Concomitant Impairment in Endothelial Function and Neural Cardiovascular Regulation in Offspring of Type 2 Diabetic Subjects

Ferdinando Iellamo, Manfredi Tesauro, Stefano Rizza, Stefano Aquilani, Carmine Cardillo, Micaela Iantorno, Mario Turriziani, Renato Lauro

Abstract—Endothelial function is impaired in first-degree relatives (FDRs) of patients with type 2 diabetes. Many states characterized by endothelial dysfunction are associated with increased cardiovascular sympathetic outflow. In this study, we investigated endothelial and autonomic nervous system (ANS) functioning in FDRs and tested the hypothesis that in basal condition, impaired endothelial function is associated with impaired cardiovascular ANS regulation. Flow-mediated endothelium-dependent and -independent vasodilation of the brachial artery was measured with high-resolution ultrasound in 27 otherwise healthy FDRs (14 men and 13 women; mean age 32 years) with normal oral glucose tolerance and in 15 age- and gender-matched control subjects. Cardiovascular ANS regulation was investigated by means of spectral analysis of heart rate and systolic blood pressure (SBP) variability. Baroreflex sensitivity was assessed by the spontaneous baroreflex sequences technique. Flow-mediated endothelium-dependent vasodilation was 9.4±1.0% in FDRs and 17.0±2.3% in control subjects \( (P=0.001) \). Low-frequency oscillations in SBP variability were 8.6±2.8 and 2.8±0.6 mm Hg in FDRs and controls, respectively \( (P=0.04) \). Baroreflex sensitivity was significantly less in FDRs than controls (22.8±2.7 versus 37.0±5.8, respectively; \( P=0.01 \)). Change in vessel diameter was inversely correlated with the low-frequency component of SBP variability \( (r=-0.40; \ P=0.014) \). In healthy FDRs of diabetic patients there is a concomitant, possibly related, impairment in endothelial and ANS functioning, which manifests, indirectly, with increase in vascular sympathetic outflow and a depressed baroreflex, vagal, control of heart rate. (Hypertension. 2006;48:1-6.)

Key Words: autonomic nervous system • baroreflex • endothelium • atherosclerosis • risk factors • diabetes mellitus

Offspring of type 2 diabetic patients feature a high risk of developing diabetes and its complications related to atherosclerotic processes over the course of their life. Endothelial dysfunction is thought to play an important role in the initiation of atherosclerosis and its progression and can be detected even in healthy people with risk factors for cardiovascular disease. Indeed, several studies reported an impaired flow-mediated vasodilation (FMD), an indicator of endothelial dysfunction, in normoglycemic first-degree relatives of type 2 diabetic patients, although the mechanism(s) underlying the impaired FMD in these still-healthy subjects has not been clarified.

Interestingly, the endothelium shares a functional antagonism with the sympathetic nervous system efferents in maintaining vessel tone. In the normal state, there is a tonic balance between the release of vasodilating factors from the endothelium and vasoconstricting factors from sympathetic nerve terminals to maintain the appropriate vessel tone. The possibility of early alterations in neural cardiovascular regulation in healthy offspring of diabetic patients has been addressed recently, but no differences in cardiac autonomic modulation in comparison to control subjects has been found in basal conditions. However, all of these studies focused on cardiac autonomic control only. No study addressed the possibility of early alterations in peripheral sympathetic outflow, which is the main regulatory mechanism to interact with the endothelial function (EF). In addition, no study in offspring of diabetic patients investigated the baroreflex control of heart rate (HR), which is a key component of cardiovascular homeostasis and carries relevant pathophysiological and prognostic information. It is known that impairment in vagal control of the heart usually precedes impairment in sympathetic cardiovascular regulation in diabetic patients and, indeed, arterial baroreflex control of sinoatrial node has been reported to be altered early in the course of diabetic disease.
Few studies have investigated possible associations between autonomic nervous system (ANS) and endothelial functioning in still-healthy but high-risk populations. Concomitant assessment of ANS and EF before cardiovascular diseases (CVDs) become clinically manifested may provide insight on the nature of CVD development.

Accordingly, in the present study, we investigated FMD of the brachial artery and ANS functioning in offspring of type 2 diabetic patients and tested the hypothesis that in basal conditions the impairment in FMD is associated with an increased peripheral sympathetic outflow and an altered baroreflex, vagal, control of the heart.

Methods

Study Population
We recruited 27 healthy subjects (age 32.3±6.3 years, 14 men and 13 women) with ≥1 parent with a diagnosis of type 2 diabetes (first-degree relatives [FDRs]) and 15 healthy subjects matched for age and sex (28.5±5.0 years; 7 men and 8 women) with no history of diabetes in any FDR as a control group. All of the participating women reported regular menstrual cycles, and none of them was receiving oral contraceptives. In female subjects, all of the experiments were performed during the first week of the menstrual cycle.

Before inclusion in the study, all of the subjects were screened by clinical history, physical examination, electrocardiography, chest x-ray, and routine chemical analyses. Exclusion criteria were history or evidence of hypertension (blood pressure [BP] >140/90 mm Hg), diabetes, cardiac diseases, peripheral vascular disease, coagulopathy, or any other disease predisposing them to vasculitis or Raynaud’s phenomenon. No subject reported inflammatory and/or infectious diseases during the 2 months preceding the study. All of the subjects were nonsmokers, and no subject was engaged in regular physical activity, defined as physical training performed on a regular basis, for ≥30 minutes, from 3 to 5 times per week. No one subject was taking drugs at the time of the study. Written informed consent was obtained from each subject, and the study was approved by the Institutional Review Board of the University of Tor Vergata.

Experimental Protocol
All of the studies were performed in the morning in a quiet room at ambient temperature (22 to 24°C). Participants were asked to refrain from drinking alcohol or beverages containing caffeine for ≥24 hours before the study. The studies were performed in the morning of 2 consecutive days.

Metabolic Evaluation
On the first day, a standard oral glucose tolerance test (OGTT) was performed in all of the participants. Venous blood was sampled at 0, 30, 60, 90, and 120 minutes to determine plasma glucose and serum insulin. The OGTT results had to be within the reference range for FDRs and control subjects. The homeostasis model assessment (HOMA) values, an index of insulin resistance,23 were also calculated. We also evaluated high-sensitive C-reactive protein as marker of inflammation.

ANS Assessment
On the second day, after the patients had sat quietly for 15 minutes, BP was measured by a sphygmomanometer in the sitting position twice, 5 minutes apart, and the measurements were averaged. Thereafter, we recorded the electrocardiographic signal from a precordial chest lead, whereas arterial BP was continuously and noninvasively measured by Finapres (OHMEDA 2003 NIBP monitor). Respiratory signal was also recorded by means of a thoracic belt. The 3 analog signals were sampled at 300 Hz per channel (MP 150 Biopac System) and stored for subsequent analyses. After the instrumentation and after a 15-minute adaptation period in supine position, patients underwent continuous BP and HR recordings for 10 minutes. Systolic (S) BP and RR interval signals were analyzed by power spectral analysis. Details of the offline analysis have been already published.24–26 Briefly, the harmonic components of RR interval and BP variabilities were evaluated by the autoregressive method. Components in the frequency band from 0.04 to 0.15 Hz were considered as low frequency (LF) and those in the range 0.15 to 0.4 Hz, which is synchronous with respiration, as high frequency (HF). LF components of RR interval and SBP variabilities are considered to be an expression of cardiac and vascular efficient sympathetic regulation, respectively, whereas the HF component of RR interval variability is considered to be an expression of cardiac vagal modulation.24–27 Oscillations <0.03 Hz were considered as very LF components. Spectral analysis of the respiratory signal was performed on the signal sampled once for every cardiac cycle. Respiratory spectra were used to assess the main respiratory frequency. The power density of each spectral component was calculated both in absolute values and normalized units.24 Baroreflex control of HR was performed by the spontaneous sequences method. Details of this technique have been described previously.26,28 This method reflects mainly vagally mediated baroreceptor-cardiac responses29 and has provided reproducible results.30

EF Test
After the recordings for ANS assessment had been performed, endothelium-dependent and -independent vasodilator function was assessed following currently published guidelines.30 Briefly, subjects lay supine on a bed and were allowed to rest for ≥10 minutes. Then, the left brachial artery was visualized 2 to 15 cm proximal to the antecubital fossa using a high-resolution ultrasound (ATL HDI 3000) with a 7.5-MHz linear array transducer. After baseline images and flow measurements were obtained, a pressure cuff applied on the upper arm was inflated to 50 mm Hg above SBP for 5 minutes. Blood flow was measured during the 15 seconds after cuff release, and arterial images for diameter measurement were acquired between 60 and 90 seconds after cuff deflation. Endothelium-dependent flow-mediated dilation (FMD) was calculated as the increase in poststimulus diameter as a percentage of the baseline diameter. After ≥15 minutes of rest, new baseline images and flow measurements were obtained, and 0.4 mg of nitroglycerin (NTG) spray were given sublingually to assess endothelium-independent vasomotor responsiveness. Blood flow and images for arterial diameter were recorded between 3 and 4 minutes after NTG administration. NTG-mediated dilation was calculated as the increase in poststimulus diameter as a percentage of the baseline diameter. For both FMD and NTG-mediated dilation, arterial diameter was measured from the anterior to the posterior endothelial-lumen interface at end diastole, at the onset of the R-wave on the ECG. Images were then coded and analyzed by an investigator blinded to image sequence and subjects' clinical data.

Statistics
Each variable was checked for normality of distribution by the Kolmogorov–Smirnov test. When normality test passed, unpaired t-test was used to compare FDRs and controls for each of the reported variables. The Mann–Whitney test was used for variables with nonnormal distributions. Within-group comparisons were performed by paired t-test and the Wilcoxon signed rank test, as appropriate. General linear model was used to correct the differences of the variables of interest for age, sex, and body mass index. All of the reported values are expressed as mean±SEM. Differences were considered statistically significant when P was <0.05.

Results

Metabolic Data
The metabolic and hemodynamic characteristics of FDRs and control subjects are reported in Table 1. There was no significant difference in fasting blood glucose and insulin levels between FDRs and controls. Similarly, no significant differences were detected in the lipoprotein profile. The
TABLE 1. Characteristics of FDRs and Control Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>FDRs</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height, cm</td>
<td>171.0±0.02</td>
<td>170.0±0.02</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>73.5±3.3</td>
<td>66.5±2.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.9±0.8</td>
<td>23.1±0.7</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>87.0±2.0</td>
<td>81.1±2.0</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>87.4±2.2</td>
<td>85.2±1.6</td>
</tr>
<tr>
<td>Fasting insulin, µg/mL</td>
<td>8.3±0.8</td>
<td>7.2±0.6</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.35±0.06*</td>
<td>5.08±0.05</td>
</tr>
<tr>
<td>C peptide, (n=12 for controls), ng/mL</td>
<td>1.34±0.08†</td>
<td>1.02±0.08</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.84±0.20</td>
<td>1.50±0.12</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>180.6±7.2</td>
<td>171.1±8.3</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>56.2±2.6</td>
<td>65.2±3.7</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>118.5±6.9</td>
<td>107.7±8.4</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>95.4±12.9</td>
<td>75.7±8.7</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>2.20±0.6†</td>
<td>0.59±0.1</td>
</tr>
<tr>
<td>RR interval, ms</td>
<td>924.4±22.5</td>
<td>946.9±28.9</td>
</tr>
<tr>
<td>Systolic arterial pressure, mm Hg</td>
<td>111.5±2.9</td>
<td>115.0±1.9</td>
</tr>
<tr>
<td>Diastolic arterial pressure, mm Hg</td>
<td>72.6±1.6</td>
<td>77.0±1.5</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM. BMI indicates body mass index; TG, plasma triglyceride; hsCRP, high-sensitive C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. *P<0.01 vs controls. †P<0.05 vs controls.

HOMA-IR index did not differ between FDRs and controls. However, FDRs still had normal but significantly higher value for HbA1c than control subjects, and C peptide was also significantly higher in FDRs. Again, FDRs had still normal but significantly higher values for blood glucose than controls subjects from 60 up to 120 minutes after oral glucose load. Similarly, insulin levels were significantly higher in FDRs from 60 up to 120 minutes in the OGTT (Figure 1), reflecting hyperinsulinemia. The area under glucose curve (AUC) during OGTT was significantly higher in FDRs than control subjects (14.4±0.6 versus 11.9±0.5 µmol/L per hour; P=0.01), whereas the difference in AUC during OGTT for insulin fell just short of statistical significance (108.2±8.9 versus 80.8±10.2 µUI/mL per hour in FDRs and controls, respectively; P=0.07). High-sensitive C-reactive protein was also significantly higher in FDRs than in control subjects.

ANS Function

RR interval, SBP, and diastolic (D) BP did not differ significantly between FDRs and control subjects (Table 1). Spectral powers characteristics and baroreflex sensitivity (BRS) data are summarized in Table 2. There were no statistically significant differences between FDRs and control subjects in the spectral indexes of cardiac autonomic regulation. On the contrary, the LF component of SBP variability was significantly higher in FDRs than control subjects, and BRS was significantly and markedly decreased in FDRs in comparison with controls.

EF

The basal diameter of the brachial artery was not different between FDRs and control subjects (3.5±0.1 versus 3.1±0.1 mm, respectively; P=0.08). Also, basal blood flow was not significantly different between FDRs and control subjects (62.7±8.8 versus 45.2±5.6, respectively; P=0.17). Occlusion of the forearm induced a significant postischemic increase in blood flow in both FDRs and control subjects, and the magnitude of the increase in hyperemic blood flow was not significantly different between FDRs and controls (378.6±52.6 versus 329.9±80.9 mL/min, respectively; P=0.6). FMD was significantly lower in FDRs than control subjects, whereas no significant tendency to a reduced NTG-induced dilation was detected (Figure 2).

TABLE 2. Spectrum Analysis of RR Interval and Systolic Arterial Pressure Variability and Spontaneous Baroreflex Sensitivity in FDRs and Control Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>FDRs</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR interval</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variance, ms²</td>
<td>4526.7±1197.4</td>
<td>4365±895.8</td>
</tr>
<tr>
<td>Low frequency, ms²</td>
<td>1170.5±345.7</td>
<td>1278.3±288.2</td>
</tr>
<tr>
<td>n.u.</td>
<td>47.9±4.8</td>
<td>53.1±4.4</td>
</tr>
<tr>
<td>High frequency, ms²</td>
<td>1612.6±504.7</td>
<td>1404.6±449.6</td>
</tr>
<tr>
<td>n.u.</td>
<td>46.7±4.5</td>
<td>44.3±4.3</td>
</tr>
<tr>
<td>LF/HF</td>
<td>2.2±0.6</td>
<td>1.7±0.4</td>
</tr>
<tr>
<td>SBP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variance, mm Hg²</td>
<td>36.9±8.8</td>
<td>21.1±4.0</td>
</tr>
<tr>
<td>Low frequency, mm Hg²</td>
<td>8.6±2.8*</td>
<td>2.8±0.6</td>
</tr>
<tr>
<td>High frequency mm Hg²</td>
<td>1.2±0.2</td>
<td>1.1±0.3</td>
</tr>
<tr>
<td>BRS, ms/mm Hg</td>
<td>22.8±2.7†</td>
<td>37.0±5.8</td>
</tr>
<tr>
<td>Respiratory frequency, Hz</td>
<td>0.26±0.01</td>
<td>0.27±0.01</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM. n.u. indicates normalized units. *P<0.05 vs controls. †P<0.01 vs controls.
Change in vessel diameter induced by forearm ischemia (ie, flow-mediated endothelium-dependent vasodilatation) was inversely and significantly correlated with the LF oscillations in SBP variability in the entire population \((r = -0.4; P = 0.014,\) by Spearman rank test\). In addition, FMD was also inversely and significantly correlated with the area under glucose and insulin curve during OGTT \((r = -0.30, P = 0.04\) and \(r = -0.29, P = 0.04,\) respectively). After adjustment for age, sex, and body mass index, FMD confirmed to be lower \((P < 0.01)\) and LF of SBP higher \((P < 0.05)\) in FDRs than in controls.

**Discussion**

The new finding of the present study is that in healthy offspring of type 2 diabetic patients there is in basal conditions an increased peripheral sympathetic outflow, which is linked to a decreased endothelium-dependent flow-mediated vasodilatation and is associated with a concomitant impairment in baroreflex, vagal, control of the heart. To the best of our knowledge, this is the first study to assess concomitantly endothelial and overall ANS functioning in FDRs of diabetic patients.

**Endothelial Function**

Baseline vessel diameter and blood flow were not significantly different between FDRs and controls; most importantly, the magnitude of hyperemic blood flow was not significantly different in FDRs and controls, suggesting that the magnitude of the shear stress stimulus was closely similar in both experimental groups. Hence, despite similar shear stress stimulus, FDRs featured a significantly reduced forearm vasodilation, indicating endothelial dysfunction in such a higher-risk population. Although we did not find significant difference in HOMA-IR, an index of insulin resistance, between FDRs and controls, nonetheless, several findings from this study suggest the presence of an hyperinsulinemic state in the FDR group. These include higher values of HbA1c and C peptide and higher values for blood glucose ≤120 minutes after OGTT concomitant with higher insulin levels (Figure 1), reflecting a relative hyperinsulinemic state. The AUC for glucose during OGTT was also significantly higher in FDRs than in control subjects, and a similar tendency was also observed for AUC for insulin. In addition, the AUCs for glucose and insulin during OGTT were inversely correlated with FMD. Overall, these findings are consistent with previous studies showing impaired flow-mediated endothelium-dependent vasodilation in FDRs of diabetic patients, possibly related, although not invariably, to hyperinsulinemia.

**ANS Function**

In line with previous studies, we did not observe significant differences in cardiac autonomic modulation between FDRs and control subjects in basal, unstimulated conditions. However, we detected a significantly greater SBP LF fluctuation in FDRs than in controls, which would indicate an enhanced efferent sympathetic outflow to peripheral vasculature in FDRs.

The finding of no significant differences in spectral indexes of cardiac autonomic modulation in basal condition associated with altered spectral index of peripheral sympathetic modulation is not surprising and is consistent with the growing evidence indicating the selectivity of autonomic regulation and the highly differentiated sympathetic responses of peripheral vascular beds. In this context, it should be outlined that the LF component of BP variability assessed in this study would reflect, although indirectly, the overall rather than regional efferent sympathetic vascular modulation.

We cannot compare our results on LF oscillations in SBP variability with those of other studies, because, to the best of our knowledge, this is the only study to assess spectral analysis of SBP variability as an indicator of efferent sympathetic vascular modulation in offspring of type 2 diabetic patients. However, our findings are consistent with that of Hijmering et al, showing that physiologically induced sympathetic activation resulted in a reduced FMD in healthy subjects.

The mechanism(s) causing this sympathetic overactivity is not known. The enhanced peripheral sympathetic outflow in FDRs might have been mediated, at least in part, by a central or peripheral neural action of insulin. Administration of dexamethasone, a drug that could exert its effects either by inhibition of the transport of insulin from the plasma to the central nervous system and/or by altering central neural peptide release, has been shown to impair the ability of insulin to stimulate sympathetic nerve activity. Finally, in both normal subjects and borderline hypertensive patients, insulin has been shown to increase sympathetic nerve activity to peripheral muscle vessels with a concomitant vasodilatation, without elevating BP. However, it should be recalled that hyperinsulinemia is not invariably associated with sympathetic overactivity in humans, as demonstrated by the normal sympathetic nerve activity in patients with insulinoma. Alternatively, because NO has been shown to inhibit central neural vasoconstrictor outflow in humans and seems to be involved in the tonic restraint of sympathetic outflow, it is possible that defect in NO synthesis might have contributed to sympathetic overactivity. The finding of a reduced flow-mediated endothelium-dependent vasodilation, which is related to NO pathways, in our group of FDRs and of an inverse significant correlation between SBP LF and FMD would support, although does not directly prove, this hypothesis.

Another new finding is the markedly depressed BRS in FDRs, implying an early impairment in baroreflex, vagal control of HR in this higher-risk population. The observation...
of a significantly reduced BRS concomitant with maintained HF oscillations, an expression of oscillatory cardiac vagal modulation,24–27 in FDRs is not totally unexpected, because BRS and HRV, although possibly related,42 are differently linked to respiratory influences, with the former being minimally affected by respiration and the latter more closely reflecting respiratory sinus arrhythmias.42 Indeed, in the present study, respiratory activity did not differ between FDRs and controls.

By its nature, this study cannot clarify the mechanism(s) responsible for the decreased BRS in FDRs, although the observed sympathetic activation may be partly responsible for this observation.26,43 An alternative hypothesis for the decreased BRS would relate to a widespread reduction in NO activity at central and peripheral sites. Indeed, inhibition of endogenous NO production with NG-nitro-L-arginine resulted in a decrease in BRS in healthy subjects44 and enhanced NO synthesis by systemic administration of l-arginine–enhanced BRS in healthy individuals.45 The presence of discrete neuronal populations that possess NO synthase at different sites within the brain stem regions involved in baroreflex modulation of HR46 would provide the anatomic and functional substrate for this hypothesis.

Limitations of the Study
Because our volunteers refused to undergo the standard technique of the euglycemic hyperinsulinemic glucose clamp, we used the indirect, although widely accepted, HOMA-IR index to assess insulin sensitivity and did not find a difference between FDRs and controls. This was not totally unexpected, because the HOMA-IR index is based on fasting insulin and glucose levels, which were not significantly different in our group of relatively young and nonobese FDRs and control subjects. However, as discussed previously, several results from the present investigation suggest that, in our group of FDRs, an hyperinsulinemic state was present. It should be also mentioned that there is some controversy as to whether endothelial dysfunction and insulin resistance always coexist.47,48

A second limitation might include the indirect method used to assess changes in autonomic function. The issue of the validity of this approach was addressed by experiments in humans,25 in whom direct recordings of muscle sympathetic nerve activity were performed during various states of autonomic regulation, as produced by graded infusions of vasodilators and vasoconstrictors. The presence of similar, coherent, oscillations at low and high frequencies in nerve activity, RR intervals, and SBP variabilities at various levels of induced pressure changes provides support for the use of LF RR and HF RR to infer the changing state of, respectively, sympathetic and vagal modulation of the sinoatrial node and of LF SBP as an index of efferent sympathetic vascular modulation.29 Finally, in the interpretation of our findings, the relatively small sample size should be considered.

Perspectives
Our results indicate the existence of a concomitant impairment in endothelial and ANS functioning in offspring of type 2 diabetic patients, characterized by a reduced endothelium-dependent FMD and an enhanced peripheral sympathetic outflow, inversely related to one another, associated with an impairment of baroreflex control of HR.

Although the mechanism(s) of how endothelial and ANS dysfunction might be connected is still to be defined, nonetheless, measures of primary prevention in such apparently normal, but higher risk population, targeted to both of these 2 regulatory mechanisms, should be strongly recommended. Among these, a primary role may be played by regular physical activity, which has been demonstrated to improve both autonomic cardiovascular regulation10,50 and EF.51,52

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Disclosures
None.

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