertension. 1999;33:1008-1012.)

Key Words: insulin resistance • nitric oxide • sodium chloride, dietary • blood pressure • hypertension, sodium dependent

Hyperinsulinemia and resistance to insulin-mediated glucose uptake (insulin resistance) are present in about 50% of patients with essential hypertension1 and in a substantial number of healthy persons.2,3 The kidney is normally responsive to the NaCl-retaining effect of insulin in insulin-resistant individuals,4–6 suggesting that the greater the degree of insulin resistance and hyperinsulinemia, the greater the amount of salt (and water) retained at any level of dietary NaCl intake. Thus, it could be postulated that the presence of insulin resistance and compensatory hyperinsulinemia would lead to NaCl retention and extracellular fluid volume (ECFV) expansion, thereby increasing the likelihood that insulin-resistant individuals would develop a salt-sensitive increase in blood pressure. Indeed, evidence has been presented in support of this hypothesis.7–10 On the other hand, not all studies have demonstrated a relationship between insulin resistance, hyperinsulinemia, and salt sensitivity.11,12 Thus, this issue remains unsettled.

Another controversy exists concerning the role of salt intake in the relationships among insulin resistance, compensatory hyperinsulinemia, and blood pressure regulation. Specifically, evidence has been presented that variation in NaCl intake will lead to changes in insulin sensitivity and/or plasma insulin concentration.13–15 However, there is also evidence that neither salt loading nor restriction had any effect on either insulin sensitivity or plasma insulin response to oral glucose.7,10 Thus, uncertainty also characterizes this aspect of the relationships among Na+ intake, insulin resistance, hyperinsulinemia, and blood pressure regulation.

If insulin resistance and/or hyperinsulinemia play a role in modulation of the effect of variations in salt intake on blood pressure, at least 2 conditions must be met. First, previous studies16–18 showing that renal NaCl retention is enhanced in response to an acute increase in insulin concentration must be extended to demonstrate that insulin-resistant and hyperinsulinemic individuals will have an impaired natriuretic response to NaCl loading. Second, for enhanced salt retention to lead to an increase in blood pressure, it is likely that a defect must also exist in the ability of an individual to compensate for the increase in ECFV associated with NaCl retention. Although

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there are many possible mechanisms to account for this postulated abnormality, the NO system appeared to us to be a likely candidate. Endogenous NO synthesis, at least in rats, induces vasodilation and natriuresis,19,20 and altered NO metabolism has been implicated in the pathogenesis of salt-sensitive hypertension.20 A blunted compensatory NO response might therefore be the reason why only some insulin-resistant individuals develop a pressor response to NaCl loading and ECFV expansion.

The current study was initiated to test the hypothesis that the natriuretic response to a high-salt diet will be impaired in insulin-resistant, as compared with insulin-sensitive, individuals and to determine whether the change in blood pressure associated with the high-salt diet will vary as a function of the degree to which the NO system is activated in response to the increase in ECFV (the less exuberant the response, the greater the increase in blood pressure). The results support this hypothesis.

Methods
Nineteen consecutive healthy volunteers were studied at the San Francisco General Hospital General Clinical Research Center (GCRC). The study was approved by the University of California San Francisco Institutional Review Board, all experimental procedures were in accordance with institutional guidelines, and all subjects gave written informed consent.

Study Procedures
A standard 75-g oral glucose tolerance test was performed on all subjects to rule out impaired glucose tolerance and diabetes. Subjects were considered healthy on the basis of results of a physical examination, routine blood chemistry tests, blood cell count, and resting 12-lead ECG. On the day of admission, subjects started either a low-salt (25 mmol/d) or a high-salt (200 mmol/d; \( \approx 80 \) mmol supplied as NaCl tablets) diet for 5 days and then switched to the other diet. The order of the diets was randomly assigned. All meals were provided by the GCRC metabolic kitchen; diets were isocaloric and consisted of 50% carbohydrate, 35% fat, and 15% protein. No alcohol, cured meats, black tea, or dietary supplements were consumed, and foodstuff antioxidant, vitamin, and K-254 intake was kept constant in both phases (K \( \approx 90 \) mmol/d). Salt intake of 200 mmol/d is within the daily consumption of many individuals of industrialized countries.21 Blood pressure was measured daily, in the early morning, on arousal, before venipuncture and orthostasis, in the supine position with an automatic oscillometric device (Dinamap 8260, Critikon). Mean arterial pressure (MAP) was calculated as 1/3 of the pulse pressure added to the diastolic pressure; the mean of the 8 values obtained on the last 2 days of each dietary phase was used. Twenty-four-hour collection of urine was performed during the experimental protocol was repeated in a similar manner. During the next 5 days, crossover to the other diet occurred and the experimental protocol was repeated in a similar manner.

Data Analysis
Results are mean±SE. Nonnormally distributed variables such as SSPG and insulin were log-transformed (for simplicity, the term “log” is omitted in the text and tables). A paired Student’s \( t \) test was used to analyze differences between means at the end of each dietary period. An unpaired Student’s \( t \) test was used to compare insulin-sensitive and insulin-resistant subjects. Simple and multiple regression analyses were used to identify potential associations among the study variables.

Results
Seventeen men and 2 women were studied. Their mean age was 43±11 (SD) years, and their mean body mass index was 25.5±0.4 kg/m\(^2\) (range, 21.1 to 28.6 kg/m\(^2\)). Table 1 lists the values of all experimental variables at the end of the low- and high-NaCl-intake period for the 19 subjects. These results indicate that the high-Na" diet was associated with significantly higher values for 24-hour urinary Na" excretion and body weight. However, MAP, SSPG, and insulin did not vary as a function of differences in salt intake. It can also be seen that the high-salt diet led to the expected decreases in PRA.

### Table 1. Experimental Variables at End of Low-Sodium (25 mmol/d) and High-Sodium (200 mmol/d) Diet Periods

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low</th>
<th>High</th>
<th>( \Delta )</th>
<th>( \Delta P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>78.8±3.1</td>
<td>79.4±3.0</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Natriuresis, mmol/d</td>
<td>12.7±2.8</td>
<td>174±14</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>82.7±2.6</td>
<td>82.9±2</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>SSPI, pmol/L</td>
<td>306±42</td>
<td>330±49</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>SSPG, mmol/L</td>
<td>8.25±1.01</td>
<td>7.83±1.00</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>270±33</td>
<td>258±32</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>ANP, pmol/L</td>
<td>4.6±0.7</td>
<td>10.3±1.3</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>PRA, ng/mL per h</td>
<td>2.8±4</td>
<td>0.41±0.8</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Aldosterone, pmol/L</td>
<td>674±75</td>
<td>92±11</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>UNO(V), (\mu)mol/d</td>
<td>1119±94</td>
<td>1353±136</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

*SSPI indicates steady-state plasma insulin.

At the end of each dietary phase (day 5), after an overnight fast and before orthostasis, blood was drawn in duplicate for measurement of fasting atrial natriuretic peptide (ANP),27 plasma renin activity (PRA),28 and aldosterone.29 After this baseline blood sample was drawn, to measure in vivo insulin action, each subject underwent an insulin suppression test.30 Subjects were given a fixed dose, simultaneous intravenous infusion of octreotide (Sandostatin) at 5 \( \mu \)g/min, glucose (240 mg/m\(^2\) per minute), and insulin (25 mU/m\(^2\) per minute). The solution was administered via continuous infusion into an indwelling Teflon catheter placed in a superficial antecubital vein. Venous blood samples were obtained from a similar catheter inserted in the contralateral antecubital vein and kept patent by a slow infusion of 0.9% NaCl in water. The infusion was given for 180 minutes, and blood was obtained every 60 minutes during the first 2 hours and every 10 minutes during the last half hour for measurement of plasma glucose and insulin. Such a rate of insulin infusion is designed to achieve a physiological, postprandial-like insulinemia of \( \approx 300 \) pmol/L. The mean value of the 4 measurements made during the last half hour was used to calculate the steady-state plasma glucose (SSPG) and steady-state plasma insulin concentrations. In healthy humans, octreotide inhibits endogenous insulin secretion and the dose of insulin infused suppresses endogenous glucose production. Under these circumstances, the higher the SSPG, the more insulin resistant the individual.

During the next 5 days, crossover to the other diet occurred and the experimental protocol was repeated in a similar manner.

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and aldosterone concentrations and the increase in ANP. In addition, an increase in UNO\textsubscript{V} of borderline significance ($P=0.06$) was observed with the high-salt diet.

To determine the variable most related to the change in weight and natriuresis associated with the difference in salt intake, age- and gender-adjusted regression analysis was performed. Because the values of ANP, PRA, aldosterone, and UNO\textsubscript{V} either increased (ANP and UNO\textsubscript{V}) or decreased (PRA and aldosterone) during the high-salt diet, the difference ($\Delta$) between the values on the 2 diets was used in this analysis. On the other hand, SSPG and insulin were similar with both diets and the mean of the 2 values for these variables was used in this analysis. The results are shown in Table 2, and it is obvious that the higher the SSPG (the more insulin resistant), the greater the increase in weight and the lesser the increase in 24-hour urinary Na\textsuperscript{+} excretion when changing from a low- to a high-Na\textsuperscript{+} diet. A similar, but somewhat weaker, relationship was seen between plasma insulin concentration 2 hours after oral glucose and increases in weight and natriuresis in response to the high-salt diet. In contrast, the dietary-induced changes in weight and natriuresis were unrelated to the associated changes in UNO\textsubscript{V}, PRA, aldosterone, and ANP.

Although MAP did not increase in response to the high-salt diet in the group as a whole, there was substantial individual variability. Table 3 presents the results of regression analysis, which was conducted to assess the contribution of the experimental variables to the salt-induced change in MAP. These results show that the change in MAP in response to the high-salt diet was related directly to the increase in body weight and inversely to $\Delta$UNO\textsubscript{V}. The latter relationship was particularly strong, as shown in the Figure. To evaluate the potential impact of differences in insulin resistance on the relationship between $\Delta$MAP and $\Delta$UNO\textsubscript{V}, subjects were divided into 2 groups on the basis of the median SSPG value of the whole group (7.7 mmol/L). The results in the Figure demonstrate that the relationship between $\Delta$MAP and $\Delta$UNO\textsubscript{V} seemed unaffected by the insulin-resistant (SSPG $>$ 7.7 mmol/L) and insulin-sensitive (SSPG $<$ 7.7 mmol/L) groups. Multiple regression analysis with $\Delta$MAP (as dependent variable) and $\Delta$UNO\textsubscript{V} and $\Delta$weight (as independent variables) was highly significant ($R=0.84$, $R^2=0.70$, $P<0.0001$), with $\Delta$weight retaining borderline significance as an independent predictor of $\Delta$MAP ($P<0.06$). Thus, in this regression model, 70% of the variance in $\Delta$MAP could be explained by dietary Na\textsuperscript{+}-induced differences in UNO\textsubscript{V} and weight.

To evaluate further the effect of insulin resistance and/or hyperinsulinemia in the response to variations in salt intake, the study population was divided into the 8 most insulin-sensitive and 8 most insulin-resistant individuals, and values of the other variables at the end of the 2 diet periods were compared. The mean SSPG values of the 2 groups place them in the highest quartile (SSPG $>$ 10 mmol/L) and lowest quartile (SSPG $<$ 4 mmol/L) of the population at large, based on results of similar measurements made by our group in more than 500 nondiabetic individuals since 1989.

Table 4 shows that insulin-resistant individuals were different than insulin-sensitive subjects in that they had higher insulin levels and lower urinary Na\textsuperscript{+} excretion rates. Such differences were seen during both high salt intake, indicating a delay in achieving Na\textsuperscript{+} balance, and low salt intake, suggesting the ability of insulin-resistant individuals to adapt faster to a state requiring maximal Na\textsuperscript{+} (and water) preservation. Consistent with these results is the finding that insulin-resistant subjects had a greater increase in weight between the low- and high-salt diets (0.82±0.09 kg) as

### Table 2. Regression Analysis Between the Change ($\Delta$) in Weight and Natriuresis in Response to Two Diets and Potential Determinants of Changes

<table>
<thead>
<tr>
<th>Variable</th>
<th>$r_{\text{Weight}}$</th>
<th>$P$</th>
<th>$r_{\text{Natriuresis}}$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta$Weight</td>
<td>-0.64</td>
<td>0.01</td>
<td>-0.50</td>
<td>0.04</td>
</tr>
<tr>
<td>$\Delta$SSPG</td>
<td>0.54</td>
<td>0.03</td>
<td>0.00</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.47</td>
<td>0.07</td>
<td>-0.36</td>
<td>0.11</td>
</tr>
<tr>
<td>$\Delta$UNO\textsubscript{V}</td>
<td>0.00</td>
<td>NS</td>
<td>0.00</td>
<td>NS</td>
</tr>
<tr>
<td>$\Delta$PRA</td>
<td>0.06</td>
<td>NS</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>$\Delta$Aldosterone</td>
<td>0.25</td>
<td>NS</td>
<td>-0.12</td>
<td>NS</td>
</tr>
<tr>
<td>$\Delta$ANP</td>
<td>0.02</td>
<td>NS</td>
<td>0.03</td>
<td>NS</td>
</tr>
</tbody>
</table>

### Table 3. Regression Analysis Between $\Delta$MAP in Response to the High-Sodium Diet and Other Relevant Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta$Weight</td>
<td>0.51</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>$\Delta$SSPG</td>
<td>0.24</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.19</td>
<td>NS</td>
</tr>
<tr>
<td>$\Delta$UNO\textsubscript{V}</td>
<td>-0.77</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$\Delta$PRA</td>
<td>0.22</td>
<td>NS</td>
</tr>
<tr>
<td>$\Delta$Aldosterone</td>
<td>0.19</td>
<td>NS</td>
</tr>
<tr>
<td>$\Delta$ANP</td>
<td>0.23</td>
<td>NS</td>
</tr>
</tbody>
</table>
change in weight. Such an observation also indicates that the lower Na\(^+\) excretion observed in insulin-resistant individuals is not the result of differences in dietary compliance. As with Na\(^+\) retention, the change in weight seems to be independent of changes in ANP, PRA, aldosterone, or UNO\(_{V}\).

The results in Table 3 support the view that the greater the degree of volume expansion (weight gain), the higher the MAP response to the high-NaCl diet. Changes in ANP, PRA, and aldosterone do not appear to contribute to the change in blood pressure (Table 3). Moreover, the results in Table 3 and Figure 1 demonstrate that the change in blood pressure observed in response to an 8-fold increase in NaCl intake was inversely correlated with the increase in UNO\(_{V}\). Although this correlation was statistically highly significant, only a small number of subjects were studied, and even though they were selected on the basis of being normotensive and without impaired glucose tolerance and expected to be representative of the normal population, it is possible that the relationships we found could be altered when examined in a larger group of subjects. Also, many pitfalls exist in the use of UNO\(_{V}\) as an index of endogenous NO production. For instance, differences in exercise level and dietary nitrate and nitrite intake might make UNO\(_{V}\) uninterpretable. However, because the dietary intake (other than NaCl) and physical activity level of all our study subjects were kept under steady and controlled circumstances, we can assume that the intrasubject variability in UNO\(_{V}\) mainly reflected a Na\(^+\)–induced change in the rate of endogenous NO production rather than day-to-day differences in exogenous NO intake. Thus, it is likely that the subjects whose blood pressure increased during high NaCl intake were unable to increase endogenous NO production and that this effect might be, as shown in rats, causally related to the pressor response observed. However, it should be noted that in those studies, nonselective inhibition of NO synthase (NOS) was used. Results of recent experiments in laboratory animals with selective inhibitors of NOS have brought into question the hemodynamic significance of the increase in UNO\(_{V}\) observed after Na\(^+\) challenge and suggest that endothelial NOS activation might generate enough NO to elicit vasodilation and a measurable blood pressure response but not a measurable increase in UNO\(_{V}\). Thus, there is the possibility that the variation in UNO\(_{V}\), seen in our experimental setting, might not be of hemodynamic significance. Therefore, our results should be interpreted cautiously and our conclusions limited to offering preliminary observational evidence that a blunted generation of NO seems to occur in salt-sensitive humans, the physiological significance of which remains to be determined.

No significant correlation was found between insulin resistance and \(\Delta\)UNO\(_{V}\) or UNO\(_{V}\) at either level of Na\(^+\) intake. Nonetheless, the mean values of UNO\(_{V}\) were lower in the most insulin-resistant as compared with the most insulin-sensitive subjects (Table 4). Although these differences were of marginal significance (\(P=0.05\) and 0.06, respectively), this observation raises the possibility that impairment of insulin-mediated skeletal muscle glucose uptake is associated not only with salt retention after NaCl loading but also with reduced generation of NO at either level of salt intake.
As a final finding of our study, it is clear that neither insulin-mediated glucose disposal nor the plasma insulin response changed in response to the 8-fold variation in Na⁺ intake. Perhaps the results most divergent from ours in this respect were those of Donovan et al., who indicated that insulin-mediated glucose uptake was 17% lower after 5 days of a high-Na diet as compared with after 5 days of a low-Na diet, suggesting that the high-Na diet modestly worsened insulin resistance. The significance of such an increment in insulin resistance in a small study (N = 8) is probably marginal. Furthermore, the difference in methodology (euglycemic clamp versus insulin suppression test) may also partly explain the disparate findings. Whether sodium intake modestly changes insulin sensitivity does not affect the other implications of our study.

In conclusion, the results of this study demonstrate that a pressor response to increased NaCl intake occurs in individuals who either do not increase or decrease urinary nitrate excretion. Such a pressor response is enhanced if more sodium and water are retained and greater volume expansion occurs, a likely consequence of insulin resistance and hyperinsulinaemia. Additional research is necessary to identify if and to what extent these observational findings are directly related to the genesis of salt-sensitive hypertension.

Acknowledgment

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References

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