Chronic Salt Overload Increases Blood Pressure and Improves Glucose Metabolism Without Changing Insulin Sensitivity

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The effect of sodium chloride salt restriction and overload on insulin sensitivity is still an open question. Some authors have shown that NaCl salt restriction increases insulin resistance, whereas others have reported the opposite. In the present study, the objective was to get some more insight on this issue by studying the influence of dietary salt content on glucose uptake in isolated adipocytes.

Male Wistar rats were fed from weaning either low (0.15%) or high (7.94%) salt diets. On the 12th week of age, weight and tail-cuff blood pressure were measured, followed 10 days later by an intravenous glucose tolerance test with concomitant insulin determinations. One week later, the rats were killed by decapitation and epididymal adipocytes were obtained for glucose metabolism evaluation.

No weight differences were observed between both groups of animals. Blood pressure was significantly higher ($P < .001$) on salt overloaded rats ($146 \pm 11$ mm Hg) than on salt restricted ones ($115 \pm 5$ mm Hg). Dietary salt content did not influence the area under the curve of plasma glucose. Area under the curve of insulin levels was lower ($P < .023$) on the high than on the low salt diet. A higher ($P < .001$) glucose uptake in the absence and in the presence of insulin was observed in adipocytes from rats on the high salt diet. The median effective concentration ($EC_{50}$) from the dose-response curves of glucose uptake was the same on both groups of animals. Glucose oxidation and incorporation into lipids was also enhanced by salt overload. High salt increased insulin receptor density ($P < .001$).

In conclusion, salt overload increased blood pressure, and high and low salt dietary content did not influence insulin sensitivity based on the unchanged $EC_{50}$ from the in vitro studies. However, insulin-independent glucose uptake, oxidation, and incorporation into lipids were enhanced in adipocytes from rats on the high salt diet. The lower levels of insulin during the glucose tolerance test on salt-loaded animals may be a consequence of the higher insulin-independent glucose uptake in that group. Am J Hypertens 1997;10:720–727 © 1997 American Journal of Hypertension, Ltd.

KEY WORDS: Salt overload, insulin resistance, blood pressure, Wistar rat.
An increase in dietary salt content is linked with higher blood pressure levels. This affirmative is based on a number of studies that have evaluated normotensive and hypertensive humans and laboratory animals, large populations, and small samples. Based on that knowledge, salt restriction has been advised by most of the recommendations for hypertension treatment. However, whether salt restriction is linked to a lower cardiovascular risk is still an open question. The few papers that studied that aspect made only an assumption that salt restriction decreases cardiovascular risk, based on the knowledge that low salt ingestion decreases blood pressure and a direct correlation observed between blood pressure levels and cardiovascular events. Only one longitudinal study addressed the influence of urinary sodium excretion on the incidence of cardiovascular events in a retrospective manner. An association between low sodium excretion and increased cardiovascular morbidity and mortality was observed. Further studies are needed for confirmation of the results obtained.

Changes in glucose metabolism associated with low salt diets were reported in response to low salt diets in normotensive volunteers, in offspring of hypertensives, and in hypertensive patients. During salt restriction, increased C-peptide and insulin levels along with unchanged blood glucose were obtained, suggesting resistance of target tissues to the insulin mediated glucose uptake. However, when euglycemic-hyperinsulinemic clamp was used to measure tissue insulin sensitivity, different results were obtained in several studies. In one report, M values (insulin dependent tissue glucose uptake) did not change after 7 days of low or high salt diet in patients with essential hypertension. In another study, lower M values were obtained in normotensives after 3 days, with overload, influences insulin sensitivity measured by two different methods in a normotensive strain of rats. One of the employed methods is the study of epididymal adipocytes, which has not previously been used in that model.

**METHODS**

In the present study, all rats received care in accordance with the guidelines for animal experimentation at our institution. All animals were maintained in an ambient environment with constant temperature (25°C) and a 12-h light/dark cycle (light on at 6 AM).

Male Wistar rats were fed, from weaning, either a low (LSD 0.15% NaCl; Harlan Teklad, Madison, WI; n = 6) or a high salt diet (HSD 7.94% NaCl, n = 6). Tail-cuff blood pressure (BP) and weight measurements, intravenous glucose tolerance test (i.vGTT) with insulin determinations, and in vitro adipocyte studies were performed on 12-week-old rats. In addition, animals were individually housed in metabolic cages for 24-h urine collection for volume, sodium, potassium, and creatinine determinations. At the end of the 24-h period, blood was collected by tail snipping, for creatinine determination. Blood pressure measurements were made in triplicate and the mean was used as the BP of that moment. Intravenous GTT was performed under anesthesia (pentobarbital 40 mg/kg intraperitoneally), after 16 h of fasting, 3 to 7 days after the metabolic cage studies. A catheter was inserted in the right jugular vein, and 0.5 mL of blood was withdrawn immediately before and 5, 15, 30, and 60 min after an intravenous glucose load (1.0 mL/kg of a 2.78 mol/L [50%] solution) for glucose and insulin measurements. Volume was replaced with saline after each obtained blood sample. After the end of the i.vGTT, the jugular catheter was removed and all the animals were returned to their cages. After 1 week, the rats were killed by decapitation. Trunk blood was collected in cold tubes containing EDTA for plasma renin activity (PRA) measurement and epididymal fat was removed for in vitro adipocyte studies. Kidney and left ventricular mass were measured. Blood samples for glucose, insulin, and PRA determinations were centrifuged at 4°C immediately after collection, and plasma was stored at −20°C until assay.

Adipocytes were isolated from the periepididymal fat according to the method described by Rodbell. The fat tissue was digested by collagenase. Once the tissue digestion was finished, isolated cells were washed and resuspended until a 5% cell concentration (lipocrit) was reached. Under these conditions, 2 to 3 × 10⁶ cells/mL are usually obtained. Cell volume and number were measured according to Di Girolamo et al. The following tests were applied to the isolated adipocytes: dose-response curve of insulin stimulated 2-deoxy-d-glucose (2DG) uptake (2DGUp), insulin receptor density, glucose incorporation into lipids, and glucose oxidation.
For the 2DGUp studies, 450 μL aliquots of the cell suspension were transferred to polystyrene test tubes containing 25 μL of porcine insulin solution (0, 1, 2, 5, 10, 25, 50, 100, 250, 500, 1000, and 5000 ng/mL). The mixture was incubated for 30 min at 37°C and afterward 25 μL of a [3H-2DG] solution (0.1 mmol/L final concentration, 0.15 μCi/tube in 20 mmol/L Hank’s/HEPES and 1% bovine serum albumin (HBB) were added. The tracer cell uptake was interrupted after 3 min by transferring 200 μL aliquots to microtubes containing 100 μL of silicone oil (dimethylpolisiloxane, density = 0.93), and by centrifuging at 11,000 rpm for 6 sec. The reaction was considered interrupted when the centrifuge was turned on. The cell pellet on top of the oil layer was collected and dipped into scintillation vials containing a cocktail for aqueous samples. Radioactivity taken up by the cells was measured in disintegrations/minute in a β-counter (Beckman Instruments Inc., model LC6000IC, Fullerton, CA). Nonspecific radioactive trapping was estimated in a separate tube in which L-[1-14C]-glucose (0.1 μCi/tube) was added instead of 3H-2DG. The radioactivity measured in the latter tube was subtracted from the 2DGUp tubes. The results were expressed per unit of cell surface area (picomoles/3 minutes/square centimeter).

Cell sensitivity to insulin was evaluated by the median effective concentration (EC50) of the dose-response curve. For binding studies, adipose cell suspension was concentrated to 10% lipocrit in HBB, pH 7.8 at 16°C. Aliquots (450 μL) were placed into polystyrene test tubes containing 25 μL of [125I]-insulin (35 pmol/mL [0.2 ng/mL] 20,000 cpm) and 25 μL of “cold” insulin in order to get a final hormone concentration of 0.035, 17.3, and 865.4 pmol/L (0.2, 100, and 5000 ng/mL). The mixture was incubated for 3 h at 16°C and insulin binding to the cells was interrupted in a way similar of that used in the 2DGUp test. Radioactive trapping was measured with a gamma counter (Abbott Laboratories, AutoLogic model, North Chicago, IL). Insulin receptor number was obtained by calculating the values of bound insulin (B) and bound to free insulin ratio (B/F) and using the Scatchard analysis of data (B/F v B plot).

For glucose oxidation, adipocytes were washed and resuspended to 5% lipocrit (as described above) in the following buffer: 131.2 mmol/L NaCl, 4.7 mmol/L KCl, 2.1 mmol/L CaCl2, 1.5 mmol/L MgSO4, 24.6 mmol/L NaHCO3, 1.1 mmol/L KH2PO4, 2.0 mmol/L glucose, and 0.1% BSA, pH 7.4 at 37°C, and gassed for 15 min with 95% O2, 5% CO2. d-[14C]-Glucose oxidation to CO2 was assayed by using a modification of a method described by Rodbell. Aliquots (900 μL) of 5% lipocrit cell suspension were transferred to 20 mL plastic scintillation vials already containing 100 μL of buffer with and without insulin (for a final concentra-

For glucose incorporation into lipids, in the same plastic scintillation vials used in the glucose oxidation assay, after collecting the 14CO2 the mixture was treated with 5 mL of Dole’s reagent (isopropanol: n-heptane: H2SO4 at 4.0:1.0:0.1 vol:vol:vol) for lipid extraction, as described previously.

For biochemical determinations, creatinine was measured by standard laboratory procedures. Plasma and urinary sodium and potassium were assayed by flame photometry. Plasma renin activity was measured by radioimmunoassay, using a commercial available kit (INCSTAR Corporation, Stillwater, MN). Plasma insulin determinations were performed in 100 μL samples by radioimmunoassay. Glucose was analyzed using the glucose oxidase method.

For calculations and statistical analysis, data is given as mean and standard deviation. Area under the curve (AUC) of plasma glucose and insulin was calculated using the GraphPad Inplot 4.03 software (Graph Pad Software, Inc., San Diego, CA). An unpaired Student’s t test was used to detect differences between group means on salt overload and restriction. Glucose and insulin levels during ivGTT were analyzed by two-way analysis of variance (ANOVA) followed by the Bonferroni multiple comparison test. The null hypothesis was rejected when the α error < .05.

RESULTS
Tail-cuff blood pressure, weight, creatinine clearance, electrolyte excretion, PRA, kidney mass (KM), and left ventricular mass (LVM) are shown in Table 1. Blood pressure was higher (P < .001) on HSD (146 ± 11 mm Hg) than on LSD (115 ± 5 mm Hg). Weight was not significantly different (P = .143) between LSD (383 ± 25 g) and HSD (362 ± 20 g). Kidney mass was higher (P < .001) on HSD, and no differences (P = .23) on LVM due to dietary salt content were detected. A higher creatinine clearance was observed on salt overload (P < .002). Urinary sodium excretion confirmed the salt ingestion of each group. Urinary potassium excretion was higher (P = .036) on HSD. PRA on LSD
(4.2 ± 1.1 ng AI/mL/h) was higher (P < .001) than on HSD (0.40 ± 0.3 ng AI/mL/h).

Plasma glucose levels during the ivGTT were not different (P > .05) between HSD and LSD, and the concomitant insulin concentrations were higher (P < .001) in salt restricted than in salt loaded rats (Figure 1).

No differences (P = .941) were observed between the area under the curve (AUC) of blood glucose on HSD (577.72 ± 84.83 mmol/L/min [10,399 ± 1,527 mg/dL/min]) and LSD (574.56 ± 38.44 mmol/L/min [10,342 ± 692 mg/mL/min]). The AUC of plasma insulin was lower (P = .023) on HSD (12.5 ± 3.5 μmol/L/min [72 ± 20 ng/mL/min]) than on LSD (18.7 ± 4.0 nmol/L/min [108 ± 23 ng/mL/min]). A smaller proportion between insulin and glucose AUC (P = .037) was obtained on HSD (0.17 ± 0.06) than on LSD (0.26 ± 0.05).

The results from the in vitro studies on isolated adipocytes are shown in Table 2. Adipocyte volume was not significantly different (P = .615) between the low and the high salt groups. All tests performed on adipocytes from rats on HSD disclosed a higher glucose metabolic rate than on cells obtained from the group on LSD. Basal (in absence of insulin) and maximal 2DGU/p were higher (P < .001) on HSD than on LSD. A higher basal and maximal glucose oxidation and incorporation into lipids was observed in adipocytes from the group on HSD. Also the receptor density (P < .001) was significantly higher in adipocytes from rats on HSD. Despite an increased glucose metabolic rate in adipocytes from HSD rats, the EC₅₀ (insulin concentration for half of the maximal uptake) was the same in cells from animals on both diets (Figure 2). Taking together all the results from the in vitro studies, differences in glucose metabolism occurred without changes in insulin sensitivity.

DISCUSSION

In the present study, blood pressure was higher in 12-week-old rats submitted to a dietary sodium chloride load started immediately after weaning, as compared with rats submitted to sodium chloride restriction. An increase of arterial pressure in normotensive strains of rats on high salt intake has been known for some decades, but not all studies have reported the same results. To our knowledge, there is no clinical study reporting the use of a diet with 7.94% NaCl content. Therefore, the increased blood pressure observed in the group of normotensive rats submitted to sodium load may not be relevant for human hypertension. Kidney mass, but not left ventricular mass, was higher in the rats on the high salt diet. The higher glomerular filtration rate measured in salt overloaded rats is in accordance with other studies. Several studies have shown a correlation between kidney weight

### TABLE 1. TAIL-CUFF BLOOD PRESSURE (BP), WEIGHT, CREATININE CLEARANCE (Cler), SODIUM (UNaV) AND POTASSIUM (UKV) EXCRETION, PLASMA RENIN ACTIVITY (PRA), KIDNEY (KM) AND LEFT VENTRICULAR (LVM) MASS IN 12-WEEK-OLD WISTAR RATS SUBMITTED, FROM WEANING, TO A LOW (LSD) OR TO A HIGH (HSD) SALT DIET

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<th>LSD</th>
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<tr>
<td>BP (mm Hg)</td>
<td>115 ± 5 (6)*</td>
<td>146 ± 11 (6)</td>
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<tr>
<td>Weight (g)</td>
<td>383 ± 25 (6)</td>
<td>362 ± 20 (6)</td>
</tr>
<tr>
<td>Cler (mL/min)</td>
<td>1.23 ± 0.29 (6)†</td>
<td>2.18 ± 0.50 (6)</td>
</tr>
<tr>
<td>UNaV (mmol/day)</td>
<td>0.32 ± 0.12 (6)*</td>
<td>11.57 ± 2.86 (6)</td>
</tr>
<tr>
<td>UKV (mmol/day)</td>
<td>0.75 ± 0.29 (6)**</td>
<td>1.08 ± 0.16 (6)</td>
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<tr>
<td>PRA</td>
<td>4.2 ± 1.1 (8)*</td>
<td>0.40 ± 0.3 (5)</td>
</tr>
<tr>
<td>KM</td>
<td>3.1 ± 0.2 (6)*</td>
<td>4.4 ± 0.4 (6)</td>
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<tr>
<td>LVM</td>
<td>2.2 ± 0.1 (6)</td>
<td>2.3 ± 0.2 (6)</td>
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Values are mean ± SD (number of rats). *P < .001 v LSD; †P < .002 v LSD; ** P = .036 v LSD.
TABLE 2. ADIPOCYTE VOLUME, BASAL AND MAXIMAL 2-DEOXY-D-GLUCOSE UPTAKE (2DGUp), 
EC50 (INSULIN FOR HALF OF THE MAXIMAL 
2DGUp), AND RECEPTOR DENSITY (R) IN 
ADIPOCYTES OF RATS SUBMITTED FROM 
WEANING TO A LOW (LSD) OR A HIGH 
(HSD) SALT DIET

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<th>LSD</th>
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<tr>
<td>Adipocyte volume (μm²)</td>
<td>243 ± 33 (6)</td>
<td>233 ± 35 (6)</td>
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<tr>
<td>Basal 2DGUp (pmol/3 min⁻¹/cm⁻²)</td>
<td>2.1 ± 0.6 (6)</td>
<td>5.1 ± 1.0 (6)**</td>
</tr>
<tr>
<td>Maximal 2DGUp (pmol/3 min⁻¹/cm⁻²)</td>
<td>6.1 ± 1.7 (6)</td>
<td>12.3 ± 1.1 (6)**</td>
</tr>
<tr>
<td>Basal glucose oxidation (nmol/10⁻⁵ cells/h⁻¹)</td>
<td>3.1 ± 1.1 (6)</td>
<td>10.9 ± 2.5 (6)**</td>
</tr>
<tr>
<td>Maximal glucose oxidation (nmol/10⁻⁵ cells/h⁻¹)</td>
<td>4.7 ± 1.8 (6)</td>
<td>14.9 ± 3.2 (6)**</td>
</tr>
<tr>
<td>Basal glucose incorporation into lipids (nmol/10⁻⁵ cells/h⁻¹)</td>
<td>7.8 ± 2.3 (6)</td>
<td>14.3 ± 2.7 (6)**</td>
</tr>
<tr>
<td>Maximal glucose incorporation into lipids (nmol/10⁻⁵ cells/h⁻¹)</td>
<td>10.5 ± 4.1 (6)</td>
<td>20.7 ± 4.1 (6)†</td>
</tr>
<tr>
<td>EC50 (μmol/L)</td>
<td>0.97 ± 0.28 (6)</td>
<td>0.97 ± 0.42 (6)</td>
</tr>
<tr>
<td>R (number/nm²)</td>
<td>3.5 ± 1.8 (6)</td>
<td>8.1 ± 2.4 (6)**</td>
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Values are mean ± SD (number of rats). *P < .05, †P < .002, **P < .001 v LSD.

and function. This correlation was observed in the early neonatal period in rats³⁸,³⁹ and in association with protein-rich diets.⁴⁰

Plasma insulin levels were lower during the ivGTT performed on the animals receiving high salt intake, whereas blood glucose concentrations were the same on both diets. Plasma insulin levels are influenced by insulin clearance, by insulin synthesis, or by both.

Meland et al have reported a lower insulin production during salt overload in hypertensive patients.⁴¹ A similar mechanism could be involved in the present study. In addition, the possible inhibition of insulin secretion by high salt may be due to the increased insulin-independent glucose uptake obtained in the in vitro determinations. Another explanation comes from the knowledge that angiotensin II decreases blood flow to the liver,⁴² the main organ in which insulin is cleared from circulation.⁴³ In the present study, the activity of the renin-angiotensin system was lower in the high salt rats, which could be the mechanism responsible for increased insulin clearance and, therefore, lower insulin blood levels. Another evidence in favor of that mechanism is the lowering effect of converting enzyme inhibitor on plasma insulin observed by Egan et al in hypertensive patients on low salt diet.⁴⁴

The higher insulin receptor density in the high salt rats may be an upregulation in consequence of the hypoinsulinemia.⁴⁵

A higher adipocyte glucose uptake was observed in Wistar rats after several weeks on salt overload, as compared with a group of animals submitted to salt restriction. That increased glucose uptake was measured in epididymal adipocytes in the absence, as well as in the presence, of various concentrations of insulin. The EC₅₀ from the dose-response curve of glucose uptake was the same in rats receiving low and high salt diets. According to other studies,⁴⁶ the EC₅₀ is a measure of insulin sensitivity and, therefore, insulin sensitivity was the same on high and low salt diets. Based on the fact that the glucose uptake dose-response curves are parallel, the higher maximal glucose uptake in adipocytes from salt overloaded rats is a possible consequence of the higher basal uptake in the absence of insulin. In other words, high salt stimulates insulin-independent glucose uptake without changing insulin sensitivity. With these results, it was demonstrated that in the presence of similar concentrations of insulin, changes in glucose uptake may occur without changes in insulin sensitivity. Data from the present study do not permit any conclusion about the mechanisms involved in the salt stimulated insulin-independent glucose uptake; however, some speculations are pertinent.

It is well established that rat adipocytes express two glucose transporter (GLUT) isoforms: GLUT-1 and GLUT-4.⁴⁶ The first is constitutively present in plasma membranes whereas the second, which is found in insulin-targeted tissues, is distributed into two cellular pools: 1) the plasma membrane and 2) the Golgi-associated vesicles. This latter pool translocates to plasma membrane under insulin stimulation and moves back to the Golgi region after insulin is washed out.⁴⁷,⁴⁸ This movement from Golgi to plasma mem-

FIGURE 2. Glucose uptake in adipocytes from Wistar rats on high (HSD, n = 6) and low (LSD, n = 6) salt diet. EC₅₀ insulin concentration for half of the maximal glucose uptake.
brane and back to Golgi is the molecular basis of the glucose transport regulation by insulin.

Our results with isolated adipocytes showed an increased basal glucose uptake rate in the high-salt diet treated rats. As our data is expressed in pmol of 2-deoxy-D-glucose transported per centimeter² of adipocyte surface area, it is strongly suggestive that a rise in glucose transporter population have taken place in plasma membrane of high salt diet adipocytes. In favor of that idea is the fact that GLUT-1 and GLUT-4 isoforms exhibit very similar kinetic properties. For example, they have very close $K_m$ values (between 1 and 5 mmol/L), and show first-order kinetics. Therefore, a reasonable explanation for the increment in basal transport rate is the increase in transporter number.

It is difficult to speculate with regard to which isoform is responsible for that effect. GLUT-1 isoform is a candidate in the sense that it is not translocatable as is GLUT-4, and the basal rate determined during the insulin-stimulated glucose transport dose-response assay is the consequence of GLUT-1 activity because, in that condition, GLUT-4 is usually almost absent in plasma membrane. An alternative explanation is the increased recycling of GLUT-4 from intracellular stores to the cellular membrane, observed in the presence of intracellular potassium depletion. In that condition, increased basal as well as maximal stimulated glucose uptake was observed in rat adipocytes. Potassium depletion may be a consequence of high sodium ingestion. Whether this occurred in our high salt rats is a question to be answered in future studies. This phenomenon may explain the in vivo but probably not the in vitro results, given that in the in vitro studies, adipocytes were maintained in the same environmental conditions, whether they came from high or low salt rats.

In addition, when insulin maximally stimulated the 2-deoxy-glucose uptake, the increment in cell response to the hormone, about 7 pmol/cm²/3 min in high salt diet adipocytes against 4 pmol/cm²/3 min in low salt diet cells (note the difference between maximal and basal 2-deoxy-glucose uptake in Table 2), almost doubled, indicating that the translocatable pools of GLUT-4 isoform was also increased.

Similar results were obtained in parallel studies involving glucose metabolism: that is, an enhancement both in basal and in maximal rates of glucose oxidation and incorporation into lipids (Table 2) on high salt diet when compared to low salt diet cells. The augmented capacity to metabolize glucose in basal conditions seemed to be a consequence of the increased basal glucose uptake, because in all the tests the basal response in high salt diet cells were 2 to 2.5 times greater than in those for low salt diets. Nevertheless, the maximal insulin effect upon glucose metabolism did not duplicate the basal response as occurred with glucose transport. As a matter of fact, the maximal effect was about 30% to 40% higher. The rise in glucose transport and, hence, the rise in glucose influx, was not proportionately utilized for oxidation or lipid synthesis. This result, although apparently discrepant, is not unusual, because even when glucose transport is elevated 150% to 200% above basal, it does not imply that a response of the same magnitude will be observed when other insulin biologic effects are determined.

When insulin interacts with its receptor, a biological signal is generated inside the cell, starting the activation of the tyrosine-kinase site in the cytoplasmic tail of the $β$-subunit of the insulin receptor. After that, a 180 kDa protein, the insulin receptor substrate 1 (IRS-1), is phosphorylated on tyrosyl residues included in the sequential motifs YMXM or YXXM and the resultant phospho-Y-IRS-1 binds to SH2-associated proteins, among which are the phosphatidyl-inositol-3'-kinase (PI3K), the growth hormone receptor binding protein 2 (GRB-2), and the phosphotyrosine phosphatase 2 (PTP-2). It is presumed that the steps following PI3K activation are linked to the pathway leading to GLUT-4 translocation to membrane. The steps leading to other biological effects are not necessarily the same. It has been shown in isolated adipocytes that their incubation with insulin and glucose simultaneously rendered them resistant to insulin. We have shown that adipocytes preincubated with glucose and insulin became resistant to insulin only in relation to glucose transport effects, but not other biological actions such as protein synthesis stimulation, glucose oxidation, and incorporation into lipids or glycogen synthase activation. A similar phenomenon could be taking place here, with high or low salt treated rats. Although blood glucose profiles in ivGTT were similar in both groups, the insulin response was more intense in salt restricted rats. This can also be interpreted as if low salt diet rats are developing a mild degree of insulin resistance. If we recognize that, after every meal, the low salt animals come up with blood glucose rises and more intense insulin secretion, then both factors could be working together in order to chronically induce postreceptor defects on the adipocyte response to insulin. The other possibility is that the amount of salt present in the diet is directly influencing the cells' ability to respond to insulin.

In addition, it is obvious that the muscle is the main territory for insulin action. Therefore, the question to be addressed is: Can the events described here for the adipocytes be extrapolated to the muscle fibers? To make such a connection, we must keep in mind that muscle response to insulin changes all the time, depending on the degree of its exertion or physical training; and the type of muscle fiber under consideration...
differentially influences its responsiveness to insulin (slow switched fibers being more responsive than fast ones). Such factors were not adequately checked in our study. Anyway, as the animals were permanently kept in small cages, we assume that they were all sedentary, both groups were under the same conditions. On the other hand, the ivGTT was designed in order to observe the in vivo regulation of the glucose homeostasis. As the differences detected in both groups were in accordance with the findings seen in isolated adipocytes, we have no reasons to discard the hypothesis that similar events could be taking place in muscular territory.

In the in vivo studies, plasma insulin levels were lower during the ivGTT performed on the animals receiving high salt intake, whereas blood glucose concentrations were the same on both diets. Plasma insulin levels are influenced by insulin clearance, by insulin production, or by both. Meland et al have reported a lower insulin production during salt overload in hypertensive patients.41 A similar mechanism could be involved in the present study. In addition, the possible inhibition of insulin production by high salt may be due to the increased insulin-independent glucose uptake obtained in the in vitro determinations. Another explanation comes from the knowledge that angiotensin II decreases blood flow to the liver,39 the main organ in which insulin is cleared from circulation.40 In the present study, the activity of the renin-angiotensin system was lower in the high salt rats, which could be the mechanism responsible for increased insulin clearance and therefore, lower insulin blood levels. Another evidence in favor of that mechanism is the lowering effect of converting enzyme inhibitor on plasma insulin observed by Egan et al in hypertensive patients on low salt diet.44 The higher insulin receptor density in the high salt rats may be an upregulation in consequence of the hypoinsulemia.43

In conclusion, chronic salt restriction, as compared with salt overload, decreases insulin-independent glucose uptake in isolated adipocytes without changes in insulin sensitivity, and increases insulin levels during an ivGTT.

REFERENCES


