Diabetic Neuropathy Is a More Important Determinant of Baroreflex Sensitivity Than Carotid Elasticity in Type 2 Diabetes

Juan Ruiz, David Monbaron, Gianfranco Parati, Sophie Perret, Erik Haesler, Claude Danzeisen, Daniel Hayoz

Abstract—The object of this study was to evaluate the contribution of carotid distensibility on baroreflex sensitivity in patients with type 2 diabetes mellitus with at least 2 additional cardiovascular risk factors. Carotid distensibility was measured bilaterally at the common carotid artery in 79 consecutive diabetic patients and 60 matched subjects without diabetes. Spontaneous baroreflex sensitivity assessment was obtained using time and frequency methods. Baroreflex sensitivity was lower in diabetic subjects as compared with nondiabetic control subjects (5.25±2.80 ms/mm Hg versus 7.55±3.79 ms/mm Hg; P<0.01, respectively). Contrary to nondiabetic subjects, diabetic subjects showed no significant correlation between carotid distensibility and baroreflex sensitivity (r²=0.08, P=0.04 and r²=0.04, P=0.13, respectively). In diabetic subjects, baroreflex sensitivity was significantly lower in subjects with peripheral neuropathy than in those with preserved vibration sensation (4.1±0.5 versus 6.1±0.4 ms/mm Hg, respectively; P=0.005). Age in nondiabetic subjects, diabetes duration, systolic blood pressure, peripheral or sensitive neuropathy, and carotid distensibility were introduced in a stepwise multivariate analysis to identify the determinants of baroreflex sensitivity. In diabetic patients, neuropathy is a more sensitive determinant of baroreflex sensitivity than the reduced carotid distensibility (stepwise analysis; F ratio=5.1, P=0.028 versus F ratio=1.9, P=0.16, respectively). In diabetic subjects with 2 additional cardiovascular risk factors, spontaneous baroreflex sensitivity is not related to carotid distensibility. Diabetic subjects represent a particular population within the spectrum of cardiovascular risk situations because of the marked neuropathy associated with their metabolic disorder. Therefore, neuropathy is a more significant determinant of baroreflex sensitivity than carotid artery elasticity in patients with type 2 diabetes. (Hypertension. 2005;46:162-167.)

Key Words: baroreflex ■ carotid arteries ■ diabetes mellitus

Atherothrombotic events and sudden cardiac death related to arrhythmia are accountable for the increased cardiovascular morbidity and mortality observed in patients with type 2 diabetes mellitus (T2DM). Cardiac dysautonomia often associated with peripheral neuropathies in T2DM contributes to the electrical instability of the myocardium. Additionally, silent myocardial ischemia has been shown to be associated with cardiac autonomic neuropathy in T2DM, thus amplifying the risk of arrhythmia and cardiac events. Therefore, detection and monitoring of autonomic dysfunction may have diagnostic and prognostic values in diabetic patients. A battery of tests has been developed to assess cardiac autonomic neuropathy. One of them relies on measurements of beat-to-beat variations in blood pressure and heart rate. The spontaneous change in cardiac cycle length per unit change in systolic pressure is an accepted measure of baroreflex sensitivity (BRS), which is highly sensitive for detecting early cardiovascular autonomic dysfunction in diabetic patients. BRS has emerged as a valuable prognostic factor for cardiovascular events in several high-risk conditions such as hypertension, myocardial ischemia, chronic stress exposure, and myocardial infarction.

T2DM is characterized by vascular wall alterations with increased intima-media thickness and/or plaque accumulation in the carotid bulb where baroreceptors are located. Therefore, alterations in carotid stiffness may reduce distensibility of the artery wall and blunt BRS. Alteration of mechanotransduction and of central nervous system processing as well as adjustment of cardiovascular responsiveness to neural outflow may contribute to decreased BRS in T2DM. In this study, we evaluated the potential contribution of carotid distensibility changes on BRS in whites with T2DM with at least 2 additional other cardiovascular risk factors. These patients are considered to be at high risk for cardio-
vascular events. Carotid distensibility was measured by automatic edge detection bilaterally at the common carotid and at the bulb level in 79 consecutive T2DM patients attending our outpatient clinic and in 60 matched control subjects. BRS assessment was obtained using the sequence technique in the time domain and the calculation of the alpha coefficient in the frequency domain.

Subjects and Methods

Subjects
We studied 79 consecutive subjects (18 females) aged 42 to 77 years (60±9; mean±SD) with T2DM recruited at the diabetes outpatient clinic of the Department of Endocrinology, Diabetology, and Metabolism of the Lausanne University Hospital. All patients presented at least 2 cardiovascular risk factors in addition to diabetes. The study was approved by the institution ethics committee. Informed consent was obtained from all subjects. All patients were asked to refrain from smoking and drinking coffee for at least 2 hours before the tests. The examination took place in the afternoon between 1:30 and 5:00 pm, at least 2 hours after a light lunch in a quiet room with a constant temperature of 21°C to 22°C. According to the American Diabetes Association/American College of Cardiology recommendations, a systematic noninvasive coronary heart disease screening test was performed in all patients.

Spontaneous BRS, heart rate variability, pulse wave velocity (PWV), and carotid distensibility of T2DM patients were compared with those of 60 non-diabetic subjects (15 females) with at least 2 cardiovascular risk factors (CVRFs). With the exception of antidiabetic medications, drug treatments were not significantly different between groups.

Clinical Examination
All studies were performed with subjects in the supine position. A clinical examination with measurements of bilateral ankle-brachial index and great toe pressure was performed after 10 minutes of rest in the supine position. Peripheral neuropathy was assessed with a tuning fork (Rydell-Seiffer 128 Hz) at the first metatarsal joint and the internal malleolus on a scale ranging from 0 to 8; (8=normal vibration detection); we defined peripheral neuropathy when the score was <4.

Carotid Artery Distensibility
An ATL HDI5000 ultrasound scanner with a L12–5 linear array probe was used to measure arterial wall dimension and movement at the common carotid and the bifurcation level. All ultrasound images were stored as cine-loop digital data file. An automatic edge detection program (HDI-Laboratory) was used to analyze off-line diameter changes during the cardiac cycle between systole and diastole. Ultrasound scanning was performed by experienced staff with the probe perpendicular to the vessel wall axis. The common carotid artery 1 to 2 cm from the bifurcation were measured in the longitudinal axis and wall displacements data were averaged over 5 cardiac cycles. Intra-individual and inter-individual variations were 3.5% and 6.4%, respectively, for the common carotid artery. Beat-by-beat internal carotid diameters changes were determined from the B-mode images analyzed by automatic edge detection program (HDI-Laboratory). Distensibility was calculated as ∆D/Dia/∆P, where ∆D is the change in internal diameter from diastole to systole, Dia is the diastolic internal diameter, and ∆P is the pulse pressure measured at the wrist by applanation tonometry (Colin).

Because of the very poor reproducibility of carotid bulb distensibility, this measure was not taken into account for the analysis. Therefore, we have limited our analysis to the straight arterial segment of the common carotids.

BRS and Heart Rate Variability
After a 15-minute baseline resting period, heart rate and blood pressure signals were recorded beat-by-beat for 10 minutes with the subjects in the supine position. During the recording period, subjects were asked to breathe regularly at a frequency of 15 breaths per minute. The digitized blood pressure signal was edited by an interactive procedure to eliminate possible artifacts. Systolic and diastolic blood pressures were identified on a beat-to-beat basis and the cardiac rhythm was derived from the blood pressure wave by computing the pulse interval, defined as the time interval between consecutive systolic peaks, identified with the help of parabolic interpolation of the blood pressure waveform.

In this study, we simultaneously estimated BRS by 2 independent and complementary methods to obtain a more comprehensive evaluation of the baroreflex function: the sequence technique, which focus on the baroreflex response to pressure transients, and the calculation of the so-called α index, which reflects the baroreflex response to rhythmic oscillations.16,17 The sequence method was described by Parati et al.18-21 ECG and blood pressure were recorded at a sampling frequency of 200 Hz. Beat-to-beat series of R-R interval from the ECG were derived for heart rate velocity analysis, as were series of systolic blood pressure and pulse interval for BRS estimation. The mean duration of R-R intervals and the standard deviation of all RR intervals were analyzed.

PWV
For PWV measurements of the aorta, Doppler and ECG signals were connected to a computer. Data acquisition was performed by means of a custom-made software, allowing simultaneous recording and displaying of both ECG and Doppler signals digitized at 1 kHz (Labview; National Instruments Corp) as previously described.22 The PWV was determined from the foot-to-foot flow wave velocity. A single investigator obtained all PWV measurements. Variation in intra-observer repeatability was 6±1%.22

Laboratory Measurements
Total cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured in serum on Hitachi automates using reagents from Boehringer, Mannheim/Roche. The HbA1c value was determined by high-performance liquid chromatography on a dDiamat Analyser System (Bio-Rad). Urinary creatinin was measured on a Hitachi automate using kits from Boehringer, Mannheim/Roche (Basel, Switzerland). Diabetic nephropathy was assessed either by 24-hour urine collection (microalbuminuria; when >30 mg/24 h) or by a albumin-to-creatinine ratio tested in the first morning urine (defined as pathological if >2.5 mg/mmol for men and >3 mg/mmol for women from at least 2 separate samples).

Statistical Analysis
Continuous variables, normally distributed, were expressed in means with SD and the Student t test was used for comparisons. The effects of hemodynamic parameters on BRS were analyzed by univariate and multivariate stepwise analyses. All statistical analyses were performed using JMP 5.0 (SAS Institute), and P≤0.05 was considered statistically significant.

Results

Population Description
The clinical characteristics of the T2DM population and the control nondiabetic subjects are described in Table 1. The arterial blood pressure in the T2DM population was close to the recommended target values for diabetic patients and their metabolic characteristics were in the normal range, with the exception of HbA1c.

In T2DM patients, a history of coronary heart disease was present in 30% of the population and stroke or transient ischemic attack was encountered in 4%. Active smoking was recorded in approximately one-third of the patients (32%) and 44% were former smokers. Background and proliferative
**TABLE 1. Clinical Characteristics of Diabetic (n=79) and Nondiabetic Subjects (n=60)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic</th>
<th>Nondiabetic</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>60±9</td>
<td>62±6</td>
<td></td>
</tr>
<tr>
<td>Diabetes duration, years</td>
<td>9 (3–22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>30%</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>76%</td>
<td>68%</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>88%</td>
<td>82%</td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>83%</td>
<td>81%</td>
<td></td>
</tr>
<tr>
<td>Microalbuminuria/macroalbuminuria</td>
<td>50%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic retinopathy</td>
<td>34%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>31.0±5.2</td>
<td>26.4±4.7</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>8.2±1.5%</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>137±19</td>
<td>136±14</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.5±1.1</td>
<td>5.0±0.6</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>0.9±0.5</td>
<td>1.8±0.5</td>
<td>P&lt;0.02</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.2±0.0</td>
<td>3.2±0.5</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.7±1.2</td>
<td>1.22±0.55</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>108.5±47.6</td>
<td>87.7±10.1</td>
<td>P&lt;0.02</td>
</tr>
</tbody>
</table>

Mean±SD.

retinopathy were observed in the same proportion of the population (17%) and microalbuminuria documented in 50% of the patients. By design, similar CVRF were observed in the control group.

Two-thirds of the patients (77.2%) were using insulin therapy and oral antidiabetes were prescribed in the following percentages according to class: biguanide 56.9%, sulfonylurea 13.9%, glinide 5%, and glitazone 12.6%. All patients were prescribed at least 1 of the following antihypertensive drugs: angiotensin-converting enzyme inhibitors 43%, AT-1 receptor blockers 40.5%, β-blockers 32.9%, diuretics 45.5%, and calcium channel blockers 29.1%. Antiplatelet agents were prescribed to 73.4% and oral anticoagulant to 7.6%. Lipid-lowering agents were given to 64.5% of the diabetic subjects in form of a statin and as fibrates in 3.7%.

**Vascular and Autonomic Function Investigations**

The majority of T2DM patients had peripheral plaques (inhomogeneous intima-media thickening >1.2 mm) as a manifestation of atherosclerosis, with the right femoral bifurcations being the most affected large artery segment demonstrating atherosclerotic plaques in 68% of the patients versus 58% for the right carotid bifurcation being the least affected segment. Only 13% of the patients were free of plaque at all 4 sites of investigations. The proportion of plaque-free subjects in the control group was 35%.

The autonomic and vascular function characteristics of the study populations are described in Table 2.

BRS in the time and frequency domains were strongly correlated ($r^2=0.46$, $P=0.0001$). For subsequent analysis, only BRS in the time domain are reported because similar results were observed in the frequency domain. In T2DM patients, BRS was inversely correlated with age ($r^2=0.09$; $P=0.02$), pulse pressure ($r^2=0.06$; $P=0.05$), systolic pressure ($r^2=0.06$; $P=0.055$), carotid-femoral PWV ($r^2=0.06$; $P=0.05$), and total cholesterol ($r^2=0.07$; $P=0.03$). No correlation was observed between left or right common carotid distensibility and BRS exploiting spontaneous fluctuations of blood pressure and heart rate period both in the time and the frequency domains ($r^2=0.04$; $P=0.13$). However, a nonsignificant negative trend between carotid-femoral PWV, a marker of aortic stiffness and carotid distensibility ($r^2=0.03$; $P=0.09$), was noted. On the contrary a moderate but significant correlation was observed in nondiabetic subjects between BRS and carotid distensibility ($r^2=0.08$, $P=0.04$). Carotid artery distensibility was negatively related to age in both groups and to the same extent (Figure 1).

In diabetic patients, BRS was not associated with the presence of coronary heart disease (CHD) evidenced by stress echocardiography, scintigraphy, and/or coronary angiograms. Patients with CHD (n=18) demonstrate a significantly higher BRS than those free of overt or silent CHD (6.6±2.8 versus 4.8±2.7 ms/mm Hg; respectively; $P=0.03$). This was independent of β-blocker treatment, which was similar in the 2 groups of patients. In male subjects, erectile dysfunction was associated with a reduced BRS (4.4±2.6 versus 6.4±2.8; $P=0.03$). Microalbuminuria was also correlated with a reduced BRS (4.4±2.7 versus 6.0±2.7; $P=0.02$). Heart rate velocity was significantly reduced in T2DM when compared with controls as expressed by a lower standard deviation (SD) of the RR intervals (32.1±19 versus 62.8±23 ms; $P<0.01$).
SD was significantly lower in subjects with a tuning fork score < 4 than in those with preserved pallesthesia (26.2 ± 24.1 versus 35.6 ± 16.3 ms; P < 0.05).

Similarly, BRS was lower in patients with peripheral neuropathy than in those with a score > 4, independent of age (4.1 ± 0.5 versus 6.1 ± 0.4 ms/mm Hg, respectively; P = 0.005). Arterial stiffness expressed as carotid-femoral PWV was higher in patients with sensitive motor alteration than in those with a score > 4, independent of age (13.9 ± 0.6 versus 11.6 ± 0.5 m/sec, respectively; P = 0.005).

Arterial stiffness indices such as carotid-femoral PWV and carotid distensibility were not significantly different between the 2 study populations (Table 2).

A positive correlation was observed between diabetic autonomic dysfunction expressed as a reduced SD of the RR intervals and sensitive motor impairment demonstrated by a reduced tuning fork score (r² = 0.29; P < 0.04).

Stepwise multivariate analysis taking into account age in nondiabetic subjects, diabetes duration, systolic blood pressure, neuropathy in diabetic subjects (tuning fork score), and carotid distensibility revealed that the neuronal alteration (peripheral neuropathy) was more sensitive than the mechanical component (carotid distensibility) on BRS (r² = 0.14 for the whole model; F ratio = 5.1, P = 0.02 versus F ratio = 1.9, P = 0.16; respectively). For the control group, the systolic blood pressure was the most significant determinant for BRS, followed by carotid distensibility (r² = 0.21; F ratio = 4.7, P = 0.03 versus F ratio = 1.4, P = 0.23; respectively).

Discussion
Increased arterial stiffness assessed by increase PWV and increased pulse pressure have been shown to relate directly with cardiovascular events.23,24 Similar prognostic information can be gained from BRS evaluation in different patient populations at risk for cardiovascular complications. Previous investigations have demonstrated that BRS is significantly related to carotid artery distensibility. A positive association between the elastic properties of the carotid artery and BRS and vascular resistance has been reported in healthy volunteers,25 pregnant women,26 hypertensive subjects,27 and nondiabetic patients with carotid artery stenosis.28 However, in this study we did not observe any correlation between BRS and common carotid artery wall distensibility in T2DM patients with at least 2 additional cardiovascular risk factors.

This T2DM population differs significantly from previously published works. We have selected high-risk subjects with T2DM who are characterized by stiffened and diseased arteries as evidenced by the elevated carotid-femoral PWV and the widespread atherosclerotic lesions documented in the femoral and carotid bifurcations. Does the absence of correlation between BRS and carotid distensibility preclude that the stiffened vessel walls have no influence on the mechanotransduction signals from the carotid artery? In the comparative nondiabetic group with similar degree of arterial stiffening as evidence by equally elevated PWV and reduced carotid distensibility, a positive but weak correlation was observed between carotid distensibility and BRS, confirming previous observations in the nondiabetic group of subjects.25–28

There are several aspects of the study that deserve clarification to properly interpret the data. First, spontaneous baroreflex sensitivity estimation using the alpha index and the sequence techniques may have a different meaning compared with the traditional phenylephrine method. However, a whole body of data supports the validity of the spontaneous indices as an estimate of baroreflex function both in experimental conditions and in clinical investigations, although it should be acknowledged that “spontaneous” and laboratory methods of the assessment of BRS offer complementary and not necessarily superimposable information.19,29

Another possible limitation of our study may be related to the use of β-blockers. The treatment that was not discontinued in this study for evident reasons may increase R-R interval and thus affect both R-R interval variability and baroreflex sensitivity parameters, with a possible impact on the interpretation of the results. However, analysis of BRS in the study population after adjustment for administration of β-blockers did not reveal any significant difference. Additionally, adjustment for other antihypertensive, oral antidiabetic, or lipid-lowering drugs did not modify the results either.

Would inadequate measurements of the elastic properties of the common carotid artery be responsible for the discrepancy results obtained here. As far as the straight segment of the common carotid artery is considered, good reproducibility data were obtained and have been previously published.30 Moreover, we observed a negative trend between local common carotid artery distensibility and a more generalized and well-validated marker of aortic stiffness expressed here as carotid-femoral PWV.

Whether the common carotid artery can be considered an adequate estimate of the carotid bulb properties for baroreflex function analysis remains a matter of debate. Previous studies have demonstrated a tight correlation between common carotid distensibility and BRS.31 Baroreceptors are located in the proximal portion of the carotid bulbs and on the aortic arch where distensibility may be preserved for a longer period than at the carotid bifurcations. The lack of data on the elastic behavior of the aortic arch precludes addressing this question; however, a positive correlation was observed in our study population between aortic stiffness and BRS after adjustment for age. However, patients having had bilateral glomus tumor removal are not able to regulate blood pressure adequately, suggesting that aortic baroreceptors may not be able to substitute for carotid sinus baroreceptors.32

Continuous radial artery blood pressure recordings calibrated on oscillometric brachial artery measurements were used to determine carotid distensibility. Whether peripheral blood pressure measurements introduce uncertainty in computing carotid distensibility is quite unlikely, although we have to acknowledge that differences between peripheral and central arterial pulse waves might occur. Central blood pressure can only be indirectly derived from transfer functions of peripheral artery waveforms, and the accuracy of which is still matter of debate.33 Moreover, beyond age 60, as a consequence of vessel wall stiffening, central and peripheral arterial pulse pressures do not differ much because of the early reflection of the pressure wave as a consequence of
vessel wall stiffening. Calibrated carotid artery tonometry would be a valuable choice to determine carotid pulse pressure, although we did not feel comfortable to use it in patients with widespread atherosclerotic plaques in the carotid arteries because of the risk of possible embolization. Furthermore, it is not possible to obtain steady wave recordings for long periods of time because of respiration and swallowing movements.

The most likely explanation for the lack of association between common carotid artery distensibility and BRS in the present study appears to be related to the diabetic autonomic dysfunction manifested by the reduced heart rate variability in T2DM patients when compared with controls. Furthermore, we also provide evidence for a sensitive motor dysfunction in addition to the autonomies, stressing the impact of diabetes on the integrity of the peripheral nervous system. In this high-risk diabetic population, BRS was 30% lower than that observed in the nondiabetic population with similar CVRF. BRS has been demonstrated repeatedly to be one of the earliest indicators of cardiovascular dysautonomia often undetected by the conventional Ewing tests. Neurological alteration of both the afferent and efferent loops of the baroreflex appears to be more important than the changes in carotid distensibility as demonstrated by stepwise regression analysis, in which the loss of vibration sensation is a more significant determinant of BRS than changes in carotid distensibility. The positive correlation observed between diabetic neuropathy documented by the loss of vibration sensation of the lower limbs and reduced BRS corroborates the neurological nature of the baroreflex impairment in T2DM patients.

Silent or overt myocardial ischemia may further aggravate cardiac autonomic dysfunction leading to severe arrhythmia. The absence of a positive correlation between BRS and CHD is not totally surprising because the aggregate of positive stress echocardiography, scintigraphy, and/or coronary angiograms does not allow teasing out macroangiopathy from microangiopathy. In the T2DM, 27% of the patients had a positive test, suggesting coronary artery disease or a positive angiogram confirming CHD. The limited number of cases does not allow any conclusion to be drawn.

Detection of BRS impairment may allow early detection of patients with metabolic syndrome prone to cardiovascular complications. The protective role of an intensive treatment aimed at correcting the metabolic disorder in patients with impaired BRS at a very early stage, before organ damage occurs, should be evaluated in prospective studies. Such an opportunity may be offered to the patients, as demonstrated by the recently published Steno 2 study, which clearly showed that tight diabetic control has a favorable impact on autonomic neuropathy. In conclusion, T2DM represents a particular condition within the spectrum of cardiovascular risk situations because of the severe neuropathy associated with this metabolic disorder. The direct link between changes of the mechanical properties of elastic arteries and the neural autonomic cardiovascular alterations observed in nondiabetic hypertensive patients was not observed in this high-risk diabetic population. Our data provide for the first time to our knowledge clear evidence that in these patients, impairment of BRS is more closely related to the neurological alteration associated with diabetes than with the reduced distensibility of the carotid arteries.

References
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