A Defect in Glycogen Synthesis Characterizes Insulin Resistance in Hypertensive Patients With Type 2 Diabetes

Anna Solini, Francesco Di Virgilio, Paola Chiozzi, Paola Fioretto, Angela Passaro, Renato Fellin

Abstract—A subgroup of patients with type 2 diabetes shows a clustering of abnormalities such as peripheral insulin resistance, hypertension, and microalbuminuria. To evaluate whether these traits reflect intrinsic disorders of cell function rather than in vivo environmental effects, we studied a group of 7 nondiabetic hypertensive subjects with an altered albumin excretion rate (AER) (HyMA+) and 3 groups of patients with type 2 diabetes: 7 with normal blood pressure and normal AER (DH−MA−), 7 with high blood pressure and normal AER (DH+MA−), and 7 with both high blood pressure and altered AER (DH+MA+). Glucose disposal was measured during an hyperinsulinemic clamp (40 mU·m²−1·min−1) with primed deuterated [6.6 2H₂] glucose infusion. In the same subjects, a skin biopsy was performed and the following parameters were investigated: glucose transport (as determined by [3H]2-deoxyglucose uptake); glycogen synthase activity (as determined by [14C] glucose incorporation from UDP-[U-14C] glucose into glycogen); glycogen phosphorylase activity (as measured by the incorporation of [U-14C] glucose 1-phosphate into glycogen); and total glycogen content. In vivo glucose disposal was significantly reduced in DH+MA− and DH+MA+, with respect to DH−MA−, HyMA+, and controls. Insulin-stimulated glucose transport was similar in the 3 groups of patients with diabetes. A significant reduction of intracellular glycogen content was observed in DH+MA− and DH+MA− compared with DH−MA− in both basal and insulin-stimulated conditions, probably because of a major impairment of glycogen synthase activity. Glycogen phosphorylase activity did not show differences between the groups. These results suggest that (1) the combination of type 2 diabetes with hypertension and altered AER is associated with impaired insulin sensitivity, and (2) intrinsic, possibly genetic, factors may account for increased peripheral insulin resistance in hypertensive microalbuminuric patients with type 2 diabetes, pointing to the reduction of glycogen synthase activity as a shared common defect. (Hypertension. 2001;37:1492-1496.)

Key Words: blood pressure ■ diabetes ■ insulin resistance ■ glycogen synthase ■ fibroblasts

Impaired insulin sensitivity with compensatory hyperinsulinemia may be a common pathogenetic factor in the development of both hypertension and type 2 diabetes. In these conditions, the main site of insulin resistance is represented by skeletal muscle, 1 of the major sites for glucose consumption. Glycogen synthesis is a major pathway of glucose disposal in skeletal muscle and is regulated by the insulin-sensitive and rate-limiting enzyme glycogen synthase. Defects in this enzyme can significantly alter the intracellular routing and metabolism of glucose and contribute to insulin resistance in muscle tissue. Skeletal muscle glycogen synthase has been shown to be stimulated little by insulin in both white subjects with type 2 diabetes and Pima Indians, as well as in relatives of patients with diabetes. A decreased insulin responsiveness of glycogen synthase in fibroblasts of patients with type 2 diabetes has also been described; this suggests that a genetically determined defect controls this pathway in different tissues.

Some authors have shown that the association of hypertension, altered albumin excretion rate (AER), or both confers a higher degree of insulin resistance to patients with type 2 diabetes; however, little is known on the intracellular glucose metabolism of this particular subgroup of patients. Consequently, the aim of this study was to investigate whether the presence of hypertension and AER could affect the degree of insulin sensitivity at the cellular level in patients with type 2 diabetes and to try to identify the site of the possible prevalent defect.

Methods

We studied 4 groups of patients: 7 nondiabetic patients with high blood pressure values and altered AER (HyMA+); 7 patients with type 2 diabetes with normal blood pressure and normal AER (DH−MA−); 7 patients with diabetes with high blood pressure values and normal AER (DH+MA−); and 7 patients with diabetes with both high blood pressure values and increased AER (DH+MA+). Seven nondiabetic subjects with normal blood pressure and matched for age, gender, and body mass index (BMI) served as controls.

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From the Department of Clinical and Experimental Medicine (A.S., A.P., R.F.) and Section of General Pathology (F.D.V., P.C.), University of Ferrara, Ferrara, Italy; and Department of Internal Medicine (P.F.), University of Padova, Padova, Italy.
Correspondence to Anna Solini, MD, PhD, Department of Clinical and Experimental Medicine, Section of Internal Medicine II, Via Savonarola, 9, I-44100 Ferrara, Italy. E-mail sli@dns.unife.it
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All diabetic patients were treated either with diet or with sulfonylureas. The diagnosis of arterial hypertension was made in agreement with the Working Group on Hypertension in Diabetes.10 Among patients with hypertension, 8 of 21 had the diagnosis at the time of the study, starting pharmacological treatment after protocol completion; the other patients were treated with calcium channel blockers (8 patients), diuretics (4 patients), and beta-blockers (1 patient). Patients on angiotensin-converting enzyme inhibitors were excluded from the study. Before enrolment, blood was drawn for determination of fasting plasma glucose, HbA1c, BUN, creatinine, and lipid profiles. Three 24-hour urine collections were obtained in the 3 months preceding the study for evaluation of AER. Estimated and fasting plasma insulin, pmol/L

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls</th>
<th>HyMA+</th>
<th>DH−MA−</th>
<th>DH+MA−</th>
<th>DH+MA+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>54±6</td>
<td>53±4</td>
<td>50±4</td>
<td>52±6</td>
<td>53±6</td>
</tr>
<tr>
<td>Gender, n (%M/F)</td>
<td>4/3</td>
<td>4/3</td>
<td>3/4</td>
<td>4/3</td>
<td>5/2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.2±3.1</td>
<td>28.1±2.5</td>
<td>27.1±2.7</td>
<td>28.3±2.8</td>
<td>28.7±5.0</td>
</tr>
<tr>
<td>Estimated lean body mass, kg</td>
<td>57.1±4.5</td>
<td>54.4±6.5</td>
<td>54.3±4.5</td>
<td>53.6±7.1</td>
<td>53.9±6.7</td>
</tr>
<tr>
<td>Diabetes duration, y</td>
<td>...</td>
<td>...</td>
<td>4±1</td>
<td>4±2</td>
<td>5±1</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>4.5±0.8</td>
<td>4.7±0.6</td>
<td>7.3±1.2**</td>
<td>7.5±1.4**</td>
<td>8.2±1.3**</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>120±3</td>
<td>160±6*#</td>
<td>123±5*∞</td>
<td>158±7*#</td>
<td>160±10*#</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>76±4</td>
<td>89±4*#</td>
<td>73±6*∞</td>
<td>88±5*#</td>
<td>90±6*#</td>
</tr>
<tr>
<td>AER, µg/min</td>
<td>10 (2–18)</td>
<td>53 (39–71)*λ</td>
<td>12 (3–17)*∞</td>
<td>13 (3–16)*∞</td>
<td>48 (22–120)*#λ</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>4.4±0.6</td>
<td>4.6±0.4</td>
<td>7.5±0.8**</td>
<td>7.4±0.7**</td>
<td>7.7±0.8**</td>
</tr>
<tr>
<td>Fasting plasma insulin, pmol/L</td>
<td>38±12</td>
<td>37±6</td>
<td>42±8</td>
<td>40±10</td>
<td>43±8</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD or median (range).

*P<0.001 vs controls; *P<0.001 vs HyMA+; #P<0.001 vs DH−MA−; ∞P<0.001 vs DH+MA−; and *P<0.001 vs DH+MA+.

All diabetic patients were superimposable for age, gender, BMI, duration of disease, and degree of metabolic control. Serum creatinine and albumin concentrations were normal in all subjects (data not shown). Blood pressure values and AER were higher in HyMA+, DH+MA−, and DH+MA+, by selection criteria. Plasma glucose and insulin concentrations did not differ in all groups of patients, both in the basal state and during clamp.

Figure 1A depicts whole-body glucose uptake in the 4 study groups and in the control group. Glucose utilization was significantly reduced in DH+MA− and DH+MA+ com-
pared with HyMA+ and DH−MA−, and controls. The hepatic glucose production, reported in Figure 1B, did not significantly differ between the groups in the basal state; insulin-mediated suppression of hepatic glucose output was uniformly impaired in hypertensive and diabetic patients with respect to normal subjects, even after adjustment for age, BMI, and fasting plasma glucose.

Basal glucose transport and phosphorylation were uniformly reduced in the 3 diabetic groups, showing a reduction with respect to nondiabetic subjects. After insulin stimulation, there was a trend for a further more pronounced reduction in DH+MA− and DH+MA+ with respect to DH−MA−, but statistical significance was not reached (Figure 2).

In Figure 3, the rate of fibroblast glycogen synthesis is shown. Interestingly, we found the same trend observed in vivo: intracellular glycogen content was reduced in all diabetic patients compared with nondiabetic subjects in both basal and insulin-stimulated conditions. However, a significant impairment was observed in DH+MA− and DH+MA+ compared with DH−MA−.

To further clarify the rate-limiting step in glycogen synthesis, we evaluated glycogen synthase and glycogen phosphorylase activities and observed a reduction in glycogen synthase activity in DH+MA− and DH+MA+ compared with DH−MA−, HyMA+, and controls (Figure 4). Glycogen phosphorylase activity did not show significant differences between the 4 groups of patients (48±6 mU/mg protein in control, 41±10 mU/mg in HyMA+, 35±8 mU/mg in DH−MA−, 33±9 mU/mg in DH+MA−, and 29±7 mU/mg in DH+MA+, P=not significant).
Finally, in a multivariate analysis (Table 2), we evaluated the potential effect of age, gender, and BMI and of the presence of diabetes, hypertension, and microalbuminuria on the main indexes of insulin-stimulated glucose metabolism, both in vivo and in vitro. Interestingly, diabetes and hypertension were the only common determinants of metabolic parameters measured during the clamp as well as those obtained at the cellular level.

**Discussion**

We, as well as other authors, have previously described a phenotypic link between type 2 diabetes, altered AER, and hypertension on the one hand and a marked impairment of peripheral insulin sensitivity on the other hand. In these subjects, skeletal muscle is presumably the main site of insulin resistance, with a relevant impairment of muscle glycogen synthesis. This condition could be either due to a proximal abnormality (ie, impaired glucose transport), to a more distal abnormality (ie, phosphorylation), or to a defective glycogen synthase activity.

The novel finding of the present work is the documentation of a link between a cluster of in vivo metabolic alterations and a cellular defect in hypertensive patients with type 2 diabetes. Moreover, we provide new information on the possible main site of the defect; we demonstrate a reduction of glycogen content in these cells that is mainly attributable to an impairment of enzymatic activity of glycogen synthase. This key enzyme, strongly activated by insulin, is regulated both allosterically, by binding of glucose 6-phosphate, and covalently, by the phosphorylation of multiple serine residues, and represents a potential site of inherited or acquired insulin resistance. Its basal and insulin-stimulated activity is usually reduced by 35% to 50% in skeletal muscle cells from patients with type 2 diabetes with respect to controls.

Primary cell cultures provide a useful model for the identification of intrinsic metabolic defects that are expressed independent of the host environment. Several authors have provided evidence that muscle cultures reflect the metabolic behavior of intact skeletal muscle and, particularly, the rates of glucose and lipid metabolism in vivo.

In the patients described in this study, skeletal muscle samples were not collected because of ethical reasons. Fibroblasts, however, are a good model to study intracellular glucose metabolism in vitro because they express glucose transporters and all the key enzymes of glucose metabolism. Moreover, even though their insulin sensitivity is lower than that of adipocytes, it is not dissimilar to that estimated in human muscle in vitro. To the best of our knowledge, no studies to date have evaluated the influence of high insulin levels per se on human fibroblasts of patients with type 2 diabetes and different degrees of insulin sensitivity correlated to different phenotypic characteristics. Our experiments add new information to the few available studies that concern the role of insulin receptors or postreceptorial alterations, respectively, in determining insulin resistance during hypertension and they also suggest that the glycogen synthase defect is not tissue specific. Because cell cultures are grown at normal glucose concentrations and undergo multiple cell doublings before experimental procedures, it is conceivable that any acquired component of defective enzymatic activity would be reversed under such circumstances; alternatively, we cannot exclude, however, that an irreversible acquired defect could persist in culture.

In regard to glucose transport, our experimental model did not reveal significant differences either in the transmembrane glucose transport or in the phosphorylation between hypertensive diabetic and normotensive diabetic patients. The discrepancy between these results and a more pronounced impairment of whole-body glucose uptake in the first group is, to our opinion, mainly because in vivo measurement is largely accounted for by glucose disposal in insulin-dependent tissues, primarily skeletal muscle. In muscle, the ability of the cells to build up glycogen is the result of activation of the glycogen synthetic pathway as well as the increase in glucose transport, which occurs mainly via the glucose transporter 4 translocation. A fibroblast is a non–insulin-dependent cell that mainly expresses the non–insulin-inducible glucose transporter 1; it is likely that in this kind of cell, the insulin effect could be more pronounced on glycogen synthesis than on transport, resulting in a much lower glycogen content. Moreover, this model cannot either exclude the coexistence of a defective hexokinase activity or allow the identification, at the level of the complex activation pathway of glycogen synthase, of the defect. For example, we do not have any information concerning the cAMP-dependent pro-
tein kinase that could either phosphorylate glycogen synthase or activate an inhibitor of phosphatase, inducing a blockage of protein phosphatase-1, an increased glycogen synthase phosphorylation, and a reduced glycogen synthase activity.17

Our results may perhaps offer a contribution in identifying the independent role of hypertension and increased urinary albumin excretion in influencing insulin resistance in humans. In patients with essential hypertension and microalbuminuria, Bianchi et al have already described a 35% reduction in insulin-mediated glucose disposal compared with their normoalbuminuric counterparts23; in these patients, the change was entirely due to impaired nonoxidative glucose disposal, whereas the ability of insulin to stimulate glucose oxidation was unaltered. Unfortunately, we were not able to evaluate patients with type 2 diabetes with normal blood pressure values and microalbuminuria, but the degree of insulin sensitivity of hypertensive microalbuminuric subjects does not seem to differ from that of control subjects matched for age and BMI.

On the other hand, the coexistence of hypertension with diabetes, with or without microalbuminuria, sets the stage for a further reduction of whole-body glucose uptake, whereas the presence of a clear defect at the level of skin fibroblasts in the same patients suggests a potential role of inherited factors in influencing the degree of insulin sensitivity, with the same abnormality probably present in different tissues. A multivariate analysis that recognizes HbA1c and systolic blood pressure as the only 2 factors independently affecting all the metabolic parameters considered supports this view. With regard to this possibility, it is also of great interest that a polymorphism in the glycogen synthase gene has been reported in association with insulin resistance in diabetic patients with current hypertension and a family history of hypertension,24 even if the glycogen synthase content was similar in specimens from patients with or without the mutation.

Nevertheless, the significance of the association between insulin resistance and microalbuminuria in our patients remains uncertain, and our results do not allow us to state whether the development of altered albumin excretion either precedes or follows the development of an insulin-resistant state. Three main hypotheses can be formulated: first, microalbuminuria and reduced insulin sensitivity could be both genetically determined and cosegregate with the hypertensive status; alternatively, insulin resistance could be causally related to microalbuminuria; and finally, both insulin resistance and altered albumin excretion could be the consequence of hypertension. Further prospective studies are requested to clarify whether a progressive increment of blood pressure values and urinary albumin loss keep up with an increasing tissue insulin resistance.

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References


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