Elevated sodium-lithium countertransport activity in erythrocytes is predictive of the development of microalbuminuria in IDDM

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Summary Pathogenetic mechanisms other than the quality of metabolic control may play a role in the development of diabetic nephropathy. Some cross-sectional studies have shown that elevated erythrocyte sodium-lithium countertransport (Na^+/Li^+ CT) activity may be linked to incipient or overt nephropathy in insulin-dependent diabetic (IDDM) patients. The aim of the present work was to ascertain if high erythrocyte Na^+/Li^+ CT activity anticipates the development of microalbuminuria in IDDM patients. Evaluation of this cation transport system was carried out in 159 normotensive, normoalbuminuric IDDM patients, who were divided into two groups: those with values above (Group A) and those with values below (Group B) the median level in the overall population ($300 \mu\text{mol}/\text{erythrocytes} \times \text{h}$). A total of 79 patients in Group A and 80 in Group B underwent periodic examinations over a similar time period (5.2 years, range 3.3–7.4 years and 5.4 years, range 3.4–7.5 years, respectively). Median sodium-lithium countertransport activity was stable when evaluated after 2 and 4 years of follow-up. Only seven patients were excluded from the protocol because changes in their sodium-lithium countertransport activity placed them on the other side of the median value with respect to their baseline measurement. Thus, 152 patients completed the study (76 in Group A and 76 in Group B). Of the 76 patients in Group A, 17 developed persistent microalbuminuria (22.3%). The number of patients in Group B showing persistent microalbuminuria was significantly lower (4 of 76; 5.2%;

$p < 0.01$). The sensitivity of erythrocyte Na^+/Li^+ CT in predicting the development of microalbuminuria was 85 % and its specificity was 55 %. Seven patients of Group A and five of Group B developed arterial hypertension. Subjects in Group A had significantly higher mean HbA_{1c} values of twice yearly measurements than those in Group B (9.6 ± 1.7 vs 8.3 ± 1.7 %, $p < 0.002$, mean $\pm$ SD) despite similar daily insulin requirements. Systolic and diastolic blood pressure levels were also evaluated every 6 months and were significantly higher in the Group A than in the Group B patients, although on average within the normal range. The odds ratio for developing persistent microalbuminuria in IDDM with elevated baseline erythrocyte Na^+/Li^+ CT activity after adjustment for gender and baseline albumin excretion rate, and mean 6 monthly plasma creatinine, HbA_{1c} and systolic and diastolic blood pressure levels was 4.2 (95 % confidence intervals 2.0–11.1). It was also found that the percentage of offspring having both parents with Na^+/Li^+ CT activity above the median value was significantly higher in Group A than in Group B (Group A vs Group B: 35 vs 19 %; $p < 0.01$). On the contrary the percentage of offspring whose erythrocyte Na^+/Li^+ CT was lower in both parents was lower in Group A than in Group B: 10 vs 38 %, $p < 0.01$). Parents of Group A offspring had arterial hypertension more frequently than those of Group B. These results indicate that erythrocyte Na^+/Li^+ CT activity is a useful diagnostic tool in identifying normotensive, normoalbuminuric

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Abbreviations: IDDM, Insulin-dependent diabetes mellitus; AER, albumin excretion rate; Na^+/Li^+ CT, sodium-lithium countertransport activity; ANOVA, analysis of variance.

patients who may be predisposed to develop persistent microalbuminuria. This disorder in the cation transport system is associated with poor metabolic control, higher blood pressure, and male sex; it also appears to be, at least partly, genetically transmitted. [Diabetologia (1997) 40: 654–661]

The Diabetes Control and Complications Trial (DCCT) [1] clearly showed that poor glycaemic control plays a primary role in determining the development of renal damage in insulin-dependent diabetes mellitus (IDDM). More recently, a threshold value of glycated haemoglobin A_{1c} (HbA_{1c}) has been identified. It was found that when HbA_{1c} is above 8.1 %, the risk of developing diabetic nephropathy increases sharply [2]. However, it has to be stressed that in the DCCT, although strict glycaemic control nearly halved the cumulative incidence rate of microalbuminuria during a 9-year follow-up, approximately 15 % of IDDM patients still developed renal complications, despite good metabolic control.

Thus, other factors appear to play an important role in the pathogenesis of diabetic nephropathy in IDDM. The development of overt proteinuria in the first 20 years of IDDM is closely correlated to the predisposition to essential hypertension [3]. Moreover, the occurrence of microalbuminuria in the first 20 years of IDDM is likewise related to three indices of predisposition to essential hypertension: family history of hypertension, adolescent blood pressure and erythrocyte sodium-lithium countertransport activity (Na⁺/Li⁺ CT) [4]. Two cross-sectional studies reported that elevated erythrocyte Na⁺/Li⁺ CT is associated with overt or incipient nephropathy in IDDM [3, 5]. These findings, however, have not been confirmed by other authors [6]. Moreover, no information is available on the importance of elevated erythrocyte Na⁺/Li⁺ CT in predicting micro- or macroalbuminuria in IDDM during prospective follow-up intervals.

The aim of the present study was to evaluate the role of erythrocyte Na⁺/Li⁺ CT as a risk factor for the development of microalbuminuria in normotensive, normoalbuminuric IDDM patients.

Subjects and methods

We recruited 171 consecutive IDDM patients attending the outpatient clinics of the Department of Internal Medicine and of the Department of Paediatrics of the University of Padova and the District Hospital of Camposampiero (Padova) between 1 October 1987 and 1 December 1992. All of them were asked to participate in a screening programme for urinary albumin excretion rate (AER) and erythrocyte Na⁺/Li⁺ CT. The selection criteria were the following: onset of diabetes before the age of 30 years, insulin treatment within the first

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3 months of diagnosis, sitting blood pressure below 140/90 mmHg [7], no antihypertensive therapy or clinical or biochemical evidence of renal diseases, serum creatinine less than 106 µmol/l, AER below 12 µg/min (mean ± 2 SD in normal control subjects in our laboratory) as median value of three daily timed urine specimens.

The 159 patients who met these requirements gave informed consent to participate in a longitudinal study whose protocol was reviewed and approved by the ethical committees of the University of Padova and of the District Hospital, whereas 12 patients did not agree to adhere to the protocol study.

Erythrocyte Na⁺/Li⁺ CT were measured in fasting venous blood specimens following methods described elsewhere [8, 9]. In the population of IDDM offspring erythrocyte Na⁺/Li⁺ CT was not significantly different between males and females (311 ± 135 µmol/l of erythrocyte per hour in males, 287 ± 154 in females, mean ± SD, NS) and was not related to gender and age (*r*: 0.04 and 0.02, NS, respectively). However, erythrocyte Na⁺/Li⁺ CT was significantly related to gender in the parents of IDDM offspring (*r* = 0.39, *p* < 0.05). Therefore, the IDDM offspring patients were divided into two groups on the basis of erythrocyte Na⁺/Li⁺ CT without matching for gender. Group A had values above and Group B levels below the median value, e.g. 300 µmol · l erythrocytes⁻¹ · h⁻¹ in the overall population. We also calculated the sensitivity positive, predicting value for the occurrence rate of microalbuminuria and the specificity negative, predicting value for the occurrence rate of microalbuminuria by adopting different cut-off points for erythrocyte Na⁺/Li⁺ CT values as described in detail elsewhere [10].

All subjects were weighed in indoor clothing without shoes, and height was also recorded. Blood pressure was measured (phases I and V) twice on the right arm to the nearest 2 mmHg using a Hawksley Random zero sphygmomanometer by two different authors of the present study (R.N. and G.M.). Measurement was made with subjects in a supine position after 10 min rest. Mean arterial pressure was calculated as the diastolic pressure plus one-third of the pulse pressure and the mean of the two measurements used for calculation. All patients were examined and blood was collected in the morning after an overnight fast. Patients' morning insulin injections were deferred until after this procedure. The same procedures were used to evaluate the clinical and biochemical features of the parents of IDDM patients. More particularly, arterial hypertension was diagnosed, when sitting blood pressure level was above 140/90 mmHg in untreated subjects or when the patients were taking antihypertensive therapy. This latter criterion was the case in the large majority of the parents.

All patients were prescribed a relatively low protein (1.0 g/day), low salt (90 mEq · 1.73 m⁻² · day⁻¹), isocaloric (2000 kcal · 1.73 m⁻² · day⁻¹) diet, which closely resembles the nutritional habits of the control population in northeast Italy. Patients were examined at baseline and every 6 months when HbA_{1c}, serum creatinine, plasma electrolytes, cholesterol, triglycerides, AER from three 24-h urine collections, body weight and

arterial blood pressure were measured. Erythrocyte Na⁺/Li⁺ CT was measured at baseline and after 2- and 4-years of follow-up. HbA_{1c} was measured by high-liquid pressure chromatography [11] and serum creatinine, triglycerides, cholesterol, sodium and potassium by automatic analyser [12]. AER was measured by radioimmunoassay, as described elsewhere [13].

Persistent microalbuminuria was defined as an AER above 20 µg/min at all subsequent measurements (at least three values every 6 months) after the first assessment. Intermittent microalbuminuria was defined as an AER not constantly above 20 µg/min at subsequent measurements (at least three values every 6 months). We also evaluated the cumulative absolute change of AER (µg/min) from baseline values during the 5.3-year follow-up period.

Statistical analysis

Standard descriptive statistics were used to describe the distribution of variables between the two patient groups. Values are given as mean ± SD or median with ranges. Comparisons between the values observed during the follow-up period in Group A and B offspring used a two-factor analysis of variance (ANOVA) with erythrocyte Na⁺/Li⁺ CT category and time (baseline and follow-up period) as the factors, when a significant interaction was shown by ANOVA. For the sake of clearness, exposition data for the follow-up period are expressed as mean values of subsequent determinations every 6 months. Chi-square tests, two-tailed Student's *t*-test or Mann-Whitney non-parametric test were used to assess the degree of statistical significance of differences. These tests were also used to analyse baseline values of offspring and their parents. As the descriptive measure of association between risk factors and each of the outcomes, odds ratios have been given together with 95% confidence intervals [14]. These odds ratios were based on the cumulative incidence rates of micro-macroalbuminuria during the 5.3-year follow-up interval (the average in the two pooled groups). Multiple logistic regression analysis was used to study the relationship between the occurrence rate of microalbuminuria (dependent variable) and the baseline rate of activity of erythrocyte Na⁺/Li⁺ CT (independent variable), while simultaneously controlling for gender and baseline AER, and the mean values of plasma creatinine, HbA_{1c} and systolic and diastolic blood pressure levels every 6 months during the follow-up period. These independent variables were chosen because of their univariate associations with the occurrence rate of microalbuminuria. The occurrence rate of persistent microalbuminuria was treated as dichotomous (AER above vs AER below 20 µg/min) and as continuous numerical variable (cumulative absolute change of AER (µg/min) from baseline. Gender and erythrocyte Na⁺/Li⁺ CT (above vs below the median value of 300 µmol · l erythrocytes⁻¹ · h⁻¹) were treated as dichotomous variables. Statistical analysis was carried out using SPSS 6.1 Base System software (SPSS Inc., Chicago, Ill., USA).

Results

Sodium-lithium countertransport activity in IDDM offspring and parents. The median value of erythrocyte Na⁺/Li⁺ CT in the IDDM patients who took part in the study (159 out of 171 interviewed patients) was 300 µmol · l erythrocytes⁻¹ · h⁻¹. Eighty patients had values higher and 79 had lower than the median

Table 1. Erythrocyte Na⁺/Li⁺ CT µmol · l erythrocytes⁻¹ · h⁻¹ in IDDM patients at baseline and at year 2 and 4 of follow-up

	Baseline	Year 2	Year 4	Coefficient of variation (%)
Group A	429 ± 122 (300–748)	430 ± 113 (303–759)	421 ± 148 (301–771)	11 ± 9
Group B	188 ± 104 (104–299)	191 ± 113 (103–289)	184 ± 96 (101–296)	9 ± 8

Values are mean ± SD and (range)

N. B. The patients were divided into two groups: A, those with values of erythrocyte Na⁺/Li⁺ CT above, and B, those with values below, the median level in the overall population (300 µmol · l erythrocytes⁻¹ · h⁻¹)

level and were assigned to Group A and Group B, respectively. Seven patients showed, after 2 or 4 years, alterations in erythrocyte Na⁺/Li⁺ CT levels which placed them on the other side of the median value with respect to their baseline measurement. These were excluded from our protocol. Twelve patients developed arterial hypertension (systolic and/or diastolic blood pressure levels above 140/90 mmHg), and were prescribed antihypertensive medication, but were not excluded from the study. The present work thus considers data on 152 IDDM patients. No change in erythrocyte Na⁺/Li⁺ CT values was observed between baseline, 2 and 4 year measurements (Table 1). The two groups of IDDM patients were comparable for age, duration of diabetes, body mass index and biochemical parameters. In particular no differences were found with regard to baseline AER and HbA_{1c} levels. Systolic blood pressure levels were slightly but significantly higher in Group A than B patients, albeit within the normal range. No significant differences were observed with regard to diastolic blood pressure. The number of males was significantly higher in Group A than B (Table 2).

Erythrocyte Na⁺/Li⁺ CT was measured in both parents of 61 out of 76 patients in Group A and 64 out of 76 patients in Group B. Their clinical features are shown in Table 3. The average age of the fathers was greater than that of the mothers in both groups. However, no difference was observed with regard to age between parents of Group A and B as overall cohorts of males and females. The number of subjects undergoing antihypertensive therapy was significantly higher in the parents of Group A than those of Group B (24 vs 11%; *p* < 0.05). Mean blood pressure levels of the parents of Group A and Group B IDDM offspring, who were not taking antihypertensive therapy were similar. Blood pressure levels tended to be slightly higher in the fathers than in the mothers of offspring IDDM patients, as were triglyceride plasma concentrations (Table 3).

Erythrocyte Na⁺/Li⁺ CT was greater in males than females among parents of IDDM offspring.

Table 2. Baseline clinical features of the IDDM patients who completed the study

	Group A	Group B	P-value
Sex (Male/female)	48/28	38/38	$p < 0.01$
Age (years)	17.3 ± 7.4	18.4 ± 6.2	NS
Duration of IDDM (years)	5.8 ± 4.2	6.4 ± 3.9	NS
Blood pressure (mmHg)			$p < 0.05$
Systolic	123 ± 17	118 ± 17	
Diastolic	75 ± 8	74 ± 16	NS
HbA _{1c} (%)	8.9 ± 2.6	8.6 ± 3.4	NS
Cholesterol (mmol/l)	4.1 ± 1.7	4.2 ± 0.8	NS
Triglycerides (mmol/l)	0.99 ± 1.13	1.03 ± 0.95	NS
Plasma creatinine (μmol/l)	70 ± 17	69 ± 24	NS
Creatinine clearance (ml · min ⁻¹ · m ⁻²)	117 ± 104	109 ± 104	NS
Insulin requirement (IU · day ⁻¹ · kg ⁻¹)	0.88 ± 0.60	0.77 ± 0.78	NS
Background/proliferative retinopathy (%)	29/0	26/0	NS
Albumin excretion rate (μg/min)	8 (1–10)	7 (2–9)	NS

Each clinical and biochemical parameter represents the mean of a single evaluation in the IDDM offspring

N.B. Group A had levels above and Group B levels below the median value erythrocyte Na⁺/Li⁺ CT (300 μmol · l erythrocytes⁻¹ · h⁻¹). Mean ± SD or median with ranges at baseline

However, parents of Group A patients had higher erythrocyte Na⁺/Li⁺ CT values on average than did those of Group B (fathers: 356 vs 266, $p < 0.05$; mothers: 277 vs 221, μmol · l erythrocyte⁻¹ · h⁻¹, $p < 0.01$). The number of IDDM patients in Group A whose erythrocyte Na⁺/Li⁺ CT was greater in both parents than the median value in the parent population (e.g. 277), was significantly higher than that in Group B (35 vs 19%). On the contrary, the number of IDDM patients in Group A whose erythrocyte Na⁺/Li⁺ CT was lower in both parents than the median value was significantly lower than that in Group B (10 vs 38%) (chi-square: $p < 0.01$). No difference was found between the two groups with respect to the number of IDDM patients who had only one parent with erythrocyte Na⁺/Li⁺ CT greater than the median value (55 vs 43 %, NS).

Table 4. Mean clinical features of IDDM patients who completed the study

	Group A	Group B	P-value
Blood pressure (mmHg)			
Systolic	124 ± 17	117 ± 17	$p < 0.05$
Diastolic	77 ± 9	73 ± 8	$p < 0.01$
HbA _{1c} (%)	9.6 ± 1.7	8.3 ± 1.7	$p < 0.002$
Cholesterol (mmol/l)	4.4 ± 0.19	4.6 ± 0.8	NS
Triglycerides (mmol/l)	1.02 ± 1.39	1.00 ± 0.95	NS
Plasma creatinine (μmol/l)	78 ± 17	74 ± 24	NS
Creatinine clearance (ml · min ⁻¹ · m ⁻²)	107 ± 87	115 ± 95	NS
Insulin requirement (IU · day ⁻¹ · kg ⁻¹)	0.92 ± 0.69	0.74 ± 0.34	NS
Background/proliferative retinopathy (%)	47/4	36/1	$p < 0.05$

Each value (except for eye evaluation) represents the average of subsequent determinations every 6 months during the follow-up period

N.B. Group A had levels above and Group B levels below the median value erythrocyte Na⁺/Li⁺ CT (300 μmol · l erythrocytes⁻¹ · h⁻¹) expressed as mean values of the measurements performed every 6 months during the follow-up period

Rate of development of microalbuminuria in IDDM offspring. During the follow-up period Group A patients had significantly higher mean HbA_{1c} values than did Group B (Table 4) despite similar daily insulin requirements. Systolic and diastolic blood pressure levels were slightly, but significantly, higher in Group A than in Group B, although within the normal range (Table 4). No differences were observed with regard to other clinical and biochemical parameters. The number of patients who developed persistent microalbuminuria during the follow-up period in the overall population of normotensive and hypertensive IDDM offspring population was significantly higher in Group A than in Group B (Table 5). No significant differences were observed with regard to the rate of development of intermittent microalbuminuria (Table 5). Twelve patients developed arterial hypertension, seven in Group A and five in Group B. The rate of occurrence of microalbuminuria was also assessed excluding these patients because of the possibility that antihypertensive treatment affects AER.

Table 3. Clinical features of the parents of IDDM offspring

		Age (years)	Body mass index (kg/m ²)	Blood glucose (mmol/l)	Triglycerides (mmol/l)	Cholesterol (mmol/l)	Mean blood pressure (mmHg)	Hypertension (%)	IDDM (%)
Parents of Group A (n = 61)	Female	46 ± 8 ^b	24 ± 7	4.72 ± 1.61	1.34 ± 0.78	4.24 ± 0.54	107 ± 12	28 ^a	0
	Male	44 ± 8	25 ± 8	4.38 ± 0.86	0.98 ± 0.70	4.11 ± 0.86	101 ± 13	20 ^a	0
Parents of Group B (n = 64)	Female	44 ± 8 ^b	25 ± 8	4.62 ± 1.12	1.21 ± 0.88	4.18 ± 0.72	102 ± 6	14	0
	Male	40 ± 16	23 ± 8	4.20 ± 1.04	0.97 ± 0.88	4.07 ± 0.96	98 ± 13	7	0

Presence of arterial hypertension and IDDM is expressed as percentage. Mean blood pressure levels were measured only in subjects not receiving antihypertensive treatment.

^a $p < 0.05$ parents of Group A vs parents of Group B; ^b $p < 0.05$ males vs females

Table 5. Development of persistent or intermittent microalbuminuria in each of the two groups

	Group A	Group B	<i>P</i> -value
Duration of follow-up (years)	5.2 (3.3–7.4)	5.4 (3.5–7.5)	NS
Persistent microalbuminuria in normotensive offspring	14 of 69 (20.2%)	4 of 71 (5.6%)	<i>p</i> < 0.01
Persistent microalbuminuria in all offspring	17 of 76 (22.3%)	4 of 76 (5.2%)	<i>p</i> < 0.01
Intermittent microalbuminuria in all offspring	2 of 76 (2.6%)	2 of 76 (2.6%)	NS

Data are mean and range of follow-up period and number of patients

N.B. Group A had levels above and Group B levels below the median value erythrocyte Na⁺/Li⁺ CT (300 μmol · l erythrocytes⁻¹ · h⁻¹)

Table 6. Operative characteristics of erythrocyte Na⁺/Li⁺ CT as predicting factor of the rate of occurrence of microalbuminuria in the overall IDDM population during the follow-up period

Cut-off value (μmol · l erythrocytes ⁻¹ · h ⁻¹)	Sensitivity (%)	Specificity (%)
0.20	90	27
0.30	85	55
0.40	70	88
0.50	55	96

Sensitivity indicates the true positive rate of development of microalbuminuria for a given cut off value;

Specificity indicates the true negative rate of development of microalbuminuria for a given cut-off value

Table 7. Results of multiple logistic regression analysis

Variable	Odds ratio ^a	95 % Confidence interval
Erythrocyte Na ⁺ /Li ⁺ CT (0 or 1)	4.2 ^b	2.0–11.1
HbA _{1c} (%)	1.6 ^c	0.7–2.3
Triglycerides (mmol/l)	0.9 ^c	0.4–1.0
Systolic blood pressure (mm Hg)	1.3 ^c	0.7–1.1
Diastolic blood pressure (mm Hg)	1.4 ^c	0.8–1.2
Baseline AER (μg/min)	0.9 ^c	0.4–1.1
Gender (0 or 1)	2.0 ^c	0.9–3.1
Plasma creatinine (μmol/l)	0.7 ^c	0.5–0.9

HbA_{1c}, systolic and diastolic blood pressure level, gender and erythrocyte Na⁺/Li⁺ CT are the independent variables. Dependent variable: Rate of occurrence of microalbuminuria (expressed as dichotomous variable)

^a Odds ratio concerning numerical and categorical values shown in parentheses. Apart from gender and baseline AER, the other variables indicate the mean value of sequential determinations every 6 months during the follow-up period.

^b *p* < 0.01; ^c NS

However, the degree of significance in the rate of development of both persistent and intermittent microalbuminuria did not vary in the two groups even if the data on these hypertensive patients were included (Table 5).

Table 6 shows the sensitivity of different cut off values of erythrocyte Na⁺/Li⁺ CT in predicting the rate of occurrence of microalbuminuria. The erythrocyte Na⁺/Li⁺ CT value of 300 μmol · l erythrocyte⁻¹ · h⁻¹ identified 17 truly positive and 3 false negative patients, e.g. it had a sensitivity of 85 %. The erythrocyte Na⁺/Li⁺ CT value of 400 μmol · l erythrocyte⁻¹ · h⁻¹, which has been previously used mainly in cross-sectional studies, identified 14 truly positive and 6 false negative patients, e.g. it had a significantly lower sensitivity (70 %, *p* < 0.05). The specificity, e.g. the number of false positive patients, was significantly lower using the 400 cut-off point (Table 6). However, sensitivity rather than specificity, is the most prominent clinical parameter in follow-up studies, focusing on diabetic complications.

The cumulative, absolute increase of AER above baseline values, during the 5.3-year follow-up period, was significantly greater in Group A than in Group B (10.8 ± 25.2 vs 2.11 ± 10.4 μg/min, *p* < 0.01). The increase of AER above baseline did not correlate with baseline AER, but was highly significantly related to the baseline erythrocyte Na⁺/Li⁺ CT (*p* < 0.002) and to gender (*p* < 0.03) and slightly, but significantly, related to the mean values of HbA_{1c} during the follow-up period (*p* < 0.04). Neither systolic nor diastolic blood pressure levels were significantly related to the occurrence rate of microalbuminuria. At the end of the follow-up interval the percentage of patients with background and proliferative retinopathy was higher in Group A (Table 4).

Table 7 shows the odds ratios from the logistic analysis used to control for the covariates. The rate of development of microalbuminuria, as a dichotomous variable, was associated with baseline erythrocyte Na⁺/Li⁺ CT after adjustment for baseline AER and gender and HbA_{1c}, triglyceride and mean blood pressure levels, expressed as average values of the sequential determinations every 6 months in each subject during the follow-up (*p* < 0.002). The odds ratio for the occurrence of microalbuminuria after adjustment for the above-mentioned confounding variables in IDDM with elevated baseline erythrocyte Na⁺/Li⁺ CT was 4.2 (95 % confidence intervals of 2.0–11.1). Similar results were observed using the cumulative absolute increase of AER above baseline as the continuous numerical variable.

Discussion

The results of the present study indicate that the risk of developing persistent microalbuminuria is greater in normotensive, normoalbuminuric IDDM patients with elevated erythrocyte Na⁺/Li⁺ CT. This risk is more evident in male patients, in keeping with the observation that diabetic nephropathy is more frequently shown by male than female IDDM patients.

Moreover, elevated patterns of erythrocyte Na^+/Li^+ CT are associated with poor glycaemic control, as suggested by higher HbA_{1c} levels. Finally, it was found that IDDM patients with elevated erythrocyte Na^+/Li^+ CT have slightly, but constantly, higher blood pressure values.

The results of the current study on the rate of occurrence of microalbuminuria in IDDM are consistent with the findings of the Microalbuminuria Collaborative Study Group [15], carried out in the United Kingdom, which reported an incidence rate of 2% per year for persistent microalbuminuria and 17% for intermittent microalbuminuria in a cohort of 137 IDDM patients.

The physiological significance of erythrocyte Na^+/Li^+ CT in vivo is not yet understood. It has been postulated that it reflects the activity of sodium/hydrogen exchange in vivo, e.g. the membrane cation transport system which reflects intracellular pH, cell growth and bicarbonate reabsorption by proximal tubules in the kidney [16]. However, more recently evidence has been provided that the erythrocyte Na^+/Li^+ CT system differs from that of several isoforms of sodium/hydrogen exchange in mammals [17]. Why this transport system is elevated in this group of IDDM patients is not entirely clear but the explanation can probably be attributed to inherited genetic, rather than to environmental, factors [18].

This view is supported by the finding that despite a 10% coefficient of variation of erythrocyte Na^+/Li^+ CT activity which may have caused the misclassification of some patients, only 7 out of 159 patients (4%) crossed over from A to B or vice versa. According to our findings, the activity of this cation appears to be stable over prolonged periods. Moreover, it has been found that parents of offspring with elevated erythrocyte Na^+/Li^+ CT also show significantly increased cation transport activities.

Erythrocyte Na^+/Li^+ CT has different kinetic abnormalities regarding both apparent affinity for external sodium (K_m) and maximal velocity of cation translocation ($V_{\max}$) [19, 20]. Both are abnormal in patients with essential hypertension [19, 20] and diabetic nephropathy [21]. Assessing erythrocyte Na^+/Li^+ CT at only one external sodium concentration (150 mmol/l) accurately estimates neither K_m nor $V_{\max}$. However, this method has been validated in many epidemiological studies (for review, see [22]) and it is definitely more practical and accurate for clinical use. Therefore, we have used this method to assess its value in the long-term prediction of the risk of developing microalbuminuria, as has already been done for assessing the risk of developing hypertension [23, 24].

A screening test has to be sensitive and not necessarily specific [10]. With the median value (300 μmol

$\cdot \text{l cells}^{-1} \cdot \text{h}^{-1}$) as a cutoff point, in the present study the sensitivity of erythrocyte Na^+/Li^+ CT in predicting the development of microalbuminuria at a 5-year follow up is 85% and its specificity 55% (Table 5). As with other screening tests, using more stringent criteria or higher cutoff values increases the specificity but lowers the sensitivity [10]. For instance, by increasing the cutoff value to 400 $\mu\text{mol} \cdot \text{l cell}^{-1} \cdot \text{h}^{-1}$, the specificity increases to 88% but the sensitivity falls to 70%. For this reason we chose a reasonably low cutoff value (300 $\mu\text{mol} \cdot \text{l cell}^{-1} \cdot \text{h}^{-1}$) for erythrocyte Na^+/Li^+ CT which gives a low false-negative rate (15%) and high negative predictive value (96%). This means that when erythrocyte Na^+/Li^+ CT is higher than 300 $\mu\text{mol} \cdot \text{l cell}^{-1} \cdot \text{h}^{-1}$ the development of microalbuminuria in a 5-year follow up is not certain but when it is lower than 300 $\mu\text{mol} \cdot \text{l cell}^{-1} \cdot \text{h}^{-1}$ the probability of renal complications is very low.

Erythrocyte Na^+/Li^+ CT has been shown to be a genetic marker indicating a predisposition to arterial hypertension in non-diabetic populations [8]. This is in keeping with our present findings showing a slight but constant elevation in blood pressure levels in Group A. Furthermore, this view is also supported by the observation that arterial hypertension was more frequently found among the parents of Group A than B offspring. It can be postulated that the haemodynamic challenge due to blood pressure levels in the normal-upper range, may contribute to determining microalbuminuria in IDDM patients, although this view has not been uniformly confirmed in the literature [6].

The significance of the association between increased erythrocyte Na^+/Li^+ CT and raised HbA_{1c} levels in the Group A patients is unclear. Impaired insulin sensitivity is linked to increased erythrocyte Na^+/Li^+ CT in insulin-dependent [25, 26] and non-insulin-dependent [27] diabetes and in essential hypertension [28]. Poor metabolic control could be explained by resistance to insulin action in patients with elevated erythrocyte Na^+/Li^+ CT. Patterns of HbA_{1c} levels constantly above 8.1% found in Group A patients could themselves lead to renal damage. In fact, it has recently been shown that the risk of developing nephropathy increases logarithmically when HbA_{1c} is above the threshold value of 8.1% in IDDM patients with [2]. Although more recently the DCCT data do not support the conjecture that a glycaemic threshold for the development of complications exists at an HbA_{1c} of 8%, this latter report still confirms the role of hyperglycaemia in the pathogenesis of renal and retinal damage in IDDM [29].

With regard to the relation between erythrocyte Na^+/Li^+ CT and gender the present study shows that the fathers of IDDM offspring had higher activities than the mothers. These findings are

consistent with previous reports that erythrocyte Na^+/Li^+ CT is higher in males than females [22]. However, this was not true among IDDM offspring. Although the reason for this observation deserves further investigation, it can be postulated that uniformly elevated circulating insulin levels conceal gender differences both in Group A and B IDDM patients, as it has been observed that insulin raises erythrocyte Na^+/Li^+ CT [20, 22]. In fact subcutaneous insulin treatment results in chronic peripheral hyperinsulinaemia along with relative under-insulinisation of the portal vein in IDDM patients [30]. It has to be pointed out that the daily insulin requirement was not significantly different in Group A and in Group B, who were followed blindly in outpatient clinics with regard to their erythrocyte Na^+/Li^+ CT values.

In summary, three different hypotheses can be postulated to explain the link between elevated erythrocyte Na^+/Li^+ CT and the development of renal damage in IDDM. First, the activity of this cation transport system could directly underscore a predisposition to the occurrence of complications at kidney level. Second, erythrocyte Na^+/Li^+ CT could be the characteristic of IDDM patients resistant to insulin action and thus exhibiting a worse metabolic control. Chronic hyperglycaemia in turn may be responsible for kidney damage. Third, erythrocyte Na^+/Li^+ CT is a marker of predisposition to arterial hypertension. According to this latter view slightly elevated blood pressure levels could play a predominant role in the pathogenesis of diabetic complications at the kidney level.

Whatever the nature of the mechanisms underlying the association between elevated erythrocyte Na^+/Li^+ CT and diabetic nephropathy, the results of the present study seem to provide some new insights. Previous cross-sectional reports [3–5] showed a link between disorders in this cation erythrocyte transport system and incipient or overt nephropathy. This association, however, has not been confirmed by other studies [6]. The present, prospective study shows that IDDM patients with erythrocyte Na^+/Li^+ CT above the median value are more at risk for developing persistent microalbuminuria. Since persistent microalbuminuria precedes the development of overt nephropathy in IDDM, it might be useful to evaluate erythrocyte Na^+/Li^+ CT in normotensive, normoalbuminuric IDDM patients. This could permit early identification of patients at risk of developing renal complications and thus becoming candidates for antihypertensive and intensive insulin treatment and meticulous metabolic control.

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