Early Autonomic Dysfunction in Glucose-Tolerant but Insulin-Resistant Offspring of Type 2 Diabetic Patients

Simona Frontoni, Daniela Bracaglia, Alessandra Baroni, Fabio Pellegrini, Michela Perna, Elena Cicconetti, Giuseppina Ciampittiello, Guido Menzinger, Sergio Gambardella

Abstract—In type 2 diabetes, both insulin resistance and hyperglycemia are considered responsible for autonomic dysfunction, but the specific role of these two abnormalities is not clear. To test the specific role of insulin resistance on autonomic dysfunction, we studied 69 glucose-tolerant offspring of type 2 diabetic patients, comparing the most insulin-resistant tertile (IR) with the most insulin-sensitive tertile (IS) and comparable control subjects, all undergoing the oral glucose tolerance test, impedance, 24-hour blood pressure and ECG monitoring, and an intravenous glucose tolerance test (IVGTT) followed by a euglycemic hyperinsulinemic clamp, with continuous blood pressure and ECG measurements. Sympathovagal balance was evaluated as low- to high-frequency ratio (LF:HF) by spectral analysis on R-R intervals. The change of systolic and diastolic blood pressure was calculated as [(day-night/d)]×100. In IR, the changes of systolic and diastolic blood pressure were significantly lower versus IS (9.2±5.0% versus 12.4±3.6%, P<0.02; 13.2±6.5% versus 17.4±5.2%, P<0.02). During the night, LF:HF fall was reduced in IR (43.1±21.0 versus 61.4±16.9, P<0.02). Hyperinsulinemia (IVGTT) rapidly and significantly increased LF:HF in IR (4.9±3.3 versus basal: 2.3±1.4, P=0.03) but not in IS. In offspring of type 2 diabetic patients with normal glucose tolerance and normal blood pressure values, insulin resistance is associated with abnormal control of blood pressure and sympathetic activation. Insulin resistance may therefore be responsible for some early derangements of the autonomic nervous system control and thus contributes to increase the incidence of arterial hypertension and/or diabetes. (Hypertension. 2003;41:1223-1227.)

Key Words: glucose ■ autonomic nervous system ■ insulin resistance ■ blood pressure monitoring ■ sympathetic nervous system

It has been recognized for some time that hypertension is approximately twice as common in diabetic subjects as in the general population.1,2 Hyperinsulinemia/insulin resistance appears to play an important role in the pathogenesis of hypertension in type 2 diabetes,3,4 mainly through a sympathetic activation, as suggested by the finding that insulin infusion increases muscle nerve sympathetic activity in healthy subjects5 and in borderline hypertensive patients.6 However, when diabetes becomes clinically evident, it is extremely difficult to investigate the association between insulin sensitivity and cardiac autonomic regulation as well as the effect of acute hyperinsulinemia on cardiac sympathovagal balance. In fact, hyperglycemia per se exerts profound effects on both parameters.7,8

Offspring of type 2 diabetic patients are a good model to study the effects of hyperinsulinemia on hemodynamic parameters and on sympathetic nervous system activity, since they are insulin resistant even when they still display a normal glucose tolerance.9–11 Moreover, they have a 30% to 40% lifetime risk of developing diabetes, and insulin resistance appears to be the best predictor. We previously demonstrated that offspring of type 2 diabetic patients have a marked predominance of sympathetic over parasympathetic nervous system activity in response to endogenous hyperinsulinemia.12 This appears to be confirmed also with exogenous hyperinsulinemia,13 at least when insulin resistance is accompanied by (the confounding) overweight. However, not all the offspring of type 2 diabetic patients inherit and display insulin resistance.14

We therefore compared insulin-resistant subjects with matched insulin-sensitive subjects, all being offspring of type 2 diabetic patients. This allowed us to identify the specific role of insulin resistance on cardiac autonomic regulation in offspring of type 2 diabetic patients, with normal and matched blood glucose tolerance, blood pressure values, and body weight. Our data support the hypothesis that early autonomic dysfunction is present in glucose-tolerant but insulin-resistant offspring of type 2 diabetic patients.
Assessment of Heart Rate Variability

A continuous ECG (Del Mar Avionics, model 563 StrataScan Holter Analysis System) was made for the 24-hour period during IVGTT and during the clamp study.

Holter tapes were scanned and spectral analyses were estimated, as previously reported.\textsuperscript{15} Time and frequency domain measures of heart rate variability were analyzed in accordance with ESC/NASPE recommendations.\textsuperscript{16} The percentage change from day to night (Δ) for low-frequency/high-frequency (LF/HF) ratio was defined as [(mean value during the day−mean value at nighttime)×100]/mean value during the day.

Methods

Subjects

Sixty-nine offspring of type 2 diabetic patients and 11 control subjects with a negative family history for type 2 diabetes were studied. Informed consent was obtained from all participants; all the investigations were performed in accordance with the principles of the Declaration of Helsinki. All subjects had a normal glucose tolerance test. Inclusion criteria were age between 30 and 55 years, either sex, with only one parent affected by type 2 diabetes, absence of neoplastic, immunologic, or other diseases able to modify glucose metabolism, either directly or by drugs with anti-insular action; normal blood pressure according to WHO criteria; and a body mass index (BMI) <30 kg/m\textsuperscript{2}. Participants used no medications.

Experimental Procedures and Protocol

On the first day, after an overnight 12-hour fast, all subjects underwent anthropometrical evaluation: BMI, weight-height (W/H) ratio, and body composition (impedentiometry, Body Fat Analyzer, BT-905, Skylark Device). An oral glucose tolerance test (OGTT) was performed. On the second day, at 8 AM, after a 12-hour overnight fast, all subjects underwent an intravenous glucose tolerance test (IVGTT, 300 mg/kg) followed by a hyperinsulinemic (40 mU/m\textsuperscript{2} per minute) glucose clamp.\textsuperscript{18} Continuous blood pressure (BP) and ECG recordings were performed throughout the IVGTT and the clamp studies to evaluate the response of BP and of sympathovagal balance, both to endogenous and exogenous hyperinsulinaemia.

BP Monitoring

Twenty-four-hour BP monitoring and BP during the clamp study were measured by the oscillometric technique (90207; Spacelabs), as previously described.\textsuperscript{16} This system has been validated by the British Hypertension Society.\textsuperscript{17} The percentage change from day to night (Δ) for BP (systolic and diastolic) was defined as [(mean value during the day−mean value at nighttime)/mean value during the day].

During the IVGTT and the clamp study, recordings were obtained at 6-minute intervals throughout the study. The mean value for the first hour and for the second hour was calculated for systolic, diastolic, and mean BP.

Clinical Characteristics of the Study Group

According to the insulin sensitivity measured as the rate of glucose infusion during the steady state of the clamp studies, offspring were divided into 3 tertiles, and the first tertile (insulin resistant, IR) was compared with the third tertile (insulin sensitive, IS). All subjects in the control group and in the offspring groups had normal glucose tolerance. The Table shows clinical characteristics of the study group. The groups were comparable for gender, age, BMI, W/H ratio, and body composition. The IR group had a higher fasting insulin concentration than the IS and control groups ($P<0.02$).

Assays and Calculations

Plasma glucose was measured in duplicate (Beckman glucose analyzer II, Beckman Instruments). Plasma insulin concentration was determined by radioimmunoassay (Sorin Biomedical; intra-assay coefficient of variation=3.2±0.2%). During the hyperinsulinemic clamp, under steady-state conditions, hepatic glucose production is suppressed, and all the infused glucose is taken up by peripheral tissues, except for a minor amount excreted in the urine. However, since the renal glucose threshold was never reached during the clamp, we did not correct the glucose metabolized for the urinary glucose excretion.

Statistical Analysis

Data are expressed as mean±SD unless otherwise indicated. Overall group comparisons and pairwise contrasts for the variables analyzed were assessed by 1-way ANOVA. Repeated-measures ANOVA was used for group comparisons over time. Significance is expressed as $F$ tests with 2-sided probability values. All the analyses were performed with the use of SAS Statistical Package Release 8.2 (SAS Institute Inc).

Results

Twelve-Hour BP Monitoring

Systolic and diastolic blood pressure (SBP and DBP) were similar in the three groups during the day, but they were significantly higher in the IR versus the IS group during the night ($P=0.0076$ and $P=0.0287$). Furthermore, the percent-

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>IS Group ($n=23$)</th>
<th>IR Group ($n=23$)</th>
<th>Control Group ($n=11$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, M/F</td>
<td>8/15</td>
<td>6/17</td>
<td>4/7</td>
</tr>
<tr>
<td>Age, y</td>
<td>38.2±9.0</td>
<td>38.5±10.5</td>
<td>35.2±9.2</td>
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<td>BMI, kg/m\textsuperscript{2}</td>
<td>25.2±4.2</td>
<td>25.9±4.0</td>
<td>24.3±3.0</td>
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<td>Lean, %</td>
<td>71.9±16.8</td>
<td>66.7±14.8</td>
<td>69.3±8.8</td>
</tr>
<tr>
<td>Fat, %</td>
<td>28.1±16.8</td>
<td>33.3±14.8</td>
<td>30.7±8.8</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>116.7±6.6</td>
<td>117.3±12.5</td>
<td>122.2±10.3</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>71.4±7.6</td>
<td>72.3±11.5</td>
<td>79.8±13.3</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L</td>
<td>46.5±17.8</td>
<td>64.3±28.2*†</td>
<td>46.8±10.3</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L</td>
<td>4.6±0.1</td>
<td>4.7±0.1</td>
<td>4.6±0.1</td>
</tr>
<tr>
<td>Whole-body glucose uptake, mmol · kg\textsuperscript{-1} · FFM · min\textsuperscript{-1}</td>
<td>86.8±35.5</td>
<td>44.7±13.1*†</td>
<td>66.5±9.4</td>
</tr>
</tbody>
</table>

Data are mean±SD or number. FFM indicates free fat mass.

*2P=0.02 IR group vs IS group; †2P<0.02 or IR group vs control subjects.
age change from day to night (Δ) of SBP and DBP was significantly reduced in the IR group both versus the IS group (P=0.0127 IR vs IS; †P=0.04 IR vs control group, ΔSBP: *P=0.01191 IR vs IS; †P<0.089 IR vs control group).

HRV

Sympathovagal balance, evaluated as LF:HF ratio at different times using a repeated measures ANOVA model, was similar in the three groups at baseline. During endogenous hyperinsulinemia (IVGTT), LF:HF significantly increased only in the IR group (4.9±3.3 versus basal: 2.3±1.4, P=0.03) but not in the IS and control groups (Figure 2). On the contrary, LF:HF ratio significantly increased in IS and control groups during the clamp (120 minutes: P=0.007 and P<0.05, respectively, versus basal) but not in the IR group. HF showed a slight but not significant reduction during the clamp study in the IS and control groups.

Lipid Profile

Total cholesterol, LDL cholesterol, and triglycerides were significantly increased in the IR group versus the IS group (P<0.05 or less), whereas HDL cholesterol was reduced, although not significantly, in the IR group versus the IS and control groups (Figure 3).

**Discussion**

It has been suggested** that insulin resistance and compensatory hyperinsulinemia are the primary events and that enhanced sympathetic activity and diminished adrenal medullary activity are important links between the defect of insulin action and the development of hypertension and associated metabolic abnormalities (impaired glucose tolerance and diabetes). It is therefore extremely interesting to study the impact of insulin resistance on sympathetic activity before the onset of impaired glucose tolerance to dissect out the (confounding) effects of hyperglycemia from the direct effects of

Figure 1. Percentage change from day to night (Δ) of SBP and DBP (see text for calculations). White bars indicate control subjects; hatched bars, IS offspring; black bars, IR offspring. ΔSBP: *P=0.0127 IR vs IS; †P=0.04 IR vs control group. ΔDBP: *P=0.01191 IR vs IS; †P<0.089 IR vs control group.

Figure 2. A, LF:HF ratio during IVGTT and clamp study. End of IVGTT is basal of clamp study. White circles indicate control subjects; hatched squares, IS offspring; black triangles, IR offspring. *P=0.03 vs basal in IR and †P=0.007 vs basal of clamp in IS; †P<0.05 vs basal of clamp in control group. B, Percentage change from day to night (Δ) of LF:HF ratio (see text for calculations). White bars indicate control subjects; hatched bars, IS offspring; black bars, IR offspring. *P=0.02 IR vs IS. †P<0.05 IR vs control group.

Figure 3. Plasma LDL cholesterol, HDL cholesterol, and triglycerides. White bars indicate control subjects; hatched bars, IS offspring; black bars, IR offspring. *P<0.05 or less, IR vs IS.
insulin resistance on the typical metabolic and/or hemodynamic abnormalities of type 2 diabetes. We chose to accurately measure insulin sensitivity in a large group of offspring of type 2 diabetic patients, since they are known to be hyperinsulinemic-insulin resistant, before the clinical onset of diabetes or even of impaired glucose tolerance.7–11 However, not all the offspring of type 2 diabetic patients are insulin resistant.14 We therefore compared insulin-resistant subjects with matched insulin-sensitive subjects, all being offspring of type 2 diabetic patients, thus identifying and studying subjects who directly inherited insulin resistance from their diabetic parent and not just a generic risk to develop diabetes. This allowed us to identify the specific role of insulin resistance on cardiac autonomic regulation in subjects with still-normal glucose tolerance, blood pressure, and body weight.

The major finding of our study was that in the group of insulin-resistant offspring of type 2 diabetic patients, we observed abnormalities in blood pressure profile and sympathetic activation. Our results indicate that insulin-resistant offspring have an abnormal circadian rhythm of blood pressure, with a reduced fall in nocturnal blood pressure, even in the presence of normal and comparable diurnal values. This finding is consistent with previous observations,20 in which similar abnormalities in blood pressure profile were associated with autonomic neuropathy in nondiabetic offspring of type 2 diabetic subjects; the role of insulin resistance, however, was not addressed. We now suggest that the key role in the development of abnormalities in blood pressure profile, in normotensive family members of type 2 diabetic patients, is played by insulin resistance.

Previous studies have quite consistently reported that hyperinsulinemia or glucose ingestion affect indicators of peripheral sympathetic activity, either plasma norepinephrine concentrations or muscle sympathetic nerve activity, suggesting that hyperinsulinemia and/or hyperglycemia might cause a predominance of sympathetic effects on the heart. In the current study, autonomic function was evaluated by spectral analysis of R-R interval on 24-hour ECG recordings and expressed as LF:HF ratio. The HF spectral component of heart rate variability (HRV) reflects parasympathetic nervous control of heart rate, and the LF spectral component of HRV is thought to be under both sympathetic and parasympathetic control, whereas LF:HF ratio probably reflects sympathovagal balance.18,26 In fact, since every change in the LF component is always related to the same change of the HF component, but in the opposite direction, it is more appropriate to consider this relation in terms of sympathovagal balance rather than to consider LF and HF separately, as independent indexes of sympathetic and vagal control. As a consequence, an increase or a decrease in this index indicates a shift of the cardiac sympathovagal balance toward the sympathetic or parasympathetic predominance, respectively.18,26

It has recently been observed that endogenous hyperinsulinemia increases LF:HF ratio in normal subjects27 and in offspring of type 2 diabetic patients.12 In our study, sympathovagal balance was studied in response both to endogenous hyperinsulinemia, obtained by IVGTT and to exogenous hyperinsulinemia, during the clamp study. We observed a significant sympathetic predominance, as expressed by a marked increase of LF:HF ratio, at the end of IVGTT, only in the IR group. This does not appear to be related to the actual insulin values during IVGTT, since they were similar in the two groups, and confirms previous data suggesting that endogenous hyperinsulinemia at plasma concentrations within the physiological range can induce a relative sympathetic prevalence. It could also be hypothesized that the small but significant mean increase in blood glucose (24.3 ± 2.5 mg/dL) in IR versus IS during IVGTT could be implicated in the observed sympathetic activation in this group. However, this difference is extremely low when compared with the increase in blood glucose within the groups during IVGTT (~ +200 mg/dL above basal). Moreover, when introducing blood glucose as a time-varying confounder into the repeated-measures ANOVA model for LF:HF during IVGTT (actually an analysis of covariance model with time-varying covariates), no significant association was found (P = 0.1659) and, above all, the significant difference between end of IVGTT and basal in the IR group was left unchanged.

It is therefore unlikely that our results could be explained by potential confounding factors. In our opinion, therefore, hyperinsulinemia could not represent the only explanation for the observed sympathetic activation, but the insulin-resistant state itself is probably associated with sensitization of the autonomic nervous system to insulin or glucose (and, possibly, other stimuli). In our study, glucose clamp was performed after the IVGTT, and the LF:HF ratio, which was increased during the IVGTT in the IR group, did not return to the basal level at the beginning of the glucose clamp. Therefore, the lack of further stimulation of sympathetic activity in IR group by the subsequent exogenous hyperinsulinemia is probably due to the fact that LF:HF ratio was already maximally increased at the end of IVGTT. These results are in agreement with the study by Laitinen and coworkers,13 who observed a similar change of LF:HF ratio and a decrease in HF power only in the offspring of diabetic patients with the insulin-resistant phenotype. In the current study, we confirm our previous observations12 that HF power tended to be lower in the IR group, although this was not statistically significant. This implies that in our hands, the increase in LF:HF ratio, in response to hyperinsulinemia, is related both to an increase in LF and decrease in HF components.

In our study, sympathovagal balance was studied not only in response to endogenous and exogenous hyperinsulinemia, but also during the day life. The main observation was that offspring with insulin resistance display a relative sympathetic prevalence during the night when compared with the insulin-sensitive group. The observation of a reduced circadian oscillation of LF:HF in the IR group probably is related to the condition of insulin resistance and confirms previous data of a sympathetic prevalence during the night in other states of insulin resistance.28 This behavior seems to play a fundamental role in the reduction of the nocturnal fall in blood pressure, observed in the same group, as already hypothesized for diabetic patients.29 This finding suggests but does not prove that insulin resistance is one of the early pathophysiological changes related to the development of autonomic dysfunction, which in turn is responsible for the
Perspectives
We suggest that insulin resistance and compensatory hyperinsulinemia and enhanced sympathetic activity are the primary events leading to the development of hypertension and/or diabetes. Prospective studies on a high-risk population are needed to confirm this hypothesis.

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