Hypertension, Non-Insulin-Dependent Diabetes, and Intracellular Sodium Metabolism

MAURIZIO TREVISAN, OLGA VACCARO, MARTINO LAURENZI, FRANCESCO DE CHIARA, MICHELE DI MURO, ROBERTO IACONE, AND ANTONIETTA FRANZESE

SUMMARY The present study was designed to investigate whether non-insulin-dependent diabetic hypertensive patients exhibit abnormalities in intracellular sodium metabolism similar to those described for essential hypertensive patients. Both normotensive and hypertensive non-insulin-dependent diabetic patients had similar average values of both Na⁺-Li⁺ countertransport and Na⁺-K⁺ cotransport compared with nondiabetic controls. Within the group of diabetic patients, hypertensive patients did not exhibit any abnormalities in either of the sodium transport pathways studied. The possible implications of these findings are addressed. (Hypertension 11: 264–268, 1988)

KEY WORDS • hypertension • diabetes • countertransport • cotransport • ion transport • erythrocytes

THE association between hypertension and altered intracellular sodium metabolism has been the focus of much scientific investigation.1–8 Hypertensive patients have been documented to have abnormalities in various aspects of the intracellular ion metabolism. The Na⁺-Li⁺ countertransport and the Na⁺-K⁺ cotransport are among the intracellular ion transport pathways that have been analyzed.3–8 On the other hand, both alteration in glucose metabolism and overt diabetes are strongly associated with hypertension.9–13 To our knowledge, no data have been presented to date on the relationship between hypertension, diabetes and sodium transport abnormalities. The present report focuses on some aspects of the intracellular sodium metabolism (Na⁺-Li⁺ countertransport and Na⁺-K⁺ cotransport) in hypertensive and normotensive diabetic patients and in a group of non-diabetic normotensive and hypertensive subjects.

Patients and Methods

Non-insulin-dependent diabetic white male patients were selected from the diabetic outpatient clinics of the Department of Internal Medicine and Metabolic Diseases of the Second Medical School in Naples, Italy. Male normotensive and hypertensive diabetic patients were matched by age (±1 year), body mass index (±1 unit), and antidiabetic treatment (orally administered hypoglycemic drugs, n = 11; insulin, n = 11). Patients with proteinuria (Albustik-positive), with serum creatinine of 1.5 mg/dl or more, or who were taking digitalis, or had other endocrine disorders were excluded, and only patients whose weight had remained stable during the past 6 months were selected. Male nondiabetic normotensive and hypertensive controls were selected and paired by age and body mass index with the diabetic patients from a population-based epidemiological study on hypertension. At the onset of the study, nondiabetic and diabetic participants were defined as hypertensive if they were taking antihypertensive medications or if they had systolic blood pressure readings of 140 mm Hg or more, diastolic blood pressure readings of 90 mm Hg or more, or both. Of the hypertensive nondiabetic participants, 14 were taking antihypertensive medications and eight were untreated. Of the untreated, five were borderline hypertensive (systolic blood pressure >140 and <160 or diastolic blood pressure >90 and <95 mm Hg or both) and three had established hypertension (SBP >160 or DBP >95 mm Hg or both). No difference was detected among these three different groups with regard to Na⁺-Li⁺ countertransport or Na⁺-K⁺ cotransport. These findings were confirmed by analyses of the data of the epidemiological study population comparing treated with untreated and borderline with established hypertensive patients.4 (Diabetic hypertensive patients were all taking antihypertensive medications.) The diabetic
patients receiving orally administered hypoglycemic drugs were all taking sulfonylureas. Antidiabetic and antihypertensive medications were not discontinued before the blood drawing because of previous evidence suggesting a lack of effect of drug treatment on Na\(^+\)-Li\(^+\) countertransport and Na\(^+\)-K\(^+\) cotransport.\(^{8,15,16}\)

Previous results from clinical and epidemiological investigations presented evidence for a difference of approximately 20% in the average level of Na\(^+\)-Li\(^+\) countertransport between hypertensive and normotensive subjects.\(^{7}\) Such an expected difference was used to calculate the sample size needed for a statistical power of 0.80 with an \(\alpha\) level of 0.05, with a two-tailed \(t\) test.

Blood pressure was measured in all of the subjects with a standard mercury sphygmomanometer. The measurements were performed by observers trained according to the Hypertension Detection and Follow-up guidelines.\(^{17}\) The first and fifth phases of the Korotkoff sounds were recorded. The values expressed here are the average of two subsequent readings 1 minute apart. Pulse was measured during the blood pressure measurement.

Plasma glucose and serum creatinine were determined by autoanalyzer. Glycosylated hemoglobin was determined with microcolumns by chromatographic method.\(^{18}\)

Venous blood was drawn regardless of the fasting state. The blood was centrifuged, and the plasma and buffy coats were removed after centrifugation at 4°C. The cells were then suspended in a preserving solution according to the method described by Canessa et al.,\(^{3}\) with some modifications. Briefly, cells were washed three times with cold isosmotic choline chloride solution (choline, 149 mM; MgCl\(_2\), 1 mM; Tris MOPS, 10 mM). Then, 1 ml of packed cells was placed in 5 ml of loading solution (LiCl, 150 mM; Tris MOPS, 10 mM) and incubated at 37°C for 3 hours. After the incubation, the cells were washed four times with isosmotic cold choline chloride solution. After the final wash, the packed cells were suspended with an approximately equal volume of cold washing solution. The hematocrit of the cell suspension was determined, and 0.7 ml of the cell suspension was added to two tubes containing 7 ml of efflux media (one with furosemide and the other one without furosemide). The tubes containing the furosemide had 7 \(\mu\)l of a 1-M furosemide solution. Then, 1.5 ml of the suspension was divided into five tubes, and the efflux part of the assay was performed as for the Na\(^+\)-Li\(^+\) countertransport. A detailed description and methodological evaluation of this method have been described elsewhere.\(^{20}\)

The maximal velocity of the Na\(^+\)-Li\(^+\) countertransport was determined according to the method described by Canessa et al.,\(^{3}\) with some modifications. Briefly, cells were washed three times with cold isosmotic choline chloride solution (choline, 149 mM; MgCl\(_2\), 1 mM; Tris MOPS, 10 mM). Then, 1 ml of packed cells was placed in 5 ml of loading solution (LiCl, 150 mM; Tris MOPS, 10 mM) and incubated at 37°C for 3 hours. After the incubation, the cells were washed four times with isosmotic cold choline chloride solution. After the final wash, the packed cells were suspended with an approximately equal volume of cold washing solution. The hematocrit of the cell suspension was determined, and 0.7 ml of the cell suspension was added to two tubes containing 7 ml of efflux media at 4°C: one tube with sodium (NaCl, 150 mM; MgCl\(_2\), 1 mM; Tris MOPS, 10 mM; glucose, 10 mM; ouabain, 0.1 mM) and one tube without sodium (choline, 148 mM; MgCl\(_2\), 1 mM; Tris MOPS, 10 mM; glucose, 10 mM; ouabain, 0.1 mM). Next, 1.5 ml of this solution was divided into five tubes. Two of the two tubes were placed at 4°C in (water and crushed ice), while three tubes were incubated at 37°C for 60 minutes (in a water bath). All of the tubes were capped. The two tubes placed at 4°C were centrifuged (for 5 minutes), and the supernatant was then aspirated and saved for the determination of the lithium concentration. At the end of the incubation (60 minutes), the three tubes were placed briefly in water and crushed ice and then centrifuged (4°C for 5 minutes) and the supernatant was then aspirated and saved for the determination of the lithium concentration.

The maximal velocity of the Na\(^+\)-K\(^+\) cotransport was determined after loading the cells with the ionophore nystatin.\(^{20,21}\) After washing the cells twice with an isosmotic solution of choline chloride, 1 ml of packed cells was placed in 5 ml of loading solution (4°C) containing 60 \(\mu\)l of freshly prepared nystatin solution (3 mg in 1 ml of dimethylsulfoxide). The cells were then incubated for 20 minutes while protected from light at 4°C. After the incubation, cells were centrifuged at 4°C for 3 minutes, and then the supernatant was aspirated and discarded and replaced with new nystatin loading solution for 10 more minutes. The final concentration of the intracellular sodium was 50 mmol/L red blood cells. After the second incubation, the cells were centrifuged at room temperature and washed four times with a warm nystatin washing solution. The removal of the extracellular sodium and potassium was then achieved by washing the cells four times with cold isosmotic choline chloride solution. The packed cells were then suspended with an approximately equal volume of cold choline chloride solution. Next, 0.7 ml of the suspension was added to two tubes containing 7 ml of cold efflux media (one with furosemide and the other one without furosemide). The tubes containing the furosemide had 7 \(\mu\)l of a 1-M furosemide solution. Then, 1.5 ml of the suspension was divided into five tubes, and the efflux part of the assay was performed as for the Na\(^+\)-Li\(^+\) countertransport.

One of every 10 specimens was submitted as a blind duplicate to the laboratory for the determination of technical error. The technical errors, expressed as the percentage of the mean of the duplicates, are as follows: Na\(^+\)-Li\(^+\) countertransport, 6.4%; Na\(^+\)-K\(^+\) cotransport, 7.2%; plasma glucose, 7%; serum creatinine, 4%; glycosylated hemoglobin, 8%.

Statistical comparisons were performed using the unpaired \(t\) test for unmatched data and the paired \(t\) test for matched data.

**Results**

The characteristics of the four groups of participants are summarized in Table 1. The average age and body mass index of the participants reflect a successful
matching procedure. As expected, plasma glucose was significantly higher in the two diabetic groups compared with the control groups, while no significant difference was detected between normotensive and hypertensive diabetic patients with regard to plasma glucose and glycosylated hemoglobin. Serum creatinine was higher in hypertensive diabetic patients than in normotensive nondiabetic controls (p < 0.01). On the average, heart rate was higher in diabetic compared with nondiabetic participants, but this difference did not reach statistical significance.

The average values of the maximal velocity of the Na⁺-Li⁺ countertransport and Na⁺-K⁺ cotransport in the diabetic participants according to their antidiabetic treatment are presented in Table 2. There was no evidence for a statistically significant difference in the intraerythrocytic ion transport pathways between diabetic patients taking orally administered hypoglycemic drugs and diabetic patients taking insulin. With regard to the Na⁺-Li⁺ countertransport, no difference was detected between normotensive and hypertensive diabetic patients (Table 3), while a significant difference was evident between nondiabetic normotensive and hypertensive participants, with the hypertensive subjects showing on average higher values than the normotensive subjects. Na⁺-K⁺ cotransport did not differ significantly among the four different groups of participants.

### Table 1. Characteristics of Diabetic and Nondiabetic Participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n = 22)</th>
<th>Hypertensive (n = 22)</th>
<th>Normotensive (n = 22)</th>
<th>Hypertensive (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>57.1 ± 4.6</td>
<td>57.3 ± 5.9</td>
<td>57.3 ± 4.9</td>
<td>57.2 ± 5.0</td>
</tr>
<tr>
<td>Body mass index</td>
<td>27.1 ± 3.2</td>
<td>27.2 ± 3.3</td>
<td>27.1 ± 3.3</td>
<td>27.2 ± 3.1</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>136.7 ± 9.7</td>
<td>151.9 ± 16.7</td>
<td>135.8 ± 13.0</td>
<td>161.4 ± 13.4</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>84.1 ± 4.9</td>
<td>90.0 ± 11.1</td>
<td>81.9 ± 7.1</td>
<td>93.2 ± 10.4</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>75.2 ± 7.7</td>
<td>75.7 ± 10.9</td>
<td>71.0 ± 11.4</td>
<td>73.4 ± 10.7</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>178.1 ± 52.6*</td>
<td>172.9 ± 58.7*</td>
<td>90.6 ± 22.4</td>
<td>93.4 ± 23.1</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.2†</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Glycosylated hemoglobin (%)</td>
<td>8.6 ± 1.3</td>
<td>9.1 ± 1.5</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are means ± SD. *p < 0.001, compared with values for nondiabetic normotensive and hypertensive controls. †p < 0.01, compared with values for normotensive controls.

### Table 2. Na⁺-Li⁺ Countertransport and Na⁺-K⁺ Cotransport in Diabetic Participants According to Antidiabetic Treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Oral hypoglycemic drugs (n = 11)</th>
<th>Insulin (n = 11)</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺-Li⁺ CT (mmol/L RBC/hr)</td>
<td>0.275 ± 0.133</td>
<td>0.265 ± 0.104</td>
<td>0.278</td>
</tr>
<tr>
<td>(0.247–0.303)</td>
<td>(0.243–0.287) NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺-K⁺ COT (mmol/L RBC/hr)</td>
<td>0.856 ± 0.345</td>
<td>0.835 ± 0.353</td>
<td>0.200</td>
</tr>
<tr>
<td>(0.782–0.930)</td>
<td>(0.760–0.910) NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. Values in parentheses represent 95% confidence intervals.

CT = countertransport; COT = cotransport; RBC = red blood cells.

### Discussion

The present investigation was designed to analyze possible interrelationships among diabetes, hypertension, and two aspects of the erythrocyte cation transport. These potential interrelationships are of interest because of 1) the epidemiological and clinical findings of an association between diabetes and hypertension, 2) the evidence linking certain abnormalities of the erythrocyte cation transport and hypertension, 3) the hypothesis that insulin could play a major role in the development of hypertension in both diabetic and nondiabetic persons by enhancing sodium retention at the kidney level, 4) the evidence presented suggesting a correlation between the Na⁺-Li⁺ countertransport in erythrocytic and proximal tubular function.22

The findings of the present report seem to indicate that non-insulin-dependent diabetic patients do not show any alteration in either Na⁺-Li⁺ countertransport or Na⁺-K⁺ cotransport. In addition, they suggest that hypertensive non-insulin-dependent diabetic patients do not exhibit abnormalities in the maximal velocity of Na⁺-Li⁺ countertransport and Na⁺-K⁺ cotransport compared with normotensive diabetic patients. With regard to the nondiabetic population, our results indicate an increased Na⁺-Li⁺ countertransport in hypertensive compared with normotensive participants, while no significant difference is evident between these two groups with regard to the Na⁺-K⁺ cotransport. Our findings of an elevated countertransport in essential hypertensive subjects are in agreement with the majority of the findings presented by other investigators from both clinical and epidemiological investigations.5,7,8,23 The findings relating the Na⁺-K⁺ cotransport activity to hypertension are less clear and complicated by a number of methodological problems. In fact, different investigators have used different techniques and have studied different aspects of this ion transport pathway. Studies have been performed either in fresh cells or in artificially loaded cells to determine both the outward and inward components of this pathway. In addition, some studies have focused on the
maximal velocity, while others have tried to determine the affinity of this transport pathway. With regard to the outward components of the Na\(^+\)-K\(^+\) cotransport from erythrocytes loaded to achieve the maximal velocity of this transport pathway, the results are contrasting. In white essential hypertensive patients, this mode of transport of the Na\(^+\)-K\(^+\) cotransport has been found to be increased, decreased, or unchanged. The studies presented to date have mostly focused on a relatively small and selected group of patients, and further studies are needed to clarify the association between the outward maximal rate of the Na\(^+\)-K\(^+\) cotransport and hypertension.

The relationship between glucose metabolism and blood pressure has also been investigated. Diabetic patients have been shown to have a higher prevalence of hypertension compared with nondiabetic controls, and several epidemiological studies have presented evidence for a strong independent relationship of either fasting or postload plasma glucose to either systolic or diastolic blood pressure levels. Common mechanisms for the development of hypertension in diabetic and nondiabetic persons have been postulated. In particular, insulin, with its sodium retention action, has been considered a possible cause of hypertension in both diabetic and nondiabetic persons. The sodium retention activity of insulin could be due to its effect on membrane ion transport. These effects are not yet clearly defined. Insulin has been postulated to have a direct effect on potassium concentration due to a hyperpolarization effect on the membrane and secondary to a decrease in the sodium to potassium permeability ratio or to a stimulation of the Na\(^+\)-K\(^+\) pump. These effects on intracellular ion transport could be responsible for the sodium retention action that insulin has at the kidney level. Recently, the results of two studies reporting on the short-term effect of insulin on the intracellular sodium-potassium metabolism in both humans and experimental animals have challenged the hypothesis that insulin has a direct effect on the intracellular metabolism of both sodium and potassium. Our findings of a lack of effect of diabetes on Na\(^+\)-Li\(^+\) countertransport confirm previous findings by Beuckelmann and Erdmann, who reported no significant difference in Na\(^+\)-Li\(^+\) countertransport between controls and a group of nine male insulin-dependent diabetic patients. The present limited evidence, therefore, seems to suggest that non-insulin-dependent diabetes does not affect either Na\(^+\)-Li\(^+\) countertransport or Na\(^+\)-K\(^+\) cotransport.

At this time the pathogenesis of hypertension in diabetes, like that of essential hypertension, remains unknown. The roles of the central nervous system, the renin-angiotensin system, and catecholamines have been investigated, but these studies have not, to date, provided any conclusive statement. Whether or not the pathophysiological mechanisms responsible for the elevation in blood pressure are similar in diabetics and nondiabetic persons still remains to be ascertained. Our data seem to suggest that hypertensive diabetic patients do not share the abnormalities in Na\(^+\)-Li\(^+\) countertransport and Na\(^+\)-K\(^+\) cotransport observed in the nondiabetic hypertensive populations. If, as suggested by some authors, these abnormalities in the intraerythrocytic sodium metabolism reflect mechanisms that are pathophysiologically responsible for the elevation of blood pressure levels in hypertension, our results would indicate that the mechanisms responsible for hypertension in nondiabetic diabetic patients are different from those responsible for hypertension in nondiabetic subjects.

Further studies are needed to clarify the hypothesized link between abnormalities in erythrocyte ion transport and the pathophysiological mechanism responsible for the elevation of blood pressure in hypertension.

### Table 3. Na\(^+\)-Li\(^+\) and Na\(^+\)-K\(^+\) Cotransport in Diabetic and Nondiabetic Participants According to Their Hypertension Status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n = 22)</th>
<th>Hypertensive (n = 22)</th>
<th>Normotensive (n = 22)</th>
<th>Hypertensive (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+)-Li(^+) CT (mmol/L RBC/hr)</td>
<td>0.25 ± 0.1*</td>
<td>0.28 ± 0.1†</td>
<td>0.28 ± 0.1†</td>
<td>0.35 ± 0.1</td>
</tr>
<tr>
<td>Na(^+)-K(^+) COT (mmol/L RBC/hr)</td>
<td>0.91 ± 0.3</td>
<td>0.79 ± 0.3</td>
<td>0.89 ± 0.3</td>
<td>0.80 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SD. Values in parentheses represent 95% confidence intervals.

CT = countertransport; COT = cotransport; RBC = red blood cells.

*† = 3.89, p < 0.05; †t = 2.72, p < 0.05; compared with values for hypertensive nondiabetic controls.

### References
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