Close Relationship Between Microalbuminuria and Insulin Resistance in Essential Hypertension and Non-Insulin Dependent Diabetes Mellitus

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ABSTRACT

The aim of this study was to investigate the relationships among insulin resistance and albumin excretion rate in 25 nondiabetic patients with essential hypertension and in 28 patients with non-insulin dependent diabetes mellitus (NIDDM). Two groups of healthy subjects matched for age, sex, and weight served as controls. Patients with essential hypertension were divided into two subgroups: without (H1) and with (H2) microalbuminuria. Diabetic patients were divided into four subgroups: those with normoalbuminuria without (NIDDM1) and with (NIDDM2) hypertension and those with microalbuminuria without (NIDDM3) and with (NIDDM4) hypertension. Whole-body glucose utilization during euglycemic hyperinsulinemic clamp (40 mU/m²/min insulin infusion) was calculated by tracer dilution techniques (6,6 ²H₂ glucose tracer continuous infusion) and was significantly lower in hypertensives with microalbuminuria than in those without (H₂ versus H₁ versus controls: 3.41 ± 0.51 versus 6.52 ± 0.62 versus 7.03 ± 0.48 mg/kg/min; mean ± SE). Whole-body glucose utilization in NIDDM patients—NIDDM₁ versus NIDDM₂ versus NIDDM₃ versus NIDDM₄ versus controls—was: 1.86 ± 0.31 versus 2.21 ± 0.39 versus 2.01 ± 0.40 versus 5.98 ± 0.77 versus 5.52 ± 0.92 mg/kg/min (mean ± SE). Whereas the first three subgroups did not differ among themselves, they had significantly lower glucose utilization than did the normotensive NIDDM.

Patients without microalbuminuria and nondiabetic controls (P < 0.01). Hypertensives with microalbuminuria had higher Vₘₐₓ of sodium-lithium countertransport (Na/Li CTT) in red blood cells than did both hypertensives without microalbuminuria and controls. It was also observed that NIDDM patients with microalbuminuria had higher Vₘₐₓ of Na/Li CTT than did NIDDM patients without microalbuminuria and controls. Both hypertensive and NIDDM patients with microalbuminuria had ultrasound organomegaly at cardiac and renal level and lipid abnormalities. In conclusion, insulin resistance and hypertension are not always associated; however, microalbuminuria is strongly related to insulin resistance and elevated Vₘₐₓ of Na/Li CTT in essential hypertension as well as in NIDDM.

Key Words: Hypertension, insulin resistance, non-insulin dependent diabetes, microalbuminuria, sodium-lithium countertransport activity, renal and cardiac hypertrophy

Insulin resistance and hyperinsulinemia have been suggested to be mechanisms linking non-insulin dependent diabetes mellitus (NIDDM) to hypertension (1). Evidence in support of this is provided by the observation that essential hypertension is an insulin-resistant state and that plasma insulin concentration and blood pressure levels are correlated in lean hypertensive subjects (2–5). Several studies have, however, found a weak or a lack of relationship between insulin concentration and blood pressure (6–13). The reasons for the discrepancies among studies are elusive. One possible explanation could be that hypertension and insulin resistance occur together in only a subset of hypertensive patients.

One of the most consistent findings in human essential hypertension has been an elevation of Vₘₐₓ of erythrocyte Na/Li countertransport (CTT) (14,15). Recently, an association between increased Vₘₐₓ Na/Li CTT and nephropathy has been found in patients with insulin-dependent diabetes mellitus (16–19). Interestingly, microalbuminuria is frequently found in patients with hypertension as well as in non-insulin-dependent diabetic patients (20–22).

This study examines the relationships between insulin resistance, microalbuminuria, and activity of
V_{\text{max}} \text{ of Na/Li CTT in patients with NIDDM and pa-
patients with essential hypertension.}

MATERIALS AND METHODS

Two groups of patients, one with essential hyper-
tension and another with NIDDM, participated in this
study. Twenty-five patients with essential hyper-
tension were examined. They had systolic blood pressure
\geq 145 \text{ mm Hg} and diastolic blood pressure \geq 90 \text{ mm Hg}
on at least three different occasions. All patients were
free from renal, liver, and endocrine diseases.
Their weight was within 15\% of ideal body weight
and had been stable for the last 6 months. Hyperten-
sive patients were divided into two groups according
to albumin excretion rate (AER): 12 patients (H_1) had
AER in the normal range (below 20 \mu g/min) and 13
patients (H_2) had AER above 20 \mu g/min. Antihyper-
tensive treatment was similar in both groups (H_1, 9
patients on angiotensin-converting enzyme inhibi-
tors and 3 on calcium antagonists; H_2, 11 patients on
angiotensin-converting enzyme inhibitors and 2 on
calcium antagonists; H_1 and H_2 patients were taking
thiazides). Ten normotensive subjects without
family history of hypertension, matched for sex, age,
and body weight, were selected and examined as
controls for the hypertensives.

Four subgroups (seven subjects each) of NIDDM
patients were examined in this study: Group 1, nor-
motensive (NIDDM_1) patients (blood pressure levels,
systolic < 140 \text{ mm Hg} and diastolic < 85 \text{ mm Hg}) with
AER less than or equal to 15 \mu g/min; Group 2, hy-
pertensive (NIDDM_2) patients (blood pressure levels,
systolic \geq 145 \text{ mm Hg} and diastolic \geq 90 \text{ mm Hg}) with
AER less than or equal to 15 \mu g/min either in the
absence of antihypertensive treatment or after 15
days of antihypertensive treatment withdrawal;
Group 3, normotensive (NIDDM_3) patients with AER
greater than or equal to 20 \mu g/min; Group 4, hyper-
tensive (NIDDM_4) patients with AER greater than or
equal to 20 \mu g/min. Ten normotensive normal sub-
jects matched for sex, age, and body weight were selected
and examined as controls for NIDDM patients.

The diagnosis of arterial hypertension (in agree-
ment with the Working Group on Hypertension Dia-
betes [23]) was made when diastolic blood pressure
measured to the nearest 2 mm Hg after 15 min of
rest in the supine position by the same observer
exceeded the above described values on at least three
eventions with a Hawksley random zero sphygmo-
manometer (12 x 35-mm cuff) (Hawksley and Sons,
Lancing, United Kingdom). Diabetes was diagnosed
when fasting plasma glucose was greater than or
equal to 7.8 mmol/L on two different occasions (24).
Patients and controls were of European origin and
were following an isocaloric diet containing 55\% car-
bohydrate, 25\% fat, 20\% protein, and approximately
150 mmol/day of sodium chloride. Patients were
evaluated by dietary history and sodium excretion
rate measurements with three 24-h collections dur-
ing the last 2 to 3 wk before being tested.

Euglycemic Insulin-Glucose Clamp

Controls, hypertensives, and NIDDM patients were
studied after an overnight fast at the plasma glucose
levels shown by each subject (baseline). Diabetic pa-
tients then received a 20-mU/kg/h insulin infusion
for 2 h to achieve euglycemia. Insulin sensitivity was
assessed by the euglycemic insulin-clamp technique
previously described by our group (25). The rate of
continuous insulin infusion was 40 mU/m^{2}/min for
180 min, during which plasma glucose concentration
was held constant at euglycemic values by a variable
infusion of exogenous glucose (20 wt/vol). Whole-
body glucose disposal and endogenous glucose
production were assessed by an isotope dilution
technique with [6,6^{2}H_{2}]glucose (98.6\% \text{H}_{2}) (Tracer
Technologies, Cambridge, MA). This tracer was
administered as a primed, constant infusion for 2 h
before the start of the insulin-clamp period and was
continued throughout the experimental period.
Tracer administration was adjusted continuously in
order to clamp circulating values of tracer glucose
enrichment by the hot glucose infusion technique
described by Finegood et al. (26). The rates of glucose
turnover in the systemic circulation were calculated
from the isotopic data by use of a two-compartment
model for non-steady-state glucose kinetics after
correction for changes in glucose levels in a distri-
bution volume of 250 mL/kg as described elsewhere
(25,26).

Serum and Urine Measurements

Glycated hemoglobin A_{1c} was measured by HPLC
(27), blood glucose was measured by enzymatic tech-
niques (28), and plasma insulin was measured by
RIA techniques (29). Plasma C peptide concentra-
tions were measured in diabetic patients and control
subjects at baseline after an overnight fast and 5 min
after a 1-mg iv glucagon injection (30). Fasting serum
was separated and stored at 4°C for determination of
lipids within 5 days. Concentrations of cholesterol
(31) and triglycerides (32) were measured by enzy-
matic colorimetric techniques. Serum creatinine
was measured by standard methods (33). Twenty-four-
hour urine collections for urinary albumin measure-
ment by RIA technique (34) were taken at least four
times over a week before the study in patients not
undergoing antihypertensive treatment. The inter-
assay and intrassay coefficients of variation were 11
and 5\%, respectively. The mean of all values for each
patient was used for calculations. For AER, the me-
Insulin Resistance and Microalbuminuria

Sodium-Lithium Countertransport in Red Blood Cells

A fasting blood sample was taken into a 20-mL heparin-treated syringe for measurement of Na/Li CT in Li⁺-loaded red blood cells, by the method of Canessa et al. (14) as modified and described in detail by our research group (22).

Ultrasound Renal and Cardiac Measurements

Kidney volumes were measured by ultrasound techniques (35). The volume of both kidneys was measured, and the mean of the two values was used for calculations. Each subject underwent a two-dimensional derived/M-mode echocardiographic study with a mechanical 2.5 MHz probe and commercially available instrumentation (Esacord 81; OTE Biomedica, Milano, Italy). According to the criteria of the American Society of Echocardiography (36), the following parameters relative to the left ventricle were obtained, each one being an average of the last three measurements: (1) left ventricle end-diastolic dimension; (2) left ventricle end-systolic dimension; (3) left ventricle diastolic posterior wall thickness; and (4) interventricular septum thickness. From these measurements, left ventricular mass index was calculated by the Devereux formula (37).

Statistical Analysis

Differences among the study groups were tested by analysis of variance. If a significant difference was found, then the differences between individual groups were tested by the Tukey test unless otherwise stated. Linear regression analysis was used to assess the degree of statistical relationship between insulin sensitivity (dependent variable) and blood pressure levels, AER, body weight, and Na/Li countertransport activity.

RESULTS

Table 1 and Table 2 show the clinical characteristics of the study groups. As can be seen, the subgroups were comparable within each study group. Patients with essential hypertension, however, were significantly younger and weighed less than patients with NIDDM.

Insulin Sensitivity

In the study group of patients with essential hypertension, those who had normoalbuminuria (H₁) had whole-body glucose utilization similar to control individuals (Figure 1). Whole-body glucose utilization was, however, significantly lower in hypertensive patients with microalbuminuria (H₂) than in hypertensive patients without microalbuminuria (H₁). (Controls versus H₁ versus H₂, 7.03 ± 0.48 versus 6.52 ± 0.62 versus 3.41 ± 0.51 mg/kg/min; mean ± SE.) Steady-state plasma insulin concentrations were 98 ± 7 versus 101 ± 7 versus 105 ± 8 μU/mL, respectively for controls, H₁ and H₂.

In the NIDDM group, whole-body glucose utilization during euglycemic clamp was normal (similar to controls) in diabetic patients who were normotensive and without microalbuminuria (Figure 2). Whole-body glucose utilization was, however, lower in all other NIDDM subgroups, i.e., hypertensive diabetic patients without (NIDDM₂) and with microalbuminuria (NIDDM₃).

Table 1. Clinical features of 10 control subjects and 25 hypertensives without (H₁) and with (H₂) microalbuminuria

<table>
<thead>
<tr>
<th></th>
<th>Controls (N = 10)</th>
<th>Hypertensives (N = 12)</th>
<th>Hypertensives (N = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>5/5</td>
<td>6/6</td>
<td>7/6</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>35 ± 3</td>
<td>37 ± 4</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>Duration of Antihypertensive Treatment (yr)</td>
<td>1.6 ± 0.3</td>
<td>1.7 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>23.7 ± 0.4</td>
<td>23.1 ± 0.3</td>
<td>23.4 ± 0.4</td>
</tr>
<tr>
<td>Blood Pressure Levels (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>121 ± 4</td>
<td>160 ± 5</td>
<td>158 ± 4</td>
</tr>
<tr>
<td>D</td>
<td>74 ± 3</td>
<td>95 ± 6</td>
<td>94 ± 4</td>
</tr>
<tr>
<td>AER (μg/min)</td>
<td>5 ± 2</td>
<td>8 ± 3</td>
<td>32 ± 6</td>
</tr>
<tr>
<td>Creatinine Clearance (mL/min/1.73 m²)</td>
<td>109 ± 7</td>
<td>111 ± 6</td>
<td>118 ± 9</td>
</tr>
</tbody>
</table>

*S, systolic; D, diastolic. Values are mean ± SE.
TABLE 2. Clinical characteristics of 10 control subjects and 28 patients with NIDDM (normotensive without (NIDDM) and with (NIDDM3) microalbuminuria and hypertensive without (NIDDM2) and with (NIDDM4) microalbuminuria)*

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>NIDDM1</th>
<th>NIDDM2</th>
<th>NIDDM3</th>
<th>NIDDM4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>6/4</td>
<td>4/3</td>
<td>3/4</td>
<td>4/3</td>
<td>4/3</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>53 ± 5</td>
<td>51 ± 4</td>
<td>52 ± 7</td>
<td>55 ± 7</td>
<td>58 ± 9</td>
</tr>
<tr>
<td>Duration of Diabetes (yr)</td>
<td>7 ± 3</td>
<td>7 ± 2</td>
<td>10 ± 3</td>
<td>11 ± 4</td>
<td></td>
</tr>
<tr>
<td>Diabetes Treatment With Oral Agents (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>27.1 ± 3.0</td>
<td>27.1 ± 4.9</td>
<td>30.2 ± 4</td>
<td>27.2 ± 3.5</td>
<td>33.2 ± 2.9</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>126 ± 4</td>
<td>130 ± 3</td>
<td>160 ± 7</td>
<td>131 ± 3</td>
<td>181 ± 3</td>
</tr>
<tr>
<td>D</td>
<td>75 ± 2</td>
<td>76 ± 4</td>
<td>95 ± 3</td>
<td>76 ± 2</td>
<td>97 ± 3</td>
</tr>
<tr>
<td>AER (µg/ml)</td>
<td>4 ± 2</td>
<td>5 ± 2</td>
<td>7 ± 2</td>
<td>79 ± 10</td>
<td>160 ± 21</td>
</tr>
<tr>
<td>Creatinine Clearance (mL/min/1.73 m²)</td>
<td>101 ± 5</td>
<td>113 ± 6</td>
<td>124 ± 8</td>
<td>120 ± 7</td>
<td>95 ± 8</td>
</tr>
</tbody>
</table>

* S, systolic; D, diastolic.

Figure 1. Mean ± SE of whole-body glucose uptake in 10 control subjects (C) and in 25 hypertensives without (H1, 12) and with (H2, 13) microalbuminuria during euglycemic-hyperinsulinemic clamp (−100 µU/mL of plasma insulin concentration with an insulin infusion rate of 40 µU/m²/min); glucose utilization was calculated by the tracer glucose dilution technique. NS, not significant.

Sodium-Lithium Countertransport Activity in Red Blood Cells

As shown in Table 3, patients with essential hypertension and microalbuminuria had significantly higher $V_{\text{max}}$ of Na/Li CTT in red blood cells when compared with controls and hypertensive patients without microalbuminuria. In normotensive and normalbuminuric NIDDM1 patients, $V_{\text{max}}$ of Na/Li CTT in red blood cells was similar to that of nondiabetic controls. In hypertensive diabetic patients without (NIDDM2) and with (NIDDM4) microalbuminuria, as well as normotensive NIDDM3 patients with microalbuminuria, $V_{\text{max}}$ of Na/Li CTT was elevated and was significantly higher when compared with that of controls and NIDDM1 patients (Table 3).

Organomegaly

Both groups of hypertensives had a higher ultrasound index of left ventricular mass than controls. Despite similar blood pressure levels, hypertensives with microalbuminuria had a more elevated value of left ventricular mass index than hypertensives without microalbuminuria (Table 3). A higher ultrasound index of cardiac mass was observed in NIDDM2 and NIDDM4 patients compared with normotensive NIDDM3 patients and controls (Table 3). Interestingly, left ventricular mass index was also found to be greater in microalbuminuric normotensive NIDDM3 patients than in controls (Table 3).
By kidney ultrasound imaging, it was shown that hypertensives with microalbuminuria had greater renal volume than hypertensives without microalbuminuria (Table 3). Similarly, as with left ventricular mass index, diabetic patients in subgroups NIDDM2, NIDDM3, and NIDDM4 had elevated values of renal volume (Table 3).

**Lipid Patterns**

No differences were found in total serum cholesterol concentrations among hypertensives and controls; however, high-density lipoprotein (HDL) cholesterol was found to be significantly lower in hypertensives with microalbuminuria when compared with hypertensives without microalbuminuria and controls (Table 4). On the contrary, serum triglyceride concentrations were higher in hypertensives with microalbuminuria and controls (Table 4).

In diabetic patients, those with microalbuminuria (without NIDDM3 and with NIDDM2 hypertension) had significantly higher serum cholesterol concentrations than did those without microalbuminuria and controls (Table 4). Similarly, serum triglyceride concentrations were elevated in microalbuminuric diabetic patients (without NIDDM3) and with [NIDDM4] in comparison with diabetics without microalbuminuria and controls (Table 4). HDL cholesterol was normal in normotensive and normoalbuminuric NIDDM1 patients, whereas in other diabetic subgroups, it was significantly lower (Table 4).

**Correlation Coefficients**

Whole-body glucose utilization rate was strongly and inversely related to Na/Li countertransport activity in red blood cells ($r = -0.69; P < 0.001$) and positively related to AER ($r = 0.51; P < 0.05$) but not to body mass index and cardiac index of organomegaly among patients with essential hypertension. In NIDDM patients, a strongly inverse relationship was observed between whole-body glucose utilization and Na/Li countertransport activity in red blood cells ($r = -0.74; P < 0.005$). A positive significant relationship was also found between Na/Li countertransport activity, blood pressure levels ($r = 0.41; P < 0.05$), and AER ($r = 0.58; P < 0.01$). This was not true for body mass index and cardiac index of organomegaly.

**DISCUSSION**

Insulin resistance has been described in essential hypertension (2–5). Both conditions are known as
TABLE 4. Lipid patterns in the study groups

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Total Serum Cholesterol (mmol/L)</th>
<th>Total Serum Triglycerides (mmol/L)</th>
<th>HDL Cholesterol (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>4.6 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>H1</td>
<td>4.7 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>H2</td>
<td>4.9 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Controls</td>
<td>4.6 ± 0.7</td>
<td>1.2 ± 0.3</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>NIDDM1</td>
<td>5.2 ± 0.5</td>
<td>1.8 ± 0.4</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>NIDDM2</td>
<td>5.2 ± 0.3</td>
<td>1.0 ± 0.4</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>NIDDM3</td>
<td>6.3 ± 0.3</td>
<td>2.9 ± 0.8</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>NIDDM4</td>
<td>6.0 ± 0.4</td>
<td>2.5 ± 0.3</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>

*Values are mean ± SE.

P < 0.05 by Tukey test after analysis of variance.

The study presented here was designed to examine the pattern of associations among blood pressure, insulin resistance, and microalbuminuria to patients with essential hypertension and patients with NIDDM. We found that insulin resistance can occur without hypertension, and conversely, among hypertensive patients, only some have insulin resistance. Elevated AER is associated with insulin resistance, irrespective of blood pressure levels. A strong relationship exists between insulin resistance and high Vmax of Na/Li CTT, and microalbuminuria. In addition, patients with insulin resistance, microalbuminuria, and high Vmax of Na/Li CTT had cardiorenal hypertrophy and dislipidemias.

The lack of a strong correlation between hypertension and insulin resistance is in agreement with several studies that found either no relationship between insulin concentrations and blood pressure or only a weak one (6-13). For example, in a group of young, nonobese, hypertensive subjects, Rocchini et al. observed that more than 40% of the subjects had normal insulin-stimulated whole-body glucose uptake (40).

It is noteworthy that, in our study, only the subgroup of hypertensives who were insulin resistant actually showed microalbuminuria and high Na/Li CTT.

Similarly, in the set of NIDDM patients, whenever insulin resistance and microalbuminuria occurred together, an abnormality in cell cation handling (high Na/Li CTT) was also present. These findings confirm and further expand the tenet of Resnick et al., who suggested that when hypertension and insulin resistance occur together, an abnormality of cellular cation handling underlies both conditions (41), rather than hyperinsulinemia being the determinant of high blood pressure, as proposed by others (42).

Two explanations can be proposed to account for the associations between high Na/Li CTT, insulin resistance, and microalbuminuria observed in a percentage of hypertensive and NIDDM patients. First, high Na/Li CTT could simply be a genetic marker not directly involved in the pathogenesis of this syndrome but still useful to identify the patients predisposed to the development of hypertension, insulin resistance, and renal damage. Alternatively, abnormalities in Na/Li CTT, which do not have any known physiologic role, could be related to alterations of other cation transport systems, which may have a significant biologic impact.

Na/Li CTT shares many features with the amiloride-sensitive Na/H antiporter (43), a membrane activity that is involved in transepithelial ion transport, intracellular pH control, cellular growth, and replication. Therefore, one can speculate that the subset of hypertensive and NIDDM patients with high Na/Li CTT may actually have alterations of the Na/H antiporter, causing changes in intracellular proton and calcium concentrations, cellular replication and volume, and renal sodium reabsorption, which in turn leads to various degrees of insulin resistance, hypertension, and renal damage.

An important role of cellular volume and replication in this complex scenario is suggested by the finding presented here that both essential hypertensive and NIDDM subjects with altered Na/Li CTT also had organomegaly at the cardiac as well as the kidney level, independently from hypertension. Recently, Devereux reported that left ventricular mass is increased in normotensive subjects with parental hypertension, suggesting that the mechanisms leading to left ventricular hypertrophy are active before the elevation of blood pressure and may be genetically determined (44). Accordingly, in an animal model of genetic hypertension, cardiac hypertrophy has been found to precede the development of hypertension (45).

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