Insulin, Sodium–Lithium Countertransport, and Microalbuminuria in Hypertensive Patients

Giuseppe Andronico, Lidia Ferrara, Mariateresa Mangano, Giuseppe Mulè, Giovanni Cerasola

Abstract—Both microalbuminuria (>0.290 nmol/min [20 µg/min]) and high sodium–lithium countertransport (SLC) in diabetic or hypertensive humans are predictive of overt nephropathy and more aggressive cardiovascular complications, perhaps induced by insulin resistance. To analyze the relationships between microalbuminuria, SLC, microalbuminuria, and insulin in essential hypertension, we studied 90 hypertensive white patients, 25 of whom had microalbuminuria and 32 of whom were healthy. When urine sampling was completed for albuminuria determination, SLC was measured; all patients then underwent standard (75 g) oral glucose load to measure basal (0 minutes) and 2-hour glucose and insulin serum levels. Glucose-insulin ratio was used as insulin sensitivity index (ISI). In both hypertensive patients with normal microalbuminuria and those with pathological microalbuminuria, plasma insulin at 120 minutes was significantly higher than in control subjects. When the patients with pathological microalbuminuria were divided into thirds on the basis of their microalbuminuria, in the lower third, we found statistically significant less fasting insulin and higher basal ISI. SLC was higher in hypertensives than normotensives and, among hypertensives, higher in the subgroup with elevated microalbuminuria. In hypertensives, we found a weak but significant correlation between SLC and microalbuminuria, independent of insulin or ISI. The prevalence of high value of SLC (≥0.383 nmol · L⁻¹ · h⁻¹) was significantly lower in hypertensives with normal rather than abnormal urinary albumin excretion. Our results indicate that in nondiabetic hypertensive whites, higher microalbuminuria is accompanied by signs of insulin resistance; moreover, a link exists between SLC and microalbuminuria, both predictive of aggressive complications of hypertension. (Hypertension. 1998;31[part 1]:110-113.)

Key Words: insulin ■ sodium–lithium countertransport ■ microalbuminuria ■ insulin resistance ■ cardiovascular risk ■ nephropathy

Microalbuminuria, defined as urinary albumin excretion (UAE) rate >0.290 nmol/min (20 µg/min), has recently been recognized in diabetes mellitus predictive of overt nephropathy and more aggressive cardiovascular complications.1-3 It has also been related to 24-hour blood pressure levels in hypertensive nondiabetic subjects4,5 and regarded as an independent risk factor for renal and cardiovascular damage.4,6 Increased sodium–lithium countertransport (SLC) activity is another marker of renal and cardiovascular complications in diabetes7,8 and in essential hypertension,9,10 where it could be linked to an insulin–resistance condition.11-13 The aim of this study is to analyze the relationships between microalbuminuria, SLC, and insulin in essential nondiabetic hypertensive whites.

Methods

We studied 90 white patients with mild to moderate essential hypertension lasting no more than 5 years and 32 healthy subjects from laboratory and ward staff with comparable body mass index, race, and gender distribution (Table 1). All gave their informed consent, and the study protocol was approved by the ethics committee of the Internal Medicine Institute of our university.

Hypertensive subjects were studied after at least 2 weeks of pharmacological washout; secondary hypertension was excluded by clinical examination and the determination of levels of serum and urinary electrolytes, plasma renin activity, urinary and plasma aldosterone, and plasma catecholamines. All subjects were free of diabetes mellitus and other major diseases except hypertension.

After an 8-day period of eating a diet with a standard content of sodium (140 to 150 mEq/d), potassium (40 to 50 mEq/d), and protein (70 g/d), 24-hour urine samples were collected to evaluate UAE. When urine sampling was completed, the subjects underwent standard 75-g oral glucose load. Blood was drawn at basal (0 minutes) time for glucose, insulin, and SLC and was drawn again at 120 minutes after the glucose load to determine glucose and insulin serum levels. Urinary albumin content was measured by radioimmunoassay (Techno-Genetics); the sensitivity of the method is <0.006 nmol/mL (0.4 µg/mL), and the variability coefficient is <0.05.

Patients were divided according to percentiles of our laboratory normal reference value for microalbuminuria; group A (normoalbuminuric; n=55) consisted of subjects under the 75th percentile (0.160 nmol/min [11 µg/min]), group B (borderline; n=10) consisted of subjects with UAE between the 75th and 95th (0.290 nmol/min [20 µg/min]) percentile, and group C (microalbuminuric; n=25) consisted of subjects with microalbuminuria above the 95th percentile. Insulin was measured by radioimmunoassay (Insstar); the glucose-insulin ratio was used as an insulin sensitivity index (ISI).

Erythrocyte SLC was determined using the technique of Canessa et al14 with slight modifications as previously described.15 Briefly, eryth-

Received February 12, 1997; first decision March 26, 1997; revision accepted July 25, 1997.

From the Internal Medicine and Hypertension Centre, University of Palermo, Italy.

Reprint requests to Giuseppe Andronico, via Campolo 92, 90145 Palermo, Italy.

E-mail androp@unipa.it
© 1998 American Heart Association, Inc.

110
erythrocytes from heparinized venous blood were washed in sodium-free cold washing solution (10 mmol/L Tris-MOPS, 75 mmol/L MgCl₂, 85 mmol/L sucrose, and 10 mmol/L glucose; pH 7.4; 4°C) and were incubated in lithium chloride at 37°C to load cells to achieve an intracellular concentration of lithium >8 mmol/L. After 2 hours of incubation, red cells were washed and hematocrit was measured, and equal aliquots were incubated in the sodium-free washing solution containing 0.1 mmol/L ouabain or in a 150-mmol/L NaCl solution containing 0.1 mmol/L ouabain, 10 mmol/L glucose, 10 mmol/L Tris-MOPS (pH 7.4; 4°C) for 30, 60, and 90 minutes. After incubation, cells were centrifuged, and lithium concentration in the supernatant was measured. All the reagents were purchased from Sigma Chemical Co. The SLC was determined from the difference in intracellular concentration of lithium and those in the medium with sodium and was expressed as millimoles per liter of erythrocytes per hour.

To perform statistical analysis of means, we used ANOVA and appropriate post hoc tests when data were normally distributed (as in case of glycemia and insulinaemia); we used the Mann-Whitney and Kruskal-Wallis tests with the Dunn’s analysis for those parameters with skewed distribution (as glucose-insulin ratio, SLC, and microalbuminuria). Spearman’s correlation was performed to determine relationships, and the partial correlation coefficient formula was applied were appropriate. P<.05 was considered significant.

### Results

Fasting insulin plasma concentrations were higher in hypertensive subjects than in normotensive patients, but this difference was not statistically significant. After oral glucose load, in comparison with normal subjects, hypertensive patients showed a significantly higher insulin response (483.72±40.19 versus 260.95±27.55 pmol/L; P=.002) and lower ISI (24.37±3.44 versus 29.67±5.15 μmol/pmol; P=.027). Plasma glucose as well as insulin levels were significantly higher at 120 minutes after glucose load, both in the group of hypertensives with clear microproteinuria and in the group with normal UAE compared with normal subjects (Table 2). Group B, the subjects with an intermediate UAE rate, did not reach a statistically different value of these parameters. When the patients with pathological UAE (group C) were divided into thirds according to microalbuminuria level, in the lower third, we found less fasting insulin and a higher basal ISI than in normotensive patients, but this difference was not statistically significant.

### Table 1. Characteristics of Patients Under Study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normals</th>
<th>All</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>n, M/F</td>
<td>14/18</td>
<td>42/48</td>
<td>26/29</td>
<td>5/5</td>
<td>11/14</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.1±0.6</td>
<td>27.9±0.4</td>
<td>27.8±0.5</td>
<td>27.7±1.1</td>
<td>28.2±0.6</td>
</tr>
<tr>
<td>Office SBP, mm Hg</td>
<td>126.0±2.0</td>
<td>169.9±0.9</td>
<td>168.7±1.2</td>
<td>170.7±2.7</td>
<td>172.1±1.2</td>
</tr>
<tr>
<td>Office DBP, mm Hg</td>
<td>75.9±1.3</td>
<td>103.7±0.5</td>
<td>103.0±0.6</td>
<td>102.5±1.1</td>
<td>105.7±0.6*</td>
</tr>
<tr>
<td>Hypertension, y</td>
<td>...</td>
<td>3.5±0.1</td>
<td>3.5±0.2</td>
<td>3.5±0.3</td>
<td>3.4±0.2</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.42±0.06</td>
<td>1.48±0.04</td>
<td>1.46±0.05</td>
<td>1.50±0.10</td>
<td>1.50±0.07</td>
</tr>
</tbody>
</table>

Normal values are expressed as mean±SEM. (To convert albumin from nmol to μg, divide by 0.0145.)

*P<.001 versus group A.

### Table 2. Microalbuminuria, Sodium, Lithium Countertransport, and Metabolic Parameters in Studied Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>All</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAE, mmol/min</td>
<td>0.071±0.006</td>
<td>0.229±0.023</td>
<td>0.095±0.004</td>
<td>0.210±0.013</td>
<td>0.532±0.042</td>
</tr>
<tr>
<td>SLC, mmol · L · h</td>
<td>0.272±0.009</td>
<td>0.329±0.010†</td>
<td>0.305±0.010*</td>
<td>0.377±0.048</td>
<td>0.360±0.018‡§</td>
</tr>
<tr>
<td>B-glucose, mmol/L</td>
<td>5.05±0.05</td>
<td>4.94±0.06</td>
<td>4.99±0.07</td>
<td>4.78±0.17</td>
<td>4.90±0.12</td>
</tr>
<tr>
<td>2h-glucose, mmol/L</td>
<td>5.04±0.11</td>
<td>5.79±0.13‡</td>
<td>5.87±0.17†</td>
<td>5.08±0.20</td>
<td>5.88±0.28†</td>
</tr>
<tr>
<td>B-insulin, μmol/L</td>
<td>92.27±5.38</td>
<td>116.98±7.29</td>
<td>114.00±9.54</td>
<td>118.89±24.37</td>
<td>122.84±12.92</td>
</tr>
<tr>
<td>2h-insulin μmol/L</td>
<td>260.95±27.55</td>
<td>483.72±40.19†</td>
<td>445.78±40.83†</td>
<td>396.78±57.71</td>
<td>601.98±109.06§</td>
</tr>
<tr>
<td>B-ISI, μmol/L</td>
<td>63.92±6.11</td>
<td>64.16±5.16</td>
<td>68.63±7.20</td>
<td>61.67±14.74</td>
<td>54.57±7.06</td>
</tr>
<tr>
<td>2h-ISI μmol/L</td>
<td>29.67±5.15</td>
<td>24.37±3.44*</td>
<td>25.80±4.83</td>
<td>14.67±1.70</td>
<td>25.09±6.06</td>
</tr>
</tbody>
</table>

See Table 1 for explanation of subjects. UAE indicates urinary albumin excretion rate; SLC, sodium-lithium countertransport; B, basal, at time 0 of oral glucose tolerance test; and 2h, at 120 minutes after glucose load of oral glucose tolerance test. UAE difference between normal and all hypertensive groups was highly (P<.001) significant, as it obviously was between the hypertensive subgroups. Values are expressed as mean±SEM. (To convert albumin from nmol to μg, divide by 0.0145.)

*P<.05, †P<.01, ‡P<.001 vs normals, §P<.05 vs group A.
and 44% in the group with pathological UAE (group C); the group with normal UAE, 45.45% in group B, with borderline UAE, 39.28% in group A. The Spearman's correlation test performed in the hypertensive group has shown a weak but significant correlation between UAE and basal insulin ($r_P=0.242$; $P=.02$). This positive correlation between UAE and basal insulin was also found in each subgroup of patients: normomicroalbuminuric group A ($r_P=0.363$; $P=.008$), intermediate group B ($r_P=0.645$; $P=.05$), and microalbuminuric group C ($r_P=0.410$; $P=.04$). Moreover, glucose-insulin ratio at 120 minutes after glucose load was negatively related with UAE rate in all hypertensive subjects ($r_P=-0.214$; $P=.04$). As expected, SLC rate was higher in hypertensive than normotensive subjects ($0.329\pm0.010$ versus $0.272\pm0.009$ mmol·L$^{-1}$·h$^{-1}$; $P=.004$) and, among hypertensive patients, higher in group C than in group A ($0.360\pm0.018$ versus $0.305\pm0.010$ mmol·L$^{-1}$·h$^{-1}$; $P=.016$) (Table 2).

The Spearman's correlation test performed in the hypertensive group has shown a weak but significant correlation between SLC and UAE, independent of insulin or ISI when analyzed by means of partial correlation coefficient ($r_P=0.31; P=.03$). The prevalence of a high value of SLC, defined as a value $\geq 0.383$ mmol·L$^{-1}$·h$^{-1}$ (97.5th percentile of values in our normal population), was 16.36% in group A hypertensives, with normal UAE, 45.45% in group B, with borderline UAE, and 44% in the group with pathological UAE (group C); the difference between groups A and C was significant ($\chi^2=5.6; P=.018$).

### Discussion

In recent years, it has become evident that humans with maturity-onset diabetes mellitus more often and rapidly develop complications of diabetes such as nephropathy if there are early signs of an UAE rate higher than normal (ie, $>0.290$ mmol/min [20 $\mu$g/min])$^{15}$; moreover, both cardiovascular morbidity and mortality are higher in this subset of diabetic patients.$^{2,16}$

Thereafter, UAE has been studied in hypertensive nondiabetic patients, and relationships between increased excretion rate and early cardiovascular complications from hypertension were found,$^{15,18}$ so that microalbuminuria can be regarded as a marker of renal and cardiovascular risk in arterial hypertension.$^{19,20}$ SLC is a mechanism of transmembrane cationic flux that has been proposed as representative of Na$^+$-H$^+$ exchange when pH$_1$ is $>7.0$.$^{21}$

Since the first report by Canessa et al,$^{15}$ many studies have shown a link between this transport system and arterial hypertension.$^{14,21,22}$ Moreover, hypertensive humans with high SLC exhibit more marked family history of cardiovascular morbidity and mortality$^{23}$ and experience early cardiovascular complications.$^{14,21-26}$

SLC has been also considered a genetic marker for hypertension,$^{7-33}$ and it is well used in studies on arterial hypertension, in spite of the fact that more than 15 years have passed since the first report,$^{14,22}$ possibly even because other more direct genetic markers for primary hypertension have not been found to date. Both microalbuminuria and SLC, on the other hand, have been linked with insulin resistance,$^{1,13}$ which characterizes the hypertensive population.

In this study, indeed, the hypertensive group, particularly after glucose load, showed higher insulin levels and lower ISI. We chose the glucose-insulin ratio as the ISI because insulin resistance produces the higher circulating insulin levels that are required to obtain a specific blood glucose value$^{12}$ and this is a practical and feasible procedure for ambulatory studies that give good estimates of insulin sensitivity.

Moreover, in our work, the highest levels of insulin and insulin resistance were in the groups of hypertensive patients with the most microalbuminuria. This finding is in accordance with the work of Bianchi et al,$^{13}$ which reports a significant relationship in hypertensive population between UAE rate and insulin area under the curve after standard glucose load. Our investigation, which has been conducted in a larger number of patients, shows in all hypertensives, independent of body mass index, a negative relationship between UAE rate and ISI both at fasting and after glucose load.

Additionally, to try to understand the weighting of the group of hypertensive patients with pathological UAE by using correlations, we performed separate tests for hypertensive subgroups. The negative relationship with ISI was found not only in the group with pathological microalbuminuria but also in the group with certain normal (<0.160 mmol/min [<$11$ $\mu$g/min]) albumin excretion; this finding suggests that in hypertensive subjects, UAE is somehow linked to insulin resistance. Because no similar correlation could be found in healthy subjects, this issue is still in doubt and deserves broader study.

Yip et al$^{34}$ recently published an article in which they report that insulin resistance occurs independently of microalbuminuria in patients with hypertension; this is in accordance with our results that show higher insulin response to glucose load also in hypertensive patients without pathological albuminuria;

### Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st Third (nmol/min)</th>
<th>2nd Third (nmol/min)</th>
<th>3rd Third (nmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAE</td>
<td>0.345±0.010</td>
<td>0.460±0.018†</td>
<td>0.761±0.057†</td>
</tr>
<tr>
<td>B-Insulin, pmol/L</td>
<td>103.68±15.86</td>
<td>87.18±17.22</td>
<td>171.48±23.53*</td>
</tr>
<tr>
<td>2h-Insulin, pmol/L</td>
<td>423.33±124.85</td>
<td>505.12±134.17</td>
<td>846.65±248.26</td>
</tr>
<tr>
<td>B-ISI, µmol/pmol</td>
<td>58.50±12.55</td>
<td>67.84±13.24</td>
<td>39.28±9.28*</td>
</tr>
<tr>
<td>2h-ISI, µmol/pmol</td>
<td>27.45±8.40</td>
<td>33.42±15.18</td>
<td>15.58±5.65</td>
</tr>
</tbody>
</table>

UAE indicates urinary albumin excretion rate; B, basal, ie at time 0 of oral glucose tolerance test and 2h, at 120 minutes after glucose load of oral glucose tolerance test. Values are expressed as mean±SEM. (To convert albumin from nmol to $\mu$g, divide by 0.0145.)

$^*P<.05$, †$P<.001$ vs 1st third.
in that work, among the hypertensives, the four subjects with microalbuminuria exhibited values of insulin resistance in its upper distribution.

In our investigation, office diastolic blood pressure in patients with normal albuminuria was lower than in patients with pathological microalbuminuria, but no correlation was found between microalbuminuria and blood pressure, probably because blood pressure measurements taken in the office are not suitable for correlation studies.

The percentage of hypertensive patients with high SLC in the present study was significantly larger in the group with microalbuminuria than in the group with normal albuminuria, and the positive correlation we found between SLC and albuminuria was independent of insulin or ISI. These results do not agree with those of Giampietro et al,38 who found no difference of Na\(^+\)/H\(^+\) exchange between hypertensive patients with pathological and normal UAE rates. We have to emphasize, however, that sodium-proton exchange and SLC do not necessarily reflect the same phenotype or genotype39 and that SLC is, between these, the most studied transport system in epidemiological investigations on predictors of hypertension.30,36,37 On the other hand, in agreement with our results, Falkner et al36 demonstrated a relationship between UAE rate and SLC in young adult African Americans with borderline to mild hypertension.

In conclusion, our data show that even in mild to moderately hypertensive whites, there is a relationship between insulin resistance and microalbuminuria and confirm that it can be linked more with genetically determined metabolic alteration, as the correlation with the SLC indicates, than with hypertension alone.

Acknowledgment

This work was supported by a grant ("60% quota") from the Italian Ministry for University and Scientific Research (MURST).

References

Insulin, Sodium-Lithium Countertransport, and Microalbuminuria in Hypertensive Patients

Giuseppe Andronico, Lidia Ferrara, Mariateresa Mangano, Giuseppe Mulè and Giovanni Cerasola

Hypertension. 1998;31:110-113
doi: 10.1161/01.HYP.31.1.110

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
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:
http://hyper.ahajournals.org/content/31/1/110

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/