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Clustering of risk factors in hypertensive insulin-dependent diabetics with high sodium-lithium countertransport

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Clustering of risk factors in hypertensive insulin-dependent diabetics with high sodium-lithium countertransport. Diabetic nephropathy is more common in patients with a positive family history of hypertension and with elevated red blood cell sodium-lithium countertransport, a marker of risk for essential hypertension. To evaluate whether there is a relationship between this cation transport system and indicators of risk of renal and cardiovascular complications in diabetic patients before the development of clinical proteinuria, we studied 31 type 1 (insulin-dependent) diabetic patients with arterial hypertension, without clinical proteinuria and 12 normotensive normoalbuminuric diabetic patients. Sodium-lithium countertransport activity was significantly higher in hypertensive patients (0.43 ± 0.03 mmol/l RBC × hr) than in normotensive patients (0.23 ± 0.03; $P < 0.001$). To better explore the nature of the association between this transport system and arterial hypertension, hypertensive patients were divided in two groups, with high (>0.41 mmol/l RBC × hr) or normal (<0.41) sodium-lithium countertransport activity. The two groups of hypertensive diabetics were similar in age, sex, body mass index and blood pressure levels. Hypertensive patients with elevated rates of sodium-lithium countertransport compared with those with normal sodium-lithium countertransport activity showed elevated glomerular filtration rate (130 ± 4 ml/min/1.73 m^2 vs. 122 ± 3; $P < 0.05$), albumin excretion rate (median 26 μg/min vs. 11; $P < 0.001$), higher fractional proximal sodium reabsorption (74 ± 1.2% vs. 71.6 ± 0.9; $P < 0.01$), exchangeable sodium pool (2937 ± 62 mmol/1.73 m^2 vs. 2767 ± 56; $P < 0.01$), larger kidney volume (317 ± 7 ml/1.73 m^2 vs. 270 ± 8; $P < 0.05$) and left ventricular mass index (122 ± 4 g/m^2 vs. 107 ± 5; $P < 0.05$). Hypertensive patients with normal sodium-lithium countertransport activity had renal parameters similar to normotensive diabetic patients, except higher left ventricular mass index and kidney volume. Hypertensive diabetic patients with elevated sodium-lithium countertransport activity also had higher levels of plasma triglycerides, lower plasma HDL-cholesterol and impaired insulin sensitivity (assessed by euglycemic insulin-glucose clamp) compared with the other two groups. In conclusion, renal, cardiac and metabolic abnormalities are prominent in hypertensive type 1 (insulin-dependent) diabetic patients with higher sodium-lithium countertransport.

Blood pressure rises early in the course of the renal disease of insulin-dependent diabetes mellitus [1, 2] and diabetic nephropathy is more common in patients with a positive family history of hypertension [3, 4]. Moreover, elevated rates of red blood cell sodium-lithium countertransport (Na^+/Li^+ CT), a transport system associated with the risk for essential hypertension [5–7], have been found in diabetic patients who developed nephropathy [4, 8], in patients with microalbuminuria [4, 9] and with raised glomerular filtration rate [10] (2 precursors of overt renal disease).

In hypertensive patients, though Na^+/Li^+ CT is on average elevated, there is considerable overlap with values in normotensive subjects with approximately 50% of hypertensives showing normal Na^+/Li^+ CT activity [6]. Population studies have confirmed the association between Na^+/Li^+ CT activity and hypertension and have provided evidence for a major locus inheritance, but this association is not complete, and it has been suggested that the locus for Na^+/Li^+ CT is a susceptibility rather than a disease gene for hypertension [11].

Some authors have reported closer associations between raised Na^+/Li^+ CT activity and family history of hypertension and cardiovascular disease than between Na^+/Li^+ CT overactivity and hypertension itself [6, 12, 13]. All these findings raise the question as to whether an elevation of Na^+/Li^+ CT characterizes a particular subset of hypertensive patients prone to target organ complications.

In the context of diabetes and hypertension, it becomes important to discover whether there is a relationship between the rates of Na^+/Li^+ CT and indicators of risk of renal and the attendant cardiovascular complications in diabetic patients prior to the development of persistent proteinuria. This may permit early identification of patients at risk of end organ complications.

We therefore studied, in nonproteinuric, hypertensive insulin-dependent diabetic patients, the rates of Na^+/Li^+ CT and related them to indexes of renal and cardiovascular risk such as albumin excretion rate, lipids, and renal functions, kidney and heart size and insulin resistance.

Methods

Patients

Thirty-one insulin-dependent diabetic patients with elevated arterial pressure, but otherwise free of any other clinically overt

complication, attending the outpatient diabetic clinics of the Department of Internal Medicine, University of Padova and two District General Hospitals in the Veneto region (Pordenone and Monfalcone) were recruited for this study on the basis of three selection criteria: 1) blood pressure levels higher than 140 mm Hg for systolic and 90 mm Hg for diastolic value in absence of anti-hypertensive treatment; 2) urine negative for albustix test (Boehringer-Manheim, Germany); and 3) duration of diabetes lower than 11 years. The patients were otherwise free of any other clinically overt complication.

A matched group of twelve normotensive diabetic patients recruited from the same clinics served as controls. Physical activity (moderate noncompetitive exercise 4 to 5 hours per week) was similar in all subjects, as well as were smoking habits and alcohol intake. All subjects were Caucasian with normal serum creatinine concentration. Blood pressure (diastolic phase V) was measured in the sitting position after 10 minutes of rest to the nearest 2 mm Hg using a random zero sphygmomanometer. Causes of secondary hypertension were excluded by a complete medical work up with a 12 lead ECG. All subjects had normal renal, liver and endocrine function and were following an isocaloric diet containing 50% carbohydrate, 25% fat and 25% protein and about 100 mmol/day sodium chloride. Timed overnight urine collections for urinary albumin measurement [14] were performed at least four times over a period not exceeding six months. Patients were admitted to a metabolic ward a week prior to the study and all anti-hypertensive medications were discontinued for at least a week prior to admission.

In the six months preceding admission all patients were changed to three insulin injections a day and followed at least once a month in order to stabilize their metabolic control. Glycosilated hemoglobin A_{1c} (HbA_{1c}) records of their metabolic control were available from case notes over the preceding three years. Daily insulin requirement was measured for 30 days after the first visit and the 30 days preceding the study. The average of the insulin requirements during the two monthly periods was then used.

A fasting blood sample was taken in a heparin-treated syringe for measurement of Na^+/Li^+ CT, as previously described by Canessa et al [5], at different times from ten to six months before each study. Studies were carried out during the last two to three days of admission in the hypertensive patients and on an outpatient basis in the normotensive diabetic controls. The day before renal function determination, the patients omitted any intermediate-acting insulin injection and received short-acting insulin doses only at 8:00 a.m. and at 12:00 a.m. At 6:00 p.m., a constant subcutaneous infusion of insulin (Actarapid Human, Novo, Rome, Italy) at a rate of 15 mU/kg/hr with a bolus MC20 insulin-infusion device (Miles, Ames, Cavenago, Mi, Italy) was started to achieve and maintain euglycemia. In the evening, all patients were given 16.2 mmol of lithium carbonate orally. Food, alcohol, coffee, tea and smoking were prohibited after 10:00 p.m. In the morning, the overnight constant insulin infusion was continued and the patients connected to an artificial pancreas (Biostator, Miles-Ames) run in mode 9:1 to maintain blood glucose at 4.7 mmol/liter with the infusion of glucose [15, 16]. All experiments were performed under comparable conditions of fluid infusion. At 7:00 a.m., a wrist vein was cannulated in a retrograde manner and the hand placed in a warming box to arterialize venous blood for blood sampling to measure hormone and metabolite levels and Na^+/Li^+ CT for a second time. Subjects drank 1 liter of tap water followed by 0.3 liter of water every 30 minutes throughout the study to induce a steady state of water diuresis at 8 to 10 ml/min. At 8:00 a.m., an indwelling catheter was placed in an antecubital vein of the opposite arm, and priming doses of 50 μCi ^{51}Cr-EDTA followed by constant infusion of 0.7 μCi/min of the tracer was given to determine GFR as previously indicated [15, 16]. Patients remained supine throughout the experiment, standing only to void. After two hours of tracer equilibration, urine was collected for four thirty minute periods. Blood was drawn at the midpoint of each urine collection. Lithium clearance and sodium excretion rate were measured simultaneously [15, 16]. For total exchangeable sodium, a dose of 60 μCi of ^{24}Na was given orally after an overnight fast and urine was collected during the subsequent 24 hours. During the last hour, four blood samples were obtained for determination of sodium specific activity [17]. On a separate day, insulin sensitivity was assessed by the use of an euglycaemic insulin-clamp technique previously reported [18, 19]. Briefly, a constant infusion of insulin (Actrapid HM, Novo, Denmark) was given at a rate of 30 mU/hr/kg for 180 minutes followed by a rate of 90 mU/hr/kg for the next 180 minutes. Plasma glucose concentration was maintained constant by a variable infusion of exogenous glucose (20% wt/vol). The amount of glucose required to maintained euglycemia equals whole body glucose disposal, when endogenous glucose production is added to this value. Endogenous glucose production was determined by isotope dilution technique using 6,6-$^2H_2$ glucose (98.6% $^2H_2$; Merk Isotopes, Montreal, Canada). This tracer was administered as a primed (2 mg/kg) constant (0.019 mg/kg/min) infusion for two hours before the start of the insulin-clamp period and was continued throughout the experimental period.

Analytical measurements and data analysis

Plasma glucose was assayed by the glucose oxidase method (Beckman Glucose Analyzer, Beckman Instruments, Fullerton, California, USA). Hemoglobin A_{1c} was determined by microcolumn chromatography (Bio Rad Laboratories, Richmond, California, USA). Plasma free insulin was measured by radioimmunoassay technique [20].

Lithium (Li^+) was determined in diluted samples by flame photometry for lithium clearance determination [16] and by atomic emission spectrophotometry for Na^+/Li^+ CT activity determination [5]. Sodium in urine and plasma was determined by flame photometry. Li^+ clearance was calculated according to standard formulas.

Rates of glucose turnover were calculated from the isotopic data using a two-compartment model for non-steady-state glucose kinetic [21, 22]. Proximal sodium reabsorption (PR_{Na}) was calculated as follows:

$$PR_{Na} = PN_{Na} \times GFR \times FR_{Li}$$

where P_{Na} is plasma sodium and $FR_{Li} = 1 - (V/GFR) \times (U_{Li}/P_{Li})$ where U_{Li}/P_{Li} is the urine to plasma ratio of lithium and V the urine flow.

Kidney and cardiac volumes were measured by an ultrasound technique [23, 24]. The volume of both kidneys was measured

and the mean of the two values used for calculations. Each subject was submitted to a 2-D derived/M-mode echocardiographic study with a mechanical 2.5 MHz probe and a commercially available instrumentation (Esacord 81, OTE Biomedica, Italy). According to the American Society of Echocardiography [24], the following parameters relative to left ventricle were obtained, each as an average of at least three measurements:

—left ventricular end-diastolic dimension (LVDd)
—left ventricular end-systolic dimension (LVSd)
—left ventricular diastolic posterior wall thickness (LVPW)
—interventricular septum thickness (IVS)

From these measurements left ventricular mass index (LVMI) was calculated according to the Devereux formula [25].

Total exchangeable sodium was measured by radioisotope dilution technique using ^{24}Na [17].

Fasting serum was separated and stored at 4°C for determination of lipids within five days. High density lipoproteins (HDL) were separated by ultracentrifugation [26]. Concentrations of cholesterol [27] and triglycerides [28] were measured by enzymatic colorimetric techniques.

The results are expressed as mean ± SEM. Analysis of variance was used for statistical evaluation of differences among groups. If a significant difference was found, differences between individual groups were tested by unpaired Student's *t*-test (unless otherwise stated) and data were subjected to Bonferroni correction for multiple comparisons. Correlation between variables was assessed by Spearman-Rank analysis.

Results

Sodium-lithium countertransport (Na^+/Li^+ CT) activity in the 31 hypertensive IDDM patients was significantly higher than that of the 12 normotensive IDDM control patients, but there was a large overlap between the two groups (Fig. 1). After six months of strict metabolic control, HbA_{1c} value decreased from 7.2 ± 0.4 to 6.2 ± 0.4 ($P < 0.01$) in normotensive and from 8.1 ± 0.3 to 6.2 ± 0.3 ($P < 0.01$) in hypertensive IDDM patients. Insulin daily requirement increased from 0.62 ± 0.08 U/kg/day to 0.75 ± 0.07 ($P < 0.05$) in normotensive and from 0.71 ± 0.05 to 0.93 ± 0.05 ($P < 0.01$) in hypertensive IDDM patients. Daily insulin requirement after six months of strict metabolic control was significantly higher in hypertensive ($P < 0.01$) than in normotensive diabetic patients. No significant change was found with regard to Na^+/Li^+ CT activity (normotensives from 0.23 ± 0.04 to 0.23 ± 0.03 mmol/liter RBC/hr; hypertensives from 0.42 ± 0.02 to 0.43 ± 0.03).

The wide range of Na^+/Li^+ CT values in hypertensive patients suggests heterogeneity among the hypertensive diabetic patients. To further explore this phenomenon we therefore compared a number of variables in hypertensive patients with supranormal Na^+/Li^+ CT with those of hypertensive patients with their countertransport activity within the normal range. In our laboratory the normal range of Na^+/Li^+ CT in 35 normal subjects without family history of hypertension ranged from 0.11 to 0.41 mmol/liter RBC/hr. Fourteen of the hypertensive diabetic patients had Na^+/Li^+ CT activity greater than the upper limit of normal range (Group H_2D; mean ± SEM; 0.55 ± 0.03) while seventeen had Na^+/Li^+ CT within the normal range (Group H_1D: 0.31 ± 0.02; $P < 0.01$). All twelve normotensive diabetic patients had Na^+/Li^+ CT activity within the normal range (Group ND).

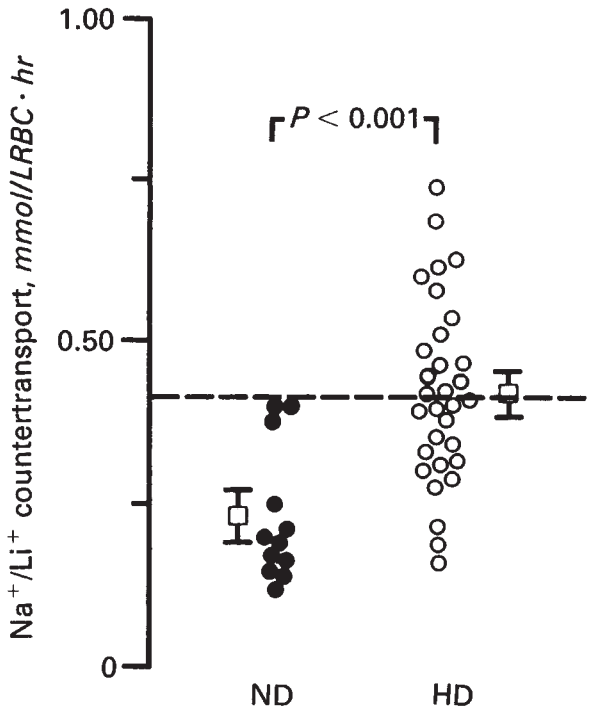

Fig. 1. *Sodium-lithium countertransport (Na^+/Li^+ CT) activity in red blood cells of twelve normotensive insulin-dependent diabetics (ND) and thirty-one hypertensive insulin-dependent diabetics (HD).* The broken line indicates the upper limit of normals (see text).

Table 1. Clinical data of 31 hypertensive type 1 (insulin-dependent) diabetic patients with high (H_2D) and normal (H_1D) Na^+/Li^+ CT activity and of 12 normotensive type 1 (insulin-dependent) diabetic patients (ND)

	H_2D	H_1D	ND
Sex (M/F)	9/5	11/6	7/5
Age *years*	27 ± 2	29 ± 2	31 ± 2
Duration of diabetes *years*	9 ± 1	8 ± 1	7 ± 1
Body mass index kg/m^2	22.7 ± 0.6	22.7 ± 0.3	22.5 ± 0.5
Systolic blood pressure *mm Hg*	164 ± 5	156 ± 4	125 ± 2
Diastolic blood pressure *mm Hg*	102 ± 2	99 ± 3	80 ± 1
Duration of antihypertensive treatment *years*	1.9 ± 0.4	2.1 ± 0.3	—

Values are given as mean ± SD.

The three groups of insulin-dependent diabetic patients were comparable for age, sex distribution, body mass index and duration of diabetes. The two groups of hypertensive diabetic patients had similar levels of blood pressure and duration of antihypertensive treatment (Table 1). When patients were recruited, they were taking the following drugs: ACE inhibitors (6 patients in Group H_2D vs. 5 in H_1D), furosemide (2 in H_2D vs. 2 in H_1D), nifedipine (7 in H_2D vs. 9 in H_1D), and thiazides (1 in H_2D vs. 3 in H_1D).

No difference were found in total cholesterol (4.81 ± 0.13 mmol/liter in H_2D; 4.73 ± 0.14 in H_1D and 4.91 ± 0.19 in ND) plasma levels. The cholesterol fraction in high density lipoproteins (HDL cholesterol) was significantly lower in H_2D (1.10 ±

Table 2. GFR, albumin excretion rate (AER), fractional proximal sodium reabsorption (FPNaR), sodium pool, mean kidney volume and left ventricular mass index (LVMI) of hypertensive diabetic patients with high (H_2D) and normal (H_1D) Na^+/Li^+CT activity and of normotensive diabetic patients (ND)

	H_2D	H_1D	ND
GFR *ml/min/1.72 m²*	130 ± 4[a,c]	122 ± 3	117 ± 2
AER *μg/min*	26 (6–69)[b,c]	11 (5–23)	6 (3–13)
FPNaR %	75.4 ± 1.2[b,c]	71.6 ± 0.9	72.5 ± 0.5
Sodium pool *mmol/1.72 m²*	2937 ± 62[b,c]	2767 ± 56	2782 ± 104
Mean kidney volume *ml/1.73 m²*	313 ± 7[a,c]	270 ± 8[d]	228 ± 18
LVMI *g/m²*	122 ± 4[a,c]	107 ± 5[d]	91 ± 2

Values are given as mean ± SD except for AER which is given as median (range).
[a] $P < 0.05$, [b] $P < 0.01$ H_2D vs. H_1D
[c] $P < 0.01$ H_2D vs. ND
[d] $P < 0.05$ H_1D vs. ND

0.03 mmol/liter) than in H_1D (1.6 ± 0.09; $P < 0.05$) and in ND patients (1.9 ± 0.08; $P < 0.01$). Plasma triglycerides were also significantly higher in H_2D compared with the other two groups of patients (1.62 ± 0.10 mmol/liter in H_2D; 1.53 ± 0.11 in H_1D; $P < 0.05$; and 1.39 ± 0.07 in ND; $P < 0.01$).

Plasma sodium concentrations were similar in the three groups (H_2D vs. H_1D vs. ND 141 ± 3 vs. 140 ± 2 vs. 139 ± 2 mmol/liter) as well as sodium excretion rate (216 ± 8 vs. 224 ± 10 vs. 198 ± 11 mmol/24 hr), but fractional proximal tubule sodium reabsorption rate was significantly higher in H_2D than in H_1D ($P < 0.01$) and in ND patients ($P < 0.01$; Table 2).

Sodium pool was significantly greater in H_2D patients than both H_1D ($P < 0.01$) and ND patients ($P < 0.01$) who showed similar values. Albumin excretion rate was significantly elevated in H_2D patients compared to H_1D and ND patients. All ND patients had albumin excretion rates lower than 20 μg/min (normal range in our laboratory: 1 to 20 μg/min) while 2 out of the 17 H_1D and 7 out of 14 H_2D patients showed an albumin excretion rate in the microalbuminuric range. The glomerular filtration rate was higher in H_2D than in H_1D ($P < 0.05$) and in ND patients ($P < 0.01$). Both H_1D and H_2D groups showed higher LVMI and mean kidney volume than ND patients, but they were significantly greater in H_2D patients than in H_1D group (Table 2).

In the fasting postabsorptive state plasma glucose concentration was similar in all groups (H_2D vs. H_1D vs. ND: 81 ± 3 mg/dl vs. 80 ± 2 vs. 79 ± 2) as well as plasma insulin concentration (21 ± 2 μU/ml vs. 22 ± 3 vs. 22 ± 3).

During glucose clamp (Fig. 2) plasma insulin levels rose to a similar plateau in the three groups during the first (H_2D 45 ± 3 μU/ml; H_1D 46 ± 3; ND 47 ± 4) and second insulin infusion period (H_2D 101 ± 7; H_1D 103 ± 4; ND 107 ± 8). Plasma glucose levels were maintained at around 85 mg/dl throughout the study in all three groups. The amount of exogenous glucose required to hold glucose level constant was significantly smaller in H_2D patients (14.2 ± 2.1 and 40.1 ± 1.9 g/160 min/1.73 m²) than in H_1D patients (18.8 ± 2.3 and 55.4 ± 5.2; $P < 0.05$) and in ND subjects (20.8 ± 2.3 and 62.4 ± 4.8; $P < 0.01$) during the low and high insulin clamp, respectively.

Whole body glucose utilization, calculated using [6.6 $^2H_2$] glucose dilution data, was significantly lower in H_2D patients during the first and second insulin plateau than in H_1D and ND patients (first plateau, 2.23 ± 0.24 mg/kg/min vs. 3.17 ± 0.26 vs. 3.39 ± 0.31, $P < 0.01$ for both, respectively; second plateau: 3.88 ± 0.34 mg/kg/min vs. 5.41 ± 0.39 vs. 5.88 ± 0.41, $P < 0.01$ for both).

Endogenous glucose production rate at baseline was similar in the three groups and averaged 2.29 ± 0.19 mg/kg/min in ND patients, 2.19 ± 0.22 in H_1D patients, and 2.27 ± 0.31 in H_2D patients. Insulin infusion promptly and similarly inhibited endogenous glucose release by about 50% during the first insulin plateau and by about 100% during the second insulin plateau in all groups (Fig. 2).

Discussion

The combination of diabetes mellitus and hypertension has a devastating effect on the kidney, the heart and the arteries [29, 30]. The insulin-dependent diabetic patients who have developed proteinuria and are prone to cardiovascular complications have been recently found to have higher rates of sodium-lithium countertransport [4, 8] and to belong to families with a higher frequency of parental arterial hypertension [3, 4]. The present study demonstrates that elevated rates of sodium-lithium countertransport identifies a subgroup of as yet uncomplicated hypertensive insulin-dependent diabetic patients in whom a striking aggregation of early risk indicators for renal and cardiovascular complications occurs. Hypertensive insulin-dependent diabetic patients with raised sodium-lithium countertransport activity showed higher albumin excretion rate, greater renal sodium reabsorption and total body exchangeable sodium pool, enlarged kidneys and hearts, worse glycemic control and a reduced insulin sensitivity. All of these parameters have been related with the later development of renal and cardiovascular complications in the general as well as in the diabetic population.

Our findings are consistent with the observation that only a subset of hypertensive or diabetic subjects are at risk of end organ complications. This risk appears to be related to the possession of an abnormal sodium-lithium countertransport activity. Other workers have suggested the possible association of elevated rates of sodium-lithium countertransport with cardiovascular complications in non-diabetic patients with hypertension or renal disease [10, 11, 31].

Microalbuminuric insulin-dependent diabetic patients have been reported to have higher rates of sodium-lithium countertransport [9]. In spite of similar levels of elevated blood pressure only 12% of the hypertensive patients with normal countertransport had marginally elevated albumin excretion rate. By contrast 50% of hypertensive patients with elevated sodium-lithium countertransport were microalbuminuric. This finding is in agreement with early reports in essentially hypertensive patients which showed that microalbuminuria only occurs in a subgroup of individuals [32, 33]. It suggests that elevation of blood pressure per se may not be sufficient to alter the albumin excretion rate and that other factors are at work.

Glomerular hyperfiltration has also been suggested to be a predictor of subsequent renal damage [34, 35], and recently it has been associated with an increased sodium-lithium countertransport activity [10]. In our study the hypertensive diabetics with high sodium-lithium countertransport also showed an increased GFR. It is of interest that these patients also had

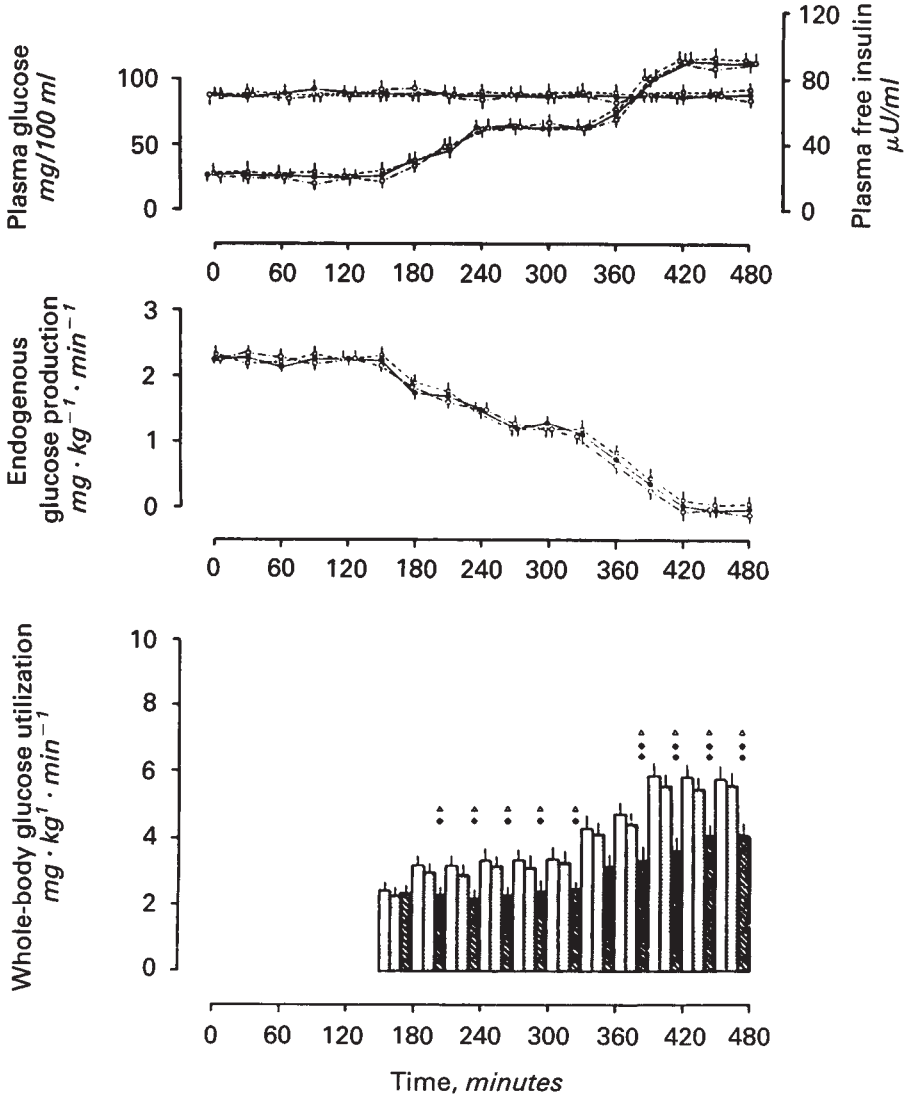

Fig. 2. *Mean ± SE for plasma glucose, free insulin concentrations, and endogenous glucose production in normotensive diabetic (–□–), in hypertensive diabetic patients with high (—●—) and normal (—○—) Na^+/Li^+ CT activity at baseline and during stepwise insulin-glucose clamp.* The bars on the lower panel indicate whole body glucose utilization rates in normotensive diabetics (□) and hypertensive diabetics with normal (▨) and high (▧) Na^+/Li^+ CT. $^*P < 0.05$ $^{**}P < 0.01$ hypertensive diabetics with high Na^+/Li^+ CT vs. ND.

nephromegaly, an increased estimated proximal sodium reabsorption and sodium pool, suggesting a more pronounced sodium retention. The abnormalities in sodium metabolism we have described resemble those reported in essential hypertensive patients with non-modulating hypertension [36] who also had higher sodium-lithium countertransport activity [37].

In our study hypertensive diabetics had echographic evidence for cardiac hypertrophy. Our findings of more pronounced left ventricular hypertrophy in patients with high sodium-lithium countertransport are interesting in that the presence of cardiac hypertrophy is well known to increase the frequency of cardiovascular morbidity and mortality [38, 39]. That cardiac hypertrophy was not simply the consequence of the mechanical effect of increased blood pressure but was more closely associated to the presence of an overactivity of sodium-lithium countertransport is supported by the observation that blood pressure levels and duration of hypertension were similar in the two groups of hypertensive diabetic patients.

The current study also demonstrates that a greater insulin resistance is a further clinical feature of these hypertensive insulin-dependent diabetic patients with high sodium-lithium countertransport. These patients also had a worse metabolic control in the years preceding the study (as testified by HbA_{1c} value) and the greater daily insulin dose required to achieve a strict metabolic control in the period immediately preceding the euglycemic clamp study. The site of impaired insulin action was the peripheral tissues and not the liver. In fact, endogenous glucose production was similar in all diabetic patients, independently of blood pressure levels, and was similarly inhibited by both moderate and high plasma insulin levels. Both diabetes and essential hypertension have been reported to be a state of insulin resistance [40, 41], a phenomenon proposed to be an independent risk factor for coronary artery disease [42]. However, only a proportion of hypertensive patients develops cardiovascular and renal complications [43], and only a subset of insulin-dependent diabetic patients develops similarly late organ damages [44]. That insulin resistance in type 1 (insulin-dependent) diabetes is influenced by a great number of variables (duration of disease, previous metabolic control, body mass index, sex, age) is well known [45], but, to date, no studies have

been addressed to the relationship between insulin resistance, arterial hypertension and sodium-lithium countertransport in insulin-dependent diabetic patients. Of particular interest is that both insulin resistance and high sodium-lithium countertransport have been found to be associated with lipid abnormalities [9, 46, 47]. All these factors which are believed to be a risk for cardiovascular disease seem therefore to cluster in the same individuals.

The nature of the association between sodium-lithium countertransport activity and insulin resistance as well as the other abnormalities we have described remain to be established. Some evidence has been provided that sodium-lithium countertransport is a mode of operation of the ubiquitous physiological sodium-hydrogen antiport [48], a cell membrane system involved in the intracellular pH regulation, in the processes leading to cell growth and replication and, at kidney level, in the tubular reabsorption of sodium [49]. Insulin stimulates the sodium-hydrogen antiport in various cell types [49] and the sodium-hydrogen antiport has been shown to be linked with calcium exchange [50]. Increase in both intracellular sodium and calcium are known not only to enhance the sensitivity of smooth muscle cells to pressor factors [51], but also to stimulate cell growth and proliferation [52]. This sequence of events could result in the cardiac and renal hypertrophy observed in our hypertensive diabetic patients with high sodium-lithium countertransport activity. Consistent with this, it is possible that insulin resistance and therefore the consequent hyperinsulinemia could cause a further elevation of sodium-hydrogen activity. That an overactivity of this system is associated with impaired glucose metabolism is supported by recent experiments in spontaneously hypertensive rats (SHR) in which the lower insulin-induced glucose uptake by brush border membrane vesicles was ascribed to an enhanced dissipation of sodium gradient across brush border membrane, secondary to a higher sodium-hydrogen antiport activity [53].

Whatever the relationship between insulin resistance and abnormality in Na^+/Li^+ CT activity, it is interesting that our data show an association between higher levels of HbA_{1c} and the occurrence of microalbuminuria in insulin-dependent diabetic patients irrespective of the presence of hypertension (**Note added in proof**). These findings are in keeping with those of Krolewski et al [4] reporting a significant positive correlation between blood glucose retrospective levels and albumin excretion rate. Moreover, the same group [54] observed more recently that insulin-dependent diabetic patients with elevated Na^+/Li^+ CT activity were to some extent protected against the development of kidney damage by a good metabolic control, despite the presence of this abnormality in cell cation transport system. All together these data support the hypothesis that a poor metabolic control can also alter kidney function in insulin-dependent diabetic patients, particularly in that cohort of patients already characterized by an elevated Na^+/Li^+ CT activity.

In conclusion, we have shown that a number of metabolic and morphological abnormalities predictive of subsequent renal and cardiovascular damage are clustering together in hypertensive insulin-dependent diabetic patients with high sodium-lithium countertransport activity. This finding clearly suggests that a high sodium-lithium countertransport activity could be a marker for those patients most at risk of renal and cardiovascular complications.

Note added in proof

Similarly, we recently observed that impaired insulin sensitivity characterizes only those patients with essential hypertension, who also have high Na^+/Li^+ CT activity (A) and cardiorenal abnormalities (B).
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