Sodium-Lithium Countertransport in Microalbuminuric Insulin-Dependent Diabetic Patients

Sharon L. Jones, Roberto Trevisan, Taimur Tariq, Andrea Semplicini, Martin Mattock, James D. Walker, Romano Nosadini, and Giancarlo Viberti

A familial predisposition to arterial hypertension has recently been suggested as one important component of the susceptibility to diabetic kidney disease. Sodium-lithium countertransport activity, a marker of risk for essential hypertension, has been found to be increased in diabetic patients with overt nephropathy. We have measured red blood cell sodium-lithium countertransport activity in 36 microalbuminuric insulin-dependent diabetic patients, a group at high risk of progression to clinical nephropathy and cardiovascular disease, and compared it with that of a matched group of 36 normoalbuminuric diabetic patients. Sodium-lithium countertransport was higher in the microalbuminuric (0.43 [95% confidence interval (CI) 0.38–0.47] mmol/l red blood cells [RBC]/hr) than in the normoalbuminuric diabetic patients (0.29 [0.25–0.33] mmol/l RBC/hr, mean difference 0.14 [0.08–0.20]; p<0.0001). Microalbuminuric patients had a higher frequency of parental hypertension than normoalbuminuric diabetic patients (56% vs. 28%, p<0.05). Sodium-lithium countertransport was related to mean arterial pressure in the microalbuminuric patients (r=0.54, p<0.001) and to daily insulin requirements in both groups (microalbuminuric patients r=0.39, p<0.05; normoalbuminuric patients r=0.42, p<0.01). In a subset of patients in whom lipoproteins were measured, sodium-lithium countertransport activity was related to total and very low density lipoprotein triglycerides (r=0.41, p<0.05 and r=0.48, p<0.05) and to apolipoprotein B (r=0.56, p<0.05), independently of body mass index, albumin excretion rate, glycemic control, and insulin dose. Thus, an overactivity of sodium-lithium countertransport occurs in microalbuminuric insulin-dependent diabetic patients and is independently associated with a higher blood pressure and a more atherogenic lipoprotein profile. (Hypertension 1990;15:570–575)
betic patients with and without microalbuminuria and to evaluate its potential contribution to the cardiovascular risk factors associated with microalbuminuria.

**Methods**

Thirty-six insulin-dependent diabetic patients identified in a screening program of urinary albumin excretion in our diabetic clinic as having persistent microalbuminuria (overnight albumin excretion rates between 20 and 200 μg/min in at least two of three collections) were compared with a group of 36 insulin-dependent diabetic patients of similar sex distribution, age (±5 years), and duration of diabetes (±3 years) randomly selected among those with albumin excretion rates in the normal range (<20 μg/min). All patients were between 16 and 60 years old and were of European origin. Their clinical features are shown in Table 1. Fifteen of the patients with microalbuminuria were treated for arterial hypertension with a variety of drugs, including diuretics, calcium antagonists, and loop diuretics. All antihypertensive drugs were discontinued for 1 week before the study, which was approved by our Ethical Committee.

Patients were asked to attend a metabolic ward between 8:30 and 9:00 AM after they had fasted from 10:00 PM the previous evening. They were weighed, without shoes, in light indoor clothing; height was measured to the nearest centimeter and a full medical history recorded. Consent was obtained from all patients before the study, which was approved by our Ethical Committee.

Patients were asked to attend a metabolic ward between 8:30 and 9:00 AM after they had fasted from 10:00 PM the previous evening. They were weighed, without shoes, in light indoor clothing; height was measured to the nearest centimeter and a full medical history recorded. After the patient had rested 10 minutes in a supine position, blood pressure (phase I/V) was measured twice within a 5-minute interval to the nearest 2 mm Hg with a random zero sphygmonanometer. Mean arterial pressure was calculated as diastolic plus one third pulse pressure. Family history of hypertension was investigated by a questionnaire that was sent to the living parent or parents; if neither parent was alive the information was regarded as unavailable. A positive history of parental hypertension was recorded if one or both parents had been diagnosed hypertensive before the age of 60 and were taking antihypertensive medication.

Morning insulin was withheld until after fasting blood samples had been drawn for measurement of glycated hemoglobin (Corning gel electrophoresis, Corning, Palo Alto, California), creatinine (Hitachi autoanalyzer, BCL, Lewes, UK), and Na⁺-Li⁺ countertransport activity as previously described. In the last 30 patients examined, fasting serum was separated and stored at 4°C for determination of lipidoprotein profile within 5 days. Low density (LDL), very low density (VLDL), and high density (HDL) lipoproteins were separated by ultracentrifugation and the subfractions of HDL by a combination of precipitation and ultracentrifugation. Concentrations of cholesterol and triglycerides were measured by enzymatic colorimetric techniques on a Cobas-Bio analyzer (Roche Diagnostica, Welwyn Garden City, UK). Apolipoproteins A1 and B were measured by immunoturbidimetry with a Cobas Bio analyzer using Orion Diagnostica antiserum and reagents (Oxoid, Basingstoke, Hants, UK). Each patient was instructed in the collection of a timed overnight urine collection to be completed on the morning of the study. Volume was measured to the nearest 2 ml, and an aliquot was taken for measurement of albumin concentration. Albumin excretion rates were expressed as micrograms per minute. A fresh midstream specimen of urine was collected for culture to exclude the presence of infection. Glomerular filtration rate was measured by [51Cr]EDTA clearance on the day of the study visit or within 6 months.

**Statistical Analysis**

Results of albumin excretion rate and of a number of lipids and lipoprotein subfractions were log-transformed before analysis. The significance of differences was tested by using unpaired Student's t test and the comparative distribution of variables by χ² test. Results are expressed as mean±SEM or mean and 95% CI unless otherwise stated. The relation between different variables was tested by univariate linear regression analysis, and multivariate analysis was used to estimate the independent associations of a number of variables.

**Results**

Urinary albumin excretion ranged between 21.3 and 198.8 μg/min, with a geometric mean of 40.6 μg/min in the microalbuminuric group compared with a range of 1.5 to 161.1 μg/min with a geometric mean of 5.2 μg/min in the normoalbuminuric group. No significant difference was found between the microalbuminuric and normoalbuminuric patients in glycemic control (HbA₁c; microalbuminuric vs. normoalbuminuric patients: 8.4±0.3% vs. 8.2±0.3%), daily insulin dose (0.8±0.1 vs. 0.7±0.1 IU/kg/24 hr), and glomerular filtration rate (120±4 vs. 115±3 ml/min/1.73 m²). The rate of Na⁺-Li⁺ countertransport was significantly higher (0.43 [95% CI 0.38–

### Table 1. Clinical Features of Microalbuminuric and Normoalbuminuric Insulin-Dependent Diabetic Patients

<table>
<thead>
<tr>
<th>Patient features</th>
<th>Diabetic patients with microalbuminuria</th>
<th>Diabetic patients with normoalbuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>No (M:F)</td>
<td>27:9</td>
<td>24:12</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>33 (16–53)</td>
<td>33 (15–58)</td>
</tr>
<tr>
<td>Duration of diabetes (yr)</td>
<td>15 (5–32)</td>
<td>13 (3–33)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24 (19–32)</td>
<td>24 (19–30)</td>
</tr>
</tbody>
</table>

Results are shown as mean (range).
cant positive correlations were found between Na⁺-Li⁺ countertransport activity and total and VLDL triglycerides (r=0.41, p<0.05 and r=0.48, p<0.01), VLDL cholesterol (r=0.43, p<0.05), and apolipoprotein B (r=0.56, p<0.05) (Figure 2). A negative correlation was found with HDL₂ cholesterol (r=-0.39, p<0.05). When each lipoprotein was entered into a multiple regression analysis as a dependent variable with Na⁺-Li⁺ countertransport activity, albumin excretion rate, HbA₁c, and insulin per kilogram as independent variables, a positive independent correlation with Na⁺-Li⁺ countertransport persisted for total and VLDL triglycerides (r=2.4, p<0.05 and t=2.3, p<0.05).

Discussion

Insulin-dependent diabetic patients with microalbuminuria, a group at high risk of overt renal disease,⁴⁻⁵,¹¹ have elevations of Na⁺-Li⁺ countertransport activity similar to those previously reported in diabetic patients with clinical nephropathy.⁸⁻¹⁰ Renal function was normal in our patients, which excluded the possibility that abnormalities of Na⁺-Li⁺ countertransport could be a consequence of renal failure. Other environmental factors such as weight, pregnancy, and thyroid disease that could contribute to the differences in Na⁺-Li⁺ countertransport²³⁻²⁶ were also excluded in our study. Our findings suggest that an increased Na⁺-Li⁺ countertransport activity is a feature of those diabetic patients susceptible to nephropathy, which is detectable before the condition is clinically overt. The activity of Na⁺-Li⁺ countertransport is bimodally distributed in the general population²⁷ and is believed to be under strong genetic influence.²⁸ It has been found to be raised in patients with essential hypertension,⁹⁻¹⁰ and it is therefore of interest that the diabetic group with higher Na⁺-Li⁺ countertransport activity also had significantly higher arterial pressures and a stronger family history of hypertension than the group with normal albumin excretion rates. That microalbuminuria is associated with higher levels of blood pressure has now been reported by several groups.⁴⁻⁵,⁶⁻¹⁰,¹¹,¹⁰ Moreover, in a small group of 12 diabetic patients with albumin excretion rates between 70 and 200 μg/min in whom the arterial pressure was not particularly elevated (diastolic arterial pressure, microalbuminuric group vs. normoalbuminuric group, 83 vs. 76 mm Hg), Krolewski et al.⁸ found higher mean levels of Na⁺-Li⁺ countertransport, which suggests that Na⁺-Li⁺ countertransport is a marker of the predisposition to hypertension in microalbuminuria. High rates of Na⁺-Li⁺ countertransport have been found to be associated not just with arterial hypertension but more specifically with subgroups of hypertensive patients⁶ with a higher frequency of hypertension and increased risk of cardiovascular disease.³¹,³² Thus, factors related to cardiovascular disease or the predisposition to it, of which Na⁺-Li⁺ countertransport activity is an indicator, could be involved in the susceptibility to renal disease in diabetic patients.
FIGURE 2. Scatterplots showing relation between sodium-lithium countertransport activity (Na⁺/Li⁺-CT) and very low density lipoprotein cholesterol (cholesterol VLDL) (upper left panel), total triglycerides (upper right panel), very low density lipoprotein triglycerides (triglycerides VLDL) (lower left panel), and apolipoprotein B (lower right panel) in 30 nonclinically proteinuric insulin-dependent diabetic patients.
Although as a group microalbuminuric diabetics had higher rates of Na\(^+-\)Li\(^+\) countertransport, there was a wide scatter of values and considerable overlap with the normoalbuminuric group. Similar overlaps in Na\(^+-\)Li\(^+\) countertransport have been shown between normotensive and essentially hypertensive patients.\(^{33}\) This distribution of values is consistent with the view that the level of biological observation provided by Na\(^+-\)Li\(^+\) countertransport is still relatively nondiscriminatory. It is, however, worth noting that only 11% of normoalbuminuric patients had Na\(^+-\)Li\(^+\) countertransport activity above the upper limits of the normal range (in our laboratory 0.12–0.41 mmol/l RBC/hr, \(n=35\)), whereas 56% of microalbuminuric patients did so. The imprecision of our discrimination may also partly derive from at least two other factors. First, albumin excretion rate has a biological variability of approximately 40%,\(^{34}\) and its predictive power is around 80%,\(^{35,36}\) which suggests that a proportion of microalbuminuric patients may not progress to overt nephropathy. Second, some of the presently normoalbuminuric patients with supranormal Na\(^+-\)Li\(^+\) countertransport may be candidates for the development of microalbuminuria at a later date. Whether in the long term Na\(^+-\)Li\(^+\) countertransport will prove a more sensitive marker of overt renal disease than microalbuminuria remains to be established. Their strong association indicates an interrelation the nature of which can only be solved by further studies. The possibility remains that the possession of a high Na\(^+-\)Li\(^+\) countertransport is peculiar to a large subset of patients in whom nephropathy develops, but that other mechanisms, independent of the phenomenon of which overactivity of Na\(^+-\)Li\(^+\) countertransport is a marker, are active in the remainder of the patients.

Although the significance of a raised Na\(^+-\)Li\(^+\) countertransport remains to be elucidated, recent evidence suggests that Na\(^+-\)Li\(^+\) countertransport is a mode of operation of the physiological sodium-hydrogen (Na\(^+-\)H\(^+\)) antiport in the proximal tubular reabsorption of sodium.\(^{40}\) Increased rates of activity of Na\(^+-\)H\(^+\) antiport may lead to renal sodium retention and reflect mechanisms that control vascular smooth muscle and mesangial cell hypertrophy and hypercontractility, which could partially be responsible for the pathophysiological and morphological changes involved in the genesis of renal damage and hypertension in diabetes. The strong association of microalbuminuria with raised blood pressure and Na\(^+-\)Li\(^+\) countertransport activity supports this contention.

Of further interest and potential pathogenic importance in the vascular damage of diabetics with renal disease is the positive relation found in this study between Na\(^+-\)Li\(^+\) countertransport activity, serum lipid levels, and insulin requirements. That Na\(^+-\)Li\(^+\) countertransport is related to serum lipid levels has also been reported in the general popu-

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**Acknowledgments**

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**References**

8. Krolewski AS, Canessa M, Warram JH, Laffel LMB, Christlieb AR, Knowler WC, Rand LI: Predisposition to hyper-

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**Footnotes and Notes**

41. Although the nature of this association has not been elucidated. We have recently reported that microalbuminuric patients show a more atherogenic lipoprotein profile than normoalbuminuric diabetic patients.\(^{37}\) Microalbuminuria has been shown to be predictive of cardiovascular mortality and morbidity both in the diabetic and nondiabetic population,\(^{12-16}\) and overactivity of the Na\(^+-\)Li\(^+\) countertransport has been associated with a history of cardiovascular morbidity and mortality in hypertensive patients with and without renal disease.\(^{33,32}\) Recent work has demonstrated an increased insulin resistance in hypertensive diabetic patients with elevation of Na\(^+-\)Li\(^+\) countertransport.\(^{33}\) It is thus possible that the strong association of Na\(^+-\)Li\(^+\) countertransport and microalbuminuria with lipid abnormalities is mediated by mechanisms that control insulin sensitivity.\(^{44}\) A positive correlation between insulin resistance and plasma concentration of VLDL triglycerides,\(^{45}\) as well as an inverse relation between plasma HDL cholesterol and insulin levels, have been described.\(^{46,47}\) Na\(^+-\)Li\(^+\) countertransport was in our study independently and more closely associated with plasma triglyceride levels, which according to a recent prospective study, appear to be a strong predictor of death from coronary heart disease\(^{48}\) although this is still a subject of debate.\(^{49}\)

The results of our study suggest that the activity of the Na\(^+-\)Li\(^+\) countertransport, which is constant through life and relatively little affected by the environment,\(^{28}\) may prove a potentially useful early marker of renal and cardiovascular complications in insulin-dependent diabetes.


**KEY WORDS** lipoproteins • sodium-lithium countertransport • microalbuminuria • blood pressure
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