Enhanced Na–Li countertransport: a marker of inherited susceptibility to type 2 diabetes

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Introduction The association between type 2 diabetes and hypertension has long been described, but the mechanisms remain unclear. Na–Li countertransport (Na–Li CT) activity is viewed as a marker of inherited pre-disposition to hypertension, especially if associated with other metabolic abnormalities.

Aim To evaluate whether enhanced Na–Li CT activity is a predictor of type 2 diabetes.

Methods Study participants were 2167 men and women, 30–70 years. Na–Li CT activity, glucose, HDL cholesterol, blood pressure, height, and weight were measured. Six years incidence of diabetes (WHO) was assessed.

Results Baseline Na–Li CT activity was significantly higher for people who developed diabetes at follow-up (n = 101) than for those who remained non-diabetic (364 ± 184 vs 300 ± 150 μmol/l RBC/h, P < 0.001). This finding was confirmed after correction for obesity, hypertension, and blood glucose. Six years’ incidence of diabetes increased across tertiles of baseline Na–Li CT activity—from 2 to 7%—with a significant linear trend (P < 0.001). In multivariate analyses Na–Li CT is a significant predictor of diabetes independent of age, BMI, HDL cholesterol, hypertension, and plasma glucose; based on exponentiation of the regression coefficient Na–Li CT higher by 154 μmol (i.e. 1 SD of the population mean) was associated with a 36% greater risk of incident diabetes.

Conclusions Prospective data from the present study show for the first time enhanced Na–Li CT activity is a significant predictor of development of diabetes in adults, thus suggesting that it could be viewed as a pre-clinical, possibly genetic, marker of inherited susceptibility to type 2 diabetes.

Keywords Countertransport, hypertension, insulin resistance

Introduction

Na–Li countertransport (Na–Li CT) is a membrane ion transport system that in vivo exchanges sodium for sodium; the system also accepts lithium, and the exchange of sodium for lithium is used in vitro for its assay.1,2 Ever since the original report from Canessa et al.,2 many clinical and epidemiological studies have confirmed that the Na–Li CT activity is enhanced in erythrocytes of patients with essential hypertension1; prospective studies have also shown that Na–Li CT is a significant predictor of blood pressure change over time and
of development of hypertension. The interest in this membrane transport system is further enhanced by the fact that its activity is highly inherited. Although acquired conditions such as pregnancy and obesity can modulate the Na–Li CT, genetic epidemiology studies suggest that 50–80% of the Na–Li CT activity can be explained by inheritance. Because these properties, even though its physiological role in vivo has not been fully clarified and the gene(s) that affect Na–Li CT activity have not been identified, elevated Na–Li flux is viewed as a marker of inherited pre-disposition to hypertension.

An association between hypertension and type 2 diabetes has long been recognized, and is also evident before the clinical onset of diabetes, but the mechanisms remain unclear. Previous studies of Na–Li CT in diabetes focus on the relation of transport system activity with hypertension and/or microalbuminuria in both type 1 and type 2 diabetes [reviewed in ref. (14)], but no study has evaluated whether Na–Li CT activity is an inherited marker of diabetes as it is for hypertension. This hypothesis is plausible as the two conditions share common pathogenic mechanisms such as impaired insulin sensitivity. In one study, conducted in an animal model of diabetes (NOD mice) the Na–H exchanger (NHE)—evaluated at the genotyope and phenotype level—was linked to diabetes susceptibility. A few years ago, after genetic linkage analysis, NHE-1 was excluded as the gene responsible for elevated SLCT activity, and as a candidate gene for essential hypertension. A more recent study has provided convincing evidence that Na–Li CT is mediated by an alternative splicing of the first isoform of NHE (NHE-1), thus renewing interest in the potential contribution of this gene to the pathogenesis of hypertension.

The aim of this work is to explore the relation of Na–Li CT activity with incidence of type 2 diabetes by using the dataset of the Gubbio population study.

Methods
Baseline examination was performed in 1983–86 as described elsewhere. Briefly a total of 5376 men and women age ≥5 years were examined; the participation rate was 92%. For the present analyses we selected participants with baseline ages 30–70 years. In this age range there were 3095 participants, of whom 638 did not attend the follow-up examination and 290 did not have valid baseline Na–Li CT measurement. Therefore, the study population is composed of 2167 participants 30–70 years old at baseline (i.e. 70% of the target cohort), with a complete baseline and follow-up dataset. Those included in the analyses (n = 2167) had comparable age (50.7 ± 11.3 vs 51.2 ± 12.1, P = 0.10) and BMI (27.2 ± 4.2 vs 27.0 ± 4.4, P = 0.47) as those not included (n = 928). At baseline age, sex, and use of medication were recorded; weight, height, and blood pressure were measured by trained observers according to a standard protocol. Participants were asked to refrain from eating or smoking for at least 2 h before examination; time since last meal was recorded. Glucose and HDL cholesterol were measured on fresh plasma. Na–Li CT was measured in red blood cells according to a slight modification of the method of Canessa within 4 days from sampling, details have been published elsewhere. Briefly erythrocytes were isolated and washed, then 1 ml of packed cells was suspended in 5 ml of loading solution (LiCl 150 mM; Tris MOPS 10 mM) and incubated for 3 h at 37°C. After the incubation, cells were washed four times and suspended in the efflux media: one tube with sodium (NaCl 150 mM, Tris MOPS 10 mM, MgCl2 1 mM, glucose 10 mM, Ouabain 0.1 mM) and one tube without sodium (choline 148 mM, Tris MOPS 10 mM, MgCl2 1 mM, glucose 10 mM, Ouabain 0.1 mM) at 4°C. The maximal velocity of the Na–Li CT was calculated by subtracting the lithium efflux in the medium with sodium from the efflux in the medium without sodium. One in every 20 specimens was submitted as a blind duplicate to the laboratory for the determination of technical error. Overall technical error was 11% of the population mean; measurements performed on batches with technical error >20% were discarded.

At second examination, among others, fasting glucose was measured for all participants and use of medications was recorded. At baseline and follow-up hypertension was defined as blood pressure ≥140/90 mmHg or use of anti-hypertensive drugs. Diabetes was defined as use of medication for diabetes or fasting plasma glucose ≥126 mg/dl (7 mmol/l). Since at baseline not all participants were examined in the fasting state, a value of ≥200 mg/dl (11 mmol/l) was used for the diagnosis of diabetes at baseline for those participants who were non-fasting. Participants with diabetes at baseline were excluded from the study.

Statistical analyses
Data are given as means and standard deviations or percentages. Means were compared by unpaired Students t-test or analysis of variance with adjustment for covariates. The relation of Na–Li CT with incident diabetes was also explored by quantile analysis with the use of sex specific tertiles of baseline Na–Li CT. Differences in proportions across tertiles were tested by chi square analysis with tests for linearity of trend. Multivariate logistic regression was used to test for independence of associations. The statistical analysis was performed with SPSS 9.0 for windows. Values of P < 0.05 two-tailed were considered statistically significant in all analyses.

Results
The study population is on average 50.7 ± 11.3 years old and slightly overweight (BMI 27.2 ± 4.2 kg/m2). Among the 2103 participants free of diabetes at baseline 101 developed diabetes at follow-up: 62 were on oral agents, 8 were on insulin alone or in combination with oral agents, 31 were treated with diet only. Incidence of diabetes was 0.8% per year, similar for men and women, and was higher with greater age and BMI. Table 3 shows baseline values for the major, well established, predictors of diabetes as well as for Na–Li CT according to incident diabetes status. Those who were non-diabetic at baseline and developed diabetes at follow-up had significantly higher Na–Li CT than those who were non-diabetic at baseline and remained non-diabetic at follow-up (364 ± 184 vs 300 ± 150 μmol/l RBC/h; P < 0.001; Table 1). Those who developed diabetes were also significantly older, had significantly higher BMI, plasma glucose and blood pressure, and significantly lower HDL cholesterol as compared to those who remained free from diabetes (Table 1).
In the multivariate logistic regression analysis performed with incident diabetes as the dependent variable and baseline Na–Li CT (continuous variable), age, BMI, HDL cholesterol, glucose, and blood pressure simultaneously entered in the model, Na–Li CT was significantly and independently associated with incident diabetes. Very similar results were obtained with the use of systolic or diastolic blood pressure in the model (Table 3). On the basis of exponentiation of the regression coefficient Na–Li CT higher by 154 µmol (i.e. 1 SD of the population mean) was associated with a 36% greater risk of incident diabetes. Changes in BMI from baseline to follow-up and hypertension at follow-up were not significantly associated with development of diabetes.

**Table 1** Baseline Na–Li countertransport and other pertinent clinical features by incident diabetes status (6 years follow-up)

<table>
<thead>
<tr>
<th>Developed diabetes at follow-up</th>
<th>Yes (n = 101)</th>
<th>No (n = 2002)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (%)</td>
<td>45</td>
<td>44</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.7 ± 8.8*</td>
<td>50.1 ± 11.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.1 ± 4.2*</td>
<td>26.9 ± 4.0</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>5.6 ± 0.8*</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.1 ± 0.3*</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>142.1 ± 19.4*</td>
<td>132.1 ± 19.4</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>81.3 ± 9.5*</td>
<td>79.2 ± 10.8</td>
</tr>
<tr>
<td>Na–Li CT (µmol/l RBC/h)</td>
<td>364.6 ± 184.8*</td>
<td>300.2 ± 149.8</td>
</tr>
</tbody>
</table>

*P < 0.001.

**Table 2** Na–Li countertransport values by diabetes status at follow-up and baseline impaired fasting glucose (IFG), obesity or hypertension—two way analysis of variance

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Na–Li CT (µmol/l RBC/h)</th>
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<tbody>
<tr>
<td></td>
<td>Diabetes at follow-up¹</td>
</tr>
<tr>
<td>IFG²</td>
<td></td>
</tr>
<tr>
<td>Yes (n = 238)</td>
<td>426 ± 132</td>
</tr>
<tr>
<td>No (n = 1865)</td>
<td>354 ± 127</td>
</tr>
<tr>
<td>Obesity³</td>
<td></td>
</tr>
<tr>
<td>Yes (n = 669)</td>
<td>377 ± 140</td>
</tr>
<tr>
<td>No (n = 1434)</td>
<td>376 ± 134</td>
</tr>
<tr>
<td>Hypertension⁴</td>
<td></td>
</tr>
<tr>
<td>Yes (n = 891)</td>
<td>385 ± 128</td>
</tr>
<tr>
<td>No (n = 1212)</td>
<td>347 ± 126</td>
</tr>
</tbody>
</table>

Among those with diabetes at follow-up 15 had IFG, 37 were obese and 42 were hypertensive. *P = ns for interaction (all comparisons).

¹ P < 0.001 for effect of diabetes (all comparisons).

² P < 0.001 for the effect of IFG.

³ P < 0.01 for the effect of obesity.

⁴ P < 0.01 for the effect of hypertension.

To account for the confounding effect of obesity Na–Li CT values were analysed by two-way analysis of variance performed according to baseline obesity (i.e. BMI ≥ 30 kg/m² yes or no) and incident diabetes (yes or no); results clearly indicate that for both obese and non-obese participants baseline Na–Li CT values were significantly higher for those individuals who developed diabetes at follow-up as compared to those who remained free from this condition (Table 2). Similar results were obtained when the analysis was performed with stratification for baseline-impaired fasting glucose (glucose < or ≥ 110 mg/dl), or baseline hypertension (blood pressure ≥ 140/90 or use of medication) (Table 2).

The relation of Na–Li CT with incident diabetes was further explored by quantile analysis. Participants were stratified according to sex-specific tertiles of baseline Na–Li CT. Six years cumulative incidence of diabetes progressively and significantly increased from tertile 1 to tertile 3 (Figure 1) with a significant linear trend (P < 0.001). The incidence of diabetes was almost 2-fold higher in tertile 3 vs tertiles 1 + 2 combined. Differences remained statistically significant after correction for BMI and blood pressure.

**Discussion**

Prospective data from the present study show for the first time that enhanced Na–Li CT activity is a significant predictor of development of diabetes in adults. No specific tests which would permit differentiation between type 1 and type 2 diabetes were performed; however, based on available data it can be estimated that up to 95% of diabetic people identified in population-based surveys of the adult population have type 2 diabetes.21 In good agreement with this estimate most people with diabetes in this study were treated with oral agents or diet alone; furthermore, analyses performed with or without the inclusion of the few insulin taking patients showed the same results. Major confounders were measured and their role accounted for in the analysis. From the coefficient of multivariate regression it can be estimated that 1 SD of the population mean increase in Na–Li CT is associated with a 36% higher risk of incident diabetes independent of BMI, plasma glucose, blood pressure, and HDL cholesterol. In this dataset age, BMI, glucose, and blood pressure, are all significant, strong, predictors of diabetes, thus confirming previous extensive evidence and conferring internal consistency to the finding.

Na–Li CT has been extensively studied in relation to hypertension and is currently viewed as a marker of inherited pre-disposition to this condition. Whether Na–Li CT activity is a predictor of type 2 diabetes as it is for hypertension is unexplored to date. We believe this is relevant as no relatively
Table 3 Multiple logistic regression analyses of the relation of Na–Li countertransport at baseline and incident diabetes (6 years follow-up)

<table>
<thead>
<tr>
<th></th>
<th>Model with systolic blood pressure</th>
<th>Model with diastolic blood pressure</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>( \beta )</td>
<td>Standard error</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>0.081</td>
<td>0.010</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>0.117</td>
<td>0.028</td>
</tr>
<tr>
<td>Na–Li CT (( \mu \text{mol/l RBC/h} ))</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.039</td>
<td>0.014</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>−0.031</td>
<td>0.012</td>
</tr>
<tr>
<td>Sex (M, F)</td>
<td>0.452</td>
<td>0.258</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>0.003</td>
<td>0.006</td>
</tr>
</tbody>
</table>

easy-to-measure marker of inherited predisposition to diabetes has been identified till now. Previous works on Na–Li CT in diabetes focus on the relation of this ion transport system activity with hypertension and/or diabetic nephropathy.\(^{14,22-26}\) Our study considerably expands current knowledge showing for the first time that, on a population basis, enhanced Na–Li CT activity is associated with incidence of type 2 diabetes.

In this study the evidence that Na–Li CT activity is enhanced years before diabetes becomes clinically evident permits to rule out the possibility that enhanced Na–Li CT activity is caused by hyperglycemia or its treatment and strongly suggests that the activity of this ion transport system may be related to key pathogenic mechanisms influencing the development of type 2 diabetes, thus opening up new perspectives for the understanding of the molecular events leading to the metabolic abnormalities of diabetes. The hypothesis that Na–Li CT could represent an operational mode of NHE has long been debated and has been recently confirmed by a molecular approach in an elegant study by Zerbini et al.\(^{16}\) who have provided convincing evidence that Na–Li CT is mediated by an alternative splicing of the first isoform of NHE (NHE-1).\(^{17}\) A few years ago, after genetic linkage analysis, NHE-1 was excluded as the gene responsible for elevated NHE-1.\(^{17}\) However, the work by Zerbini et al.\(^{16}\) renews interest in the potential contribution of this gene to the pathogenesis of hypertension.

Both impaired insulin secretion and impaired insulin action play key roles in the development of type 2 diabetes.\(^{27}\) To our knowledge there is no evidence of a relation between Na–Li CT activity and impaired insulin secretion, whereas an inverse relation between Na–Li CT activity and insulin sensitivity has been demonstrated in both diabetic and non-diabetic hypertensive people.\(^{28,29}\) Furthermore a number of observational studies, including this one, have documented enhanced Na–Li CT activity in conditions described as components of the insulin resistance syndrome (i.e. hypertension, obesity, high triglycerides, and low HDL cholesterol).\(^{1-6}\) We did not measure triglycerides. However, with the use of a modified ATP III\(^{30}\) definition for the metabolic syndrome (i.e. coexistence of three among high blood pressure, high blood glucose, low HDL or obesity) we found that Na–Li CT values were significantly higher in people with, than without, the syndrome 376 \(\pm 130\) vs 322 \(\pm 129\) \(\mu \text{mol/l RBC/h}\).

Available evidence therefore strongly suggests that the increased risk of diabetes associated with enhanced Na–Li CT activity may be partly mediated by the relation of this ion transport system with insulin resistance. Whether enhanced Na–Li CT has a role in the development of insulin resistance, or it is only a marker of this condition remains to be answered. It has been suggested that Na–Li CT activity may indeed relate to insulin resistance because of a underlying sharing process affecting membrane physiology and vesicle trafficking. It is known that alkylation of thiols associated with cell membrane/cytoskeleton affects both membrane fluidity and Na–Li kinetics.\(^{31-33}\) A further working hypothesis involves the lipid composition of cell membranes; this is known to have profound effects on physiological and pathophysiological processes in the body by influencing membrane properties, metabolic signalling, gene expression, and energy expenditure. A specific, common, fatty acid pattern in the serum lipid esters as well as in cell membranes has been described in insulin resistance; in particular, a reduced proportion of polyunsaturated fatty acids and an increased proportion in saturated fatty acids lead to reduced membrane fluidity and worsening of insulin resistance.\(^{34}\) These abnormalities are also associated with elevated Na–Li CT activity in normal lipidic, hypertensive patients.\(^{35,36}\) Finally a ‘ionic’ hypothesis has been formulated suggesting that defects of ion handling at the cellular level—of which Na–Li CT may be a marker—result in lowered intracellular pH which, in turn, would influence vesicle trafficking and the complex insulin signalling cascade.\(^{37}\)

In conclusion, results of this study indicate that increased Na–Li CT activity can be viewed as a pre-clinical, possibly genetic, marker of pre-disposition to type 2 diabetes. Although the mechanisms of the association are far from clear, common cell membrane abnormality(ies) influencing both Na–Li CT and insulin resistance is a plausible explanation. Based on current knowledge Na–Li CT may be a marker of abnormalities in cell membrane/cytoskeleton function that in other cells may be causally related to diseases. Therefore, characterization of the molecular mechanisms regulating Na–Li CT kinetics at the cellular level may provide valuable insights into the pathogenesis of type 2 diabetes and other conditions clustering with hyperglycemia and increased cardiovascular risk.

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**KEY MESSAGES**

- The association between type 2 diabetes and hypertension has long been described, but the mechanisms remain unclear.
- Na–Li countertransport activity (Na–Li CT) is viewed as a marker of inherited pre-disposition to hypertension, especially if associated with other metabolic abnormalities.
- Prospective data from the present study show for the first time enhanced Na–Li CT activity is a significant predictor of development of diabetes in adults, thus suggesting that Na–Li CT activity could be viewed as a pre-clinical, possibly genetic, marker of inherited susceptibility to type 2 diabetes.

**References**


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