Red Blood Cell Sodium-Proton Exchange in Hypertensive Blacks With Insulin-Resistant Glucose Disposal

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To define the potential pathogenic role of hyperinsulinemia as a mediator of alterations in sodium transport, we have examined red blood cell Na⁺-H⁺ and Na⁺-Li⁺ exchanges in a young adult black population characterized for blood pressure and insulin-mediated glucose disposal. Normotensive and mildly hypertensive blacks (blood pressure, 120±2/76±2 and 139±3/94±2 mm Hg, respectively) with a mean age of 26.1 years were studied for insulin sensitivity with the euglycemic hyperinsulinemic clamp (molar index of insulin sensitivity, M/I=moles glucose metabolized/insulin in milliliters of plasma). Na⁺-H⁺ exchange (U=mmol/L cell • h) was measured before and after the insulin clamp as a function of cell pH to determine the maximum transport rate. In the normotensive subjects, 18 were insulin sensitive (M/I=9.37±0.6x10⁴) and 4 were insulin resistant (M/I=3.64±0.6x10⁴). In the hypertensive subjects, 4 were insulin sensitive (M/I=9.15±1.1x10⁴) and 16 were insulin resistant (M/I=3.02±0.3x10⁴). The maximum rate of Na⁺-H⁺ exchange was significantly higher in all hypertensive vs normotensive individuals (35±3 vs 23±3 U, P<.005). Na⁺-H⁺ exchange activity was higher in insulin-resistant vs insulin-sensitive hypertensive subjects (40±3 vs 20±2 U, P<.001) but not in insulin-resistant normotensive subjects. Na⁺-Li⁺ exchange was not different in hypertensive and normotensive individuals but was higher in all insulin-resistant compared with all insulin-sensitive subjects (0.26±0.03 vs 0.16±0.02 U, P<.01). Na⁺-Li⁺ exchange also was higher in insulin-resistant vs insulin-sensitive normotensive subjects (0.35±0.03 vs 0.15±0.02 U, P<.001) and in insulin-resistant hypertensive subjects vs insulin-sensitive normotensive subjects (0.24±0.03 vs 0.15±0.02 U, P<.001). A stepwise multiple regression analysis for all variables revealed that Na⁺-H⁺ exchange was not different in hypertensive and normotensive individuals but was higher in insulin-resistant compared with all insulin-sensitive subjects (0.26±0.03 vs 0.16±0.02 U, P<.01). Na⁺-Li⁺ exchange also was higher in insulin-resistant vs insulin-sensitive normotensive subjects (0.35±0.03 vs 0.15±0.02 U, P<.001). Several mechanisms have been proposed to explain the association of insulin resistance with hypertension. A potential pathogenic role has been ascribed to hyperinsulinemia as a mediator of enhanced renal sodium (Na⁺) retention. However, investigations on Na⁺ transport mechanisms linking impaired insulin-dependent glucose disposal, hypertension, obesity, and Na⁺ retention have not yet been conducted. Numerous studies have shown that insulin can stimulate Na⁺-H⁺ exchange in skeletal muscle and other cells. Insulin-mediated enhancement of Na⁺-H⁺ exchange in renal tubular cells could result in a net increase in renal Na⁺ reabsorption.

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fraction of white, but not in black, hypertensive subjects. We have also shown that incubation of human RBCs with physiological concentrations of insulin increased the \( V_{\text{max}} \) of Na\(^+\)-H\(^+\) and Na\(^+\)-Li\(^+\) exchanges.

These findings raise two questions: Is RBC Na\(^+\)-H\(^+\) exchange altered in black hypertensive subjects with insulin-resistant glucose disposal? Is there a relation between the activity of the Na\(^+\)-H\(^+\) exchange and insulin sensitivity in this population? The present study was designed to answer these questions.

**Methods**

**Subjects**
Participants in this study were all young adult black men and women enrolled from a larger population of young adult blacks that has been under study and previously described. In each subject, growth and blood pressure data had been recorded since the newborn period and through childhood, adolescence, and young adulthood. Based on average seated blood pressure measurements obtained with a mercury column sphygmomanometer over the preceding 5 years, subjects were classified as normotensive (systolic<135 mm Hg, diastolic<85 mm Hg) or borderline hypertensive (systolic\(\geq\)135 mm Hg, diastolic\(\geq\)85 mm Hg, or both). At enrollment for this study, no subject was taking or had been taking antihypertensive medication. Before enrollment, each subject provided written informed consent to the protocol, which had been approved by the Human Studies Committee of the Medical College of Pennsylvania and Hahnemann University.

All subjects had an oral glucose tolerance test using a standard 75-g oral glucose challenge after an overnight fast. Subjects with insulin-dependent diabetes mellitus were excluded. Subjects who met the clinical criteria for non-insulin-dependent diabetes mellitus were also excluded.

**Hyperinsulinemic Euglycemic Clamp Technique**
Subjects reported to the research center after an overnight fast. All studies were begun at 9 AM. An angiocatheter (18- or 20-gauge) was placed in the right forearm for infusion, and a second (22-gauge) angiocatheter was placed in a vein in the left hand or wrist. The hand was warmed with a heating blanket to "arterialize" the blood sample from this catheter. During the study, the subject either slept or was distracted with television. After venous access was established and the subject had rested, a blood sample was withdrawn for determination of fasting plasma glucose, fasting plasma insulin concentration, and RBC transport studies.

Insulin sensitivity was determined by the hyperinsulinemic euglycemic clamp technique. An insulin "prime" was infused over 1 minute followed by a constant infusion of 30 mU/m\(^2\) per minute for 120 minutes. The insulin infusion was calculated to achieve clamped insulin concentrations of 430 to 575 pmol/L. During the insulin infusion, euglycemia was maintained with a variable infusion of 20\% glucose solution. Plasma glucose was measured every 10 minutes (Glucostat, Yellow Springs Instruments Co, Yellow Springs, Ohio). The plasma glucose concentration was used in a negative feedback equation as described by DeFronzo et al to adjust the glucose infusion rate. With this technique, plasma glucose was maintained within 5\% of the fasting concentration throughout the study. The glucose and insulin infusions were delivered by a previously calibrated syringe pump (Harvard 22, Boston, Mass). The insulin infusion solution was made with regular insulin (Novolin Regular, ER Squibb & Sons Inc) and dissolved in normal saline with 40 mEq/L KCl.

Euglycemic hyperinsulinemia was maintained for 2 hours. In the second hour, during steady-state hyperinsulinemia, samples were withdrawn every 10 minutes for subsequent measurement of plasma insulin concentration. The quantity of exogenous glucose used to maintain euglycemia was calculated as the mean of the glucose infusion rate during the second hour of hyperinsulinemia. In previous studies, Ferrannini and Groop have shown that euglycemic hyperinsulinemia completely suppresses hepatic glucose output at clamped insulin concentrations greater than 360 pmol/L (50 \(\mu\)U/mL) in normal volunteers. We have also demonstrated in this population of young adult blacks, including both obese and non-obese subjects, that during the second hour of steady-state hyperinsulinemia from 360 to 645 pmol/L, hepatic glucose production is completely suppressed. Therefore, when the clamp is performed at insulin concentrations greater than 360 pmol/L, hepatic glucose production, as in this study, is essentially zero. The glucose infusion rate (millimoles per kilogram per minute times 10\(^3\)) then reflects total insulin-stimulated glucose metabolism (M) during steady-state hyperinsulinemia. M was adjusted for level of steady-state insulinenia achieved in each case to establish a molar index of insulin sensitivity, or moles per glucose metabolized per insulin in 1 mL plasma (M/I); it is expressed as a unitless ratio. At the end of the study period, a urine sample was tested for glucosuria. The subject remained in the research center until he or she had consumed a meal and his or her blood glucose was normal after withdrawal of the intravenous infusion. Plasma samples for insulin concentration were frozen at \(\sim\)80°C and later measured with a standard radioimmunoassay (Diagnostic Products Corp, Los Angeles, Calif). Blood for the RBC Na\(^+\) transport assays was drawn into heparinized tubes and centrifuged at 2000g for 4 minutes at 4°C. The plasma and buffy coat were removed by aspiration and the RBCs suspended in 1 mL plasma (M/I); it is expressed as a unitless ratio. At the end of the study period, a urine sample was tested for glucosuria.

**Red Blood Cell Preparation**
The next morning, RBCs were washed three times with ice-cold choline wash solution containing (mM) choline chloride, 149; MgCl\(_2\), 1; and Tris-3-[N-morpholino]propanesulfonic acid (MOPS), 10, pH 7.4 (4°C), and then were resuspended to approximately 50\% with choline wash solution. Hematocrit and hemoglobin (optical density at 540 nm) and Na\(^+\) concentration (atomic absorption spectroscopy) were determined on the suspension after appropriate dilution with 0.02\% Acationox detergent (American Scientific Products, Boston, Mass) in double-distilled water.
**Measurement of Red Blood Cell Na⁺-H⁺ Exchange Activity**

Na⁺-H⁺ exchange was measured as net Na⁺ influx driven by an outward H⁺ gradient (ΔpH, 8.0 to 6.0). We previously reported that this component of Na⁺ influx is equal to H⁺ efflux stimulated by external Na⁺ and therefore estimate 1:1 exchange of Na⁺ for H⁺. Approximately 60% to 80% of this flux component is inhibited by 1 mM amiloride and high-affinity amiloride analogues such as dimethyl-amiloride and isopropyl-amiloride. Because human RBCs have a very low permeability to amiloride and high concentrations induce lysis, we do not use amiloride to assay the antiporter activity in human RBCs. The antiporter activity of human RBCs, as in the case of the sodium-potassium pump, is 100 times less than that of kidney cells, but it can be measured with great precision because of the low passive permeability of RBCs and the availability of large numbers of cells for every assay.

**Preparation of acid-loaded red blood cells.** As previously described, the precision of the measurements of net RBC Na⁺ gain with atomic absorption spectrophotometry is enhanced by using the nystatin procedure to prepare sodium-depleted cells (<1 mmol of sodium per liter of cells) before the acid loading step (pH varying from 6.0 to 7.0). All solutions were prepared in deionized, double-distilled water, and the osmolality was determined with a freezing point osmometer. Washed packed cells were incubated for 20 minutes at 4°C at 15% hematocrit in cold nystatin loading solution containing 40 μg/mL nystatin and protected from the light. Nystatin loading solution contained 150 mM KCl and 50 mM sucrose. The cell suspensions were then warmed to 37°C and stirred for 5 minutes. The RBCs were washed four times with warm nystatin washing solution, which had the same composition as the nystatin loading solution, with 1 mM KH₂PO₄ buffer, 10 mM glucose, and 0.1% albumin added. RBCs with six different pH values between 6.0 and 7.0 were incubated at 37°C for 10 minutes in six different acid loading solutions that contained (mM) KCl, 170; MgCl₂, 0.15; ouabain, 0.1; bumetanide, 0.1; glucose, 10; sucrose, 40; and Tris-2-[N-morpholino]-ethanesulfonic acid (MES), 20 (adjusted to pH 5.8, 6.0, 6.3, 6.5, 6.8, and 7.0 at 37°C). To avoid cell swelling, the osmolality of the acid loading solutions was 360 mOsm.

After the 10-minute preincubation with different acid loading solutions, 4,4'-disothiocyanatostilbene-2,2'-disulfonic acid (DIDS) (to inhibit the anion exchanger) and neptazane (to inhibit carbonic anhydrase) were then added to final concentrations of 100 and 200 μM, respectively, to “clamp” pH. The RBCs were incubated at 37°C for an additional 20 minutes and then were washed three times with ice-cold pH wash solution containing 170 mM KCl, 0.15 mM MgCl₂, and 40 mM sucrose. The RBCs were then resuspended to approximately 50% hematocrit with pH wash solution and stored on ice until used. Aliquots of the cell suspensions were used for determination of hemoglobin, hematocrit, and intracellular Na⁺ content. The pH was determined with a pH electrode in a cell lysate made with 4 vol of 0.02% Acationox detergent. The RBC Na⁺ content was determined by atomic absorption spectroscopy with suitable standards. The cation content of the acid-loaded cells was expressed per liter original volume.

**Na⁺ influx measurements.** RBCs of each different pH were incubated in two Na⁺ media with pH (pH₈) 8.0 and 6.0; both media contained (mM) NaCl, 150; KCl, 20; MgCl₂, 0.15; ouabain, 0.1; neptazane, 0.4; glucose, 10; sucrose, 40; bumetanide, 0.01; and either Tris-MOPS, 10 (pH 8.0 at 37°C), or Tris-MES, 10 (pH 6.0 at 37°C). At pH₈, 8.0, an outward H⁺ gradient is imposed, and at pH₈, 6.0, the Na⁺-H⁺ exchange is inhibited because the Kₐ for external Na⁺ is increased. The difference between Na⁺ influx at pH₈, 8.0 and pH₈, 6.0 (ΔpH, 8.0 to 6.0) represents the net Na⁺ influx (millimoles per liter of cells per hour [U]) driven by an outward H⁺ gradient. Initial transport rates were determined using duplicate samples of 200 μL at 1, 6, and 11 minutes for pH₈, 8.0 medium and at 1 and 20 minutes for pH₈, 6.0 medium, because under such conditions the flux is inhibited and the low transport rates are linear up to 30 minutes. Net Na⁺ influx was determined from the regression of cell Na⁺ content vs time of the six samples.

**Measurements of Na⁺-Li⁺ Exchange**

For these experiments, RBCs were loaded with Li⁺ (lithium, 8, sodium, 0.4 mmol/L cell) using the nystatin procedure as previously described. Na⁺-Li⁺ exchange was measured by external Na⁺-stimulated Li⁺ efflux. Lithium-loaded cells were incubated at 4% hematocrit in choline and Na⁺ efflux medium containing (mM) choline chloride or NaCl, 150; MgCl₂, 1.0; glucose, 10; ouabain, 0.1; and Tris-MOPS, 10 (pH 7.4 at 37°C). Duplicate samples were taken at 10, 25, and 40 minutes. The samples were centrifuged immediately (2000g, 5 minutes, 4°C) and the supernatant carefully removed and stored for Li⁺ determination by atomic absorption spectroscopy using appropriate standards. Initial rates of net Li⁺ efflux were determined from the regression of Li⁺ concentration in the media expressed in millimoles per liter of cell vs time using the ENZFITTER software program. The correlation coefficient of this regression line was always higher than .985.

**Statistical Analysis**

Analysis of variance, Student's t test, and linear regression analysis were performed in a computer facility of the Brigham and Women's Hospital with a computer (Digital Equipment Corp, Maynard, Mass) running a CLINFO software statistical program (Bolt, Beranek and Newman, Cambridge, Mass). Data are reported as mean±SEM. The null hypothesis was rejected at a value of P<.05. The stepwise multiple
Regression analysis was performed in a computer facility of the Medical College of Pennsylvania. Although plasma insulin concentrations are not normally distributed, the logarithm of plasma insulin is normally distributed. The metabolic variables, including plasma insulin concentration, insulin-stimulated glucose disposal (M), and insulin sensitivity index (M/I), were also analyzed with a log transformation.

### Chemicals

NaCl, MgCl₂, dibutylphthalate, and glucose were obtained from Fisher Scientific Co, Fairlawn, NJ. Ouabain, Tris, MES, MOPS, DIDS, nystatin, and albumin (bovine fraction V) were purchased from Sigma Chemical Co, St Louis, Mo, and KCl from Mallinckrodt, Inc, St Louis, Mo. Neptazane was from Lederle Laboratories, Division of the American Cyanamid Co, Pearl River, NJ. Choline chloride (ammonia-free) was obtained from Calbiochem, Behring Diagnostics, San Diego, Calif, and bumetanide was obtained from Leo Laboratories, France.

### Results

**Characteristics of the Study Population**

Data were obtained from 42 young adult black subjects, including 22 normotensive subjects and 20 mildly hypertensive subjects. Characteristics of the study population are presented in Table 1. Mean age was similar in the normotensive and mildly hypertensive subjects. Both systolic and diastolic blood pressures were significantly greater in the hypertensive subjects ($P<.001$). Body mass index (BMI) ranged from 18.5 to 35.8 kg/m² in normotensive and from 21.3 to 42.1 kg/m² in hypertensive subjects. Mean BMI was greater in hypertensive subjects ($P<.001$). There was no difference between the two blood pressure groups in fasting plasma glucose concentrations. Fasting plasma insulin concentration was significantly greater in hypertensive subjects ($P<.05$). The ratio of plasma insulin to glucose was also significantly greater in hypertensive subjects ($P<.01$). These data demonstrate fasting hyperinsulinemia in the mildly hypertensive subjects.

**Insulin-Sensitive Glucose Disposal of Normotensive and Hypertensive Blacks**

With the use of the insulin clamp protocol, the insulin infusion rate resulted in a range of steady-state hyperinsulinemia. In normotensive nonobese subjects, the mean ($±SD$) insulin sensitivity index (M/I) was 8.4±1.8×10⁻⁴. We then selected a level of 1.5 SD below the mean to be the lower level of M/I×10⁻⁴ for an insulin-sensitive normotensive subject. This value is 5.7. We then defined as an insulin-sensitive response a molar index of insulin sensitivity greater than 5.7×10⁻⁴. Subjects having a molar index of insulin sensitivity less than 5.7×10⁻⁴ less than 5.7 were classified as insulin resistant.

To determine the reliability and reproducibility of this criterion for assignment of insulin sensitivity or insulin resistance, we repeated the euglycemic hyperinsulinemic clamp procedure in 10 subjects, including 5 normotensive and 5 hypertensive individuals. The first procedure was performed at an insulin infusion rate of 40 mU/m²·min and the second at an insulin infusion rate of 20 mU/m²·min. Analysis of the computed molar index of insulin sensitivity from the high-insulin infusion clamp and the low-insulin infusion clamp for each case demonstrated a highly significant correlation ($r=.793$, $P<.006$). With a categorical assignment of the molar index of insulin sensitivity ≥5.7×10⁻⁴ and of <5.8×10⁻⁴ for insulin resistance, there was 100% agreement in assignment of insulin sensitivity or resistance between the first and second euglycemic hyperinsulinemic clamp studies. These results demonstrate reliability in the insulin sensitivity classification. The replication of insulin sensitivity index indicates that hepatic glucose production is suppressed at each level of hyperinsulinemia.

Results of the insulin clamp data are also provided in Table 1. In all cases, the plasma glucose was clamped within 5% of the fasting plasma glucose concentration during steady-state hyperinsulinemia. The insulin infusion resulted in a range of steady-state hyperinsulinemia. Mean plasma insulin concentration during steady-state hyperinsulinemia was higher in the hypertensive group compared with the normotensive group (575±42 and 438±40 pmol/L, respectively; $P<.001$). Despite greater levels of insulinemia in the hypertensive subjects, the glucose infusion rate (M) necessary to maintain euglycemia was lower in the hypertensive compared with the normotensive group (2.3±0.03 and 3.0±0.03 mmol·kg⁻¹·min⁻¹, respectively). Although the difference in glucose infusion rate did not reach statistical significance, when M was corrected for level of steady-state insulinemia, the molar index of insulin sensitivity was markedly lower in hypertensive subjects (4.11±0.7×10⁻⁴ vs normotensive subjects, 8.37±0.9×10⁻⁴; $P<.01$).

Table 2 provides the data from the euglycemic hyperinsulinemic clamp study for the subjects in this study grouped according to insulin sensitivity. Each subject was designated insulin sensitive if the molar index of insulin sensitivity derived from the clamp study was ≥5.7×10⁻⁴, and the subject was designated insulin resistant if the molar index of insulin sensitivity was <5.7×10⁻⁴. With this definition, 18 normotensive sub-
TABLE 2. Euglycemic Hyperinsulinemic Clamp Data in Young Blacks

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Insulin-sensitive</td>
<td>Insulin-resistant</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>4/14</td>
<td>2/2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.0±0.9</td>
<td>27.2±3.5</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>120±2</td>
<td>120±14</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>75±2</td>
<td>76±5</td>
</tr>
<tr>
<td>Fasting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>4.8±0.1</td>
<td>4.9±0.5</td>
</tr>
<tr>
<td>Plasma insulin (pmol/L)*</td>
<td>64±6</td>
<td>120±44</td>
</tr>
<tr>
<td>Molar I/G ratio (×10⁻⁹)*</td>
<td>15.4±1.9</td>
<td>25.6±7.8</td>
</tr>
<tr>
<td>Clamp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma insulin (pmol/L)†</td>
<td>380±37</td>
<td>524±59</td>
</tr>
<tr>
<td>Insulin range (pmol/L)</td>
<td>194-796</td>
<td>352-610</td>
</tr>
<tr>
<td>M (mmol/kg·min·1cp)†</td>
<td>3.3±0.3</td>
<td>1.9±0.4</td>
</tr>
<tr>
<td>Molar M/I (×10⁹)†</td>
<td>8.9±0.7</td>
<td>3.7±0.7</td>
</tr>
</tbody>
</table>

BMI, body mass index; BP, blood pressure; I/G, ratio of plasma insulin to glucose; M, glucose infusion rate; I, insulin; M/I, molar index of insulin sensitivity. Values are mean±SEM; n=number of subjects. *P<.01, †P<.001, insulin-resistant vs insulin-sensitive subjects.

Of the mildly hypertensive subjects, 4 were insulin sensitive and 4 were insulin resistant. Of the mildly hypertensive subjects, 4 were insulin sensitive and 16 were insulin resistant. Fasting plasma glucose concentration did not differ among the groups. Although fasting plasma insulin concentration and the insulin-glucose ratio were higher in the hypertensive subjects (Table 1), these parameters were significantly elevated in the insulin-resistant cases, in both normotensive and hypertensive individuals (P<.01).

Note that in Table 2, as expected, insulin-resistant normotensive and hypertensive subjects had significantly higher BMI values than insulin-sensitive normotensive subjects. However, insulin-sensitive hypertensive subjects had a similar elevation of BMI as insulin-resistant hypertensive subjects. Within each blood pressure group (normotensive or hypertensive), systolic and diastolic blood pressures did not differ significantly between insulin-resistant and insulin-sensitive cases (Table 2).

Kinetics of Red Blood Cell Na⁺-H⁺ Exchange in Normotensive and Hypertensive Blacks

Fig 1 depicts the cell pH activation of Na⁺-H⁺ exchange in a representative normotensive and hypertensive black. As the intracellular pH decreases, Na⁺-H⁺ exchange is activated. The maximum transport rate, Vₘₐₓ, of 26 U was reached between pHj 6.2 and 6.0 in the normotensive subject and of 48 U in the hypertensive subject.

The dependence of the Na⁺-H⁺ exchange on intracellular H⁺ was analyzed using the Hill equation as previously reported. The intercept of the x axis at y=0 gives log [H⁺]₅₀, which is the logarithm of the substrate concentration that yields 50% of the Vₘₐₓ, which for the sake of simplicity we named Kₘ. The Kₘ and nₐₚ values were similar in both subjects.

When the activity of the Na⁺-H⁺ exchange was examined in the entire population, Vₘₐₓ was significantly 50% of the Vₘₐₓ, Kₘ.

FIG 1. plots show kinetics of activation of red blood cell Na⁺-H⁺ exchange by cell pH in a normotensive (○) and hypertensive (■) young black. Panel A: Na⁺-H⁺ exchange activity as a function of cell pH in both subjects. Panel B: Hill plot of measured velocity of Na⁺-H⁺ activity as a function of cell H⁺ concentration according to the equation:

\[-\log \frac{v}{V_{\text{max}}} = n_{\text{app}} \log [H⁺] - \log K'\]

where \(v\) represents the measured Na⁺-H⁺ exchange activity, \(V_{\text{max}}\) the estimated maximum rate from the H₁ activation curve, and \(H⁺\) the H⁺ concentration in the cell (H₁). The slope gives the Hill coefficient (nₐₚ), and the intercept at the x axis at y=0 gives \(\log [H⁺]₅₀\), ie, the logarithm of the substrate concentration that yields 50% of the \(V_{\text{max}}, Kₘ\).
higher in hypertensive (35.9±3.6 U, n=19) than in normotensive (23.8±3.6 U, n=22, P<.005) subjects. The $K_m$ for $H_i$ values to activate $Na^+-H^+$ exchange were similar in both groups (6.37±0.045 vs 6.30±0.048). The Hill coefficient ($n_{HPP}$) exhibited slightly higher values in hypertensive blacks (2.8±0.27 vs 2.27±0.19), but this difference did not reach statistical significance.

$Na^+-H^+$ Exchange Activity and Insulin-Sensitive Glucose Disposal

When normotensive and hypertensive subjects were subdivided according to their insulin sensitivity as determined by the M/I×10^-4 values (Table 2), the $V_{max}$ of $Na^+-H^+$ exchange was significantly higher in the insulin-resistant hypertensive subjects compared with all other groups (Fig 2 and Table 3). Insulin-resistant normotensive and insulin-sensitive hypertensive subjects did not show an elevation of the $V_{max}$ of $Na^+-H^+$ exchange as did insulin-resistant hypertensive subjects. The $K_m$ and $n_{HPP}$ values were not different among groups (Table 3).

We also performed measurements of cell pH activation of $Na^+-H^+$ exchange in a blood sample obtained after the 2 hours of insulin clamp (Table 3, postclamp data). The enhanced antiporter activity exhibited by the insulin-resistant hypertensive subjects was observed during both fasting (preclamp) and hyperinsulinemia. However, the $V_{max}$ of $Na^+-H^+$ exchange had a significantly lower value after the insulin clamp in insulin-resistant hypertensive subjects and did not change in the other groups.

When we computed the $V_{max}$ of $Na^+-H^+$ exchange in all subjects grouped according to insulin sensitivity, we found that the antipporter activity was significantly higher in insulin-resistant subjects (33.7±4.2 U, n=19) compared with insulin-sensitive subjects (18±2.8 U, n=22, P<.005).

$Na^+-H^+$ Exchange Activity and Body Mass Index

As shown in Table 1, overall hypertensive subjects had higher BMI values than normotensive subjects. However, compared with insulin-sensitive normotensive subjects, BMI was also higher in insulin-resistant normotensive and insulin-sensitive hypertensive subjects. Although BMI was higher in these two groups, the $Na^+-H^+$ exchange activity was not higher (Table 3). Of all hypertensive subjects (insulin-resistant and insulin-sensitive), only the hypertensive subjects with insulin resistance showed increased $Na^+-H^+$ exchange activity. We questioned whether high $Na^+-H^+$ exchange activity is the result of additional impairment in insulin sensitivity imposed by greater adiposity. To examine this point, we matched normotensive and hypertensive subjects with normal BMI (20 to 26 kg/m²) and elevated BMI (26 to 39 kg/m²) and determined their $V_{max}$ of $Na^+-H^+$ exchange (Table 4). We found that in subjects with normal BMI, the $V_{max}$ of $Na^+-H^+$ exchange was 45% higher in hypertensive than in normotensive subjects. An elevation of BMI from 23 to 31 kg/m² increased the...
Vmax of Na+-H+ exchange in hypertensive subjects from 30.8 to 50.6 U but not in normotensive subjects. In high-BMI hypertensive subjects, Na+-H+ exchange increased 2.3-fold over values of normotensive subjects with normal BMI. In addition, among subjects with high BMI (Table 4) hypertensive subjects had a significantly higher Vmax of Na+-H+ exchange than normotensive subjects (P<.005). These results indicate that (1) elevated Na+-H+ exchange activity in hypertensive subjects is not the consequence of a high BMI but of both high blood pressure and impaired insulin-resistant glucose disposal, (2) increased BMI enhances Na+-H+ exchange activity in hypertensive but not in normotensive subjects, and (3) blunted insulin-resistant glucose disposal, high BMI, and high blood pressure interact to increase the activity of Na+-H+ exchange.

Na+-Li+ Exchange Activity and Insulin-Sensitive Glucose Disposal

Measurements of the activity of Na+-Li+ exchange were also performed as usual at pH1 and pH0 7.4. At this pH, the intracellular H+ sites are not occupied, and the transport activity provides an estimation of the silent 1:1 Na+-Na+ exchange that is 100 times lower than Na+-H+ exchange.

There was no difference in mean Na+-Li+ exchange between normotensive (0.20±0.026 U, n=18) and hypertensive (0.23±0.030 U, n=19) subjects. However, as shown in Figs 3A and 3B, Na+-Li+ exchange was elevated in insulin-resistant hypertensive (0.24±0.036 U, n=15) compared with insulin-sensitive normotensive (0.157±0.021 U, n=14, P<.001) subjects. The four insulin-resistant normotensive subjects (0.35±0.033 U, n=4) exhibited a significantly higher Na+-Li+ exchange activity than insulin-sensitive normotensive subjects (P<.001). The insulin-sensitive hypertensive group had Na+-Li+ exchange activity similar to insulin-resistant hypertensive subjects (Fig 3B). When we computed the Na+-Li+ exchange activity in all subjects grouped according to insulin sensitivity (Fig 3C), we found that this antiporter activity was significantly higher in insulin-resistant (0.26±0.030 U, n=19) compared with insulin-sensitive (0.16±0.02 U, n=18, P<.01) subjects.

We also examined the activation kinetics of Na+-H+ exchange in subjects with normal and elevated Na+-Li+ exchange. Subjects, normotensive and hypertensive, with a normal range in Na+-Li+ exchange activity (0.148±0.017 U, n=13) had an Na+-H+ exchange Vmax of 36.7±4.5 U and a Hill coefficient of 2.87±0.25. Subjects, normotensive and hypertensive, with a high Na+-Li+ exchange activity (0.37±0.017 U, n=10) had an Na+-H+ exchange Vmax of 28.1±4.5 U (NS) and a Hill coefficient of 1.7±0.22 (P<.01). Thus, normal Na+-Li+ exchange activity was associated with elevated Na+-H+ exchange activity, and elevated Na+-Li+ exchange was associated with reduced Hill coefficients for cell pH activation of Na+-H+ exchange. These observations are similar to those we previously reported in white hypertensive subjects.

Relation Between Insulin Sensitivity and Na+-H+ Exchange Activity

Regression analysis on the entire sample showed a significant negative correlation of Na+-H+ exchange activity with blood pressure group (r=.42, P<.01) and a significant negative correlation of Na+-H+ exchange with log M/I×10^-4 (r=-.42, P<.0045, Fig 4B) and BMI (r=-.49, P<.008, Fig 4A) but not with fasting insulin concentration (Fig 4C). As expected, there was a significant negative correlation of the index of insulin sensitivity, M/I×10^-4, with BMI in the whole population (r=-.84, P<3×10^-7).

It is well established in the literature, as we have found in our population of urban blacks, that there is a very strong relation between obesity and measures of insulin resistance. In the analysis, it was necessary to deal with the marked colinearity of obesity (in terms of BMI), blood pressure, insulin sensitivity, and the insulin sensitivity of the obese cases. To examine the relations among blood pressure, insulin sensitivity, and Na+-H+ exchange, we entered all variables, including Na+-H+ exchange, blood pressure, BMI, plasma insulin levels, and insulin sensitivity, into a series of stepwise multiple regression analyses. Whenever we perform the stepwise multiple regression analysis with any parameter of insulin sensitivity (M, M/I, plasma insulin concentration) as the dependent variable, the first and strongest correlate of all the independent variables is BMI. For example, in this sample, multiple R=.7862 and P<.001. Because of the dominant effect of BMI on insulin sensitivity, it is necessary to adjust for adiposity (BMI) to examine effects of the other variables on insulin sensitivity in normotensive (panel A) and hypertensive (panel B) blacks. Insulin-resistant hypertensive blacks had significantly higher values than insulin-sensitive normotensive blacks (P<.001). Panel C shows that Na+-Li+ exchange was elevated in all insulin-resistant subjects (0.26±0.030 U, n=19) compared with all insulin-sensitive subjects (0.16±0.02 U, n=18, P<.01). ○, Insulin-sensitive normotensive subjects; ★, insulin-resistant normotensive subjects; ●, insulin-sensitive hypertensive subjects; ■, insulin-resistant hypertensive subjects.
sensitivity. We have successfully done this in other analyses by performing the stepwise regression on cases having a BMI less than 28 kg/m². However, this maneuver is not appropriate in this sample, when there are already two very small groups.

When mean blood pressure was taken as the dependent variable, the insulin-sensitive group emerged as the most significant correlate in step one of the analysis (multiple R = .427, P = .007). No other variable added to the significance. When Na+-H+ exchange was the dependent variable, the strongest significant correlate was blood pressure group (multiple R = .419, P = .008).

Again, no other variable including BMI added significance to the model. These analyses confirm the presence of elevated V_max for Na+-H+ exchange in mildly hypertensive blacks with insulin-resistant glucose disposal.

**Discussion**

We have studied in RBCs a Na+ transport system that may provide a link between Na+ retention and insulin-resistant hypertension. The kinetics of the RBC Na+-H+ antiporter provide important insights into the relation between cellular Na+ handling, insulin sensitivity, and hypertension in blacks. Our study also permits some comparison of the functional status of RBC Na+-H+ exchange activity between blacks and whites. In both comparison of the functional status of RBC Na+-H+ exchange and the hypertensive groups, Na+-H+ exchange is higher in hypertensive than normotensive individuals. Normotensive blacks had a significantly lower Na+-H+ exchange activity than normotensive whites (20.9 ± 2.5 U, n = 22, vs 47 ± 4 U, n = 46; P < .001), as previously observed for Na+-Li+ exchange.17-20 Hypertensive blacks also had lower Na+-H+ activity than hypertensive whites (35.9 ± 3.6, n = 19, vs 60.3 ± 4.9 U, n = 42; P < .005). However, whites were older and tended to have greater body weight than the younger blacks (26 vs 45 years). In hypertensive whites, RBC Na+-H+ exchange exhibited lower n_{app} values for cell pH activation16 and higher V_{max} of Na+-Li+ exchange than in hypertensive blacks.17-20 Furthermore, in black and white hypertensive individuals, we found two types of alterations of Na+-H+ and Na+-Li+ exchange but with different proportions.18 One type has elevated Na+-H+ exchange activity with normal values of Na+-Li+ exchange activity. Another type has elevated Na+-Li+ exchange with normal Na+-H+ exchange maximal activity but with reduced Hill coefficients for cell pH activation. The first type is more frequent in blacks and the second in whites.

As demonstrated in this study, the young adult blacks with only mild hypertension have impaired insulin-stimulated glucose utilization, as evidenced by the significantly lower molar index of insulin sensitivity. Insulin resistance is therefore defined in this study as a suboptimal response of glucose disposal to insulin. The euglycemic hyperinsulinemic clamp methodology also detected some hypertensive subjects who were insulin sensitive and some normotensive subjects who were insulin resistant. This observation indicates that insulin resistance is not just a sequela of the hypertension.

The hypertensive blacks also have a higher fasting plasma insulin concentration and a higher molar ratio of insulin to glucose, although fasting plasma glucose concentration is normal. Because of a cellular defect in insulin-mediated glucose metabolism, a greater quantity of insulin is necessary to achieve metabolic control of glucose.32 Chronic hyperinsulinemia could be compensatory for peripheral cellular insulin resistance and may affect blood pressure regulation through control of Na+ excretion28 or through stimulation of vascular smooth muscle growth and increased peripheral resistance.33 Experimental work in dogs,28 rats,34 and in normal men3 has shown that insulin augments renal Na+ reabsorption. In vitro studies have shown that insulin can modulate the antiporter activity for Na+ transport in skeletal muscle15 and renal tubular cells.12 However, the effect of insulin resistance and associated hyperinsulinemia on Na+ transport in humans has been unknown. Although the role of insulin was not specifically addressed in earlier investigations on Na+-sensitive blood pressure in blacks, it is plausible that insulin may contribute to the
enhanced blood pressure response to Na+ loading reported for hypertensive blacks.35-36

Our data clearly demonstrate that insulin-resistant hypertensive subjects exhibit a twofold increase in $V_{\text{max}}$ of Na+-H+ exchange compared with insulin-sensitive subjects, both normotensive and hypertensive. The two-fold increase in $V_{\text{max}}$ of Na+-H+ exchange exhibited by insulin-resistant hypertensive subjects compared with insulin-sensitive normotensive and hypertensive subjects was accompanied by fasting insulin levels significantly higher in insulin-resistant hypertensive subjects than in insulin-sensitive normotensive subjects. The differences persisted in RBCs collected after the prolonged period of insulin clamp. Notably, in insulin-resistant hypertensive subjects, the Na+-H+ activity decreased significantly after the clamp; although many complex interactions may occur after the prolonged period of insulin clamping, these findings might be explained by the well-known downregulation of RBC insulin receptors after 2 hours of exposure to high hormone levels. In addition, our observations suggest that the enhanced antiporter activity could be directly mediated by the hormone levels. However, data analysis demonstrated that the $V_{\text{max}}$ of Na+-H+ exchange correlated significantly with insulin-sensitive glucose utilization (M/I) and BMI but not with fasting plasma insulin concentration. Moreover, insulin-resistant normotensive subjects did not exhibit increased $V_{\text{max}}$ of Na+-H+ exchange. Possible interpretations of these patterns are that (1) increased activity of Na+-H+ exchange cosegregated with blood pressure in the presence of insulin resistance, (2) hypertensive subjects might be hyperresponsive to insulin levels, and (3) the defective glucose disposal is the stimuli for an enhanced Na+-H+ exchange activity. In this study, insulin-resistant hypertensive blacks had a significantly higher BMI than insulin-sensitive normotensive blacks. However, a high BMI was not consistently associated with an elevated $V_{\text{max}}$ of Na+-H+ exchange. Insulin-resistant normotensive subjects who were hyperinsulinemic and obese did not exhibit enhanced Na+-H+ antiporter activity. Similarly, insulin-sensitive hypertensive subjects with increased BMI levels compared with insulin-resistant hypertensive subjects did not show elevated Na+-H+ exchange activity. Although the sample of insulin-resistant normotensive subjects is small, the results are similar to the data on obese normotensive blacks described by Saad et al.37

In the present study population, we also analyzed the $V_{\text{max}}$ of Na+-Li+ exchange at pH 7.4. Hypertensive compared with normotensive blacks did not have higher Na+-Li+ exchange, as was previously reported in hypertensive whites.17,19,20 However, all insulin-resistant normotensive subjects had significantly higher levels of Na+-Li+ exchange than insulin-sensitive normotensive subjects. When both normotensive and hypertensive subjects were categorized for insulin resistance, we found that insulin-resistant subjects had significantly higher values of Na+-Li+ exchange than insulin-sensitive subjects. Furthermore, all subjects with Na+-Li+ exchange activity higher than 0.30 U were insulin resistant. This observation suggests that there could be racial differences in the alterations of both exchange pathways in insulin-resistant hypertension. In blacks, insulin-resistant hypertension is associated with elevated (>30 U) Na+-H+ exchange and normal (<0.30 U) Na+-Li+ exchange. In hypertensive whites, there is more commonly an increase in Na+-Li+ exchange (>0.4 U) with normal values of Na+-H+ exchange. According to recent studies by Doria et al.,38 hypertensive whites with elevated Na+-Li+ exchange had insulin-resistant glucose disposal compared with hypertensive whites with normal values. Furthermore, black hypertensive type II diabetics have been shown to have higher Na+-Li+ exchange compared with normotensive and hypertensive nondiabetic blacks.39 It seems, therefore, that several insulin-resistant states exhibit alterations of either Na+-Li+ or Na+-H+ exchange transport modes. In blacks, elevated Na+-H+ exchange could be a marker of insulin-resistant hypertension, whereas elevated Na+-Li+ exchange appears to be associated with insulin resistance independently of blood pressure and may precede the development of hypertension.

Recently, we have shown that both exchange modes, Na+-H+ and Na+-Li+, are regulated by physiological levels of insulin that induce a marked increase in the $V_{\text{max}}$ and $K_m$ for external Na+.21-22 Therefore, it is possible that the alterations found in hypertensive subjects may reflect abnormal responsiveness to insulin. Increased Na+-H+ exchange activity can occur by an increase in the number of transport sites or by increased turnover induced by increased phosphorylation activated by cytosolic Ca2+ and protein kinases A and C.40-42 Modulation of the antiporter activity is tissue specific for agonists, including angiotensin II,43,44 epidermal growth factor, and platelet-derived growth factor,45 which mobilize Ca2+ and activate protein kinase C; however, parathyroid hormone appears to inhibit the renal antiporter activity via cyclic AMP–dependent protein kinase.46 In the case of human RBCs, physiological doses of insulin can occupy the insulin receptors and stimulate Na+-H+ exchange21,22 independently of the action of insulin on glucose metabolism, because the glucose transporter isoform GLUT-3 is not present in the membrane.47 Thus, the simplicity of the human RBC is offering the unique opportunity of unraveling the connection between insulin and the enhanced Na+-H+ exchange activity found in human hypertension. The increased Na+-H+ exchange activity might not be determined only by the hyperinsulinemic state but also by the synergic interaction with cytosolic Ca2+, another antiporter modulator, which has been reported to have elevated values in RBCs of hypertensive patients.48

In summary, our studies reveal that increased Na+-H+ exchange activity as measured in RBCs is present in black hypertensive subjects with insulin-resistant glucose disposal. Our results also demonstrate that despite impaired insulin-mediated glucose disposal in hypertensive subjects, the cellular Na+ gain via enhanced activity of Na+-H+ exchange is not blunted.

Acknowledgments

Supported by grants HL-35664, HL-42120, and HL-31802 from the National Heart, Lung, and Blood Institute. The technical assistance of Anda Spalvins, BS, in the red blood cell transport assays and of Harvey Kushner, PhD, in the stepwise multiple regression analysis is gratefully acknowledged.
References


Red blood cell sodium-proton exchange in hypertensive blacks with insulin-resistant glucose disposal.
M Canessa, B Falkner and S Hulman

Hypertension. 1993;22:204-213
doi: 10.1161/01.HYP.22.2.204

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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