Original Article

Diurnal Blood Pressure Abnormalities Are Related to Endothelial Dysfunction in Patients with Non-Complicated Type 1 Diabetes

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Patients with diabetes have an increased cardiovascular morbidity and mortality despite interventions to prevent these outcomes. Abnormalities in diurnal blood pressure patterns are also associated with excess cardiovascular mortality. The aim of this study was to determine the effects of diurnal blood pressure patterns on endothelial function and oxidative stress in patients with uncomplicated type 1 diabetes mellitus. Thirty-two normotensive and normoalbuminuric type 1 diabetic patients (21 dipper and 11 nondipper) and 37 healthy (27 dipper and 10 nondipper) volunteers underwent 24-h ambulatory blood pressure monitoring. Their endothelial functions were evaluated using flow mediated dilatation (FMD) and by measuring nitric oxide and thiobarbituric acid reactive substances (TBARS). Dippers were defined as subjects who exhibited an average reduction in both systolic and diastolic blood pressure of greater than 10% between day and night periods. Nondipper type 1 diabetic patients and controls had nighttime systolic and diastolic blood pressure values that were significantly higher than those of dipper diabetic patients ($p<0.05$) and dipper controls ($p<0.01$). Values of FMD for nondipper diabetic patients (5.12±2.2%) were significantly lower than those in dipper diabetic patients (10.19±2.5%, $p<0.01$), nondipper (10.08±2.9%, $p<0.001$) and dipper controls (11.76±3.8%, $p<0.001$). Additionally, levels of TBARS in the dipper diabetic group and dipper controls were significantly lower than those in the nondipper diabetic group ($p<0.05$). In conclusion, only type 1 diabetic patients with a nondipping pattern of blood pressure exhibited changes that may lead to endothelial dysfunction and atherosclerosis. (*Hypertens Res* 2008; 31: 2065–2073)

***Key Words*: ambulatory blood pressure monitoring, endothelial function, diabetes, nondipper, oxidative stress**

Introduction

Patients with diabetes suffer from increased cardiovascular morbidity and mortality despite interventions to prevent these outcomes (*1*, *2*). Hypertension, hyperlipidemia and microangiopathy are some of the risk factors for increased incidence of vascular events in diabetic patients (*3–5*).

Ambulatory blood pressure measurements have been shown to predict vascular events more accurately than office blood pressure or random blood pressure measurements (*6*, *7*). The diurnal pattern of blood pressure is normally characterized by a nighttime drop in blood pressure (*8*). When this diurnal pattern is disturbed, there is a tendency for elevations of nighttime blood pressure; this phenomenon is referred to as a nondipping pattern. High night-to-day (N/D) ratios are observed in diabetic patients (*8–10*) and have been shown to be associated with microvascular complications (*11–13*).

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Changes in diurnal blood pressure pattern are also associated with increased cardiovascular mortality (*6*, *14*, *15*).

Endothelial dysfunction is one of the earliest signs of vascular complications. It is linked to diminished production/availability of nitric oxide and/or an imbalance in the relative contribution of endothelium-derived relaxing and vascular contracting factors (*16*). Several methods have been used to evaluate endothelial function and, of these, flow-mediated vasodilatation is a validated tool that is frequently employed. Endothelial dysfunction as determined by impaired flow-mediated vasodilatation is very common in diabetic patients with micro- or macrovascular complications (*17–19*).

Increased oxidative stress is thought to be the common pathway in the pathogenesis of vascular complications of diabetes as well as in endothelial dysfunction. Autonomic neuropathy is one of the complications that have been shown to result from oxidative stress (*20*).

The aim of this study was to determine the effect of diurnal blood pressure patterns on endothelial function and oxidative stress in normotensive patients with uncomplicated type 1 diabetes mellitus. On the basis of these data we tested the hypothesis that an altered diurnal blood pressure rhythm is the first stage in the development of vascular changes in patients with type 1 diabetes.

Methods

We included in our study thirty-two normotensive normoalbuminuric type 1 diabetic patients (21 dipper, 11 nondipper) who had been followed at our institution's outpatient endocrinology clinic and 37 healthy volunteers (27 dipper, 10 nondipper). All the subjects were non-smokers. All the female subjects were premenopausal, and all were tested in the early follicular phase of their menstrual cycle. None of the patients received any antihypertensive or other medication, aside from insulin. All subjects underwent a screening program that included medical history, physical examinations, ECG and laboratory testing, including blood glucose, hemoglobin A1c, blood urea nitrogen, serum creatinine, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, uric acid and liver transaminase levels. Two or more timed overnight urine collection events were conducted to measure urinary albumin excretion. Fundoscopic eye examinations were performed to assess retinopathy. Patients were evaluated for neuropathy on the basis of their medical history and neurological examinations, including orthostatic blood pressure changes (normal: <10 mmHg change in systolic blood pressure after 3 min standing). Blood samples were collected for biochemical analyses, and plasma and serum samples were stored immediately at -20°C for determination of thiobarbituric acid reactive substances (TBARS) at the end of the study.

A Spacelab 90207 device (Spacelab, Redmont, USA) was used to monitor ambulatory blood pressure (ABPM) over a 24 h period. Blood pressure measurements were recorded at 20-min intervals during daytime hours (07:00 AM–11:00 PM) and at 30-min intervals during the night (11:00 PM–07:00 AM). These measurements were confirmed by a manual sphygmomanometer reading both at the beginning and at the end of the 24-h period. Subjects were asked to record their sleeping hours and activities while awake. Diurnal variations in blood pressure were assessed using these data. Nighttime was defined as the period during which the subjects were asleep. Mean blood pressure was calculated for both daytime and nighttime. Nondippers were defined as subjects with an average reduction in either systolic or diastolic blood pressure of less than 10% from day to night, and all others were classified as dippers (*21*).

The study protocol was approved by the local research ethics committee, and all subjects gave written informed consent.

Determination of Endothelial Function

Flow-mediated vasodilatation was assessed by a noninvasive method described by Celermajer *et al*. (*22*), and Yufu *et al*. (*23*). This approach evaluates endothelial function using post-ischemic (forearm) vasodilatation, causing enhanced flow in the proximal brachial artery. This measurement was carried out using a high-resolution ultrasound system (GE Vingmed; System Five, Horten, Norway) and an 8 MHz linear-array transducer. After 10 min of rest before the first scan, increased flow was induced by deflating a pneumatic tourniquet after a 5-min suprasystolic arterial forearm compression (at least 50 mmHg above systolic blood pressure). The post-ischemic scan was performed 45 to 60 s after cuff deflation. Further scans were performed at rest and 4 min after sublingual administration of 0.4 mg glyceryl trinitrate (GTN) as a direct nitric oxide donor in order to examine endothelium-independent dilatation. The time interval between the first and second measurements was 20 min to allow for vessel recovery.

Vessel diameters were analyzed on the basis of still images captured across an arterial length of >1 cm. The difference in lumen diameter between rest and reactive hyperemia, expressed as percentage change, was regarded as the endothelium-dependent vasodilatation (flow-mediated dilatation; FMD). The percentage change in brachial artery lumen diameter after GTN was regarded as the endothelium independent vasodilatation. Intraobserver and interobserver coefficients of variation in measuring the baseline brachial artery diameter were 3.6% and 4.1%, respectively. Intraobserver and interobserver variability for reactive hyperemia were 0.975 and 0.951, respectively; $p<0.0001$.

Assays

Thiobarbituric Acid Reactive Substances

Oxidative stress was measured by malondialdehyde (MDA) levels assayed for products of lipid peroxidation following the

Table 1. Demographic and Clinical Characteristic of Patients and Controls

	Type 1 diabetic patients		Controls		
	Dipper (n=21)	Nondipper (n=11)	Dipper (n=27)	Nondipper (n=10)	
Male/female	8/13	7/4	13/14	4/6	n.s.
Age (years)	28.09±4.57	32.6±8.22	31.04±7.09	30.40±5.27	n.s.
BMI (kg/m^2)	22.25±2.41	23.02±2.10	23.94±2.00	22.31±2.88	n.s.
Diabetes duration (years)	8.2±6.3	11.3±6.3	—	—	n.s.
HbA1c (%)	7.26±1.23*,†	7.81±1.25#,§	5.38±0.20	5.42±0.29	
Total cholesterol (mg/dL)	169.0±8.3	176.7±9.7	165.1±4.8	176.2±36.8	n.s.
HDL-cholesterol (mg/dL)	57.6±14.8	56.7±7.7	50.8±12.3	58.8±9.0	n.s.
Triglyceride (mg/dL)	82.8±59.9	87.8±39.5	80.1±25.8	72.8±13.9	n.s.
LDL-cholesterol (mg/dL)	94.88±30	98.7±31.5	98.1±21.1	102.9±35.9	n.s.
Albuminuria (mg/d)	10.8±5.3	18.0±9.5	11.3±3.7	14.6±7.9	n.s.

*p<0.001 dipper DM *vs*. dipper controls; †p<0.001 dipper DM *vs*. nondipper controls; #p<0.001 nondipper DM *vs*. dipper controls; §p<0.001 nondipper DM *vs*. nondipper controls. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; DM, diabetes mellitus.

Table 2. Ambulatory Blood Pressure (BP) Measurements and Diurnal Variations

	Type 1 diabetic patients		Controls	
	Dipper (n=21)	Nondipper (n=11)	Dipper (n=27)	Nondipper (n=10)
Systolic BP (mmHg)				
24 h	113.1±7.9†	120.7±5.4	113±6.1*	115.4±4.3
Daytime	118.4±7.8	124.1±5.5	119.3±5.3	118.9±4.0
Nighttime	100.5±6.2‡	115±7.5	104.1±5.0*	111.5±5.0#,§
Diastolic BP (mmHg)				
24 h	68.4±4.0‡	76.0±3.8	70.2±2.5*	72.0±2.5
Daytime	76.7±4.0	79.2±3.6	77.1±3.1	75.1±3.2
Nighttime	59.9±5.1‡	72.8±4.0	63.1±2.9**	68.7±2.9#,§
Pulse pressure (mmHg)				
24 h	41.0±4.2	45.0±3.7	41.6±4.7	42.2±5.2
Daytime	41.6±4.8	45.8±3.2	42.2±4.7	42.6±4.7
Nighttime	40.5±4.2	44.8±4.4	40.9±5.4	43.0±6.6
Heart rate (beats/min)				
24 h	76.8±4.7	79.8±4.6	72.7±6.1*	79.0±4.9
Daytime	83.2±6.3	85.3±5.0	78.1±7.3*	80.0±6.0
Nighttime	70±6.5	73.8±5.3	66.7±5.3*	69.1±3.3
Diurnal blood pressure decline (%)				
Systolic	15.0±3.1‡	6.0±1.0	12.8±2.9**	6.8±2.3#,§§
Diastolic	21.7±5.7‡	8.1±2.5	18.0±3.9**	9.6±2.2#,§§
Pulse pressure	2.4±8.0	2.0±5.3	2.7±8.5	2.7±5.3
Heart rate	15.5±10.0	14.2±5.7	14.3±3.2	13.2±7.5

*p<0.01 and **p<0.001 nondipper DM *vs*. dipper controls; †p<0.05 and ‡p<0.001 nondipper DM *vs*. dipper DM; #p<0.001 dipper DM *vs*. nondipper controls; §p<0.05 and §§p<0.01 dipper controls *vs*. nondipper controls. DM, diabetes mellitus.

formation of TBARS, as described previously (*24*). Serum samples were studied and duplicated. A standard graph of 1,1,3,3-tetraethoxypropane (TEP) was used for normalization purposes. Lipid peroxidation was assessed in terms of MDA equivalents, and the results were expressed in units of μmol/L MDA/mL serum. Intra- and interassay coefficients of variation were 4.8% and 5.1%, respectively.

Nitric Oxide

Measurements were made using a nitric oxide colorimetric assay (Boehringer Mannheim, Mannheim, Germany) based on nitrogen monoxide quantification using nitrate substrates on microtiter plates in serum samples (*25*). The intra-test variance was ≤10% and the inter-test variance was ≤20%.

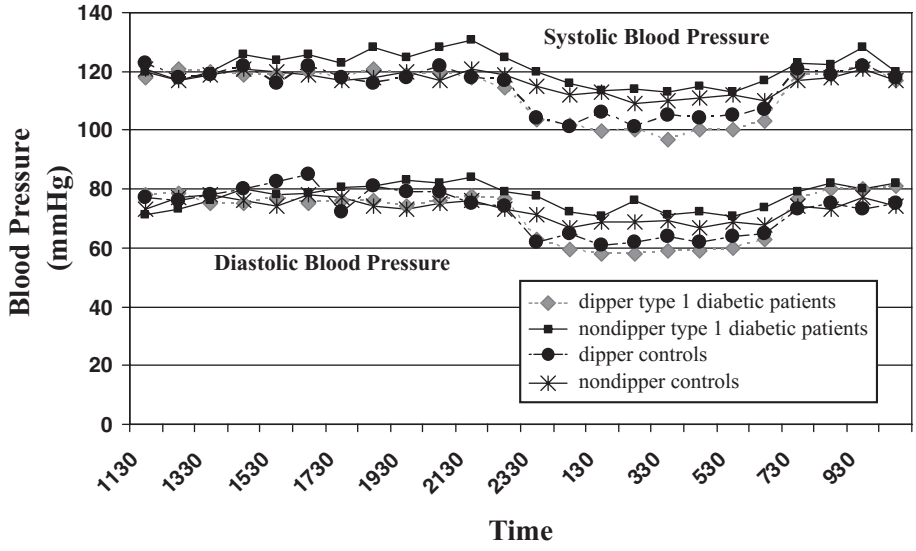

Fig. 1. *Twenty-four–hour systolic and diastolic blood pressure profiles of dipper and nondipper type 1 diabetic patients and controls. Nighttime systolic (nondipper DM vs. dipper DM, $p < 0.001$; nondipper DM vs. dipper controls, $p < 0.001$; nondipper controls vs. dipper controls, $p < 0.01$; nondipper controls vs. dipper DM, $p < 0.001$) and diastolic blood pressure values of nondipper diabetic subjects and nondipper controls (nondipper DM vs. dipper DM, $p < 0.001$; nondipper DM vs. dipper controls, $p < 0.001$; nondipper controls vs. dipper controls, $p < 0.05$; nondipper controls vs. dipper DM, $p < 0.001$) were increased compared with dipper diabetic patients and dipper controls.*

Other Measurements

Serum cholesterol and triglycerides were measured using enzymatic colorimetric assays (Roche Diagnostics, Mannheim, Germany). Albuminuria was measured in 24-h urine samples with the chemiluminescence method (expressed as the mean of three consecutive measurements) (Dade Behring, Newark, USA) (intraassay coefficient of variation [CV]: 4.3%; interassay CV: 4.4%). Serum and urine creatinine levels were assessed with a calorimetric kit (Boehringer Mannheim) using the Jaffe method and a Hitachi autoanalyzer.

Statistical Analysis

All calculations were performed using a commercially available program (INSTAT II) running on a personal computer. We used Tukey–Kramer ANOVA to assess normally distributed variables and Kruskall–Wallis ANOVA tests for nonnormally distributed variables. We analyzed the differences between the dipper and nondipper diabetic groups and the controls. Comparisons between two groups were made using the Student's *t*-test or the Mann–Whitney *U*-test as appropriate. Frequencies were compared with Fisher's exact test, and correlation analyses were calculated using the Pearson test. Stepwise linear regression analysis was used with FMD chosen as the dependent variable in the SPSS software package. All values are reported as mean±SD or as median (range). A two-tailed *p*-value of less than 0.05 was considered significant.

Results

The demographic and clinical characteristics of the subjects are listed in Table 1. There was no significant difference in age, gender and BMI values of dipper controls, nondipper controls, and dipper and nondipper type 1 diabetic patients. We observed similar HbA1c levels in dipper and nondipper diabetic groups; however, diabetic subjects had significantly increased HbA1c levels as compared with the controls. Diabetes durations were similar in dipper and nondipper diabetic groups (Table 1). None of the subjects had evidence of diabetic retinopathy or neuropathy except for the altered diurnal blood pressure rhythm in the nondipper type 1 diabetic patients. No subject had any signs or symptoms of vascular disease. The electrocardiographs of both patients and controls did not show any ischemic changes. Creatinine clearance and urinary albumin excretion rates were similar in all groups.

The blood pressure profiles of all subjects are summarized in Table 2. Although 24-h systolic and diastolic blood pressure levels of all subjects were in the normotensive range as defined in an earlier report (*26*), nondipper diabetic patients and nondipper controls had significantly increased nighttime systolic and diastolic blood pressure values as compared with dipper diabetic patients and dipper controls (Fig. 1). Twenty-four–hour diastolic and systolic blood pressure levels of nondipper diabetic patients were significantly higher than those of dipper diabetic patients and dipper controls. Diurnal systolic and diastolic blood pressure changes were significantly

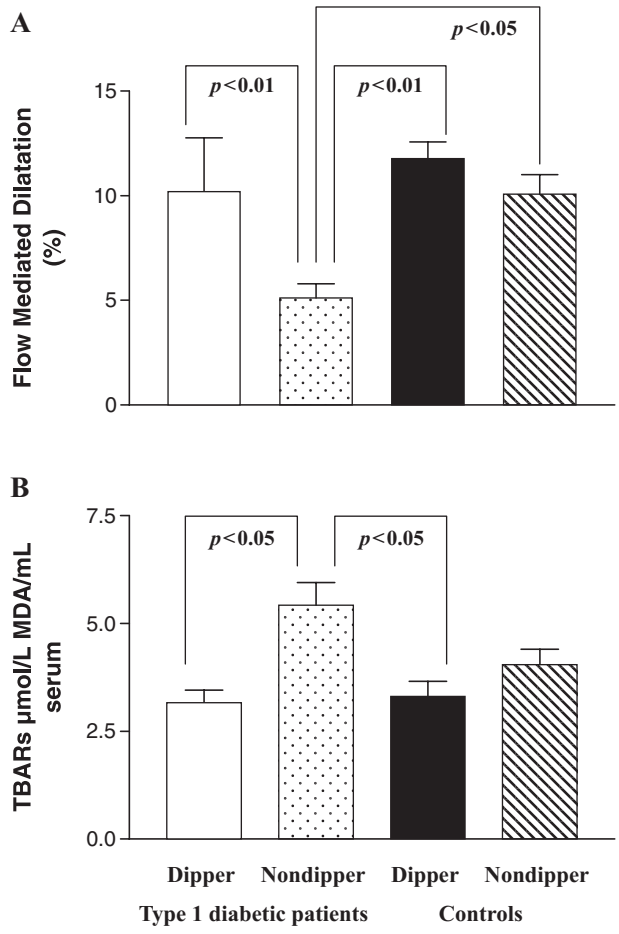

Fig. 2. *Measurements of A: flow mediated dilatations and B: TBARS (thiobarbituric acid reactive substance) levels of dipper and nondipper type 1 diabetic patients and controls.*

lower in the nondipper control and nondipper diabetic group when compared with dipper controls and the dipper diabetic group. Heart rates were higher in the nondipper diabetic group and nondipper controls, both during the day and at night, than in the dipper controls.

Total cholesterol, LDL, HDL and triglyceride levels of diabetic patients were comparable to controls (Table 1). Baseline diameters of the brachial artery were not different between dipper controls (3.4±0.3 mm), nondipper controls (3.2±0.3 mm), dipper (3.2±0.4 mm) and nondipper type 1 diabetic patients (3.5±0.3 mm). Similar increases in peak flow velocities were achieved after cuff deflation in dipper (102±31%) and nondipper diabetic patients (102±22%), and in dipper (101±38%) and nondipper controls (104±23%). However, values of FMD in nondipper diabetic patients were significantly lower than those in dipper diabetic patients, and the same was true for the dipper and nondipper controls (Fig. 2A). Sublingual administration of 0.4 mg GTN induced similar vasodilatation in dipper (16.1±6.9%) and nondipper diabetic patients (15.4±2.7%), as well as in dipper controls (16.6±4.3%) and nondipper controls (14.2±5.1%). TBARS levels in the dipper diabetic group (3.17±1.28) and dipper controls (3.31±1.71) were significantly lower than those in the nondipper diabetic group (5.43±1.93 µmol/L MDA/mL serum) ($p<0.05$) (Fig. 2B). TBARS levels in the nondipper controls (4.05±1.12 µmol/L MDA/mL serum) were not significantly different from those in other groups. Nitric oxide levels of the nondipper diabetic group (1.64±0.30 µmol/L), the dipper diabetic group (1.79±0.36 µmol/L), the dipper controls (1.89±0.27 µmol/L) and the nondipper controls (1.77±0.37 µmol/L) were similar.

We observed a significant correlation between FMD values and nighttime systolic blood pressure levels, nighttime diastolic blood pressure levels, and the values of diurnal systolic and diastolic blood pressure changes (Fig. 3). There were also significant correlations among TBARS levels and diurnal systolic ($r=-0.43$; $p<0.0005$) and diastolic ($r=-0.37$; $p<0.005$) blood pressure changes, nighttime systolic blood pressure levels ($r=0.35$; $p<0.005$) and nighttime diastolic blood pressure levels ($r=0.30$; $p<0.05$). A significant correlation among HbA1c levels and values of FMD ($r=-0.44$; $p<0.0005$) and TBARS levels ($r=0.26$; $p<0.05$) was also observed.

A linear regression model was created to determine the predictors of endothelial function measured in terms of FMD. LDL-cholesterol, HbA1c, TBARS, urinary albumin excretion rate, age, BMI and dipping status were included in the model. Stepwise regression analysis showed that dipping status and HbA1c levels were the only significant predictors of endothelial function ($p<0.001$ $r=0.579$, adjusted $r^2=0.259$).

Discussion

In this study, we evaluated normotensive, normoalbuminuric type 1 diabetic patients who had no apparent complications. In this distinctive cohort, type 1 diabetic patients exhibiting an altered diurnal blood pressure rhythm had endothelial dysfunction and increased oxidative stress as confirmed by impaired flow-mediated vasodilatation and by increased lipid peroxidation products, respectively.

Ambulatory blood pressure measurements in type 1 diabetic patients can reveal an altered circadian blood pressure rhythm that is characterized by a decrease in normal nocturnal drop of blood pressure. It has previously been demonstrated that this alteration is especially evident in micro- and macroalbuminuric patients and shows a correlation with the progression of diabetic nephropathy (*27*). Nighttime elevations in blood pressure have also been shown to be related to the development and progression of diabetic retinopathy in normotensive, normoalbuminuric type 1 diabetic patients (*28*, *29*). Our nondipper type 1 diabetic patients were all normotensive, although nighttime and 24-h systolic and diastolic blood pressure measurements in nondipper type 1 diabetic patients were higher than those in dipper diabetic patients and in dipper controls. None of the diabetic subjects had diabetic

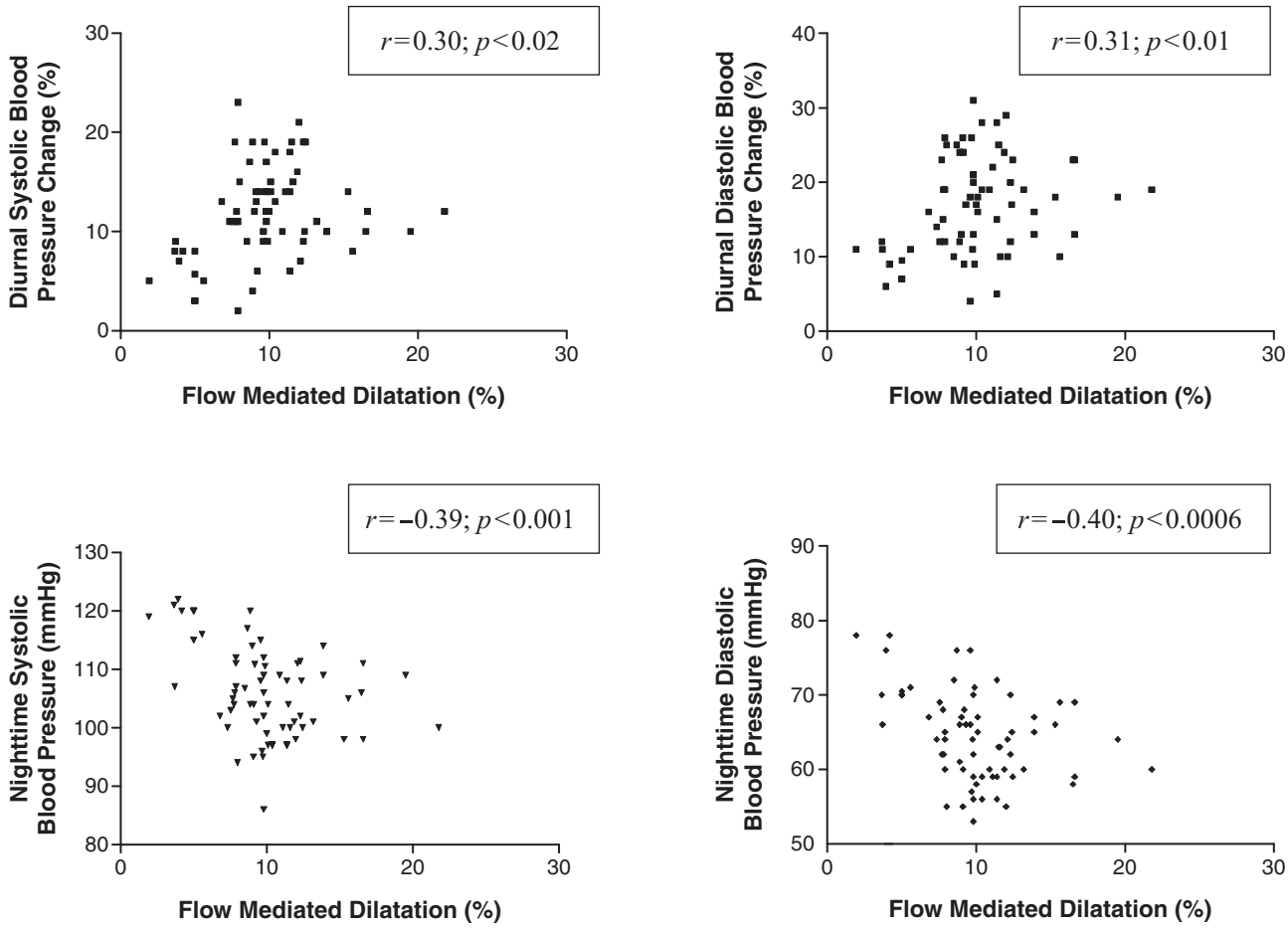

Fig. 3. *Correlations among measurements of flow-mediated dilatation and nighttime systolic blood pressure levels, nighttime diastolic blood pressure levels, and extent of diurnal systolic and diastolic blood pressure changes.*

complications, we did not find any significant differences in urinary albumin excretion rates, and the patients were all within the normoalbuminuric range. We observed similar HbA1c levels in dipper and nondipper diabetic groups. However, diabetic subjects had significantly increased HbA1c levels as compared with the controls.

Several mechanisms have been proposed for the observed nighttime elevations in blood pressure of type 1 diabetic patients. Autonomic neuropathy has been emphasized (*30*). Sympathetic overactivity has been suggested as a feature of the nighttime increase in blood pressure in these patients (*31*). Disturbances in parasympathetic function, which are seen in the early phase of diabetic autonomic neuropathy, are thought to be one of the causes of this sympathetic overactivity (*32*). In our study, we concluded that an increased heart rate during the day and night, found only in diabetic patients with an altered diurnal blood pressure rhythm, may indicate some subtle autonomic dysfunction in the nondipper diabetic group.

Patients with an altered circadian blood pressure pattern have been shown to suffer from higher mortality and morbidity for vascular events as compared with patients with normal rhythms (*7*, *33*, *34*). Attenuation of the nighttime fall in blood pressure has been shown to be associated with an increased risk of left ventricular hypertrophy, increased intima media thickness (*35*) and increased cardiovascular mortality (*36*).

Patients who exhibit coronary artery disease together with a nondipping pattern of diurnal blood pressure had higher levels of von Willebrand factor, D-dimer, fibrinogen, and soluble D-selectin, along with impaired flow-mediated vasodilation, as compared with patients who exhibited a dipping pattern (*37*). In essential hypertension, nondipping blood pressure profile has been shown to be associated with endothelial dysfunction (*38*, *39*).

Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis (*40*). Reduced production and bioavailability of nitric oxide as a result of oxidant stress have been implicated as a cause of impaired vasodilator capacity of the endothelium (*40*, *41*). NO is normally produced from L-arginine by endothelial nitric oxide synthase (NOS) in the vasculature (*20*). NO is considered to be a vasculoprotective molecule because it mediates endothelium-dependent vasorelaxation, displays antiproliferative properties, and inhibits

platelet and leukocyte adhesion to the vascular endothelium (*20*). If NOS lacks its L-arginine substrate or one of its cofactors, it may produce O_2^- instead of NO. This ion immediately reacts with NO, generating cytotoxic $ONOO^-$ (peroxynitrite) that alters the function of biomolecules by nitrating proteins and by causing lipid peroxidation. These changes also decrease NO bioavailability, thereby causing impaired relaxation and inhibition of the antiproliferative effects of NO and resulting in oxidation of tetrahydrobiopterin (an important cofactor for NOS). This in turn causes further uncoupling of NOS, leading to production of more atherogenic free radicals instead of NO (*20*). In our study, we did not observe statistically significant differences in measured NO levels, despite the fact that the nondipper diabetic group had the lowest NO levels. This may be due to our measurement of nitrate levels that reflected whole-body NO synthesis, not endothelial NOS alone. In this manner, our protocol limited the sensitivity of our assay to endothelial NOS. However, in the subjects we evaluated, values of FMD (a reflection of endothelial NOS bioactivity) were significantly decreased only in nondipper type 1 diabetic patients. Endothelial dysfunction was also observed in hypertensive patients (*42*). Hypertensive patients who exhibit a nondipping pattern have a greater impairment of endothelial function than patients who exhibit a dipping pattern. Higashi *et al*. evaluated the effects of diurnal blood pressure variation on forearm blood flow (FBF) response to intra-arterial acetylcholine infusion (an endothelium-dependent vasodilator) in hypertensive patients. Acetylcholine stimulates NO production, and daily NO production has been shown to be decreased in nondipper hypertensive patients (*39*). Increased oxidative stress was observed in diabetic patients and causes the generation of free radicals. This in turn contributed to the development of diabetic complications. Levels of a certain product of lipid peroxidation—TBARS—are an indirect measure of free-radical production that have been shown to be consistently elevated in diabetic patients who present microvascular and macrovascular complications (*43*). Hypertensive patients with diminished nocturnal blood pressure depression were found to have TBARS levels that were significantly higher than dippers (*44*). In this study, we also included a healthy nondipper normotensive group in order to clarify whether endothelial dysfunction is caused solely by a nondipping pattern and is unrelated to the presence of type 1 diabetes or, alternatively, whether the presence of type 1 diabetes tends to be accompanied by a nondipping blood pressure pattern. Of our population of normotensive type 1 diabetic patients, only patients who exhibited a nondipping pattern presented increased TBARS levels and suffered from impaired endothelial function as detected by Doppler ultrasound. Sublingual administration of GTN induced similar vasodilatation in the forearm in the four groups examined in our study. The latter result suggests that the function of arterial smooth muscle was not impaired in nondipper type 1 diabetic patients. Dipper diabetic patients had FMD values and TBARS levels similar to those of dipper and nondipper controls. Furthermore, stepwise regression analysis of our data showed that dipping status and HbA1c levels were the only significant predictors of endothelial function in our study population. All these findings indicate that there are subtle changes in the pathways leading to atherosclerosis even in uncomplicated normoalbuminuric normotensive type 1 diabetic patients. An altered diurnal blood pressure rhythm seems to be one of the main determinants of this process. Positive correlation between the values of FMD and the extent of diurnal systolic and diastolic BP change, and the negative correlation between the values of FMD and the nighttime systolic and diastolic BP levels support the hypothesis that increased blood pressure load observed in nondipper uncomplicated diabetic patients might be an early step in the pathway that ultimately leads to atherosclerosis. The findings of Perrin *et al*. provide further evidence for the importance of diurnal blood pressure change on development of diabetic glomerulopathy as evaluated using serial biopsies in 29 normoalbuminuric type 1 diabetic patients. They reported that 10 patients who developed microalbuminuria exhibited significant increases in their nighttime diastolic blood pressure and decreases in systolic and diastolic blood pressure dipping during 6 years of follow-up (*45*).

In conclusion, type 1 diabetic patients with a nondipping pattern of blood pressure fluctuation tend to exhibit endothelial dysfunction. Whether endothelial dysfunction is the reason for the increased cardiovascular mortality in these patients remains to be confirmed by large-scale studies. Restoring a normal circadian pattern of blood pressure may improve endothelial function and, in the long run, help prevent cardiovascular complications.

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