

Circadian Variation of Blood Pressure and Endothelial Function in Patients With Essential Hypertension: A Comparison of Dippers and Non-Dippers

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OBJECTIVES	The purpose of this study was to evaluate the relationship between the circadian blood pressure (BP) rhythm and endothelial function in patients with essential hypertension.
BACKGROUND	Hypertension is associated with alterations in resistance artery endothelial function. Patients with a non-dipper circadian pattern of BP have a greater risk of cerebrovascular and cardiovascular complications than do patients with a dipper circadian pattern.
METHODS	We evaluated the forearm blood flow (FBF) response to intra-arterial acetylcholine (ACh), an endothelium-dependent vasodilator, and isosorbide dinitrate (ISDN), an endothelium-independent vasodilator, infusion in 20 patients with non-dipper hypertension and 20 age- and gender-matched patients with dipper hypertension. The FBF was measured using a mercury-filled Silastic strain-gauge plethysmograph.
RESULTS	The 24-h systolic BP, as well as nocturnal systolic and diastolic BPs were higher in non-dipper patients than in dipper patients. The 24-h urinary excretion of nitrite/nitrate and cyclic guanosine monophosphate was lower in non-dippers than in dippers. The response of FBF to ACh was smaller in non-dippers than in dippers (25.1 ± 3.1 vs. 20.2 ± 3.0 ml/min/100 ml tissue, $p < 0.05$). The FBF response to ISDN was similar in dippers and non-dippers. The FBF response to ACh was similar in the two groups following intra-arterial infusion of the nitric oxide (NO) synthase inhibitor N ^G -monomethyl-L-arginine.
CONCLUSIONS	These findings suggest that endothelium-dependent vasodilation is blunted through a decrease in NO release in non-dippers compared with patients who have dipper hypertension. (J Am Coll Cardiol 2002;40:2039–43) © 2002 by the American College of Cardiology Foundation

Hypertension is associated with altered endothelial nitric oxide (NO) release. Several lines of evidence have shown that endothelium-dependent vasodilation evoked by NO release in brachial (1–3), coronary (4), renal (5), and small arteries (6) is impaired in patients with essential hypertension, which is a risk factor for cardiovascular and cerebrovascular disease. It has been postulated that endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis and plays an important role in maintaining hypertension.

Studies have shown that 24-h ambulatory blood pressure monitoring (ABPM) is a better predictor of subsequent complications than spot measurements of blood pressure (BP) (7,8). In addition, individuals with a non-dipper

circadian pattern of BP, in which the nocturnal decline in BP is diminished or absent, are at increased risk for cerebrovascular and cardiovascular complications than are individuals with a dipper circadian rhythm (9,10). However, little is known about the relationship between the circadian rhythm of BP, especially nocturnal BP, and endothelial function in patients with essential hypertension. We hypothesized that patients with the non-dipper pattern would have greater impairment of endothelial function than would patients with the dipper pattern.

To evaluate endothelial function in patients with dipper and non-dipper patterns of hypertension, we compared the endothelium-dependent vasodilation induced by intra-arterial acetylcholine (ACh) and endothelium-independent vasodilation induced by isosorbide dinitrate (ISDN) infusions in the presence and absence of the NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) in dippers and non-dippers.

METHODS

Subjects. One hundred eight inpatients with essential hypertension who were identified by office BP measurements

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Abbreviations and Acronyms

ABPM	= ambulatory blood pressure monitoring
ACh	= acetylcholine
ANOVA	= analysis of variance
BP	= blood pressure
FBF	= forearm blood flow
ISDN	= isosorbide dinitrate
L-NMMA	= N ^G -monomethyl-L-arginine
NE	= norepinephrine
NO	= nitric oxide
NOx	= nitrite/nitrate

underwent 24-h ABPM. From among patients demonstrating circadian BP variation, 20 non-dippers (13 men and 7 women; mean age 52.6 ± 9.7 years) and 20 dippers (13 men and 7 women; mean age 50.4 ± 8.9 years) matched for age, gender, body mass index, lipid profile, glucose metabolism, and smoking habit were enrolled in this study. Spot BP measurements were obtained in the Outpatient Clinic of Hiroshima University Faculty of Medicine. Hypertension was defined as an untreated systolic BP ≥ 140 mm Hg and/or a BP ≥ 90 mm Hg in the sitting position on at least three different occasions. Patients with secondary forms of hypertension were excluded by appropriate clinical and biochemical examinations. None of the patients had a history of cardiovascular or cerebrovascular disease, hypercholesterolemia, diabetes mellitus, liver disease, or renal disease. The study protocol was approved by the Ethics Committee of the Hiroshima University Faculty of Medicine. Informed consent for participation was obtained from all subjects.

24-h ABPM. Antihypertensive agents were withheld for at least two weeks prior to data collection, and subjects were placed on a regular diet that contained 170 mmol/day of NaCl one week prior to data collection to allow the systemic sodium balance and BP to stabilize. The dietary content of potassium (100 mmol/day) and calcium (40 mmol/day) was kept constant throughout the study. The caloric intake was 40 cal/kg daily. Meals were prepared in the Hiroshima University Hospital kitchen. Rigid compliance to the diet was confirmed by measuring the 24-h urinary excretion of sodium, chloride, and potassium throughout the study. The ABPM was performed from day 6 to day 7 of the dietary period using a TM2420 (AND Co., Tokyo, Japan) device, a noninvasive ambulatory BP monitor that was attached to the upper left arm. The BP was measured based on Korotkoff sounds during stepwise deflations (3.0 ± 1.0 mm Hg/step) of the cuff. The within-run precision of BP and heart rate measurements were ± 4.0 mm Hg and $\pm 5.0\%$, respectively. The reliability of this device had been previously confirmed (11). Both BP and heart rate measurements were obtained at 30-min intervals. A non-dipper pattern was defined as a difference in the mean BP of less than 10% between the daytime (6:00 AM to 9:00 PM) and nighttime hours (9:00 PM to 6:00 AM). Patients were confined to their

ward during the study and were maintained on a regular schedule; they were awakened at approximately 6:00 AM, and the room light was turned off at 9:00 PM. The 24-h urinary excretions of norepinephrine (NE), nitrite/nitrate (NOx), and cyclic guanosine monophosphate (cGMP) were determined during ABPM.

Endothelial function. Forearm vascular responses to ACh (Daiichi Pharmaceutical, Tokyo, Japan) and ISDN (Eisai Pharmaceutical, Tokyo, Japan) were evaluated from day 7 of dietary period after detachment of ABPM device in all subjects. The study began at 8:30 AM. Subjects fasted the previous night for at least 12 h. They were kept in the supine position in a quiet, dark, air-conditioned room (temperature 22°C to 25°C) throughout the study. A 23-gauge polyethylene catheter (Hakkow, Okayama, Japan) was inserted into the left brachial artery for the infusion of ACh and ISDN and for the recording of arterial pressure with an AP-641G pressure transducer (Nihon Kohden, Tokyo, Japan) under local anesthesia (1% lidocaine). Another catheter was inserted into the left deep antecubital vein to obtain blood samples.

After the patients were placed for 30 min in the supine position, we measured forearm blood flow (FBF) and arterial BP. Then, the effects of the ACh and ISDN on forearm hemodynamics were measured. Both ACh (7.5, 15, and 30 $\mu\text{g}/\text{min}$) and ISDN (0.75, 1.5, and 3.0 $\mu\text{g}/\text{min}$) were infused intra-arterially for 5 min at each dose using a constant rate infusion pump (Terfusion STG-523, Termo, Tokyo, Japan). The FBF was measured during the last 2 min of the infusion. The infusions of ACh and ISDN were carried out in a random order. Each study proceeded after the FBF had returned to baseline.

Furthermore, after a 30-min rest period, an NO synthase inhibitor, L-NMMA, was infused intra-arterially at a dose of 8 $\mu\text{mol}/\text{min}$ for 5 min while the basal FBF and arterial BP were recorded, and ACh (7.5, 15, and 30 $\mu\text{g}/\text{min}$) was administered.

Measurement of FBF. The FBF was measured using a mercury-filled Silastic strain-gauge plethysmograph (EC-5R, D.E. Hokanson, Bellevue, Washington) as previously described (2,3). Forearm vascular resistance was calculated as the mean arterial pressure divided by FBF. The FBF was calculated by two independent observers blinded to the study protocol from the linear portions of plethysmographic recordings. The intraobserver coefficient of variation was $3.0 \pm 2.1\%$. We confirmed the reproducibility of FBF responses to ACh and ISDN on two separate occasions in 10 healthy male subjects (mean age 24 ± 4 years). The coefficients of variation were $6.2 \pm 3.8\%$ and $4.6 \pm 2.9\%$, respectively.

Analytical methods. Routine chemical methods were used to determine serum concentrations of total cholesterol, creatinine, glucose and electrolytes, and urinary electrolytes. Plasma renin activity and plasma concentration of angiotensin II were assayed by radioimmunoassay. Plasma and urinary concentrations of NE were measured by high-

Table 1. Clinical Characteristics of Dipper and Non-Dipper Patients

Parameter	Dipper (n = 20)	Non-Dipper (n = 20)
Body mass index (kg/m ²)	23.1 ± 2.3	23.2 ± 2.1
Heart rate (beats/min)	62.5 ± 8.9	61.0 ± 9.3
Total cholesterol (mmol/l)	4.4 ± 0.2	4.3 ± 0.2
Serum glucose (mmol/l)	5.0 ± 0.1	4.9 ± 0.1
Plasma renin activity (ng AngI/ml/h)	0.81 ± 0.36	1.19 ± 0.22
Plasma angiotensin II (pg/ml)	17.4 ± 9.8	18.6 ± 9.1
Plasma norepinephrine (nmol/l)	1.01 ± 0.25	1.06 ± 0.28
Urinary norepinephrine (μg/d)	83 ± 25	96 ± 31
Urinary sodium (mmol/d)	163 ± 39	165 ± 42
24-h ABPM		
24-h SBP (mm Hg)	133.9 ± 12.1*	148.4 ± 12.5
24-h DBP (mm Hg)	87.6 ± 8.4	91.6 ± 8.6
Daytime SBP (mm Hg)	145.6 ± 11.9	149.7 ± 12.1
Daytime DBP (mm Hg)	91.6 ± 7.8	92.7 ± 8.7
Nighttime SBP (mm Hg)	127.2 ± 10.3*	144.8 ± 12.3
Nighttime DBP (mm Hg)	75.4 ± 8.2*	89.3 ± 8.3
FBF (ml/min/100 ml tissue)	4.4 ± 1.1	4.3 ± 1.0
Smoker (number of subjects)	6	5

All results are presented as mean ± SD. *p < 0.05 versus nondipper. ABPM = ambulatory blood pressure monitoring; DBP = diastolic blood pressure; FBF = forearm blood flow; SBP = systolic blood pressure.

performance liquid chromatography. Plasma and urinary concentrations of cGMP were measured by radioimmunoassay using a cGMP kit (Yamasa Shoyu, Choshi, Japan). Plasma and urinary concentrations of NOx were assayed by colorimetric methods using NOx assay kits (Cayman Chemical, Ann Arbor, Michigan).

Statistical analysis. Results are presented as the mean ± SD. Values of p < 0.05 were considered statistically significant. Comparisons of variables were carried out by the one-way analysis of variance (ANOVA) followed by the Bonferroni correction. Comparisons of time-course curves of parameters during the infusions of ACh and ISDN were analyzed by two-way ANOVA for repeated measures on one factor followed by the Bonferroni correction for multiple-paired comparisons. The data were processed using the software packages StatView IV (SAS Institute, Cary, North Carolina) or Super ANOVA (Abacus Concepts, Berkeley, California).

RESULTS

Clinical characteristics of dippers and nondippers. The baseline clinical characteristics of the 20 patients with the dipper pattern and the 20 patients with the non-dipper pattern are summarized in Table 1. Both the 24-h systolic BP and the nocturnal systolic and diastolic BPs were higher in non-dippers than in dippers. The other parameters were similar between the two groups.

Endothelial function in dippers and nondippers. The intra-arterial infusion of ACh increased FBF in a dose-dependent manner in both groups. The response of FBF to

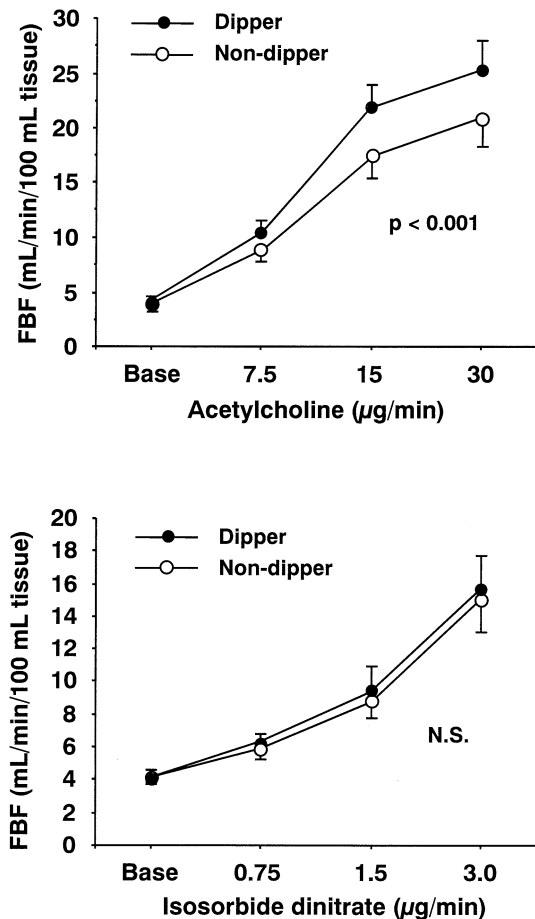


Figure 1. Line graphs show the forearm blood flow (FBF) response to intra-arterial acetylcholine (top panel) and isosorbide dinitrate (bottom panel) infusion in patients with dipper and non-dipper essential hypertension.

ACh was smaller in non-dipper patients than in dipper patients (p < 0.001; Fig. 1, top panel). The intra-arterial infusion of ISDN also increased FBF in a dose-dependent manner in both groups. The FBF response to ISDN was similar in dippers and non-dippers (Fig. 1, bottom panel). No change in the arterial BP or heart rate occurred with the intra-arterial infusion of either ACh or ISDN in either group.

The 24-h urinary excretions of NOx and cGMP were lower in nondipper patients than in dipper patients (Fig. 2). **Effects of L-NMMA on the forearm vascular response to ACh infusion.** The intra-arterial infusion of the NO synthase inhibitor L-NMMA decreased basal FBF in both groups. Changes in the basal forearm vascular responses to L-NMMA infusion were similar in the two groups. The L-NMMA infusion also decreased the FBF response to ACh infusion in both groups. There was no significant difference in the change in the FBF response to ACh after L-NMMA infusion between the two groups (Fig. 3). No change in arterial BP or heart rate occurred during the infusion of L-NMMA in either group.

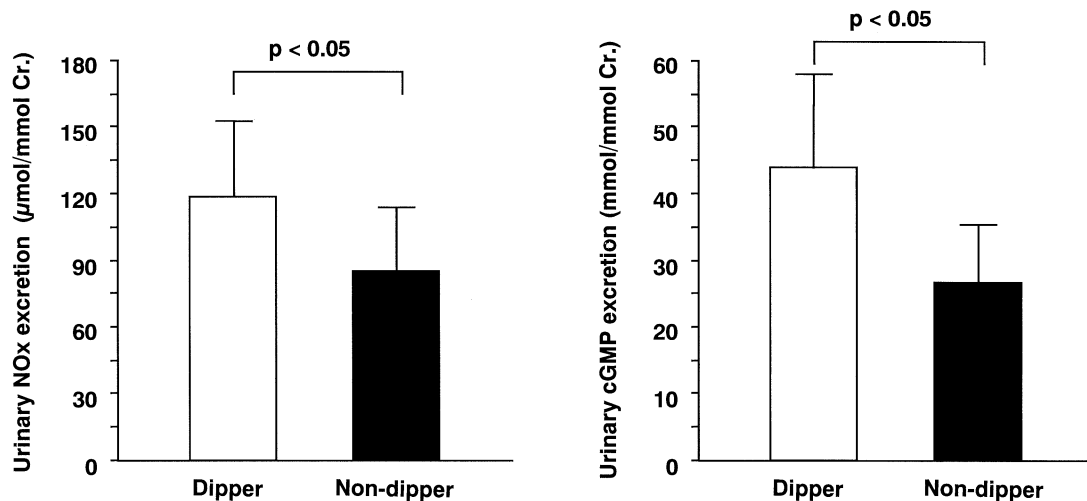


Figure 2. Bar graphs show the 24-h urinary excretion of nitrite/nitrate (NOx) and cGMP in patients with dipper and non-dipper essential hypertension.

DISCUSSION

The forearm vascular response to ACh, but not ISDN, infusion is impaired in non-dippers, indicating that endothelium-dependent vasodilation of the brachial artery is selectively impaired in non-dipper hypertension. Intra-arterial infusion of the NO synthase inhibitor L-NMMA abolished the greater FBF response to ACh in dippers. In addition, the 24-h urinary excretion of NOx and cGMP, indices of NO production, was less in non-dippers than in dippers. These findings suggest that endothelium-dependent vasodilation induced by ACh, ACh-stimulated NO production, and daily NO production are blunted in non-dipper hypertension.

Endothelial function becomes progressively more impaired as BP increases (12,13). In the present study, the nocturnal BP was higher in non-dippers than in dippers, whereas the daytime BP was similar in the two groups. Although BP was similar in dippers and nondippers during

measurement of the FBF response (8:30 AM to 12:00 PM), an inappropriate reduction in nocturnal BP may contribute to impaired endothelium-dependent vasodilation in non-dippers.

Although the precise mechanisms by which shear stress stimulates NO release from vascular endothelial cells are not fully known, the endothelium is sensitive to shear stress and can respond to changes in shear stress associated with small increases in fluid viscosity (14). Recently, Lip et al. (15) demonstrated that plasma viscosity correlates both with the mean daytime and mean nocturnal systolic BPs, suggesting that changes in BP may affect the plasma viscosity. Therefore, the diminished nocturnal decline in BP in non-dippers seen in the present study may be linked to increased nocturnal plasma viscosity, resulting in decreased shear stress and NO production.

A potent vasoconstrictor, NE attenuates endothelium-dependent vasodilation (16). Previous studies have shown that abnormalities in autonomic function, especially in the sympathetic nervous activity, inhibit the nocturnal decline in BP (17,18). However, both plasma and urinary NE concentrations were similar in dippers and nondippers. Therefore, it is unlikely that differences in the FBF response to ACh between dippers and non-dippers can be explained by differences in sympathetic activity.

Clinical implications. Perticone et al. (19) reported that when patients with essential hypertension were divided into three groups based on the magnitude of the FBF response to ACh infusion, the lower response group (poor endothelial function) had a higher prevalence of cardiovascular events during a mean follow-up of 31.5 months (range 4 to 84 months). Several investigators have demonstrated that an abnormal decline in nocturnal BP affects the prognosis for cardiovascular disease or stroke in hypertensive patients (9,10). Endothelial dysfunction, in addition to initiating atherosclerosis, contributes to its progression. Thus, it is likely that impaired endothelial function contributes to the

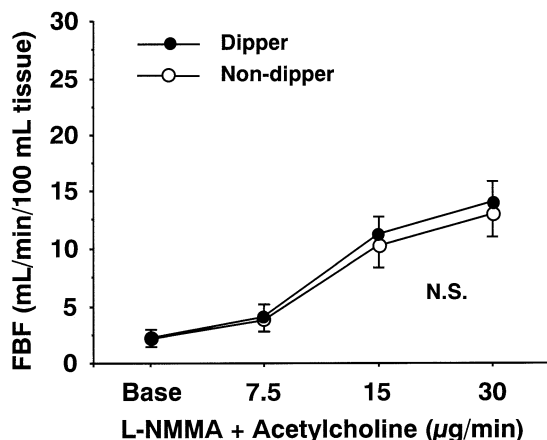


Figure 3. Line graphs show the forearm blood flow (FBF) response to intra-arterial acetylcholine infusion in the presence of the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) in patients with dipper and non-dipper essential hypertension.

higher prevalence of cardiovascular and cerebrovascular complications in non-dippers. Hypertensive patients also have endothelial dysfunction compared to normotensive subjects (1-6) and a worse prognosis (8-11). Treatment of hypertension has been associated with an improvement in outcome. The non-dippers probably represent a worse course of hypertension with more endothelial dysfunction. The aim of antihypertensive therapy is ultimately to prevent cardiovascular and cerebrovascular complications and renal dysfunction. Therefore, it is clinically important to individualize treatment, including both pharmacologic and non-pharmacologic therapy, to improve endothelial function and restore a normal circadian pattern of BP in patients with essential hypertension. Treatment of non-dippers to render them dippers could partially normalize their endothelial function and partially improve their prognosis.

We wish to emphasize that sodium intake should be regulated when assessing diurnal BP variation, because changes in sodium intake affect the nocturnal BP in hypertensive patients, particularly salt-sensitive hypertensive patients. In the present study, we maintained a constant sodium intake (170 mmol/day) for one week before data collection. We previously confirmed that this amount of sodium does not affect BP, even in patients with salt-sensitive essential hypertension (20).

Study limitations. In the present study, endothelium-dependent vasodilation induced by intra-arterial ACh infusion was impaired even during the day in non-dippers. Measuring the FBF response to ACh when the BP is lowest (2:00 AM to 4:00 AM) may permit more specific conclusions to be drawn about the relationship between endothelial function and the circadian rhythm of BP. However, the ACh infusion study cannot be performed when patients are sleeping.

In conclusion, endothelium-dependent vasodilation induced by ACh is impaired in non-dippers to a greater extent than dippers. Additionally, NO production per se is reduced in non-dippers.

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