Original Article

Pattern of 24-Hour Ambulatory Blood Pressure Monitoring in Type 2 Diabetic Patients with Cardiovascular Dysautonomy

Claudia R.L. CARDOSO[1)], Nathalie C. LEITE[1)], Ludmilla FREITAS[1)], Saulo B. DIAS[1)], Elizabeth S. MUXFELD[1)], and Gil F. SALLES[1)]

The pathophysiological mechanisms linking cardiovascular dysautonomy to mortality are unclear. The aim of this study was to investigate the pattern of 24-h ambulatory blood pressure (BP) monitoring (ABPM) in diabetic patients with cardiovascular autonomic neuropathy (CAN). We evaluated 391 type 2 diabetic patients in a cross-sectional study. Five clinical tests of CAN were performed: heart-rate variation during deep breathing, the Valsalva maneuver, and standing, and BP variation during handgrip and standing. Patients were considered to have initial CAN if one heart-rate test was abnormal or two were borderline, and to have definite or severe CAN if at least two tests were abnormal. Differences between patients with and without CAN were assessed by bivariate tests and ANCOVA. Of the 391 patients, 230 (59%) presented clinical CAN, of whom 53 had definite or severe involvement. Patients with CAN were older, had diabetes of longer duration, and had an equal prevalence of hypertension but used more antihypertensive drugs than those without CAN. On ABPM, patients with definite or severe CAN had higher systolic BP (SBP) and pulse pressures (PP) than those without CAN, particularly in the nighttime (SBP: 128±18 *vs.* 117±16 mmHg, $p=0.007$; PP: 58±13 *vs.* 50±11 mmHg, $p=0.003$) and early morning (SBP: 140±18 *vs.* 131±17 mmHg, $p=0.05$) after adjustment for potential confounders, as well as a higher prevalence of the systolic nondipping pattern (75.5% *vs.* 50.9%, $p=0.021$). In conclusion, type 2 diabetic patients with more severe CAN have higher SBP and PP, especially during the nighttime and early morning, as well as a higher prevalence of nondipping status. This unfavorable 24-h ABPM pattern may contribute to the increased cardiovascular risk of diabetic patients with dysautonomy. (*Hypertens Res* 2008; 31: 865–872)

***Key Words*: ambulatory blood pressure monitoring, cardiovascular autonomic neuropathy, type 2 diabetes**

Introduction

Cardiovascular autonomic neuropathy (CAN) is associated with increased mortality in several conditions, including diabetes, hypertension, and post-myocardial infarction (*1*–*3*) as well as in middle-aged and elderly populations (*4*, *5*). This increased mortality risk appears to be more marked in patients with diabetes, hypertension, or cardiovascular diseases (*1*).

In type 2 diabetic patients, the mechanisms by which CAN causes increased mortality are unclear, but are probably multifactorial. Part of this augmented risk can be attributed to the elevated risk of life-threatening ventricular arrhythmias caused by sympathetic-vagal imbalance (*1*, *6*). The pattern of 24-h blood pressure (BP) observed in patients with CAN may also be involved in this increased mortality risk (*7*, *8*). Not

From the [1)]Department of Internal Medicine, University Hospital Clementino Fraga Filho, Medical School, Federal University of Rio de Janeiro, Rio de Janeiro, Brasil.

This study was supported by research grants (to G.S. and C.C.) from the Brazilian National Research Council (CNPq).

Address for Reprints: Gil F. Salles, Ph.D., Department of Internal Medicine, University Hospital Clementino Fraga Filho, Medical School, Federal University of Rio de Janeiro, Rua Croton 72, Jacarepaguá, Rio de Janeiro, Brasil, CEP: 22750–240. E-mail: gilsalles@hucff.ufrj.br

Received October 2, 2007; Accepted in revised form December 3, 2007.

only is the average BP level evaluated during a 24-h period a better marker of cardiovascular morbidity and mortality than the casual office BP measurement (*9*, *10*), but also the pattern of BP variability over the 24-h period may be important, particularly the nocturnal BP reduction and the early morning BP surge (*11*). Although some previous investigations have demonstrated the prognostic value of the nocturnal BP fall and the morning BP surge (*8*, *12*, *13*), their roles in the prediction of cardiovascular events over average BP levels in hypertensive and diabetic patients are still a matter of controversy (*11*, *14*). It seems possible that different BP variability patterns between day and night may cause different types of organ damage and cardiovascular events (*12*).

Therefore, regarding the impact of the presence of CAN on cardiovascular morbidity and mortality in diabetic patients and the potential roles of BP level and variability patterns over the 24-h period, we aimed to investigate the differences in ambulatory BP monitoring (ABPM) parameters between type 2 diabetic patients with and those without clinical CAN.

Methods

Study Patients and Baseline Procedures

This was a cross-sectional study with a cohort of 391 type 2 diabetic patients enrolled from August 2004 to August 2007 in the outpatient clinic of a tertiary care university hospital. Exclusion criteria to enter the cohort were a body mass index >40 kg/m^2, serum creatinine ≥ 180 mmol/L, and the presence of any serious concomitant disease, such as hepatic, pulmonary, or cancerous. All patients gave written informed consent, and the local ethics committee previously approved the study. All patients underwent a standard protocol that included a complete clinical examination, with particular attention to the presence of micro- and macrovascular degenerative complications, five clinical tests of cardiovascular autonomic function, a laboratory evaluation, resting 12-lead ECG, 24-h ABPM, and a 2-D echocardiogram. Only patients in sinus rhythm on ECG entered this study.

Office BP was measured three times using a digital oscillometric BP monitor (HEM-907 XL, Omron Healthcare, Kyoto, Japan) with a suitably sized cuff. The first measure was discarded, and the BP considered was the mean between the two last readings. Pulse pressure (PP) was calculated as systolic BP (SBP) minus diastolic BP (DBP). Arterial hypertension was diagnosed if mean SBP ≥ 140 mmHg or DBP ≥ 90 mmHg, or if antihypertensive drugs had been prescribed.

Coronary heart disease was diagnosed by clinical or electrocardiographic criteria (Minnesota codes: 1.1–1.3, 4.1–4.4, or 5.1–5.3), or by positive ischemic stress tests. Cerebrovascular disease was diagnosed by history and physical examination and peripheral arterial disease by systolic ankle-brachial index <0.9. Diabetic retinopathy was evaluated by an ophthalmologist. A diagnosis of nephropathy required at least two urinary albumin excretion rates ≥ 30 mg/24 h or proteinuria ≥ 0.5 g/24 h, or confirmed reduction of the glomerular filtration rate (creatinine clearance <1 mL/s or serum creatinine >130 μmol/L). Peripheral neuropathy was ascertained by clinical examination (knee and ankle reflex activities, feet sensation with the Semmes-Weinstein 5.07 [10 g] monofilament and vibration, using a 128 Hz tuning fork).

The cardiovascular autonomic function tests performed were heart-rate variation during deep breathing, Valsalva maneuver, and standing, and BP variation during handgrip and standing. The protocol of the clinical tests and the cutoff values for considering each test result as normal, borderline, or abnormal were those described and validated by Ewing *et al*. (*15*). Briefly, in the deep breathing test the patient breathes deeply at 6 breaths/min, and the difference between the shortest and longest RR interval (in beats/min) during each cycle is obtained. In the Valsalva maneuver test, the patient blows against a mouthpiece connected to a manometer at 40 mmHg for 15 s and then releases the exhalation. The ratio of the longest RR interval obtained just after the release and the shortest RR during strain is the response considered. In the heart-rate response to standing, the ratio of the longest RR interval measured around the 30th beat and the shortest RR interval around the 15th beat after standing up is obtained. In the BP response to the standing test, BP is measured in the supine subject and after 2 min standing; the difference between lying and standing SBP is obtained. In the BP response to the sustained handgrip test, BP is measured before and during handgrip maintained at 30% of the maximum voluntary force during 3 min using a hand dynamometer; the difference between the highest DBP measured immediately before the release of the handgrip and the DBP before starting is obtained. All five tests were repeated three times, and the mean value for each test was calculated. Table 1 shows the cutoff mean values considered to define each test as normal, borderline, or abnormal. Patients were diagnosed as having initial CAN if one heart-rate test was abnormal or if two were borderline. Involvement was considered definite if two or three heart-rate tests were abnormal, and severe if, in addition, at least one BP test was also abnormal. These clinical cardiovascular autonomic function tests have been validated and recommended by the American Diabetes Association for CAN diagnosis (*15*, *16*).

Laboratory evaluation included fasting glycemia, serum creatinine, glycated hemoglobin, and lipid profile. Urinary albumin excretion rate (UAER), proteinuria, and creatinine were evaluated from a sterile 24-h urine collection. Two-dimensional transthoracic echocardiography (Sonoline G60S; Siemens, Munich, Germany) was performed by the same experienced observer. Left ventricular mass was calculated by Devereux's formula (*17*) and indexed to body surface area (LVMI).

ABPM was recorded using Mobil O Graph equipment (version 12, Numed, Sheffield, UK), which was approved by the British Society of Hypertension. All patients used their prescribed antihypertensive medications during ABPM. A read-

Table 1. Threshold Values for Considering Each Autonomic Function Test as Abnormal or Borderline and Results of All Diabetic Patients

Autonomic function tests	Threshold values		Results (mean±SD)	% borderline	% abnormal
	Borderline	Abnormal			
HR variation					
Deep breathing	11–14 bpm	≤10 bpm	13±17	181 (46.3%)	107 (27.4%)
Valsalva maneuver	1.11–1.20	≤1.10	1.35±0.27	83 (21.2%)	45 (11.5%)
Standing	1.01–1.03	≤1.00	1.12±0.11	42 (10.7%)	12 (3.1%)
BP variation					
Standing	11–29 mmHg	≥30 mmHg	2±18	76 (19.4%)	25 (6.4%)
Handgrip	11–15 mmHg	≤10 mmHg	18±10	89 (22.8%)	69 (17.6%)

HR, heart rate; BP, blood pressure.

ing was taken every 15 min throughout the day and every 30 min at night. The parameters evaluated were mean 24-h, daytime, nighttime, and early morning (2 h after awakening) SBP, DBP, and PP; nocturnal SBP and DBP reduction; and morning SBP and DBP surge. Nocturnal BP reduction was considered at well-known abnormal values, and patients were classified as nondippers if either SBP or DBP reductions were <10% (nondipping SBP-DBP) and also if only the nocturnal SBP fall was <10% (nondipping SBP). Morning BP surge was defined as the absolute difference between the mean BP in the early morning and the mean nighttime BP, and also as the percent increase of mean early morning BP in relation to mean nighttime values. The nighttime period was ascertained for each individual patient from registered diaries.

Statistical Analysis

Statistics were analyzed by SPSS 13.0. Continuous variables were described as means±SD. Bivariate comparisons between diabetic patients without CAN (the reference category) and patients with initial or definite/severe CAN were performed by the unpaired *t*-test in normally distributed data and by the nonparametric Mann-Whitney test in asymmetrically distributed data. Categorical data were compared by the χ^2 test. Comparisons of continuous ABPM parameters between patients without CAN and patients with initial or definite/severe CAN were performed by crude unadjusted ANOVA with the post-hoc Dunnett's two-sided test (in which the subgroup without CAN was the reference category) and by analysis of covariance (ANCOVA) allowing adjustments for possible confounding variables (age, gender, diabetes duration, presence of hypertension and antihypertensive treatment, and diabetic micro- and macrovascular complications). Adjustment for antihypertensive treatment was performed by including into the analysis each class of drug (diuretics, angiotensin converting-enzyme inhibitors, β-blockers, and calcium channel blockers) as well as the number of antihypertensive drugs in use. For categorical ABPM parameters (dipping status, either systolic or systolic-diastolic), a multivariate logistic regression was used to adjust for the same covariates. A two-tailed p value <0.05 was regarded as statistically significant.

Results

Autonomic Function Test Results and Baseline Characteristics

Table 1 shows the cutoff values used for considering the five autonomic function tests as borderline or abnormal, as well as the results for all patients. Overall, 230 patients (58.9%) presented CAN, including 177 (45.3%) with initial involvement (one heart-rate abnormal test or two borderlines), 39 (10.0%) with definite (two or three heart-rate abnormal tests), and 14 (3.6%) with severe involvement (at least two heart-rate abnormal tests plus one abnormal BP test). Table 2 shows baseline data of patients according to grades of cardiovascular autonomic neuropathy (absent, initial, and definite or severe involvement). Patients with CAN were older, had a longer duration of diabetes and an equal prevalence of arterial hypertension, and used more antihypertensive drugs than patients without CAN. They also had higher prevalences of peripheral arterial disease and diabetic retinopathy and nephropathy than those without CAN. Glycemic control was similar in both groups. Left ventricular mass index, serum triglycerides, and UAER were higher in patients with CAN.

Comparisons of ABPM Parameters between Patients with and without CAN

Table 3 presents the crude and adjusted comparisons of office BP and ABPM parameters between patients without cardiovascular dysautonomy and patients with initial CAN. Patients with initial CAN had higher unadjusted office, 24-h, daytime, nighttime, and early morning SBP and PP than those without CAN. They also had a lower nocturnal SBP and DBP falls but a nonsignificant greater prevalence of the nondipping pattern and an equal morning BP surge relative to patients without CAN. Overall, unadjusted BP level differences between diabetic patients without CAN and those with initial CAN were

Table 2. Baseline Characteristics of Patients without Cardiovascular Autonomic Neuropathy and with Increasing Grades of Autonomic Involvement

Variables	Patients without CAN (n=161)	Patients with initial CAN (n=177)	Patients with definite or severe CAN (n=53)
Age (years)	58.5±10.4	62.7±8.7*	61.5±10.0
Gender (male, *n* (%))	57 (35.4)	70 (39.5)	16 (30.2)
BMI (kg/m^2)	29.7±4.9	29.4±4.9	29.8±5.3
Smoking status (*n* (%))			
Current	12 (7.5)	10 (5.6)	3 (5.7)
Past	58 (36.0)	74 (41.8)	20 (37.7)
Never	91 (56.5)	93 (52.5)	30 (56.6)
Diabetes duration (years)	9.4±8.6	10.0±8.1	14.6±9.8*
Arterial hypertension (*n* (%))	136 (84.5)	158 (89.3)	47 (88.7)
Diabetes treatment (*n* (%))			
Metformin	121 (75.2)	146 (82.5)	42 (79.2)
Sulfonylureas	80 (49.7)	73 (41.2)	23 (43.4)
Insulin	59 (36.6)	77 (43.5)	25 (47.2)
Hypertension treatment			
Number of drugs	2.2±1.4	2.6±1.4‡	2.9±1.2‡
Diuretics (*n* (%))	94 (58.4)	130 (73.4)‡	45 (84.9)*
ACE inhibitors (*n* (%))	124 (77.0)	150 (84.7)	49 (92.5)†
Calcium channel blockers (*n* (%))	43 (26.7)	61 (34.5)	25 (47.2)‡
β-Blockers (*n* (%))	66 (41.0)	102 (57.6)‡	31 (58.5)†
Macrovascular complications (*n* (%))			
Coronary heart disease	22 (13.7)	32 (18.1)	13 (24.5)
Cerebrovascular disease	15 (9.3)	21 (11.9)	9 (17.0)
Peripheral arterial disease	18 (11.2)	41 (23.2)‡	19 (35.8)*
Microvascular complications (*n* (%))			
Retinopathy	45 (28.0)	64 (39.8)†	26 (49.1)‡
Nephropathy	37 (23.0)	55 (32.2)†	25 (47.2)‡
Peripheral neuropathy	40 (24.8)	62 (35.0)†	19 (35.8)
Laboratory variables			
Fasting plasma glucose (mmol/L)	8.5±3.4	8.7±3.7	9.3±3.5
HbA1c (%)	7.7±1.7	7.6±1.7	8.3±2.3
Serum cholesterol (mmol/L)	4.97±1.12	5.07±1.30	5.47±1.10†
HDL-cholesterol (mmol/L)	1.09±0.29	1.07±0.29	1.09±0.31
LDL-cholesterol (mmol/L)	3.10±0.96	3.08±1.10	3.36±1.00
Serum triglycerides (mmol/L)	1.76±1.14	2.12±1.68†	2.24±1.28†
Serum creatinine (μmol/L)	75±20	80±25‡	90±30*
UAER (mg/24 h)	36±89	174±544‡	197±514†
LVMI (g/m^2)	113±39	130±44*	129±39†
Heart rate (bpm)	76±14	74±14	81±17

Values are means±SD or absolute numbers (%). Comparisons between patients with initial or definite/severe CAN and patients without CAN (the reference category): †$p<0.05$, ‡$p<0.01$, *$p<0.001$. CAN, cardiovascular autonomic neuropathy; BMI, body mass index; ACE, angiotensin-converting enzyme; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; UAER, urinary albumin excretion rate; LVMI, left ventricular mass indexed to body surface.

more noticeable in the nighttime and early morning. Nevertheless, after full adjustment for potential confounders (age, gender, diabetes duration, presence of hypertension and antihypertensive treatment, and diabetic micro- and macrovascular complications), all differences between patients without CAN and with initial CAN disappeared, except for office SBP and PP.

Table 3 also shows the nonadjusted and adjusted comparisons between patients without CAN and those with definite or severe autonomic involvement. In general, the abnormalities observed in ABPM parameters in patients with initial autonomic involvement were accentuated in the subgroup with

Table 3. Crude and Adjusted Comparisons of Office Blood Pressure and ABPM Parameters between Patients without Cardiovascular Autonomic Neuropathy and with Increasing Grades of Autonomic Involvement

Variables	Patients without CAN (n=161)	Patients with initial CAN (n=177)	p value[a]	Patients with definite or severe CAN (n=53)	p value[a]
Average office BP					
SBP (mmHg)	143±25	153±27*	0.044	148±24	0.70
DBP (mmHg)	85±12	85±14	0.66	83±14	0.07
PP (mmHg)	58±19	67±21*	0.019	65±21†	0.46
Average 24-h BP					
SBP (mmHg)	126±14	131±18‡	0.28	133±15‡	0.05
DBP (mmHg)	73±10	74±11	0.42	74±9	0.62
PP (mmHg)	53±10	57±11*	0.25	59±11*	0.019
Average daytime BP					
SBP (mmHg)	129±14	133±18†	0.31	134±15†	0.10
DBP (mmHg)	75±10	75±11	0.48	75±9	0.80
PP (mmHg)	54±10	57±11‡	0.46	59±11‡	0.10
Average nighttime BP					
SBP (mmHg)	117±16	124±20*	0.13	128±18*	0.007
DBP (mmHg)	67±11	69±11	0.31	69±11	0.25
PP (mmHg)	50±11	55±12*	0.13	58±13*	0.003
Nocturnal BP reduction					
SBP (%)	9.1±7.1	6.6±8.8‡	0.23	5.0±8.3‡	0.025
DBP (%)	10.5±8.7	8.3±9.0†	0.44	7.3±9.4†	0.14
Non-dipping SBP (n (%))	82 (50.9)	107 (60.5)	0.88	40 (75.5)‡	0.021
Non-dipping SBP-DBP (n (%))	98 (60.9)	121 (68.4)	0.84	42 (79.2)†	0.06
Average early morning BP					
SBP (mmHg)	131±17	137±21‡	0.35	140±18‡	0.05
DBP (mmHg)	78±12	78±13	0.44	78±12	0.57
Morning surge					
SBP (mmHg)	14±13	13±14	0.33	12±13	0.55
DBP (mmHg)	10±9	9±9	0.99	9±9	0.72

Values are adjusted means±SD or absolute numbers (%). Crude non-adjusted comparisons between patients with initial or definite/severe CAN and patients without CAN (the reference category): †$p<0.05$, ‡$p<0.01$, *$p<0.001$. [a]Comparisons adjusted for age, gender, diabetes duration, presence of hypertension and antihypertensive treatment, and micro- and macrovascular complications by analysis of covariance (for continuous variables) or by multivariate logistic regression (for categorical variables). CAN, cardiovascular autonomic neuropathy; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

definite or severe CAN. In particular, patients with definite or severe CAN had significantly higher nighttime SBP and PP, higher early morning SBP, lower nocturnal SBP reduction, and a higher prevalence of the nondipping pattern than those without CAN, even after full statistical adjustment. The presence of definite or severe CAN is associated with a nearly three-fold greater chance of being a systolic nondipper (adjusted odds ratio: 2.62, 95% confidence interval: 1.16–5.92).

Discussion

This study shows that type 2 diabetic patients with more severe cardiovascular autonomic neuropathy have higher ambulatory SBP and PP, particularly during nighttime and early morning, lower nocturnal SBP falls, and a higher prevalence of the nondipping pattern than patients without cardiovascular dysautonomy, even after adjusting for other potential confounders, such as age, gender, diabetes duration, presence of arterial hypertension and antihypertensive treatment, and presence of diabetic micro- and macrovascular complications. This adverse ABPM profile may contribute to the increased cardiovascular morbidity and mortality observed in diabetic patients with cardiovascular autonomic neuropathy. As far as we know, this is the first large study to completely describe the 24-h ABPM profile, including mean BP levels and temporal variability patterns, of type 2 diabetic patients stratified according to increasing severity of cardiovascular autonomic involvement.

Average BP obtained in ambulatory monitoring has been shown to be more accurate in predicting cardiovascular outcomes than casual office BP (*9*, *10*, *18*). So, finding higher BP

levels on ABPM in diabetic patients with CAN is probably related, at least in part, to their increased cardiovascular risk. Nevertheless, it is still unclear which parameter of ambulatory BP—its level or its temporal pattern—is more important to the occurrence of cardiovascular damage and for the prediction of cardiovascular events. Possibly, different kinds of organ damage and cardiovascular events are affected differently by BP amplitudes and variability patterns (*12*, *19*).

Nighttime SBP has been related not only to organ damage and cardiovascular mortality in hypertensive patients (*9*, *18*, *20*), but also to the presence of micro- and macrovascular complications in diabetic patients (*21*, *22*), suggesting that altered ambulatory BP is involved in the development and progression of microvascular and macrovascular damage. The present study confirmed this association, as diabetic patients with CAN had higher prevalences of peripheral arterial disease and diabetic retinopathy as well as different stages of nephropathy (reflected by higher serum creatinine and increased UAER) than those without CAN. More worrisome, this subgroup of patients also had a worse ABPM profile, independent of the presence of these degenerative complications. These data together show that these patients have a cluster of unfavorable factors that probably act in conjunction with each other to increase cardiovascular risk. The higher nighttime SBP level observed in patients with CAN is one of the contributing factors to the increased cardiovascular risk of these patients. Chronic exposure to high nocturnal BP levels, possibly in a background of autonomic dysfunction, may accelerate structural atherosclerotic changes and increase the risk of thrombotic cardiovascular events (*11*). Studies in hypertensive individuals (*18*), in diabetic patients (*14*), and also in general populations (*9*) showed that the nighttime SBP more accurately predicted future cardiovascular events than the daytime SBP, especially in hypertensive treated patients (*23*). Also, chronotherapy for nocturnal hypertension—that is, the administration of at least one antihypertensive drug at bedtime instead of in the morning—was demonstrated to be able to control nighttime BP levels and to reverse the nondipping pattern, thus potentially improving cardiovascular prognosis (*24*).

Elevated PP is considered an indirect indicator of increased arterial stiffness, particularly in older individuals. Diabetic patients are thought to have increased arterial stiffness and PP, possibly due to accelerated vascular aging (*25*). Office PP has been associated with increased cardiovascular mortality in diabetic patients (*26*). On ABPM, PP has been related to the presence of micro- and macrovascular complications (*21*, *27*) and to the occurrence of fatal vascular events (*14*). Our results show that patients with CAN, after adjusting for potential confounders, still have higher office and nighttime PP than patients without CAN. This elevated PP may provide an additional cardiovascular risk to patients with CAN, since increased SBP augments end-systolic myocardial wall stress and promotes cardiac hypertrophy, while reduced DBP reduces coronary perfusion, therefore favoring myocardial ischemia (*28*).

In diabetic patients, the nondipping pattern has been associated with several adverse factors, such as different grades of nephropathy (*21*, *29*), autonomic neuropathy (*29*), and postprandial hyperglycemia (*30*). In our patients, the relatively high prevalence of the nondipping pattern (58.6%) is probably explained by the presence not only of CAN but also of other chronic degenerative diabetic complications, particularly nephropathy and macrovascular disease, and by the severity of arterial hypertension; 87% of our patients were hypertensives, using a median of three antihypertensive drugs. Recently, nocturnal BP reduction was demonstrated to be an active phenomenon mediated by autonomic counterregulatory systems that induce an active download state of the baroreflex sensivity threshold (*31*). So, impaired circadian modulation of sympathovagal activity is probably involved in the reduced nocturnal BP fall observed in diabetic patients (*32*). Our findings support this hypothesis by showing a progressive lower nocturnal SBP fall in relation to increasing severity of CAN. Nakano *et al*. (*8*) reported initially that a reversed circadian BP rhythm was associated with the occurrence of vascular events. Nevertheless, on a second analysis (*14*) of the same cohort of type 2 diabetic subjects, they reported that 24-h PP and nighttime SBP were independent predictors of fatal and nonfatal cardiovascular events, respectively, instead of nocturnal BP fall. This reinforces that it is still unknown which parameter of ambulatory BP is more prognostically important in diabetic patients. Another problem when evaluating dipping or nondipping status is the limited reproducibility of nocturnal BP variability. This limitation is related to changes in day activities and sleep quality, besides the "regression to the mean" phenomenon that occurs when repeating ABPM in patients previously classified as extreme dippers or nondippers and risers (*33*, *34*). Otherwise, the reproducibility of dipping status was recently reported to be more reliable in diabetic than in nondiabetic hypertensive patients (*35*).

Cardiovascular events tend to have higher incidences in the early morning hours, and morning BP or the early morning BP surge may contribute to this phenomenon (*36*). Morning SBP has been shown to inversely correlate with baroreflex sensitivity (*37*) and to be a strong risk marker for stroke in elderly subjects (*38*). The morning SBP surge has also been reported as a predictor of cerebrovascular events (*12*, *13*). In the present study, although early morning SBP was significantly higher in patients with CAN, since these patients also presented greater nighttime BP levels, no significant difference in the morning BP surge was observed between the subgroups, whether evaluated as the absolute difference between early morning and nighttime BP values or as the percentage increase in relation to mean nighttime BP level. Also, the absence of a standard definition of morning BP surge precludes comparisons between different studies and groups of patients.

The main limitation of this study is the rather uncertain

influence of antihypertensive drug treatment on autonomic function tests and also on ABPM profile. In thesis, as the clinical tests of heart-rate variation mainly investigate the integrity of parasympathetic heart enervation, they are not affected by antihypertensive drugs such as β-blockers. Even though we fully adjusted the associations between clinical CAN and ABPM parameters to all antihypertensive drug classes and also to the number of antihypertensive drugs in use during ABPM, we cannot rule out a residual influence of these drugs on these relationships. Another study limitation is inherent in its cross-sectional design, which precludes causal and temporal inferences. However, one of the strengths of this study is the relatively large number of patients evaluated, which reinforces that the ambulatory BP pattern in patients with CAN is frequently worse, and this imposes an additional burden of cardiovascular risk on these patients.

In conclusion, this study provides evidence that type 2 diabetic patients with more severe CAN have higher ambulatory SBP and PP, especially during the nighttime and early morning, and a greater prevalence of the nondipping pattern than patients without CAN. This adverse profile of 24-h ABPM may contribute to the increased cardiovascular morbidity and mortality of diabetic patients with dysautonomy. Future prospective studies are necessary to evaluate whether the progression or regression of diabetic autonomic dysfunction is associated with evolutive changes in the pattern of ambulatory BP and, most importantly, which ABPM parameter best predicts cardiovascular prognoses in type 2 diabetic individuals, particularly in those high-risk patients with cardiovascular autonomic neuropathy.

Acknowledgements

The authors would like to thank Marco Antonio de Couto for his technical assistance in performing the autonomic function tests.

References

1. Gerritsen J, Dekker JM, TenVoorde BJ, *et al*: Impaired autonomic function is associated with increased mortality, especially in subjects with diabetes, hypertension, or a history of cardiovascular disease: the Hoorn study. *Diabetes Care* 2001; **24**: 1793–1798.
2. Suarez GA, Clark VM, Norell JE, *et al*: Sudden cardiac death in diabetes mellitus: risk factors in the Rochester diabetic neuropathy study. *J Neurol Neurosurg Psychiatry* 2005; **76**: 240–245.
3. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ: Baroreflex sensivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. *Lancet* 1998; **351**: 478–484.
4. Dekker JM, Shouten EG, Klootwijk P, Pool J, Swenne CA, Kromhout D: Heart rate variability from short term electrocardiographic recordings predicts mortality from all-causes in middle-aged and elderly men: the Zutphen Study. *Am J Epidemiol* 1997; **145**: 899–908.
5. Tsuji H, Venditti FJ Jr, Manders ES, *et al*: Reduced heart rate variability and mortality risk in an elderly cohort: the Framinghan Heart Study. *Circulation* 1994; **90**: 878–883.
6. Wheeler SG, Ahroni JH, Boyko EJ: Prospective study of autonomic neuropathy as a predictor of mortality in patients with diabetes. *Diabetes Res Clin Pract* 2002; **58**: 131–138.
7. Töyry J, Niskanen L, Mäntysaari M, Länsimies E, Uusitupa M: Occurence, predictors and clinical significance of autonomic neuropathy in NIDDM: ten-year follow-up from diagnosis. *Diabetes* 1996; **45**: 308–315.
8. Nakano S, Fukuda M, Hotta F, *et al*: Reversed circadian blood pressure rhythm is associated with occurrences of both fatal and nonfatal vascular events in NIDDM subjects. *Diabetes* 1998; **47**: 1501–1506.
9. Dolan E, Stanton A, Thijs L, *et al*: Superiority of ambulatory over clinic blood pressure measurement in predicting mortality: the Dublin Outcome Study. *Hypertension* 2005; **46**: 156–161.
10. Clement DL, Buyzere ML, De Bacquer DA, *et al*: Prognostic value of ambulatory blood-pressure recordings in patients with treated hypertension. *N Engl J Med* 2003; **348**: 2407–2415.
11. Bilo G, Parati G: Temporal blood pressure patterns and cardiovascular events: "good night" or "good morning"? *J Hypertens* 2006; **24**: 1703–1705.
12. Metoki H, Ohkubo T, Kikuya M, *et al*: Prognostic significance for stroke of a morning pressor surge and a nocturnal blood pressure decline. *Hypertension* 2006; **47**: 149–154.
13. Kario K, Pickering TG, Umeda Y, *et al*: Morning surge in blood pressure as a predictor of silent and clinical cerebrovascular disease in elderly hypertensives. *Circulation* 2003; **107**: 1401–1406.
14. Nakano S, Ito T, Furuya K, *et al*: Ambulatory pressure level rather than dipper/nondipper status predicts vascular events in type 2 diabetes. *Hypertens Res* 2004; **27**: 647–656.
15. Ewing DJ, Martyn CN, Young RJ, Clarke BF: The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care* 1985; **8**: 491–498.
16. Boulton AJM, Vinik AI, Arezzo JC, *et al*: Diabetic neuropathies. A statement by the American Diabetes Association. *Diabetes Care* 2005; **28**: 956–962.
17. Devereux RB, Reichek N: Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. *Circulation* 1977; **55**: 613–618.
18. Staessen JA, Thijs L, Fagard R, *et al*: Predicting cardiovascular risk using conventional *vs*. ambulatory blood pressure in older patients with systolic hypertension. *JAMA* 1999; **282**: 539–546.
19. Metoki H, Ohkubo T, Kikuya M, *et al*: Prognostic significance of night-time, early morning, and daytime blood pressures on the risk of cerebrovascular and cardiovascular mortality: the Ohasama Study. *J Hypertens* 2006; **24**: 1841–1848.
20. Suzuki Y, Kuwajima I, Aono T, *et al*: Prognostic value of nighttime blood pressure in the elderly. *Hypertens Res* 2000; **23**: 323–330.
21. Fogari R, Zoppi A, Malamani GD, Lazzari P, Albonico B, Corradi L: Urinary albumin excretion and nocturnal blood pressure in hypertensive patients with type II diabetes mellitus. *Am J Hypertens* 1994; **7**: 808–813.
22. Knudsen ST, Poulsen PL, Hansen KW, Ebbehoj E, Bek T,

Mogensen CE: Pulse pressure and diurnal blood pressure variation: association with micro and macrovascular complications in type 2 diabetes. *Am J Hypertens* 2002; **15**: 244–250.
23. Boggia J, Thijs L, Hansen TW, *et al*: Prognostic accuracy of day *versus* night ambulatory blood pressure: a cohort study. *Lancet* 2007; **370**: 1219–1229.
24. Hermida RC, Ayala DE, Fernandez JR, Calvo C: Chronotherapy improves blood pressure control and reverts the nondipper pattern in patients with resistant hypertension. *Hypertension* 2008; **51**: 69–76.
25. Ulrich P, Cerami A: Protein glycation, diabetes and aging. *Recent Prog Horm Res* 2001; **56**: 1–21.
26. Schram MT, Kostense PJ, Van Dijk RA, *et al*: Diabetes, pulse pressure and cardiovascular mortality: the Hoorn study. *J Hypertens* 2002; **20**: 1743–1751.
27. Tanaka M, Babazono T, Takeda M, Iwamoto Y: Pulse pressure and chronic kidney disease in patients with type 2 diabetes. *Hypertens Res* 2006; **29**: 345–352.
28. Safar ME, Levy BI, Struijker-Boudier H: Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. *Circulation* 2003; **107**: 2864–2869.
29. Spallone V, Maiello MR, Cicconetti E, *et al*: Factors determining the 24-h blood pressure profile in normotensive patients with type 1 and 2 diabetes. *J Hum Hypertens* 2001; **15**: 239–246.
30. Pistrosch F, Reissmann E, Wildbrett J, Koehler C, Hanefeld M: Relationship between diurnal blood pressure variation and diurnal blood glucose levels in type 2 diabetic patients. *Am J Hypertens* 2007; **20**: 541–545.
31. Sayk F, Becker C, Teckentrup C, *et al*: To dip or not to dip. On the physiology of blood pressure decrease during nocturnal sleep in healthy humans. *Hypertension* 2007; **49**: 1070–1076.
32. Kondo K, Matsubara T, Nakamura J, Hotta N: Characteristic pattern of circadian variation in plasma catecholamine in type 2 diabetic patients. *Diabet Med* 2002; **19**: 359–365.
33. Dimsdale JE, Heeren MM: How reliable is nighttime blood pressure dipping? *Am J Hypertens* 1998; **11**: 606–609.
34. Cuspidi C, Meani S, Salerno, *et al*: Reproducibility of nocturnal blood pressure fall in early phases of untreated essential hypertension: a prospective observational study. *J Hum Hypertens* 2004; **18**: 503–509.
35. Cuspidi C, Meani S, Lonati L, *et al*: Short-term reproducibility of non-dipping pattern in type 2 diabetic hypertensive patients. *J Hypertens* 2006; **24**: 647–653.
36. White WB: Cardiovascular risk and therapeutic intervention for the early morning surge of blood pressure and heart rate. *Blood Press Monit* 2001; **6**: 63–72.
37. Eguchi K, Tomizawa H, Ishikawa J, *et al*: Factors associated with baroreflex sensitivity: association with morning blood pressure. *Hypertens Res* 2007; **30**: 723–728.
38. Kario K, Ishikawa J, Pickering TG, *et al*: Morning hypertension: the strongest independent risk factor for stroke in elderly hypertensive patients. *Hypertens Res* 2006; **29**: 581–587.