Kidney International, Vol. 44 (1993), pp. 127–133

Sodium-lithium countertransport and triglycerides in diabetic nephropathy

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Sodium-lithium countertransport and triglycerides in diabetic nephropathy. Elevated erythrocyte sodium-lithium countertransport (SLC) activity is an intermediate phenotype of essential hypertension among Caucasians, and is controversially associated with nephropathy in Type 1 (insulin-dependent) diabetes. Hypertriglyceridemia is a frequent concomitant of elevated SLC in the general population, and may be found in diabetic nephropathy. The present study was designed to investigate the influence of kidney disease, serum triglycerides and blood pressure on the interindividual variability of SLC in Type 1 diabetes. SLC and fasting major serum lipids were studied in 35 Type 1 diabetic patients with persistently elevated urinary albumin excretion and in a group of patients matched for age, sex and duration of diabetes, but with normoalbuminuria. SLC was elevated in patients with clinical nephropathy ($N = 10$; median: 420 μmol $\cdot$ $l_{RBC}^{-1} \cdot hr^{-1}$) and in patients with microalbuminuria ($N = 25$; median: 405 μmol $\cdot$ $l_{RBC}^{-1} \cdot hr^{-1}$) compared with normoalbuminuric patients (median: 296 μmol $\cdot$ $l_{RBC}^{-1} \cdot hr^{-1}$; $P < 0.01$ vs. both groups). Hypertriglyceridemia and hypercholesterolemia were found only among patients with macroalbuminuria. Analysis of covariance indicated that the association of elevated SLC with kidney disease ($P < 0.006$ in all models) was largely independent of serum triglycerides, but also of total cholesterol, insulin dose and measures of glycemic control. Only diastolic blood pressure was positively associated with SLC ($P < 0.02$) independently from nephropathy ($P < 0.005$) also after restricting analysis to the normoalbuminuric patients. Kidney disease and raised blood pressure remain major concomitants of elevated SLC in Type 1 diabetics.

The incidence pattern of clinical proteinuria in Type 1 (insulin-dependent) diabetes mellitus and the observation of a family clustering of this long-term complication strongly suggest that the development of diabetic kidney disease may be explained by the interaction between the metabolic abnormalities of diabetes with a genetic background of susceptibility [1–3]. The nature of the predisposing genes is not known, but higher blood pressure and prevalence of cardiovascular disease have been reported in the parents of patients with diabetic nephropathy, and this suggests that an inherited predisposition to essential hypertension and/or to cardiovascular disease may be involved in conferring the risk of diabetic renal disease [4–6]. The presence of elevated erythrocyte sodium-lithium countertransport activity (SLC) in Type 1 diabetic patients with kidney disease initially appeared to provide further evidence in favor of this hypothesis [5, 7, 8]. SLC is distributed in a bimodal pattern in the general population [9, 10], where elevated activities find a major concomitant in essential hypertension [9, 11–14]. Furthermore, segregation analyses among Caucasian pedigrees have suggested that up to 80% of the inter-individual variability in SLC can be explained by genetic effects, restricting to about 20% the relevance of environmental factors in the prediction of SLC at statistical modeling of cross sectional data [15].

The initial assumption that the genetic component in SLC may not be entirely blurred by the metabolic abnormalities of diabetes [5, 7, 8] was directly supported by the finding of elevated SLC in the parents of Type 1 diabetic patients with nephropathy [16]. These findings were then questioned by a number of studies, either failing to detect parental components in the elevated SLC associated with diabetic nephropathy [17], or just rejecting the presence of the phenomenon [18, 19]. Recent updates from three groups seem to consolidate the existing contrasts [20–22], but no studies have directly addressed the possible reasons for the controversy to date, and several questions remain unanswered.

In particular, hypertriglyceridemia is a known concomitant of elevated SLC in the general population [23] and in selected non-diabetic individuals [24–26]. Furthermore, serum triglycerides may represent part of the environmental component in SLC, as changes in serum triglycerides concentration may be paralleled by changes in SLC [24, 27]. Hypertriglyceridemia occurs frequently also in overt diabetic renal disease [28], and the hypothesis that this factor may explain why only some studies detected higher SLC in diabetic nephropathy has been repeatedly postulated [18–20, 29]. Indeed, the measurement of plasma lipids was neglected by all [5, 7, 16, 17, 21, 22] but one [8] of the studies reporting elevated SLC in diabetic nephropathy, where a positive association of SLC with serum triglycerides was described in a subset of patients without clinical proteinuria. Whether such a finding was independent of the presence of microalbuminuria remained, however, unsolved.

Therefore, this study was designed to examine the contribution of kidney disease, major serum lipids concentration and blood pressure to the inter-individual variability of SLC among patients with Type 1 diabetes.

Received for publication November 4, 1992
and in revised form February 3, 1993
Accepted for publication February 4, 1993

Methods

Patient selection criteria

All Type 1 (insulin-dependent) diabetic patients we considered for this study attended the Diabetes Outpatient Clinic of our Institute between 1987 and 1989, and were of Caucasian origin. Forty-three patients with a urinary albumin excretion rate (AER) persistently more than the current consensus threshold for the diagnosis of microalbuminuria [30] in timed overnight, nitrite dipstick-negative urine collections were thus identified. After excluding two patients with persistent urinary sediment abnormalities, 10 patients with clinical proteinuria and 25 with microalbuminuria accepted participation in the investigation. These were matched for age, sex and duration of diabetes to thirty-five out of a total number of 374 patients with normoalbuminuria, without knowledge of other clinical features. Such criteria were deemed appropriate to select normoalbuminuric patients with a relatively low risk of developing diabetic kidney disease. All patients gave their informed consent to participate in the study, which was approved by our local Ethical Committee.

Clinical measurements

Every patient attended a morning visit, when clinical features were checked and a fasting blood sample was collected. Body weight was obtained in light indoor clothing, and height was measured without shoes. Body mass index (BMI) was calculated as usual, and expressed as kg/m^2. The currently prescribed daily insulin dose was recorded. Resting blood pressure (Korotkoff phases I/V) was measured once on the right arm by ordinary mercury sphygmomanometry with approximation to the nearest 5 mm Hg. The diagnosis of arterial hypertension relied upon the finding of systolic blood pressure ≥160 mm Hg and/or diastolic blood pressure ≥95 mm Hg and/or current antihypertensive treatment. Mean blood pressure was calculated as the sum of diastolic blood pressure recordings with 1/3 pulse pressure. Eight patients were receiving anti-hypertensive medication with one or two of a variety of drugs, including angiotensin converting enzyme inhibitors, calcium channel blockers, diuretics and beta blockers. One patient with overt nephropathy was also receiving bezafibrate. All patients were otherwise treated only with insulin, were studied in ordinary glycemic control and were not suffering of any disease other than long-term complications of diabetes. In particular, none among our patients was known to suffer from thyroid disorders, nor was any woman pregnant or taking oral contraceptives at the time of the investigation. Anti-hypertensive treatment was not withdrawn before the study, but the usual morning dose was postponed until after the visit.

Sodium-lithium countertransport

SLC was measured according to the method originally described by Canessa et al [11] with minor modifications, as previously described [7]. By this method, the net exchange of intracellular lithium for extracellular sodium is extrapolated by measuring the difference in lithium efflux rate from lithium-loaded erythrocytes into media without significant amounts of sodium (passive lithium backdiffusion) and into media containing sodium at physiological concentration (passive backdiffusion and Na/Li exchange). Lithium efflux was measured in duplicate erythrocyte suspensions characterized by a low final hematocrit (3 to 4%). Aliquots for the measurement of extracellular lithium concentration were drawn at baseline (zero min) and after 15 and 30 minutes of incubation at 37°C. Lithium concentration in the supernatants was measured by atomic absorption spectrophotometry (Perkin-Elmer 4000), with LiCl at concentrations up to 0.050 mmol/liter as the reference standard in bidistilled water. Preliminary experiments showed that fresh efflux buffers do not differ from distilled water at the absorption wavelength of lithium. Lithium efflux rates were obtained by calculating the linear regression coefficients of lithium concentration upon incubation time. After correcting for cell volume, these were finally expressed as $\mu mol \cdot l_{RBC}^{-1} \cdot hr^{-1}$. Correlation coefficients were higher than 0.98 in all of the experiments.

In our laboratory, observations in normotensive, normoglycemic, young members of Hospital Staff range from 115 to 536 $\mu mol \cdot l_{RBC}^{-1} \cdot hr^{-1}$ ($N = 28$; median: 249 $\mu mol \cdot l_{RBC}^{-1} \cdot hr^{-1}$), perhaps reflecting the lower component in the bimodal distribution [9]. The average day to day intraindividual coefficient of variation with this method approaches 11%, which also accounts for short-term biological variability, and compares with that described by other Laboratories using the same technique [10, 11, 31].

Other measurements

Urinary albumin concentration was measured by a commercial radioimmunoassay (Phadebas Albumin RIA, Pharmacia, Uppsala, Sweden). Glycated hemoglobin (HbAIc) was measured by ion-exchange HPLC (Diamat, Bio-Rad, Richmond, Virginia, USA; normal range: 4.0 to 6.0%). Routine clinical chemistries were measured by enzymatic colorimetric techniques on autoanalyzer (COBAS FARA II, Roche, Basel, Switzerland). The diagnosis of hypertriglyceridemia was considered when serum triglycerides concentration was found above the upper limit of normal in our laboratory (1.9 mmol/liter = 170 mg/dl). Serum total cholesterol concentration was judged normal up to 5.17 mmol/liter (200 mg/dl) and frankly elevated above the conservative threshold of 6.20 mmol/liter (240 mg/dl); intermediate concentrations qualified as borderline hypercholesterolemia.

Statistical analysis

Results were analyzed by a commercial software package (JMP®, SAS Institute, Cary, North Carolina, USA) and are presented as mean with standard deviation unless otherwise indicated. Comparisons in normally distributed variables were addressed by one-way analysis of variance, adjusting for multiple comparisons by Tukey-Kramer test. Rank transformation was considered in context with all analyses including variables that were found to deviate significantly from the normal distribution. Univariate associations between continuous variables were examined by linear models, and interactions with nominal effects were addressed by analysis of covariance. Comparisons in categorical variables were made by contingency table analysis. The null hypothesis was rejected below the conventional (two-tailed) 5% level.

Table 1. General clinical features of Type 1 diabetic patients with normal urinary albumin excretion, microalbuminuria and macroalbuminuria

Renal diagnosis	Normoalbuminuria	Microalbuminuria	Macroalbuminuria	*P*
Number	35	25	10	—
Age *years*	34.1 ± 11.8	31.6 ± 12.1	36.5 ± 8.8	NS
Gender (M/F ratio)	1.5	1.7	1.5	NS
Duration of diabetes *years*	19.2 ± 7.3	16.5 ± 8.6	21.3 ± 8.5	NS
Age at the onset of diabetes *years*	14.8 ± 9.8	15.2 ± 8.6	15.2 ± 6.9	NS
Median AER *μg/min*	4.5	42.4	547	—
(range)	(2.0–15.7)	(20.1–138.9)	(213–1,773)	
Serum creatinine *μmol/liter*	78 ± 25	75 ± 16	118 ± 51[a]	0.0002
Body mass index *kg/m²*	22.3 ± 2.8	22.9 ± 2.7	23.5 ± 2.6	NS
Systolic blood pressure *mm Hg*	126.0 ± 16.1	134.2 ± 18.9	132.9 ± 10.5	NS
Diastolic blood pressure *mm Hg*	79.5 ± 8.1	83.0 ± 10.6	84.3 ± 8.5	NS
Mean blood pressure *mm Hg*	95.1 ± 9.4	100.0 ± 10.4	100.5 ± 5.7	NS
Anti-hypertensive therapy (P/A)	1/34	1/24	6/4[b]	0.0001
Arterial hypertension %	8.6	28.0	60.0[c]	0.0021

Values are mean ± SD or median with range in parentheses. Abbreviations are: M, male; F, female; P, present; A, absent.
P, overall significance either at analysis of variance or at contingency table analysis, as appropriate.
[a] $P < 0.01$ vs. both non-proteinuric groups after adjusting for multiple comparisons by Tukey-Kramer test
[b] $P < 0.005$ vs. both non-proteinuric groups
[c] $P < 0.002$ vs. patients with normoalbuminuria

Table 2. Metabolic features and major serum lipids concentration of Type 1 diabetic patients with normal urinary albumin excretion, microalbuminuria and macroalbuminuria

Renal diagnosis	Normoalbuminuria	Microalbuminuria	Macroalbuminuria	*P*
Number	35	25	10	—
HbAIc %	8.4 ± 1.8	8.6 ± 1.2	9.6 ± 2.4	NS
Blood glucose *mmol/liter*	12.6 ± 6.4	14.5 ± 7.6	16.3 ± 6.6	NS
Daily insulin dose *IU/24 hr*	43.6 ± 12.5	48.7 ± 13.8	40.7 ± 8.7	NS
Median total cholesterol *mmol/liter*	4.96	4.67	5.42	NS
(range)	(3.30–5.73)	(2.80–6.35)	(3.67–10.64)	
Hypercholesterolemia (P/B/A)	0/13/22	1/8/16	4/2/4[a]	0.0006
Median triglycerides *mmol/liter*	0.97	0.97	1.66	NS
(range)	(0.26–1.96)	(0.28–3.18)	(0.56–5.40)	
Hypertriglyceridemia (P/A)	1/34	3/22	4/6[b]	0.005

Values are mean ± SD or median with range in parentheses. Abbreviations are: P, present; B, borderline; A, absent.
P, overall significance either at analysis of variance or at contingency table analysis, as appropriate.
[a] $P < 0.03$ vs. both non-proteinuric groups
[b] $P < 0.01$ vs. patients with normoalbuminuria

Results

Clinical features of patients

The general clinical features of patients are shown in Table 1 according to the level of urinary albumin excretion. Age, gender, duration of diabetes and age at the onset of diabetes were comparably distributed in patients with microalbuminuria and in patients with macroalbuminuria; in line with selection criteria, these clinical features compared with those of normoalbuminuric patients. Serum creatinine was normal (less than 133 μmol/liter) in all patients without proteinuria; mean serum creatinine was significantly elevated only among patients with macroalbuminuria, although only three among them had clinically increased levels. Body mass index was more than 28 kg/m² only in two patients (one with normal AER and one with microalbuminuria) but was otherwise normal and comparably distributed in the three groups. Patients with microalbuminuria showed a threefold prevalence of arterial hypertension compared with the normoalbuminuric patients, but this difference did not reach statistical significance, nor did the differences in blood pressure recordings. Likewise, systolic and diastolic blood pressure recordings were not significantly higher among the macroalbuminuric patients. The prevalence of arterial hypertension was, however, significantly elevated in patients with macroalbuminuria, in association with a significantly higher occurrence of cases receiving antihypertensive treatment.

The metabolic features of patients are displayed in Table 2. No significant differences in HbAIc levels, fasting glycemia and daily insulin dose appeared to characterize patients with raised urinary albumin excretion compared with normoalbuminuric controls. Fasting serum lipids were largely normal and comparable in normoalbuminuric and microalbuminuric patients. Significantly higher occurrence of hypercholesterolemia and hypertriglyceridemia appeared to characterize only the patients with macroalbuminuria. However, both total serum cholesterol and triglycerides distributions significantly deviated from normality (Shapiro-Wilk test, $P < 0.0001$ for both variables) and differences in their continuously distributed values failed to reach significance after rank transformation.

Sodium-lithium countertransport activity

As SLC is known to follow a bimodal distribution in the general population [9, 10], all statistical analyses conservatively

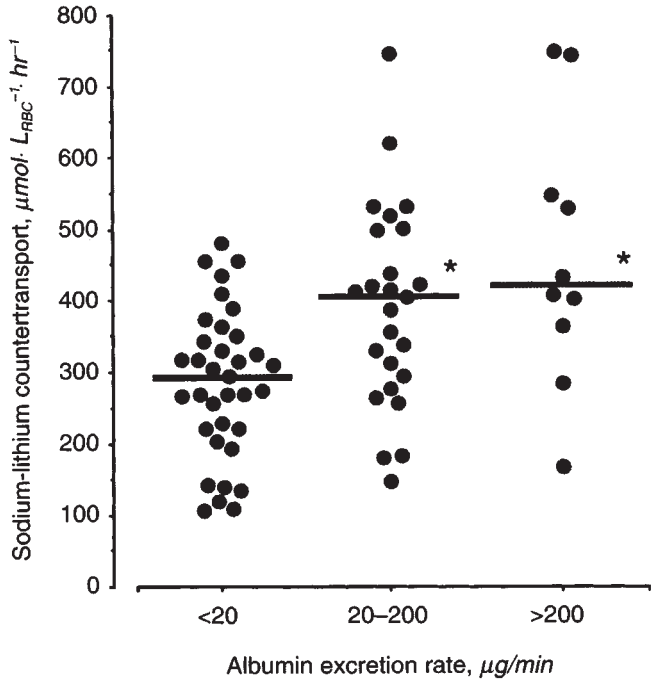

Fig. 1. *Scatterplot of erythrocyte sodium-lithium countertransport activity (SLC) in Type 1 diabetic patients with normoalbuminuria (AER < 20 μg/min), microalbuminuria (AER = 20–200 μg/min) and overt nephropathy (AER > 200 μg/min).* Horizontal lines indicate the median values. SLC was elevated in both groups with elevated urinary albumin excretion (analysis of variance after rank-transformation: $P = 0.0007$; also see Table 3). * $P < 0.01$ vs. patients with normoalbuminuria, accounting for multiple comparisons by Tukey-Kramer test.

rested upon prior rank transformation. Mean SLC was 286 ± 102 μmol · l_{RBC}^{-1} · hr^{-1} (median 296) in patients with normal urinary albumin excretion. Significantly higher values comparably characterized both patients with microalbuminuria (392 ± 141 μmol · l_{RBC}^{-1} · hr^{-1}; median: 405; $P < 0.01$ after accounting for multiple comparisons) and patients with macroalbuminuria (463 ± 185 μmol · l_{RBC}^{-1} · hr^{-1}; median: 420; $P < 0.01$), although relevant overlaps with the normoalbuminuric group were manifest (Fig. 1). Simple regression analyses, as appropriately restricted to each subset of patients, failed to document significant correlations of SLC with BMI, blood glucose, HbAIc, daily insulin dose, total cholesterol and triglycerides.

To investigate the combined influence of renal diagnosis, triglycerides, blood pressure and other clinical variables on SLC, data were examined by analysis of covariance. This method permits examination of how the interaction of one or more continuously distributed variables with nominal effects, such as the presence versus the absence of kidney disease, may explain the interindividual variability of SLC.

Table 3 lists the levels of significance obtained in each model where the effects of single covariates were compounded with that of renal diagnosis. Each covariate was entered using the native measurement units, but in the case of serum triglycerides, total cholesterol and SLC, where rank transformed values were used as previously justified. Only diastolic blood pressure contributed to increase significantly the predictivity of the whole model, independent of the effect of renal diagnosis. Among the remaining study variables, duration of diabetes, mean blood pressure, serum triglycerides concentration and presence of hypertriglyceridemia appeared to associate with some improvement in the overall model fit, but their partial effect remained non-significant. In line with these observations, the effect of renal diagnosis upon rank transformed SLC remained highly significant in all models, and significantly elevated values in SLC were found in both groups of patients with elevated AER after adjusting for the effect of each covariate (Table 3).

Larger models, where up to four covariates were added to renal diagnosis, also failed to produce significant increases in the overall model predictivity. After forcing HbAIc, daily insulin dose, BMI and serum triglycerides into the model ($r^2 = 0.20$, $F = 3.7$; $P = 0.004$), having microalbuminuria or macroalbuminuria remained the only independent predictor of SLC ($F = 4.8$, $P = 0.012$), while triglycerides still failed to reach a significant effect ($F = 3.6$; $P = 0.063$). Findings were substantially similar in other models, where these variables were entered in smaller numbers but different combinations, when blood glucose was entered instead of HbAIc, or when the presence of hypertriglyceridemia, rather than the continuous distribution of triglycerides, was considered.

The observation of a weak but independent association of diastolic blood pressure with SLC suggested exploration of this relationship in greater detail. Analysis of covariance models was designed here with blood pressure as the dependent variable, to allow the adjustment of SLC for the observed influence of renal diagnosis. A small but significant proportion of the interindividual variability in diastolic blood pressure was explained by SLC (F ratio = 5.3, $P = 0.024$; whole model $r^2 = 0.12$; $P = 0.043$), but not by the presence of nephropathy. No significant effects were observed upon systolic or mean blood pressure. When the effects of age, gender, body mass index and antihypertensive treatment were also taken into account, the overall prediction of diastolic blood pressure was weakly increased ($r^2 = 0.15$), and only the effect of SLC was significant ($F = 4.34$, $P = 0.0415$). This set of variables was more effective in the prediction of systolic blood pressure ($r^2 = 0.44$) and mean blood pressure ($r^2 = 0.28$), most likely for a strong, independent effect of age upon both ($F = 38.2$, $P < 0.00001$ and $F = 11.4$, $P = 0.0013$, respectively). In these models, SLC appeared to have a significant effect upon mean blood pressure ($F = 6.0$; $P = 0.017$), but not systolic blood pressure ($F = 3.7$, $P = 0.06$). Receiving antihypertensive treatment and renal diagnosis were not shown to associate significantly with blood pressure in any of these models, perhaps in line with an effective reduction in blood pressure values among patients treated for hypertension.

To obviate the confounding effects of antihypertensive treatment and renal abnormalities upon blood pressure in this set of analyses, the effects of age, gender, body mass index and SLC upon blood pressure were finally investigated by restricting analysis to the subset of patients with normoalbuminuria, after the exclusion of the single case receiving antihypertensive treatment. A positive, independent and significant contribution of SLC to arterial pressure was confirmed (Fig. 2, Table 4). Insulin dose, blood lipids and measures of glycemic control all failed to add and explain systemic arterial pressure among these patients.

Table 3. Independent analysis of covariance models, examining the contribution of clinical or metabolic covariate regressors to the effect of renal diagnosis upon erythrocyte sodium-lithium countertransport activity (SLC) in 70 Type 1 diabetic patients with normoalbuminuria, microalbuminuria and macroalbuminuria

Covariate	Whole model fit		Effects in model		Adjusted SLC[a]		
			Covariate	Renal diagnosis			
	r^2	P	P	P	Normoalbuminuria	Microalbuminuria[b]	Macroalbuminuria[b]
None	0.19	0.0007	—	0.0007	26.8 ± 3.1	42.2 ± 3.7	49.0 ± 5.8
Age	0.22	0.0008	NS	0.0006	27.0 ± 3.1	41.6 ± 3.7	49.9 ± 5.8
Gender	0.20	0.0020	NS	0.0008	26.6 ± 3.2	41.8 ± 3.8	48.7 ± 5.9
Duration of diabetes	0.23	0.0007	NS	0.0007	27.7 ± 3.1	41.9 ± 3.8	50.2 ± 5.8
BMI	0.19	0.0032	NS	0.0015	26.9 ± 3.2	42.0 ± 3.9	48.7 ± 6.0
Systolic blood pressure	0.20	0.0023	NS	0.0016	26.9 ± 3.2	41.9 ± 3.8	48.8 ± 5.9
Diastolic blood pressure	0.26	0.0002	0.02	0.0031	27.7 ± 3.1	41.3 ± 3.6	47.4 ± 5.7
Mean blood pressure	0.23	0.0066	NS	0.0006	27.7 ± 3.2	41.1 ± 3.7	47.8 ± 5.8
Blood glucose	0.22	0.0011	NS	0.0014	26.3 ± 3.3	41.9 ± 3.7	48.1 ± 5.9
HbAIc	0.20	0.0024	NS	0.0027	27.9 ± 3.2	42.7 ± 3.8	47.6 ± 5.9
Daily insulin dose	0.19	0.0024	NS	0.0008	26.8 ± 3.2	42.3 ± 3.8	48.8 ± 5.9
Cholesterol	0.21	0.0015	NS	0.0019	27.2 ± 3.1	42.5 ± 3.7	46.7 ± 6.2
Hypercholesterolemia	0.20	0.0043	NS	0.0043	29.7 ± 4.6	44.9 ± 4.8	48.7 ± 5.9
Triglycerides	0.23	0.0006	NS	0.0025	27.5 ± 3.1	42.1 ± 3.6	46.6 ± 5.9
Hypertriglyceridemia	0.23	0.0007	NS	0.0058	32.6 ± 4.6	46.8 ± 4.6	50.2 ± 5.8

[a] Values are the least square means ± SE of the rank scores of SLC activity in each group of patients, as adjusted for the contribution of each corresponding covariate

[b] $P < 0.05$ vs. normoalbuminuric patients in all models

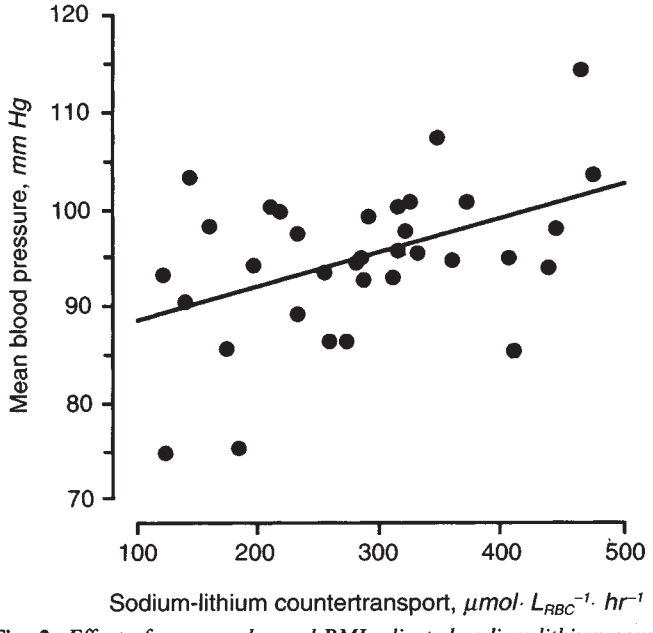

Fig. 2. *Effect of age, gender and BMI-adjusted sodium-lithium countertransport activity (SLC) upon mean blood pressure among 34 Type 1 diabetic patients with normal urinary albumin excretion.* To show usual SLC measurement units, this scatterplot was obtained by entering native SLC values into the model. The statistical effect of SLC ($F = 7.2$; $P = 0.012$) was similar to that calculated after rank-transformation (Table 4)

Table 4. Multiple regression analyses exploring the effect of age, gender, body mass index and erythrocyte sodium-lithium countertransport activity (SLC) upon blood pressure in 34 Type 1 diabetic patients with normoalbuminuria and no treatment for hypertension

Response	Whole model fit		Effects in model			
			Age	Gender	BMI	SLC
	r^2	P	P	P	P	P
Systolic blood pressure	0.40	0.0040	0.0021	NS	0.0423	0.0429
Diastolic blood pressure	0.29	0.0358	NS	NS	NS	0.0188
Mean blood pressure	0.40	0.0037	0.0118	NS	0.0199	0.0101

Discussion

This study supports previous evidence that SLC may be elevated in patients with overt nephropathy [5, 7, 17] and that comparably high SLC may already characterize patients with microalbuminuria [8], the only subclinical abnormality of the kidney presently known to reflect renal prognosis in Type 1 diabetes mellitus [32–34]. In line with previous observations, we also found that hypertriglyceridemia and hypercholesterolemia may occur more often in patients with clinical nephropathy [28], but not in microalbuminuria, where abnormalities in serum lipids may be more subtle, and be found only by examining their subfractions [28, 35, 36]. This pattern of results seems to directly rule out the possibility that hyperlipidemia may explain the finding of elevated SLC among our patients with microalbuminuria, but corroborates the idea that both abnormalities may coexist in patients with overt nephropathy [18–20, 29]. However, statistical modeling indicated that elevated SLC was significantly predicted by the presence of renal disease, rather than by hyperlipidemia, also among these patients. Thus, although we cannot exclude that the association of SLC with serum triglycerides may turn significant after examining more patients, diabetic kidney disease seems to predict elevated SLC independent of serum major lipids concentrations, suggesting that this phenomenon may not merely suffer a major environmental influence from serum triglycerides, as previously postulated [18–20, 29].

Differences in glycemic control and insulin dose are also

unlikely to explain the association of nephropathy with elevated SLC. Neither glycemia and glycated hemoglobin explained SLC in our patients, nor in any other report where these univariate correlations were investigated [5, 7, 8, 19]. This is possibly restricting to less than 5% the univariate effect of ordinary outpatient glycemic or HbAIc levels upon the interindividual variability in SLC, in line with the hypothesis that relatively worse glycemic control may be just an independent concomitant of elevated SLC in patients with nephropathy [5, 21]. Although daily insulin dose was similar among our groups of patients, this is a very indirect measure of the biodistribution and bioavailability of insulin, and 24-hour profiles of serum-free insulin levels have been reported to be more often elevated in diabetic nephropathy [37]. Insulin is known to affect cellular sodium metabolism, and careful studies of the activation kinetics of SLC, as observed in truly saturating conditions, have recently shown that insulin can acutely induce reductions in the affinity of this exchanger for extracellular sodium [38]. Therefore, although serum-free insulin concentrations were not measured in our patients, these mechanisms would be consistent with a higher chance of underestimating SLC, if anything, among the patients with nephropathy.

Importantly, higher blood pressure appeared to reflect higher SLC among our patients, independent of the concomitant effects of attained age and BMI, but also independent of the presence of nephropathy. While other studies either failed to document any relationship between blood pressure and SLC in Type 1 diabetic patients [19, 20, 39, 40], did not single out the independent effect of increased urinary albumin excretion [41], or described the presence of a positive association only among the patients with diabetic kidney disease [8, 17], our finding is largely consistent with that reported in a recent outpatient clinic survey [22]. Furthermore, such an effect was still detectable among our patients after restricting analysis to those with normoalbuminuria, who were likely to represent a subset at relatively low risk of developing nephropathy. This is in line with the hypothesis that elevated SLC may reflect a higher risk of developing arterial hypertension in Type 1 diabetic patients who do not develop nephropathy [21], and further supports the view that the metabolic alterations of Type 1 diabetes may not totally confound the pathophysiology of this established concomitant of arterial hypertension in the general population.

Thus, taking together these observations, we would like to speculate that the presence of kidney disease, eventually leading to hyperlipidemia, and raised blood pressure may find a major concomitant in elevated SLC also among Type 1 diabetic patients. Indeed, our findings seem to reflect those of cross sectional studies in the general population, where a similar independence in the statistical associations of blood pressure and serum triglycerides with SLC was described by relying upon similar laboratory techniques [12–14, 23, 27]. Although the correlation of SLC with serum triglycerides appeared to be restricted to the higher occurrence of elevated SLC and hypertriglyceridemia in overt nephropathy, we cannot exclude that quantitative correlations may become manifest after studying larger cohorts of patients. An indirect mechanism which may explain a higher occurrence of raised serum triglycerides concentration among patients with elevated SLC could be represented by insulin-resistance. The reduction in peripheral sensitivity to insulin action characterizing essential hypertension [42], perhaps more often associated with elevated SLC [43], may be relevant to explain susceptibility to hypertriglyceridemia in non-diabetic individuals. Recently, a similar association of elevated SLC with some degree of insulin resistance and raised serum triglycerides has been reported in Type 1 diabetic patients without clinical proteinuria, albeit independent of hypertension and microalbuminuria [44]. Alternatively, the hypertriglyceridemia of overt diabetic renal disease may more simply reflect the insulin-resistance of chronic renal disease [45]. Finally, whether changes in the composition of the erythrocyte membrane lipid bilayer, perhaps bound to excess lipid peroxidation [19], may primarily relate with higher SLC independent of the suggested effect of serum triglycerides [25], is not known.

In conclusion, although we cannot rule out environmental influences other than those considered in this study, raised blood pressure and kidney disease remain major independent concomitants of elevated SLC in Type 1 diabetes. Ordinary glycemic and major serum lipids dysregulations do not seem suitable candidates to explain these associations. Evidence of a significant parental component remains the only other concomitant of SLC so far detected by others among Type 1 diabetic patients [16, 17]. Thus, the hypothesis that the genetic determinants of SLC may partly explain susceptibility to diabetic kidney disease cannot be presently dismissed. Among the several issues that remain to be answered, the adoption of uniform laboratory techniques to measure Na/Li exchange may represent the *sine qua non* to interpret controversial findings [38]. The relevance of these observations to renal prognosis in Type 1 diabetes remains to be established by prospective studies.

Acknowledgment

Part of this work was presented in preliminary form at the 26th Annual Meeting of the European Association for the Study of Diabetes, Copenhagen, 10–13 September 1990.

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