Dietary Sodium Restriction Impairs Endothelial Effect of Insulin

Carmine Vecchione, Carmine Morisco, Luigi Fratta, Luigi Argenziano, Bruno Trimarco, Giuseppe Lembo

Abstract—Hyperinsulinemia and high salt intake represent two independent cardiovascular risk factors. However, it is still unknown whether the change in dietary salt intake may affect the ability of insulin to stimulate whole-body glucose uptake and to modulate endothelial function. Regarding this latter issue, we have recently demonstrated that insulin enhances endothelial-mediated $\alpha_2$-adrenergic vasorelaxation. In overnight-fasted, freely moving Wistar-Kyoto rats (10 to 12 weeks old), we assessed whole-body glucose uptake (in milligrams per kilogram per minute) during a euglycemic-hyperinsulinemic clamp (insulin infusion rate, 3 mU·kg$^{-1}$·min$^{-1}$) after 3 weeks of normal (NSD, 2% NaCl), high (HSD, 6% NaCl), and low (LSD, 0.6% NaCl) sodium diet. Three days after the clamp study, rats were killed to assess $\alpha_2$-adrenergic vasorelaxation evoked by UK 14,304 (10$^{-9}$ to 10$^{-6}$ mol/L) in aortic rings in control conditions and after insulin exposure (100 $\mu$U/mL). Different sodium intakes did not modify the mean blood pressure or the insulin-stimulated whole-body glucose uptake (NSD: 14±1.2, n=16; HSD: 15.4±1.7, n=14; LSD: 14.8±0.8, n=14; NS). In contrast, we confirmed the ability of insulin to enhance $\alpha_2$-adrenergic vasorelaxation during NSD and HSD (Δ% of maximal relaxation, NSD: from 32±3% to 58±3.4%, n=9, P<0.01; HSD: from 33±3.8% to 59±3.5%, n=8, $P<0.01$), but this effect was impaired during LSD (Δ% maximal relaxation, from 36±1.5% to 36±3.4%, n=8, NS). In conclusion, our data demonstrate that in Wistar-Kyoto rats, changes in dietary salt intake do not modify the insulin-stimulated whole-body glucose uptake. In contrast, LSD impairs the insulin potentiation of $\alpha_2$-adrenergic vasorelaxation, thus suggesting that dietary salt restriction provokes an impairment of insulin effect on endothelial function. (Hypertension. 1998;31:1261-1265.)

Key Words: sodium chloride ■ vasorelaxation ■ glucose uptake ■ blood pressure ■ aortic rings ■ glucose clamp technique

Several epidemiological studies have suggested that distinct factors related to dietary habits, such as salt intake and insulin, may have a major impact on blood pressure homeostasis.1-6 Actually, both the amount of sodium contained in a dietary regimen and the physiological rise of the pancreatic hormone in response to food intake are able to determine profound neuroendocrine adjustments that can influence the cardiovascular regulation.7-9 So far, the relationship between salt intake and insulin action has been the object of several studies that have tried to determine the effect of insulin on sodium metabolism. In particular, it has been revealed that insulin affects sodium metabolism by acting on sodium reabsorption at the renal tubular level.10 In contrast, it is still unclear whether changes in dietary salt intake influence insulin action. In this regard, it is known that changes in sodium intake may produce important consequences on arterial blood pressure,11,12 and this in turn is inversely related to insulin sensitivity.13 To verify whether an interaction between sodium intake and insulin sensitivity does exist independently by sodium influence on blood pressure, we examined the insulin effect in WKY under different sodium diet regimens. This normotensive rat strain is resistant to blood pressure change induced by modification of sodium intake. We focused our attention both on insulin-mediated glucose uptake and on the effect of the hormone on vascular tone. On this latter issue, rat aortic rings represent a reliable model to explore the vascular effects of insulin14-17; in particular, in this system we have recently demonstrated that the hormone is able to selectively sensitize the endothelial $\alpha_2$-adrenergic–evoked vasorelaxation.18

We therefore investigated the effects of three different levels of sodium diet regimen on insulin-stimulated whole-body glucose uptake and on insulin modulation of endothelial $\alpha_2$-adrenergic vasorelaxation, which is a target of insulin vascular action.

Methods

Experimental Animals

Most studies were conducted in 69 male WKY (Charles River Laboratory) aged 10 to 12 weeks. The animals were housed two per
Sodium Intake and Insulin Sensitivity

Studies on Aortic Rings

Three days before the euglycemic-hyperinsulinemic clamp study, all rats were anesthetized with an intraperitoneal injection of a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), and polyethylene catheters (PE-50) were inserted into a femoral artery in a femoral vein. Both catheters were filled with heparinized saline solution (100 μL/mL) and exteriorized subcutaneously at the interscapular area. After the surgery, the animals were housed in single cages and were allowed to recover.

Blood Pressure Measurement

Direct intrafemoral arterial pressure was measured in conscious freely moving rats after an overnight fast. At 8 AM the arterial catheter was connected to a pressure transducer (Statham P23db) through an extension of polyethylene (PE-50) tubing. After a resting period of at least 30 minutes, arterial blood pressure and heart rate were measured in each animal over a 30-minute period and recorded on a Gould polygraph at a speed of 100 mm/s. Heart rate was obtained from the arterial pressure pulse.

Euglycemic-Hyperinsulinemic Clamp Study

On the same day of blood pressure measurement, insulin-mediated whole-body glucose uptake was determined in freely moving rats with the euglycemic-hyperinsulinemic clamp technique. Insulin dissolved in rat plasma was administered through the femoral vein with an infusion pump at the dose of 3 mU·min⁻¹·kg⁻¹. Arterial blood glucose levels were measured twice in 15 minutes before the clamp study at 10-minute intervals during the insulin infusion. To maintain basal glucose levels, a glucose solution (33%) was infused at variable rates in the same femoral vein, according to the plasma glucose concentration. Plasma insulin levels were measured at the beginning and after 120 minutes of insulin infusion.

Studies on Aortic Rings

On the day of experiments on vascular function, the rats were weighed and then decapitated. The thoracic aorta was dissected out from each rat and placed in cold Krebs-Henseleit bicarbonate buffer solution with the following composition (mmol/L): NaCl 118.3, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, and glucose 5.6. The aorta was cleaned of the adhering perivascular tissue and cut into rings 3 mm long. Aortic rings were suspended in isolated tissue baths filled with 20 mL Krebs’ solution continuously bubbled with a mixture of 5% CO₂/95% O₂ (pH 7.37 to 7.42) at 37°C. One end of the aortic ring was connected to a tissue holder and the other to an isometric force transducer. The signal was passed to a Gould pressure processor and then acquired in a computerized system with Gould’s DASA (Data Acquisition and Signal Analysis). The analysis of the generated curves was performed with View II software (Gould Instruments), and the sensitivity of the system was 5±1 mg of tension generated. The rings were equilibrated for 90 minutes in the unstretched condition, and the buffer was replaced every 20 minutes. The length of the smooth muscle was increased stepwise in the equilibration period to adjust passive wall tension to 2 g. This tension was found optimal for contractions of aorta from WKY by testing the contractions to norepinephrine (10⁻⁵ mol/L). Once the basal tension was established, the length of the rings was not altered thereafter.

The following drugs were used: acetylcholine, phenylephrine (Sigma Chemical Co), UK 14,304 (Research Biochemicals International), and sodium nitroprusside (Malesci). Drugs were prepared daily in distilled water. Concentrations of the drugs are reported as the final molar concentration in the organ bath.

To verify whether changes in sodium intake may affect endothelial or smooth muscle relaxations per se, dose-response curves to acetylcholine (10⁻⁶ to 5⋅10⁻⁵ mol/L), UK 14,304 (10⁻⁸ to 5⋅10⁻⁶ mol/L [a selective α₁-agonist]), and sodium nitroprusside (10⁻⁹ to 10⁻⁷ mol/L) were performed in vessels precontracted with phenyl-ephrine (10⁻⁶ mol/L). Because relaxations induced by UK 14,304 have been demonstrated to be the targets of vascular insulin action, dose-response curves to UK 14,304 were repeated after 30 minutes of exposure to human regular insulin (100 μU/mL). This dose was chosen to reproduce levels of the hormone commonly observed in pathophysiological conditions associated with insulin resistance. Moreover, previous studies have revealed that higher levels of insulin do not show any difference from that used in the present study.

Plasma Glucose, Plasma Insulin, and Serum and Urinary Sodium

Sodium concentration in urine was measured by an autoanalyzer (Beckman System 2A). Plasma glucose concentration was determined by the glucose oxidase method (Beckman Glucose Analyzer). Plasma insulin was measured by radioimmunoassay (Instar).

Statistical Analysis

Data are expressed as mean±SE. Vasorelaxation evoked by UK 14,304, acetylcholine, and sodium nitroprusside is expressed as percent inhibition of the contraction evoked by phenylephrine. Concentration of vasorelaxant agonists producing half maximal inhibition of the phenylephrine contractile effect (ED₅₀) and maximum relaxation effect were estimated by nonlinear regression analysis from log concentration-response curves and expressed as ED₅₀ and percent maximal relaxation (GraphPad Prism).

Statistical evaluation of the data was carried out by two-way ANOVA with Bonferroni’s t test for multiple comparisons and Student’s t test. Differences were considered to be statistically significant at P<0.05.

Results

Urinary Na⁺ and K⁺ Excretion, Body Weight, Blood Pressure, Plasma Glucose, and Plasma Insulin

As expected, the different sodium regimens were able to modify urinary sodium excretion. In particular, during HSD the sodium excretion was eightfold greater than that measured during LSD (20 935±2330 versus 2648±352 μmol/24 h, P<0.01) in keeping with the change in dietary sodium calculated by food intake. The sodium excretion of rats receiving LSD was 3890 ±489 μmol/24 h, significantly different from excretion observed during HSD and LSD, but the magnitude of these changes did not strictly correspond to that expected, likely reflecting a diverse appetite for food at this level of salt intake. In contrast, the urinary potassium excretion was not significantly affected by change in sodium intake.
diet regimen (NSD, 3117 ± 414 μmol/24 h; HSD, 3401 ± 253 μmol/24 h; LSD, 2907 ± 181 μmol/24 h; NS).

After 3 weeks, body weight was 325 ± 5 g in the group treated with NSD and was not significantly different from that measured during both HSD (334 ± 2 g) and LSD (314 ± 9 g), respectively. However, the body weight of the HSD group was significantly higher (P < 0.01) than that observed in the LSD group. Furthermore, arterial blood pressure (mean blood pressure [mm Hg]: NSD, 127 ± 7; HSD, 124 ± 1; LSD, 129 ± 4; NS), glucose ([mmol/L] NSD, 5.38 ± 0.2; HSD, 5.33 ± 0.1; LSD, 5.45 ± 0.1; NS), and insulin plasma levels ([pmol/L] NSD, 214 ± 14; HSD, 193 ± 14; LSD, 208 ± 22; NS) were similar in the three study groups.

**Euglycemic-Hyperinsulinemic Clamp Study**

As shown in Table 1, plasma insulin levels increased in the high physiological range during the clamp study, while glucose levels were maintained adequately at their basal levels on whole-body glucose uptake. In contrast, dietary salt restriction abolishes the insulin sensitization of α2-adrenergic vasorelaxation in rats receiving an NSD, and this sensitizing effect of the hormone was comparable in magnitude in the group that received HSD. In contrast, insulin did not modify the vasorelaxation evoked by UK 14,304 in the LSD group (Figure 2 and Table 2).

**Discussion**

In this study, we explored the effect of change in sodium intake on two important targets of insulin action: the stimulation of whole-body glucose uptake and the modulation of vascular endothelial function. Our results clearly demonstrate that in normotensive WKY, changes in sodium diet regimens are not able to influence the effect of physiological insulin levels on whole-body glucose uptake. In contrast, dietary salt restriction abolishes the insulin sensitization of α2-adrenergic vasorelaxation exhibited at normal and high dietary sodium intake.

The mechanism by which low salt diet impairs the insulin effect on vascular function has yet to be clarified. We have recently reported that insulin affects heterogeneously endothelium-dependent relaxations. Specifically, the hormone amplifies the α2-adrenergic pathway, while it does not interfere with other endothelial-mediated responses. A potential explanation of the effect of dietary salt restriction on insulin sensitization of α2-adrenergic vasorelaxation may be a general impairment of endothelial function. On this issue, our

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**TABLE 1. Effect of Euglycemic-Hyperinsulinemic Clamp on Glucose Disposal Rate During NSD, HSD, and LSD**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NSD</th>
<th>HSD</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose, mg/dL</td>
<td>98 ± 4</td>
<td>93 ± 2</td>
<td>97 ± 2</td>
</tr>
<tr>
<td>Plasma insulin, μU/mL</td>
<td>81 ± 3</td>
<td>89 ± 9</td>
<td>83 ± 4</td>
</tr>
<tr>
<td>Glucose utilization rate, mg/kg per min</td>
<td>14.0 ± 1.2</td>
<td>15.4 ± 1.7</td>
<td>14.8 ± 0.8</td>
</tr>
</tbody>
</table>

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![Figure 1. Dose-response curves in aortic rings from WKY to acetylcholine and sodium nitroprusside during normal (□, n=9), high (▲, n=8), and low (◇, n=8) salt diets.](image-url)
results clearly demonstrate that changes in dietary sodium regimens are unable to affect the whole endothelial vasorelaxation. This suggests that low sodium intake selectively influences insulin action on endothelial function. The hypothesis that dietary salt restriction may modify insulin receptor signal does not seem appropriate because, in our work, low salt diet does not interfere with insulin-mediated skeletal muscle glucose uptake, which has an insulin receptor signal transduction pathway similar to the endothelial ones. However, the intracellular molecular events generated by insulin receptor activation, which allow the insulin sensitization of $\alpha_2$-adrenergic endothelial vasorelaxation, may have distinct features compared with the molecular transduction for the insulin action on the intermediary metabolism. Thus, low salt intake may specifically interact with insulin postreceptor molecular events, which account for insulin vascular action. In this regard, our data do not allow any definitive conclusion, but a recent study demonstrating in young normotensive subjects that low sodium intake reduces the vasorelaxant effect of insulin may lend further support to this hypothesis.

Our conclusions on insulin-stimulated glucose uptake during different sodium diet regimens are not limited by a recent report by Sechi et al., which demonstrated in the same rat strain that an increase in dietary salt intake impairs insulin-stimulated glucose utilization. Their study is not comparable to ours because they used a more pronounced high salt diet and performed the clamp technique in anesthetized rats. On the other hand, there is also evidence in humans indicating that dietary NaCl restriction evokes a hyperinsulinemic response, whereas dietary salt load decreases plasma insulin levels, suggesting that dietary salt intake may affect insulin sensitivity. It is important to note that in these latter studies, the measure of insulin sensitivity was mainly derived from the plasma levels of insulin, which is only an indirect index of the insulin sensitivity, whereas in our study, the insulin sensitivity was measured by hyperinsulinemic-euglycemic clamp technique.

A potential limitation of our study is that we explored the whole-body glucose uptake under the stimulation of a single insulin dose, which was titrated to reach levels of hormone close to those observed postprandially. Therefore, we cannot exclude the possibility that pharmacological insulin levels mediating the maximum glucose disposal might be differently regulated by sodium intake. However, it has to be considered that spontaneously hypertensive rats show their insulin resistance when physiological levels of the pancreatic hormone are tested, whereas no difference in insulin-stimulated whole-body glucose uptake is evident at high pharmacological levels of insulin. A further potential limitation is that our study population was restricted to male rats, and therefore our conclusions cannot be broadened to females. On this issue, it has been reported that the effects of insulin on vascular function are more pronounced in females than in males, suggesting that sex hormones can in some manner affect insulin vascular action. Furthermore, estrogens positively influence endothelial function, which is also the target

### Table 2: Effect of Insulin on Vasorelaxant Response to UK 14,304 During NSD, HSD, and LSD

<table>
<thead>
<tr>
<th></th>
<th>NSD</th>
<th>HSD</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK 14,304</td>
<td>ED$_{50}$, mol/L</td>
<td>$\Delta$Max, %</td>
<td>ED$_{50}$, mol/L</td>
</tr>
<tr>
<td>Control</td>
<td>$3.68 \pm 1 \times 10^{-7}$</td>
<td>$32 \pm 3$</td>
<td>$5.94 \pm 1 \times 10^{-7}$</td>
</tr>
<tr>
<td>Insulin</td>
<td>$1.18 \pm 3 \times 10^{-7}$</td>
<td>$58 \pm 3$</td>
<td>$1.79 \pm 3 \times 10^{-7}$</td>
</tr>
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*P<0.05 when compared with control conditions.
of the vascular effects of insulin. For these reasons, to examine the interaction between sodium intake and insulin-mediated effects, we decided to study only male rats to make an evaluation without confounding factors such as sex hormones.

It is known that dietary sodium restriction causes a reduction of cardiac output and a simultaneous increase of vascular resistance, and the latter represents the mechanism by which blood pressure is adequately maintained. Several neuroendocrine factors sustain the increase of vascular resistance during low salt diet regimens, and we can speculate that in this latter condition, the lack of insulin vasorelaxant effect in this condition may contribute to the increase in vascular tone. In other words, the impairment of vascular effects of insulin may represent one of the physiological events that are involved during dietary sodium restriction and play a role in blood pressure homeostasis.

It is noteworthy to remember that we recently reported\(^1\) that in a genetic model of hypertension such as spontaneously hypertensive rats, the impairment of insulin vascular action is present also during the NSD regimen. This abnormal recruitment of a vascular mechanism present in normotensives only during sodium restriction may play a contributory role in the development of hypertension.

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