# Sodium-Lithium Countertransport Activity and Insulin Resistance in Normotensive IDDM Patients

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**In insulin-dependent diabetes (IDDM), an overactivity of sodium-lithium countertransport ($Na^+/Li^+$ CT) has been associated with the risk of nephropathy and hypertension, two conditions of insulin resistance. We investigated the sensitivity to insulin with a hyperinsulinemic (≈719 pM [≈100 μU/ml]) euglycemic clamp in two groups of normotensive nonproteinuric IDDM patients; 12 (10 men, 2 women) had high $Na^+/Li^+$ CT activity (mean 0.47, range 0.42–0.68 mmol/L red blood cells [RBC]/h, group 1) and 12 (9 men, 3 women) had normal $Na^+/Li^+$ CT activity (mean 0.24, range 0.12–0.31 mmol/L RBC/h, group 2). The two groups were similar in age (mean ± SE 36 ± 2 vs. 33 ± 1 yr), duration of diabetes (19 ± 3 vs. 18 ± 2 yr), body mass index (26 ± 0.8 vs. 24 ± 0.6 kg/m$^2$), arterial blood pressure (systolic/diastolic 121 ± 4/79 ± 2 vs. 122 ± 3/77 ± 2 mmHg), and glycemic control ($HbA_1$ 8.5 ± 0.4 vs. 8.0 ± 0.4%). Albumin excretion rate (AER) ranged between 4.7 and 148 (geometric mean 14) μg/min in group 1 and between 2.7 and 93 (geometric mean 11) μg/min in group 2. There were four *microalbuminuric patients* (AER >30 μg/min) *in each* group. Whole-body glucose uptake was significantly reduced on average in group 1 compared with group 2 (41.6 ± 2.2 μmol · kg$^{-1}$ · min$^{-1}$ [7.48 ± 0.4 mg · kg$^{-1}$ · min$^{-1}$] vs. 49.6 ± 2.2 μmol · kg$^{-1}$ · min$^{-1}$ [8.93 ± 0.4 mg · kg$^{-1}$ · min$^{-1}$, $P = 0.03$), but some overlap existed between the two groups. Hepatic glucose output, as measured by isotope dilution with [3-$^3$H]glucose, was similarly suppressed in both study groups. The proportion of echocardiographically determined left ventricular hypertrophy was significantly higher in patients with high $Na^+/Li^+$ CT (33 vs. 0%, $P = 0.046$). Triglycerides (TG), the ratio of low-density lipoprotein (LDL) to high-density lipoprotein (HDL), and apolipoprotein B (apoB) were higher in group 1 (median [range] TG 0.90 [0.51–1.64] vs. 0.52 [0.37–1.26] mM, $P = 0.002$, LDL-HDL ratio 3.36 [1.43–5.72] vs. 2.28 [1.21–3.33], $P = 0.019$, apoB 1.06 [0.54–1.16] vs. 0.80 [0.61–1.33] g/L, $P = 0.03$). In clinically nonproteinuric normotensive IDDM patients, increased $Na^+/Li^+$ CT activity is associated with reduced insulin sensitivity, left ventricular hypertrophy, and lipid disturbances. These abnormalities either singly or in combination may contribute to vascular damage in a subset of patients with IDDM. *Diabetes* 41:610–15, 1992**

In insulin-dependent diabetic (IDDM) patients, several lines of evidence suggest a link between essential hypertension, or a predisposition to essential hypertension, and diabetic kidney disease (1,2). The red blood cell *sodium-lithium countertransport* ($Na^+/Li^+$ CT), a marker of essential hypertension (3), has been reported by some, though not all, authors (2,4,5) to be elevated in IDDM patients with microalbuminuria or clinical proteinuria and arterial hypertension (6). Microalbuminuric and persistently proteinuric diabetic patients also have higher lymphocyte $Na^+/H^+$ antiport activity than normoalbuminuric diabetic patients (7). An overactivity of this cell membrane sodium-transport system may be involved in the pathogenesis of arterial hypertension (8).

Arterial hypertension was recently associated with peripheral insulin resistance, and suggestions of a pathophysiological connection between these two phenomena have been made (9–11). Insulin resistance, though a well-established feature of insulin-dependent diabetes mellitus (12–14), is not a universal finding in these patients, who have various degrees of insulin sensitivity (14). The cause of such heterogeneity and its possible clinical consequences are poorly understood.

To further investigate this issue, we studied peripheral

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TABLE 1
Clinical features of insulin-dependent diabetic patients with normal or high sodium-lithium countertransport ($Na^+/Li^+$ CT)

| | Patients with normal $Na^+/Li^+$ CT | Patients with high $Na^+/Li^+$ CT |
|---|---|---|
| Sex (M/F) | 9/3 | 10/2 |
| Age (yr) | 33 (24–38) | 36 (24–50) |
| Duration of diabetes (yr) | 18 (6–25) | 19 (6–28) |
| Body mass index ($kg/m^2$) | 24 (21–29) | 26 (22–30) |
| Systolic blood pressure (mmHg) | 122 (106–139) | 121 (101–139) |
| Diastolic blood pressure (mmHg) | 77 (60–89) | 79 (69–89) |
| $Na^+/Li^+$ CT (mmol/LRBC/h) | 0.24 (0.12–0.31) | 0.47 (0.42–0.68) |

Values are means with ranges in parentheses. RBC, red blood cells.

insulin sensitivity with a euglycemic insulin-clamp technique, in combination with [3-$^3$H]glucose turnover, in normotensive nonproteinuric IDDM patients with elevated $Na^+/Li^+$ CT activity, a group believed to be at risk of hypertension and renal disease (2,4,5), and compared them with similar patients with normal $Na^+/Li^+$ CT activity. Furthermore, we assessed left ventricular mass to test whether left ventricular hypertrophy, which is usually found in IDDM patients with proteinuria and hypertension (15,16), was present in the patients with high $Na^+/Li^+$ CT before the appearance of these clinical manifestations.

## RESEARCH DESIGN AND METHODS

IDDM patients were recruited from our outpatient clinic by the following criteria: no arterial hypertension (blood pressure <140/90 mmHg), no clinical proteinuria (albumin excretion rate <150 μg/min in at least 2 consecutive overnight collections), age between 18 and 55 yr, age at diagnosis of diabetes <31 yr, and body weight within 20% of ideal.

Twelve patients with $Na^+/Li^+$ CT activity above the upper limit of normal (the normal range in our laboratory is 0.11–0.41 mmol/L red blood cells (RBC)/h measured in 35 normotensive subjects with no family history of hypertension) were matched with a group of 12 patients of similar age, sex, duration of diabetes, body mass index and blood pressure and with $Na^+/Li^+$ CT in the normal range (Table 1). All patients were white and of European origin. Patients were following their diabetic weight-maintaining diet, which was similar in both groups, and taking no drugs except insulin. They had no endocrine, liver, renal, or metabolic disease, and none of the female subjects were on oral contraceptives or estrogens. The two groups had similar levels of physical activity, and no subject engaged in competitive sports. All patients were asked to avoid heavy physical exercise on the day before the study. All patients gave written consent after explanation of the nature, purpose, and potential risks of the study, which was approved by the Ethics Committee of Guy's Hospital.

Patients were admitted to a metabolic ward the evening before the study, and their evening dose of insulin was replaced by a variable i.v. infusion of insulin to maintain overnight euglycemia. Studies began at 0800 after a 10- to 12-h overnight fast. Polyethylene cannulas were inserted into an antecubital vein for infusion of 20% wt/vol glucose and into a forearm vein for infusion of insulin and [3-$^3$H]glucose. A wrist vein in the opposite arm was cannulated in a retrograde manner and the hand placed in a warming box (60°C) for blood sampling. Use of the hot box ensured arterialization of venous blood within 20 to 40 min (17).

Peripheral insulin sensitivity was assessed by the use of a euglycemic insulin-clamp technique as described by DeFronzo et al. (18). Briefly, a primed constant infusion of insulin (Actrapid, Novo Industri, Copenhagen) was administered at a rate of 575 $pmol \cdot m^{-2} \cdot min^{-1}$ (80 $mU \cdot m^{-2} \cdot min^{-1}$) for 3 h, during which the blood glucose concentration was held constant at 5 mM by variable glucose infusion rate with a Harvard pump (model 22, Harvard Apparatus, South Natick, MA). The individual controlling the glucose infusion rate was unaware of the patient's $Na^+/Li^+$ CT status. Endogenous glucose production was determined by isotope dilution with [3-$^3$H]glucose (Amersham, Aylesbury, UK), which was administered as a primed (30 μCi) continuous (0.3 μCi/min) infusion for 2 h before the start of the insulin-clamp period and was continued throughout the experiment.

Before the start of the study, fasting blood samples were obtained for measurements of free insulin, creatinine, potassium, lipid profile, and glycated hemoglobin. Arterial blood pressure (phase I/V) was measured twice, at 2-min intervals, to the nearest 2 mmHg with patients supine by the same observer (JBLF) with a random zero sphygmomanometer. The mean of the two blood pressure measurements was used for calculation. During the study, blood samples were obtained every 5 min for plasma glucose determination and every 15 min for measurements of tritiated glucose specific activity and free insulin.

Echocardiograms were performed within 1 wk of the insulin clamp and were recorded and analyzed by one observer (JBC) unaware of the patient's clinical features. A phased-array system (Hewlett-Packard, 77020 A, Andover, MA) was used with a 2.5-MHz duplex probe. Sector scans with color-flow mapping were used to screen for wall motion or valvular abnormalities. Left ventricular M-mode recordings were then taken with the patient positioned carefully so that the septum was perpendicular to the ultrasound beam. Measurements of internal diameter and wall thickness of the left ventricle were made at end-diastole according to the recommen-

TABLE 2
Daily insulin requirement, $HbA_1$, plasma potassium ($K^+$), albumin excretion rate (AER), serum creatinine, and glomerular filtration rate (GFR) in insulin-dependent diabetic patients with normal or high sodium-lithium countertransport ($Na^+/Li^+$ CT)

| | Patients with normal $Na^+/Li^+$ CT | Patients with high $Na^+/Li^+$ CT |
|---|---|---|
| Insulin dose ($U \cdot kg^{-1} \cdot 24\ h^{-1}$) | 0.69 (0.4–1.2) | 0.69 (0.4–1.1) |
| $HbA_1$ (%) | 8.0 (5.5–10.7) | 8.5 (5.5–10.3) |
| $K^+$ (mM) | 4.1 (3.5–5.1) | 4.0 (3.4–4.5) |
| AER (μg/min) | 11 (2.7–93) | 14 (4.7–148) |
| Creatinine (μM) | 87 (64–108) | 88 (78–106) |
| GFR ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$) | 121 (100–140) | 117 (76–154) |

Values are means with ranges in parentheses, except for AER, which are geometric means with ranges in parentheses.

dations of the American Society of Echocardiography (19).

Determinations of red blood cell $Na^+/Li^+$ CT activity, albumin excretion rate, and glomerular filtration rate were made within 1–2 mo of the insulin-clamp studies.

Plasma glucose was assayed by the glucose oxidase method (Yellow Springs Glucose Analyzer, Yellow Springs Instruments, Yellow Springs, OH). Plasma free insulin was determined by radioimmunoassay as previously described (20). Glycated hemoglobin was measured by electroendosmosis (Corning Chemical, Palo Alto, CA). Urinary albumin was determined by radioimmunoassay (21). Serum concentrations of cholesterol and triglycerides were assayed by enzymatic colorimetric techniques (cholesterol oxidase/peroxidase-amidopyrine and glycerol phosphate oxidase/peroxidase-amidopyrine, Boehringer, Mannhein, Germany) with a Cobas-Bio centrifugal analyzer (Roche, Welwyn Garden City, UK). High-density lipoproteins (HDL) were separated by ultracentrifugation (22). Low-density lipoprotein (LDL) concentration was calculated with the Friedewald equation (23). Concentration of apolipoprotein B (apoB) was measured by immunoturbidimetry with a Cobas-Bio analyzer and Orion Diagnostica antiserum and reagents (Oxoid, Basingstoke, UK). Serum creatinine was measured by an automated method (Hitachi Autoanalyser, distributed by Boehringer BCL, Lewes, UK) and glomerular filtration rate by $^{51}$Cr-EDTA clearance (24). Sodium-lithium countertransport activity in red blood cell was determined by the method of Canessa (3) as previously described by our laboratory (5). [3-$^3$H]Glucose concentrations in plasma were measured in triplicate on the supernatants of 1M perchloric acid extracts of plasma samples after the radiolabeled water was removed by evaporation. Diluted aliquots of the tracer infusates were processed in the same way to determine recovery and obtain a precise measure of the tracer infusion rate (25).

Glucose disposal rate (expressed as milligrams per kilogram per minute) was calculated over 30-min intervals as the glucose infusion rate, adjusted for deviations from the target plasma glucose level of 5 mM (18,25). At steady state, achieved during the last 30 min of each experiment, whole-body glucose uptake equals glucose disposal rate when endogenous glucose production is suppressed. The steady-state disposal rate for the last 30 min of each study was used for comparison. The equation of Steele (26) was used for calculation of glucose appearance (Ra) from the tracer data. The rate of endogenous glucose production was calculated by subtracting the glucose infusion rate from Ra. This model produces negative estimates of endogenous glucose production in the presence of high levels of insulin (27). Indeed, in our study, negative rates of endogenous glucose release were obtained during the last 30 min in both groups. These measurements were taken only to indicate that endogenous glucose output was completely suppressed, and were not used further.

Left ventricular mass was calculated with the formula of Devereux and Reichek (28): left ventricular mass (in grams) = $1.04\ ([LVID + VST + PWT]^3 - [LVID]^3) - 13.6$, where LVID is left ventricular internal diameter, VST is the ventricular septal thickness, and PWT is the posterior wall thickness. This value was corrected for height because of the described association between left ventricular mass and height in control subjects (29). Left ventricular hypertrophy was defined as a value of left ventricular mass ≥2 SDs above the mean for a healthy reference group as reported by the Framingham Heart Study. The cutoff values for left ventricular hypertrophy were 143 and 102 g/m for men and women, respectively (29).

The two groups were compared with two-tailed unpaired Student's *t* test for normally distributed variables. Variables not normally distributed (for example, serum lipoproteins and steady-state whole-body glucose uptake) were analyzed with Mann-Whitney *U* test. Glucose disposal rate during the insulin-clamp study was calculated as the area under the curve (30). The Fisher exact test was used for analysis of discrete variables. *P* values <0.05 were considered significant. All data are mean ± SE unless otherwise stated.

## RESULTS

The two groups of patients had similar daily insulin requirements, blood glucose control, plasma potassium, albumin excretion rate, plasma creatinine, and glomerular filtration rate (Table 2). Four patients in each group had albumin excretion rate >30 μg/min; the rest had albumin excretion rate <15 μg/min. The patients with high $Na^+/Li^+$ CT had significantly higher serum triglycerides (median [range] 0.90 [0.51–1.64] vs. 0.52 [0.37–1.26] mM, $P = 0.002$), low-density lipoprotein (LDL)-high-density lipoprotein (HDL) ratio (3.36 [1.43–5.72] vs. 2.28 [1.21–3.33], $P = 0.019$), and apoB (1.06 [0.54–

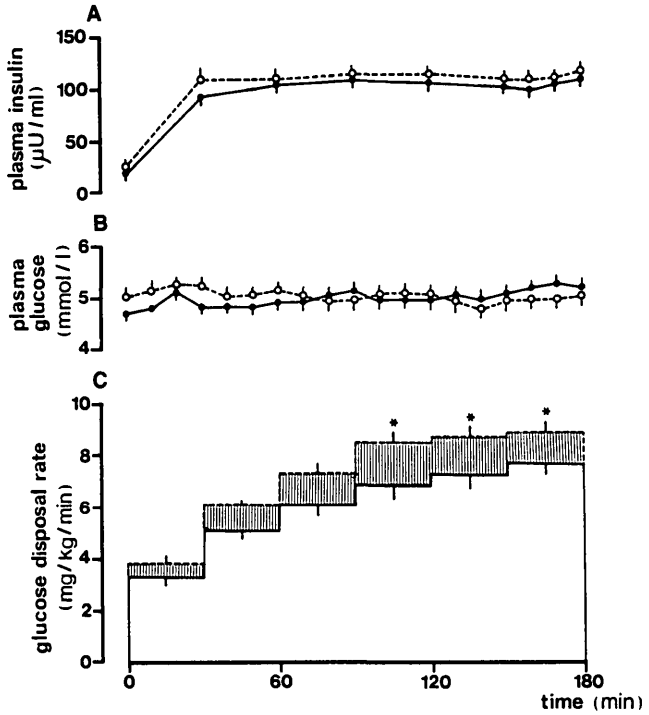


**FIG. 1. Mean ± SE plasma free insulin (*A*), plasma glucose (*B*), and insulin-stimulated glucose disposal rate (*C*) in 12 insulin-dependent diabetic patients with high (*solid circles*) and 12 insulin-dependent diabetic patients with normal (*open circles*) $Na^+/Li^+$ countertransport.**

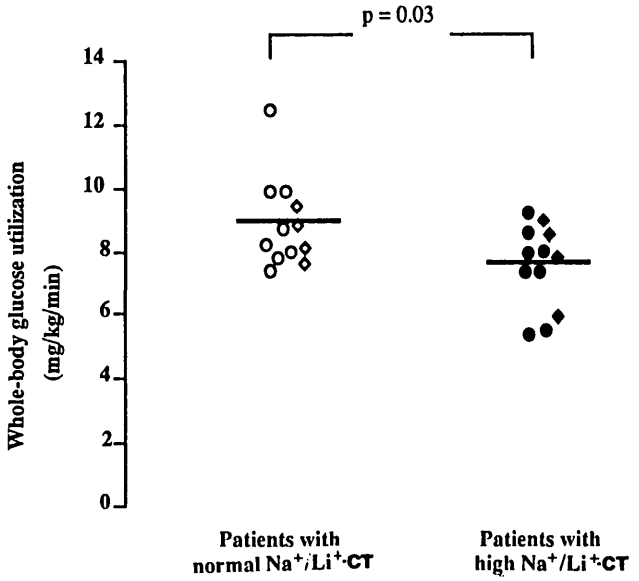


**FIG. 2. Whole-body glucose uptake during steady-state euglycemic insulin clamp in 12 insulin-dependent diabetic patients with normal $Na^+/Li^+$ countertransport (CT; *open symbols*) and high $Na^+/Li^+$ CT (*solid symbols*). *Circles*, normoalbuminuric patients. *Diamonds*, microalbuminuric patients.**

1.16] vs. 0.80 [0.61–1.33] g/L, $P = 0.003$) than patients with normal $Na^+/Li^+$ CT.

The results of the euglycemic insulin-clamp studies are given in Fig. 1. Insulin infusion promptly increased plasma free insulin levels similarly in both groups (763 ± 14 pM [106 ± 2 μU/ml] vs. 734 ± 14 pM [102 ± 2 μU/ml], NS). Euglycemia at 5 M, coefficient of variation 9%, was sustained throughout the studies by variable glucose-infusion rate. Mean glucose-disposal rate was significantly lower in patients with high $Na^+/Li^+$ CT than in patients with normal $Na^+/Li^+$ CT activity, starting from the 90-min time points ($P < 0.05$). Whole-body glucose uptake, calculated at steady state in the last 30 min of the study, was impaired in patients with high $Na^+/Li^+$ CT compared with patients with normal $Na^+/Li^+$ CT (41.6 ± 2.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ [7.48 ± 0.4 $mg \cdot kg^{-1} \cdot min^{-1}$] vs. 49.6 ± 2.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ [8.93 ± 0.4 $mg \cdot kg^{-1} \cdot min^{-1}$], $P = 0.03$; Fig. 2). This difference in insulin-stimulated glucose uptake remains significant even when the microalbuminuric patients are excluded from the calculation (40.1 ± 2.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ [7.36 ± 0.5 $mg \cdot kg^{-1} \cdot min^{-1}$] vs. 50.5 ± 3.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ [9.09 ± 0.6 $mg \cdot kg^{-1} \cdot min^{-1}$], $P = 0.04$), suggesting that different sensitivity to insulin cannot be accounted for by this group of patients. At steady state, glucose hepatic production was suppressed similarly in both groups (−1.89 ± 0.3 and −1.66 ± 0.4 $mg \cdot kg^{-1} \cdot min^{-1}$, respectively). Although mean values for whole-body glucose uptake differed significantly between the two groups, there was overlap of individual values, implying some heterogeneity within the groups.

Left ventricular mass was higher in patients with high than in patients with normal $Na^+/Li^+$ CT (105 ± 31 vs. 90 ± 17 $g/m^2$), but this difference was not statistically significant ($P = 0.15$). However, three men and one woman (33%) in the group with high $Na^+/Li^+$ CT had left ventricular hypertrophy, whereas no patients had abnormal left ventricular mass among those with normal $Na^+/$

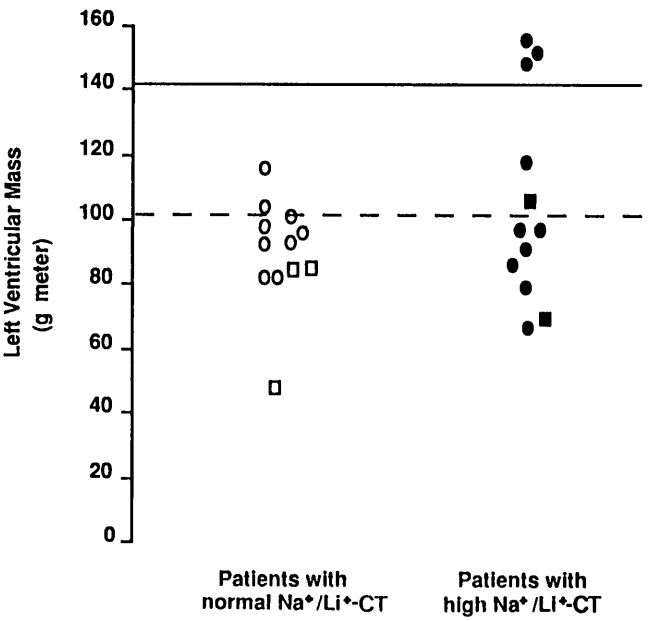


**FIG. 3. Left ventricular mass in 12 insulin-dependent diabetic patients with normal (*open symbols*) and high (*solid symbols*) $Na^+/Li^+$ countertransport (CT). *Squares*, female patients; *Circles*, male patients. *Dashed* and *solid lines*, upper limit of normal for female and male subjects, respectively. Percentage of abnormal left ventricular mass was significantly higher in patients with high $Na^+/Li^+$ CT ($P = 0.046$, Fisher's exact test).**

$Li^+$ CT ($P = 0.046$, Fisher exact test; Fig. 3). Two of the four patients with ventricular hypertrophy were at the lower end of the distribution for whole-body glucose uptake, indicating greater insulin resistance.

## DISCUSSION

Increased rates of $Na^+/Li^+$ CT and insulin resistance have been associated with essential hypertension (3,9,10). Increased $Na^+/Li^+$ CT has also been found in diabetic patients with renal disease (4), and insulin resistance occurs in both IDDM and uremia (13,31). Our study demonstrates that, as a group, normotensive nonproteinuric IDDM patients with elevated $Na^+/Li^+$ CT have significantly reduced peripheral sensitivity to insulin, suggesting a link between the two phenomena that predates any overt sign of organ or tissue dysfunction. Some heterogeneity, however, seems to exist within this group of patients with raised $Na^+/Li^+$ CT activity.

Hyperinsulinemia, presumably resulting from insulin resistance, has been implicated in the risk for cardiovascular complications in the general population (32), and insulin sensitivity in IDDM patients is an independent factor in the progression of vascular disease (33). A relevant finding of our study is an increase in serum triglycerides, LDL-HDL ratio, and apoB in the group of diabetic patients with elevated rates of $Na^+/Li^+$ CT. These lipid abnormalities, which are recognized risk factors for cardiovascular disease, could be the consequence, in our patients, of the reduced peripheral sensitivity to insulin (11). We cannot exclude, however, that higher triglycerides, as such, may contribute to the difference in insulin resistance (34,35). The cause-effect relationship between insulin resistance and changes in triglycerides in the physiological range remains to be elucidated.

Previous studies, in the general population (36,37) and in IDDM patients with various albumin excretion rates (5), consistently reported an association between $Na^+/Li^+$ CT rates and lipid abnormalities. Moreover, $Na^+/Li^+$ CT activity was elevated in a subgroup of patients with hypertensive renal disease (38,39) and in patients with essential hypertension at greater risk of renal and cardiovascular complications (40,41). Insulin resistance could constitute the pathophysiological basis for these phenomena. Of further importance is the finding that the patients with high $Na^+/Li^+$ CT were more likely to have left ventricular hypertrophy, an independent risk factor for cardiovascular complications (42,43), in the absence of hypertension or proteinuria. That cardiac hypertrophy may precede the development of hypertension was shown in humans (43) and in the spontaneously hypertensive rat model (44,45), which also displays increased activity of smooth muscle cell $Na^+/H^+$ exchanger (46) and insulin resistance (47). The processes responsible for cardiac hypertrophy could also contribute to the development of hypertrophy in the smooth muscle cells of resistance vessels and in the smooth muscle–like cells of the mesangium, leading to vascular and renal damage in a susceptible subset of IDDM patients.

Rates of $Na^+/Li^+$ CT are under strong genetic control (48,49), and their elevation in IDDM patients may identify a subgroup at higher risk of cardiovascular and renal complications. Our findings suggest that insulin resistance, possibly with ensuing hyperinsulinemia, could be one of the mechanisms for the increased susceptibility to vascular damage of this subgroup. An alternative explanation, however, is that elevated $Na^+/Li^+$ CT and insulin resistance are concomitant manifestations of another disturbed mechanism that leads to vascular damage. The nature of this common mechanism can only remain speculative now, but it is of interest that enhanced cell growth and $Na^+/H^+$ antiport activity is found in diabetic patients with nephropathy and high $Na^+/Li^+$ CT (50). In vivo in humans, growth was reported to induce insulin resistance (12) and be associated with an increase in blood pressure (51). Hypertrophic processes of vascular and mesangial cell were strongly implicated in the pathogenesis of the renal and vascular damage (52).

In summary, in clinically nonproteinuric, normotensive IDDM patients, increased $Na^+/Li^+$ CT is associated with reduced insulin sensitivity and atherogenic lipid disturbances. These abnormalities, either alone or in combination, may contribute to renal disease, vascular damage, and hypertension in a subset of IDDM patients.

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