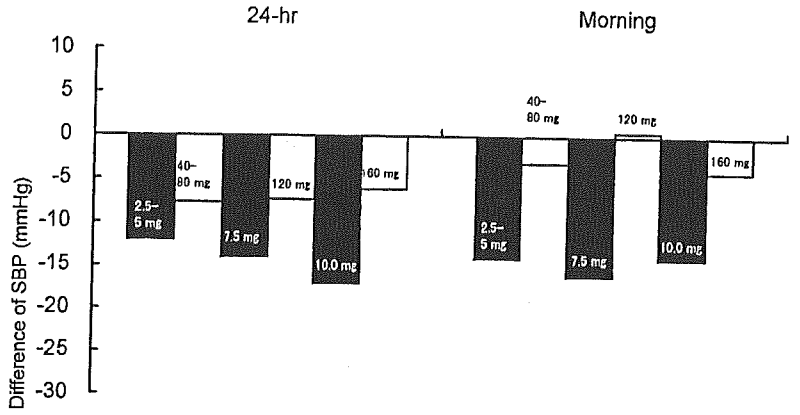


**FIG. 2.** Effects of amlodipine and valsartan on 24-hour and morning systolic blood pressure (SBP) by each dose. **Black bars,** amlodipine group; dose **(from left to right):** 2.5 to 5 mg ($n$ = 21), 7.5 mg ($n$ = 8), 10.0 mg ($n$ = 9). **White bars,** valsartan group; dose **(from left to right):** 40 to 80 mg ($n$ = 12), 120 mg ($n$ = 7), and 160 mg ($n$ = 19).

greater for amlodipine than for valsartan (both $P < .01$; Fig. 2).

To study the effect of age on the BP lowering effect, we separated the study patients into a younger age group (<65 years) and older group (≥65 years). The BP lowering characteristics of valsartan and amlodipine were essentially the same in each age group. In addition, there were no gender differences in the BP lowering effect of the two drugs.

To study the BP lowering characteristics of BP in relation to baseline BP, we graphed the scatter plot of the baseline and the reduction of 24-h BP for each group (Fig. 3). A baseline BP-dependent BP reduction was found in the amlodipine group but was not found in the valsartan group for 24-h and morning SBP.

## Discussion

In this study, a once-daily morning dose of amlodipine significantly reduced morning BP and morning BP surge. In comparison, a once-daily morning dose of the long-acting ARB valsartan did not significantly reduce morning BP and morning BP surge in hypertensive patients, and its BP lowering effect on 24-h ambulatory BP was weaker and more heterogeneous independent of baseline BP level than was amlodipine. This difference was significant independent of the dose of each drug.

Twenty-four-hour BP (mean of BP throughout a 24-h period) is the most important predictor of hypertensive target organ damage (brain, heart, and kidney) and subsequent fatal and nonfatal cardiovascular events (stroke and coronary artery disease). In a recent prospective study on treated hypertensive patients, 24-h BP was an independent predictor of cardiovascular events independent of clinic BP.[12] A significant BP lowering effect as indicated by 24-h BP and morning BP is well established from our previous study in hypertensive patients.[13] In one recent study using ABPM, the BP lowering effect of valsartan 80 mg/day was comparable to that of amlodipine 5 mg in hypertensive patients.[10] However, in our study, the mean doses were 6.4 mg/day for amlodipine and 124 mg/day for valsartan, and the BP lowering effect was clearly less effective in the valsartan group than in the amlodipine group. Ethnic char-

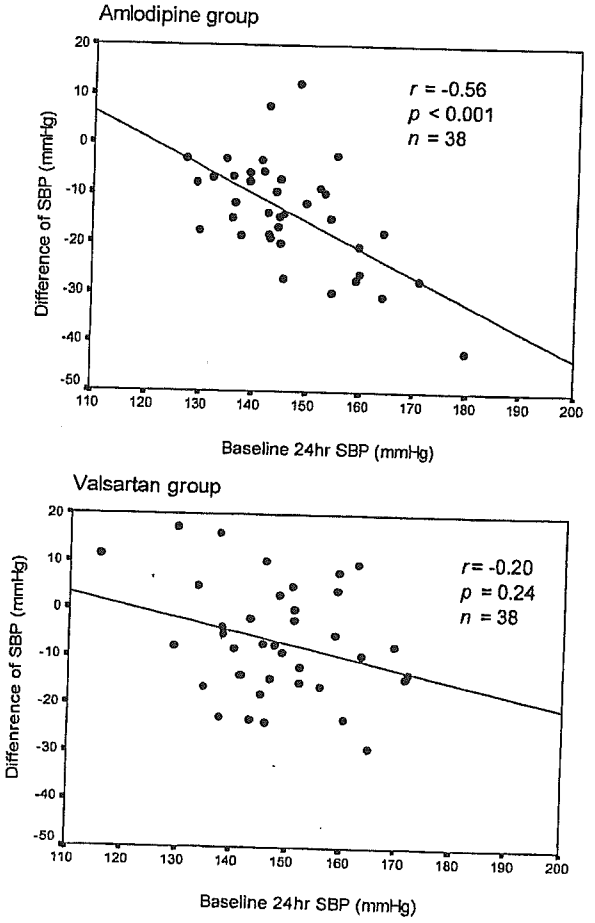


**FIG. 3.** Effects of amlodipine and valsartan on 24-h and morning systolic blood pressure (SBP) by each dose. **Solid lines** indicate linear regression lines.

acteristics or differences in salt intake between Asian (ie, Japanese) and white patients and may partly account for these inconsistent results. In Japan, stroke is much more common than coronary artery disease as compared the incidence in Western countries,[5] and the benefit of BP reduction for stroke is greater than that for coronary artery disease. In fact, as shown in the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT), relatively lower BP lowering effect of an angiotensin-converting enzyme (ACE) inhibitor was associated with increased cardiovascular risk, particularly for stroke, when compared with amlodipine.[14] In addition, the protective effect of RAAS blockade on hypertensive target organs seems limited under the BP control and is insufficient. Although a beneficial effect of valsartan in addition to ACE inhibitors on the prognosis of congestive heart failure patients whose BP is only moderately increased has been clearly demonstrated,[15] the insufficient BP lowering effect of valsartan monotherapy seems to be less beneficial than amlodipine for preventing cardiovascular events in hypertensive patients.

Recently we showed that the morning BP surge was significantly associated with clinical stroke risk in hypertensive patients.[9] This association was independent of age, 24-h BP level, and silent cerebral infarcts,[9] which are powerful predictors of clinical stroke events.[5] In addition to morning BP surge, morning BP level is also an important predictor of stroke events in hypertensive patients.[9] Because it has recently been demonstrated that, in addition to circulating factors, tissue RAAS of the cardiovascular system exhibits diurnal variation,[16] possibly in relation to a clock gene,[17] it was unexpected that valsartan did not significantly reduce morning BP level. As both valsartan and amlodipine reduced the lowest night BP to a similar degree, the morning BP surge was slightly increased in the valsartan group. This may be due to the shorter half-life of valsartan than that of amlodipine, particularly in nonresponders.

This lower BP reduction may not be generalized to all ARBs, such as telmisartan and irbesartan, given that the BP lowering effect of valsartan is weaker than that of these drugs.[18,19] However, even in the case of valsartan, when used in combination with diuretics, the BP lowering effects would be increased.[20] In the following large clinical trials in which diuretics have been permitted, benefits of RAAS blockers have been clearly demonstrated. The Perindopril pROtection aGainst Recurrent Stroke Study (PROGRESS) has demonstrated that stroke prognosis has been demonstrated only when the long-acting ACE inhibitor perindopril is used with diuretics in stroke survivors.[21] The Losartan Intervention For Endpoint reduction in hypertension (LIFE) trial has also clearly demonstrated that the benefit of ARB losartan was greater than that of β-blockers in high-risk hypertensive patients with left ventricular hypertrophy.[22] In our study, BP reduction is dependent on baseline BP level in the amlodipine group, whereas this BP response is heterogeneous in the valsartan group (as depicted in Fig. 3), indicating that there are responders and nonresponders for RAAS blockers. With regard to nonresponders, combination therapy with diuretics would be initiated or other classes of antihypertensive medication used.

In conclusion, amlodipine monotherapy controlled ambulatory BP throughout a 24-h period including the morning hours, whereas valsartan monotherapy was limited to controlling ambulatory BP, particularly morning BP, in hypertensive patients. Combination therapy with diuretics or other classes of antihypertensive medications would be necessary to achieve target BP levels.

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*Original Article*

# Greater Change of Orthostatic Blood Pressure Is Related to Silent Cerebral Infarct and Cardiac Overload in Hypertensive Subjects

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**Greater change of postural blood pressure (BP) is often seen in elderly hypertensives and is recognized as a risk factor for cognitive decline and poorer cerebrovascular outcome, but its clinical significance still remains to be clarified. We performed a head-up tilting test, ambulatory BP monitoring, and brain MRI in 59 hypertensives and 27 normotensive subjects. We measured plasma atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) levels at rest to assess cardiac burden. The 59 hypertensive patients were classified into 3 groups: an orthostatic hypertension (OHT) group with orthostatic increase in systolic BP (SBP) $\geq$10 mmHg ($n$=16); an orthostatic hypotension (OHYPO) group with orthostatic SBP decrease $\leq -10$ mmHg ($n$=18); and an orthostatic normotension (ONT) group with neither of these two patterns ($n$=25). A group of 27 normotensive subjects (NT) was also included as a control. Plasma BNP (72±92 *vs.* 29±24 pg/ml, $p<0.05$) and BNP/ANP ratio (4.6±3.3 *vs.* 2.4±1.5, $p<0.05$) were significantly higher in the OHYPO than in the NT group. The BNP/ANP ratio was also higher in the OHT than in the NT group (5.1±3.9 *vs.* 2.4±1.5, $p<0.01$). The number of silent cerebral infarct (SCI), prevalence of SCI and number of multiple SCIs was the highest in the OHT group, followed in order by the OHYPO, ONT and NT groups. Blood pressure and left ventricular mass index were not significantly different among the 3 hypertensive groups. In conclusion, hypertensive patients with greater change of postural BP (OHT and OHYPO) were shown to have increased risk of advanced silent brain lesions and greater cardiac burden. (*Hypertens Res* 2004; 27: 235–241)**

***Key Words*: orthostatic hypertension, orthostatic hypotension, silent cerebral infarcts, brain natriuretic peptide**

## Introduction

In most hypertensive subjects, blood pressure (BP) shows minimal variation from a supine to standing position due to an autoregulatory mechanism, even in elderly individuals. Orthostatic hypotension (OHYPO) is often found in older hypertensives with autonomic nervous dysfunction, and has been shown to contribute to dizziness, falls, syncope, and coronary heart disease (*1*, *2*). On the other hand, there have been only a few reports on orthostatic hypertension (OHT), defined as a greater BP increase from a supine to standing position. OHT is reported to involve abnormal circadian BP rhythm (*3*, *4*), to be associated with coronary heart disease (*2*) and silent cerebrovascular disease (*5*, *6*), and to result in impaired neurobehavioral function. However, the clinical significance of OHT and OHYPO in hypertensive patients has not been fully investigated in routine clinical practice.

In the present study, therefore, we performed a head-up tilting test (HUT) in 59 older hypertensive patients and 27 normotensive subjects, and investigated the relationship between orthostatic BP change and cardiac or cerebrovascular load.

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## Methods

### Patients

We studied a total of 86 outpatients (mean age, 67.6 years; range, 48–86 years), 59 of whom were hypertensive and 27 of whom were normotensive. This study was performed from December 1996 to May 1997 in Saga Prefecture, Japan. Hypertensive patients were consecutively selected according to the following criteria: 1) diagnosis of essential hypertension with an average clinic systolic BP (SBP) of ≥140 mmHg and/or average clinic diastolic BP (DBP) of ≥90 mmHg (average for each patient on two or more occasions); and 2) age of 40 years or greater. No patient had taken any antihypertensive medication for at least 14 days before the HUT and ambulatory BP monitoring (ABPM) study. We did not include subjects with possible diabetes mellitus (fasting glucose >110 mg/dl and/or hemoglobin A1c >6.2%), renal failure, hepatic damage, secondary or malignant hypertension, ischemic heart disease or other cardiac disease, congestive heart failure, arrhythmias including atrial fibrillation and other arrhythmias, stroke (including transient ischemic attacks), or other severe concomitant disease. Written informed consent was obtained from all subjects. This study was approved by the Research Ethics Committee, Department of Cardiology, Jichi Medical School, Japan.

### Study Protocol

After BP and pulse rate (PR) were measured with an interval of 1 min at baseline and after the subjects had been in a supine position for 10 min, they were positioned upright on a tilt table at an angle of 70° for 15 min. Two patients who developed presyncope during HUT were not included in this study.

For all patients, orthostatic BP and PR change were calculated as follows: the average value over the 2 to 9 min during tilting (8 points) minus the average in the supine position during the 1 to 5 min just before the tilting (5 points). We classified hypertensive patients into 3 groups, an OHT group with orthostatic SBP increase ≥10 mmHg ($n=16$), an OHYPO group with orthostatic SBP decrease ≤−10 mmHg ($n=18$), and an orthostatic normotension (ONT) group with neither of these two patterns ($n=25$). A group of 27 normotensives (NT group) was also included as a control.

In our previous study, the correlation coefficients of the orthostatic BP increases in the first HUT and second HUT were 0.61 for SBP and 0.47 for DBP (both $p<0.001$) in the 55 hypertensives (*6*). Of the 13 OHT patients with an orthostatic SBP increase of >20 mmHg in the first HUT, 10 (77%) had an orthostatic SBP increase of >10 mmHg. Therefore, the reproducibility of the HUT of our protocol was relatively good. However, the reproducibility of the BP change remains to be established in a future study.

### Measurement of BP and Heart Rate

Clinic BP was measured after resting for at least 5 min in the sitting position by a single physician with a standard mercury sphygmomanometer at each clinical visit. An automatic ABPM with electric-powered cuff inflation (TM2421; A&D, Tokyo, Japan), which recorded BP and PR every 30 min for 24 h, was used. This device was attached just after the tilting test. The ambulatory BP data were obtained using the oscillometric method. Each subject recorded his or her own daily activities.

### Brain MRI

Brain MRI was carried out in 41 of the 59 hypertensives (69%) and in 13 of the 27 normotensives (48%) using a superconducting magnet with a main strength of 0.5 T (MRT50GP, Toshiba, Tokyo, Japan) within 3 months of their HUT and ABPM. We performed brain MRI for those who agreed. The brain was imaged in the axial plane at a 7-mm slice thickness. $T_1$-weighted images were obtained using a short spin-echo pulse sequence with a repetition time of 470 ms and an echo time of 15 ms. $T_2$-weighted images were obtained using a long spin-echo pulse sequence with a repetition time of 4,000 ms and echo time of 120 ms. The matrix size was 256×256 pixels. A silent cerebral infarct (SCI) was defined exclusively as a low signal intensity area (≥3 mm, but all were <15 mm in size) on $T_1$-weighted images that was also visible as a hyperintense lesion on $T_2$-weighted images. The MRI images of the subjects were randomly stored and interpreted by reviewers blind to the subjects' names and characteristics. The interclass (non-SCI=0, one SCI=1, multiple SCIs=2) $\kappa$ statistics were 0.70 and 0.80 for intereader and intrareader, respectively, in our laboratory (*6*). Patients with 3 or more SCIs were considered to have multiple SCIs. Periventricular hyperintensity (PVH) was considered present if visible as a hyperintense area on proton-density and $T_2$-weighted images, without prominent hypointensity in $T_1$-weighted scans. Advanced PVH was defined as a PVH grade ≥3, as described previously (*7*).

### Neurohumoral Factors

Blood collection was performed after 10 min in the supine position just before the tilting, and after 15 min of tilting in all 86 subjects. Blood samples were immediately centrifuged at 3,000 rpm for 15 min, and plasma was decanted and stored at −80 °C until analysis. Catecholamines were measured with high-pressure liquid chromatography (Hitachi, Tokyo, Japan). Antidiuretic hormone (ADH) levels were determined using a competitive radioimmunoassay (Mitsubishi Kagaku, Tokyo, Japan). The N-terminal component of proatrial natriuretic peptide (NT-ANP) and brain natriuretic peptide (BNP) were measured from unextracted plasma using highly sensitive, noncompetitive immunoradiometric assays (Shiono-

**Table 1. Baseline Characteristics of the Patients Studied**

| | Hypertension group | | | NT group ($n$=27) |
|---|---|---|---|---|
| | OHT ($n$=16) | ONT ($n$=25) | OHYPO ($n$=18) | |
| Gender male (%) | 6 | 32 | 33 | 18 |
| Age (years) | 69±8.6 | 66±8.4 | 71±7.0 | 66±8.6 |
| BMI (kg/m$^2$) | 25.6±3.5** | 23.7±3.2 | 23.5±4.4 | 22.4±2.4 |
| Smoker (%) | 6.2 | 24.7 | 31.6 | 9.0 |
| Total cholesterol (mmol/l) | 4.9±1.0 | 5.4±0.8 | 5.1±0.8 | 4.9±0.7 |
| Hematocrit (%) | 39.2±3.3 | 40.1±3.3 | 40.4±3.7 | 39.0±3.5 |
| Serum creatinine (mg/dl) | 0.79±0.09 | 0.77±0.12 | 0.84±0.16 | 0.79±0.16 |
| LVIDd (mm) | 46±4.2 | 47±6.2 | 48±5.6 | ND |
| IVS (mm) | 9.5±2.1 | 9.3±1.5 | 10±2.1 | ND |
| PWT (mm) | 9.7±1.3 | 9.7±1.3 | 10±2.1 | ND |
| LV mass index (g/m$^2$) | 115±33 | 118±35 | 135±26 | ND |
| LV ejection fraction (%) | 76±4.9 | 75±4.7 | 76±5.2 | ND |
| Clinical SBP (mmHg) | 177±15** | 170±11** | 173±9.4** | 131±12 |
| Clinical DBP (mmHg) | 95±11** | 94±13** | 92±11** | 80±8.3 |
| Clinical PR (beats/min) | 69±9.8 | 72±15* | 70±16 | 64±10 |
| 24-h SBP (mmHg) | 149±14** | 145±14** | 150±12** | 117±9.8 |
| 24-h DBP (mmHg) | 83±6.7** | 85±9.0** | 82±7.7** | 69±7.8 |
| 24-h PR (mmHg) | 65±5.9 | 65±6.3 | 64±10.4 | 65±7.4 |
| SD of daytime SBP (mmHg) | 20±7 | 18±5 | 17±5 | 17±5 |
| SBP before tilting (mmHg) | 141±15**‡ | 145±16** | 154±13**‡ | 123±12 |
| SBP after tilting (mmHg) | 155±16**† | 144±14** | 133±15*† | 123±14 |
| DBP before tilting (mmHg) | 80±9.3† | 87±8.9** | 83±7.5** | 75±9.3 |
| DBP after tilting (mmHg) | 89±9.6**‡ | 91±10** | 78±7.0† | 79±10 |
| SBP change by tilting (mmHg) | 14±5.7**† | −1.0±5.3 | −21±9.4**† | −0.5±6.9 |
| PR change by tilting (beats/min) | 7.6±7.8 | 7.6±7.7 | 5.2±6.1 | 6.4±4.4 |

OHT, orthostatic hypertension; ONT, orthostatic normotension; LV, left ventricular; OHYPO, orthostatic hypotension; NT group, normotensive control group; BMI, body mass index; LVIDd, LV internal dimension at end-diastole; IVS, interventricular septal thickness; PWT, posterior wall thickness; SBP, systolic blood pressure; DBP, diastolic blood pressure; PR, pulse rate; ND, no data. *$p$<0.05, **$p$<0.01 *vs.* NT group; †$p$<0.05, ††$p$<0.01 *vs.* ONT group ; ‡$p$<0.01 *vs.* OHYPO group.

RIA; Shionogi Inc., Osaka, Japan). All these assays were performed at the Special Reference Laboratory (Tokyo, Japan).

### Other Measurements

The body mass index (BMI) was calculated as weight (kg)/height$^2$ (m$^2$). The left ventricular (LV) wall thickness and LV end diastolic and systolic diameters were measured with an ultrasonic echocardiograph (SSD 500CV, ALOKA, Tokyo, Japan). LV mass index (LVMI) was calculated using the formula introduced by Devereux (*8*).

### Statistical Analysis

All statistical analyses were carried out using Stat View software, version 5.0 (SAS Institute Inc., Cary, USA). The $\chi^2$ test was used to calculate proportions. One-way analysis of variance (ANOVA) was performed to detect differences in mean values among groups. After ANOVA, Fisher's PLSD test was performed to detect differences in mean values among the four groups (OHT, ONT, OHYPO and NT group). These data are expressed as the mean±SD or prevalence. Values of $p$<0.05 were considered to indicate statistical significance.

## Results

### Clinical Characteristics of Patients

As shown in Table 1, age was the highest in the OHYPO group, followed by the OHT, ONT and NT groups, but was not significantly different among groups. BMI was the highest in the OHT group, followed by the OHYPO, ONT and NT groups. LVMI was higher in the OHYPO than in the ONT and OHT groups, but these differences were not significant. BP parameters were not significantly different among the 3 hypertensive groups but were significantly lower in the NT control group. The SD of daytime SBP was not significantly different among the 4 groups (mean±SD of daytime

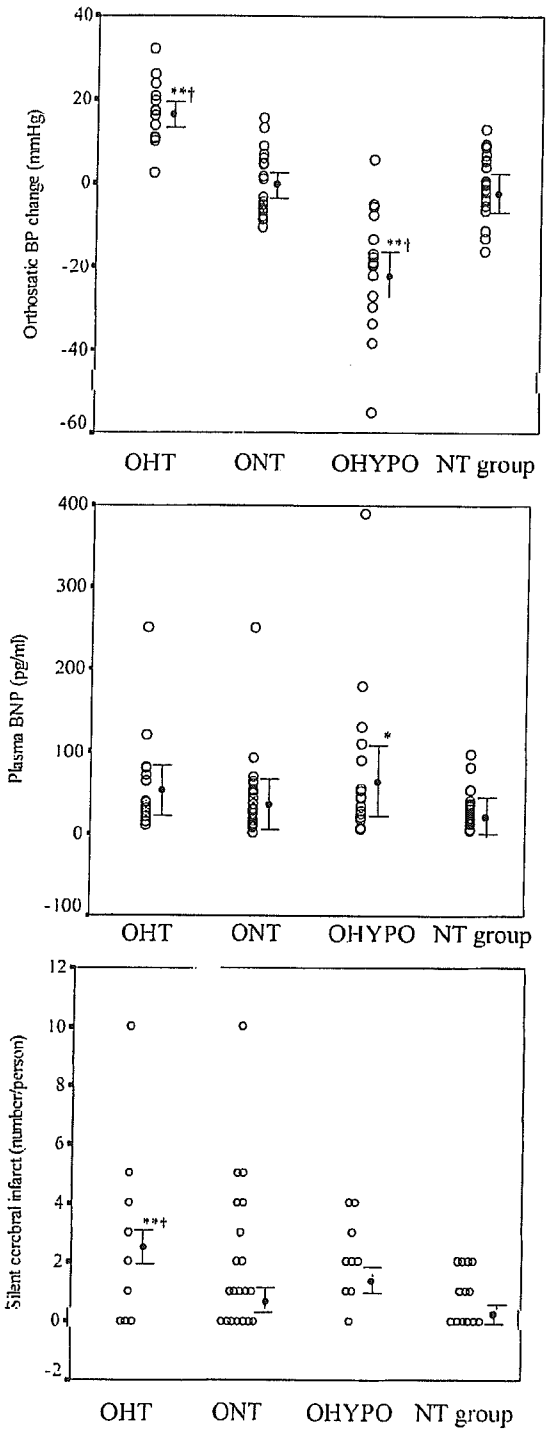


**Fig. 1.** *The orthostatic BP change (A), BNP (B), and the number of silent cerebral infarct (C) in each of the 4 groups. Values are the mean±SD. OHT, orthostatic hypertension; ONT, orthostatic normotension; OHYPO, orthostatic hypotension; NT, normotension.* *p<*0.05*, **p<*0.01* vs. *NT group;* †p<*0.05* vs. *ONT.*

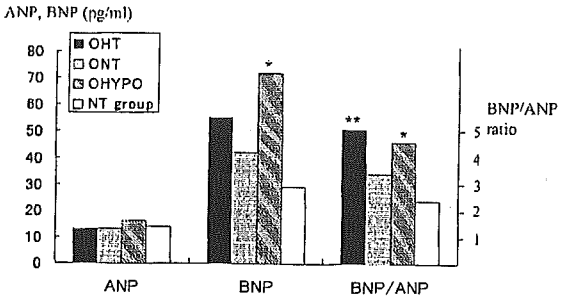


**Fig. 2.** *Baseline neurohumoral factors before tilting test. OHT, orthostatic hypertension; ONT, orthostatic normotension; OHYPO, orthostatic hypotension; NT, normotension.* *p<*0.05*, **p<*0.01* vs. *NT group.*

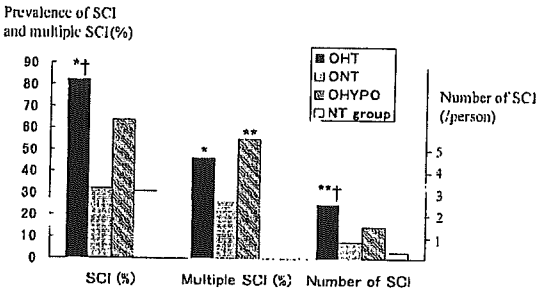


**Fig. 3.** *Silent cerebral lesions detected by MRI. OHT, orthostatic hypertension; ONT, orthostatic normotension; OHYPO, orthostatic hypotension; NT, normotension.* *p<*0.05*, **p<*0.01* vs. *NT group;* †p<*0.05* vs. *ONT. Multiple SCI was defined as SCI* ≥ *3.*

SBP: OHT 20±7 mmHg; ONT 18±5 mmHg; OHYPO 17±5 mmHg; NT group 17±5 mmHg). SBP before tilting was the highest in the OHYPO group, followed by the ONT, OHT and NT groups. SBP change by tilting was significantly higher in the OHYPO and OHT than in the ONT and NT groups (Fig. 1A).

### Neurohumoral Factors

As shown in Figs. 1B and 2, plasma BNP and the BNP/atrial natriuretic peptide (ANP) ratio were significantly higher in the OHYPO than in the NT group. The BNP/ANP ratio was also significantly higher in the OHT than in the NT group. There were no significant differences in resting ANP, PRA, adrenaline, noradrenaline, or ADH among the four groups. There were also no significant differences in the after/before tilting ratio of these parameters (data not shown.).

### Silent Cerebrovascular Lesions

In Figs. 1C and 3, the average number of SCI per person was significantly higher in the OHT than in the NT and ONT

groups. The average number of SCI was the highest in the OHT, followed by the OHYPO, ONT, and NT groups. The prevalence of SCI was significantly higher in the OHT than in the ONT or NT groups. The prevalence of multiple SCIs was significantly higher in the OHYPO and OHT than in the NT group. The prevalence of advanced PVH (data not shown) was the highest in the OHT, followed by the OHYPO, ONT and NT groups, but the differences were not statistically significant.

## Discussion

In this study, we enrolled 59 uncomplicated older hypertensive patients whose daily activities and neurological status were normal, and 27 age- and gender-matched NT controls. The most important finding was that the OHT and OHYPO subjects showed higher plasma BNP levels and advanced SCI independent of the 24-h BP in an uncomplicated hypertensive population.

Twenty-four hour BP level is a powerful independent determinant of LV hypertrophy (*9*, *10*). In the present study, we determined the BNP levels based on the difference in orthostatic BP change independent of 24-h BP levels. The fact that clinic BP and 24-h BP levels were similar among the different orthostatic groups may have been due to differences in the measurements of BP intervals. Orthostatic BP is useful for making short-term estimates of BP variability, whereas we used ABPM to estimate BP at 30-min intervals.

In previous reports, the clinical usefulness of a HUT has been documented (*11*, *12*). Frohlich *et al.* studied a group of persistently hypertensive patients both in recumbent and standing postures and found that those whose orthostatic rise in DBP was greater than normal also showed excessive increases in peripheral resistance during tilting and excessive increases in DBP after Valsalva maneuver (*11*).

### Cardiac Overload

BNP is a sensitive indicator of LV mass (*13*), ventricular wall stress (*14*) and hemodynamic pressure overload (*15*) before the development of LV hypertrophy (*16*), and so is BNP/ANP ratio (*17*, *18*). In our study, BNP was significantly greater in the OHYPO than in the NT group, and the BNP/ANP ratio was also greater in the OHYPO and OHT groups than in the NT group (Figs. 1B and 2). This indicates that hypertensive patients who have a greater orthostatic BP change have cardiac overload, irrespective of their similar 24-h SBP levels. Although SD of daytime SBP and other background characteristics were not significantly different among the 4 groups, very short term BP variability with postural change might have affected cardiac overload. The relatively higher LVMI in the OHYPO group compared to the OHT and ONT groups may indicate longstanding cardiovascular overload. In our previous report, the OHT and OHYPO subjects had a higher frequency of ECG-LV hypertrophy (*6*). Frequent episodes of BP variability during daily activities may contribute to cardiac overload and future clinical events (*1*, *19*, *20*).

Salt intake status and body fluid volume affect ANP and BNP levels in essential hypertension (*21*). Because we do not have any data on salt intake, fluid intake, or body fluid status to assess ANP and BNP in this series, we consider this one of the study limitations.

### Silent Cerebrovascular Lesions

The prevalence of SCI in hypertensive patients ($n$=41) was 53.7%, and in the NT group ($n$=13) the prevalence was 31%. In our hypertensive population, the prevalence of SCI was comparable with that of other previous reports (ranging from 41% to 54%) (*6*, *7*, *22*, *23*).

The prevalence of SCI was highest in the OHT group, followed by the OHYPO, ONT and NT groups. In the OHYPO group, the prevalence of multiple SCIs was higher than the prevalence of only one or two SCI. This is because the percentage of patients with multiple SCIs was relatively higher among OHYPO patients with SCI (6 of 7 patients) than among OHT patients (5 of 9 patients). We have recently shown the U-curve relationship between orthostatic BP change and SCI (*6*). Although the characteristics of the subjects studied were different from those in our previous report, we confirmed that OHT was a strong risk factor for SCI and multiple SCIs. Although the mechanisms of the association between orthostatic BP dysregulation and higher prevalence of SCI remain unclear, we suggest that a hemodynamic mechanism contributes to the higher prevalence of SCI. Previous reports have also shown that OHYPO, which is associated with a greater prevalence of multiple SCIs and LVMI, is also associated with higher risk for cerebrovascular damage (*24*).

Furthermore, we found a higher prevalence (36%) of advanced PVH in the OHT group than in the other groups. Advanced PVH is common in older persons, but is considered to be a pathological feature associated with increased risk for the onset of dementia, cognitive impairment, stroke or death (*25*). Higher SBP variability has been shown to be a contributing factor to the development of PVH (*26*) and cognitive impairment (*27*). Our results are consistent with these previous findings.

### The Pathophysiologic Difference between OHT and OHYPO

BP increase on standing heightens $\alpha$ adrenergic reactivity (*6*) to orthostatic stress, leading to an accentuated increase in vascular resistance and BP that may be due, at least in part, to the increase in baroreflex sensitivity. However, previous studies have reported that resting sympathetic activity was lower in patients with orthostatic hypertension than in those with orthostatic hypotension (*6*, *28*). Orthostatic hyper-

tension has been associated with silent cerebrovascular disease (*6*), carotid intima-media thickness (*29*), and future development of hypertension in young adults with normal BP (*28*). In the present study, SBP before tilting was lower in OHT subjects (141 mmHg) than in the other 2 hypertensive groups. This result is consistent with previous reports (*5*, *6*). SBP levels before tilting in OHT subjects were within normal range. The clinic BP and 24-h BP were similar among the OHT, ONT and OHYPO groups in the present study, and were also similar among these three groups in previous studies (*5*, *6*). In patients with OHT, there may be a significant increase in BP in a sitting or standing position in daily life. Although sympathetic activation can be driven by a standing position in OHT, sympathetic activity and BP in a recumbent position may be relatively lower in patients with OHT than in those with ONT or OHYPO (*6*).

In a previous study, baroreflex sensitivity was reduced in OHYPO (*30*) as well as essential hypertension (*31*). Reduction of baroreflex sensitivity is a normal phenomenon by aging and that contribute to a decreased responsiveness to sympathetic stimulation but that is affected by underlying diseases or the medication status. Masaki *et al.* reported an association between orthostatic hypotension and several frailty measures (*32*) and showed that orthostatic hypotension is a marker for causes a general lessening of physical strength (*32*). Teramoto reported a pathophysiological overlap between postprandial hypotension and OHYPO, involving such effects impaired vasoconstriction, increased vascular wall rigidity, decreased baroreflex function and increase in potential dehydration (*33*).

By beat-to-beat analysis of the orthostatic change in blood pressure, OHT might demonstrate a transient dip in BP prior to the orthostatic increase of BP (although this has not yet been reported). Such a dip could become a trigger for an orthostatic rise of BP through responsive sympathetic nervous system overshoots.

In our study, a higher prevalence of females and obese patients was observed in the OHT group. Sympathetic reactivity to postural change might be increased in these patients. Factors associated with exaggerated sympathetic reactivity have been reported in young persons, in men, in obese persons, and passively in black persons (*28*). There is also a previous report (*5*) that the rate of OHT was higher in females than males.

In conclusion, individuals who have greater orthostatic change of BP were shown to have higher plasma BNP levels and higher prevalence of SCI, which implies cardiovascular overload in hypertensive subjects.

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*Original Article*

# Effects of Bedtime *vs.* Morning Administration of the Long-Acting Lipophilic Angiotensin-Converting Enzyme Inhibitor Trandolapril on Morning Blood Pressure in Hypertensive Patients

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**Cardiovascular events occur most frequently in the morning. To study the effects of the long-acting lipophilic angiotensin-converting enzyme (ACE) inhibitor trandolapril on morning blood pressure (BP), we performed ambulatory BP monitoring (ABPM) before and after administration of trandolapril just before going to bed (bedtime-administered group: $n$=17) or in the morning (morning-administered group: $n$=20) in 37 hypertensive patients. Both sets of ABPM data were available in 30 patients. The 24-h systolic BP (SBP) levels were significantly decreased by 7.2 mmHg in the morning-administered group ($p$=0.02) and by 5.2 mmHg in the bedtime-administered group ($p$=0.04). In the bedtime-administered group, prewaking SBP (the average of the 2-h SBP values just before waking) and morning SBP (the average of the 2-h SBP values just after waking) were significantly decreased by 11 mmHg ($p$=0.005) and by 8.4 mmHg ($p$=0.03), respectively. On the other hand, in the morning-administered group, the reduction of prewaking SBP (3.9 mmHg, n.s.) and morning SBP (6.6 mmHg, n.s.) did not reach the level of statistical significance. However, the differences in the reductions of prewaking and morning SBPs between the two groups were not statistically significant. There was no additional reduction of the nighttime lowest BP in either administration group. In conclusion, bedtime administration of the long-acting ACE inhibitor trandolapril seems to be a safe and effective means of controlling morning BP in hypertensive patients without an excessive fall in nocturnal BP. (*Hypertens Res* 2004; 27: 15–20)**

***Key Words*: angiotensin-converting enzyme inhibitor, hypertension, morning blood pressure surge, morning hypertension, nocturnal blood pressure**

## Introduction

Clinical cardiovascular events and subclinical target organ damage are closely associated with blood pressure (BP) variation independent of BP level (*1–10*). Diurnal BP variation is determined by various genetic (*11*, *12*), and environmental factors, including psychological and physical activities (*13–27*). Cardiovascular events occur more frequently in the morning, and BP also exhibits diurnal variation with increases in the morning (morning surge) (*28–30*). Previously, morning BP surge was reported to be associated with cardiac hypertrophy in hypertensive patients (*31*). Recently, we showed that the morning BP surge was significantly associated with clinical stroke risk in hypertensive patients (*32*). This association was independent of age and 24-h BP level.

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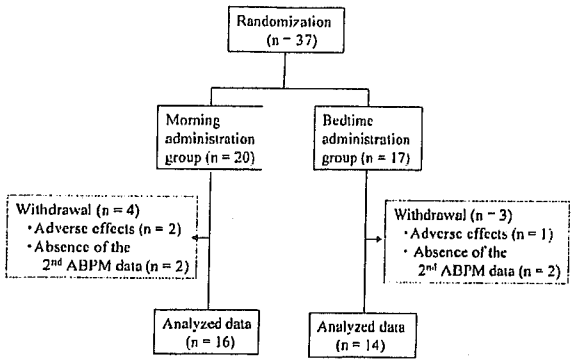


**Fig. 1.** *Selection of study subjects.*

Thus, an antihypertensive medication that was more specific for morning BP surge would be useful for the prevention of cardiovascular events in hypertensive patients.

The renin-angiotensin-aldosterone system is activated in the morning, and may contribute to morning BP surge (*29*) and morning increase in cardiovascular risk. Long-acting angiotensin-converting enzyme (ACE) inhibitors have been reported to lower the ambulatory BP without disruption of its diurnal variation (*33*). However, a specifically timed administration of the long-acting lipophilic ACE inhibitor trandolapril just before going to bed may achieve a greater reduction of morning BP in hypertensives.

## Methods

### Patient Selection

This study was a multicenter open-label randomized study of the effects of morning *vs.* evening administration of trandolapril on ambulatory BP. The subjects included older Japanese hypertensive patients at four Japanese hospital clinics. The entry period was February 2001 to January 2003. A total of 37 patients were recruited for this study (Fig. 1). The entry BP criteria were as follows: 1) average seated clinic systolic BP (SBP) $\geq$140 and $<$180 mmHg and/or diastolic BP (DBP) $\geq$90 and $<$110 mmHg during the follow-up period (3–5 weeks); and 2) 24-h SBP $\geq$130 mmHg and/or 24-h DBP $\geq$80 mmHg as shown by baseline first ambulatory BP monitoring (ABPM). To be included in the study, patients had to be $\geq$40 years of age, had to have been diagnosed with essential hypertension, and had to have no history of other significant medical disorders, including diabetes, renal failure (serum creatinine $\geq$2.0 mg/dl), atrial fibrillation, or any clinically overt cardiovascular disease. They were all fully ambulant. The body mass index was calculated as weight (kg)/[height (m)]$^2$.

### Study Design

Each patient was studied for a maximum of 12 weeks, with an observation period of 2–4 weeks, and a treatment period of up to 8 weeks. After the observation period, patients were started on 1 mg of trandolapril, taken at bedtime (bedtime-administration group), or just after breakfast (morning-administration group). After 4 weeks of treatment the dosage was increased to 2 mg of trandolapril unless the patient's BP had already been reduced to below 150 mmHg in systole and 90 mmHg in diastole, or side effects had occurred. Following this dosage adjustment, patients remained on treatment for another 4 weeks. Informed consent was obtained, and the study was approved by the Research Ethics Committee of the Department of Cardiology, Jichi Medical School.

We excluded seven patients for whom the second ABPM recordings were not obtained: four of these patients refused the second ABPM, two developed cough, and one developed dizziness during the titration period (Fig. 1).

### ABPM

Noninvasive ABPM was carried out two times on a weekday with one of two automatic ABPM devices (TM-2421 or TM-2425; A&D Co. Inc., Tokyo, Japan), which recorded BP and pulse rate by the oscillometric method every 30 min for 24 h. The first ABPM was performed at the end of the observation period, and the second ABPM at the end of the 8-week treatment period. The interval between the first and second ABPMs was 8 weeks.

Twenty-four hour BP was defined as the average of all BP readings over 24 h (*34*). The subjects were all ambulant during the day, and no subjects reported staying in bed after waking. Sleep BP was defined as the average of BPs from the time when the patient went to bed until the time he/she got out of bed, and awake BP as the average of BPs recorded during the rest of the day (*4*, *7*). Morning BP was defined as the average of BPs during the first 2 h after the wakeup time (four BP readings) (*32*). The nighttime lowest BP was defined as the average BP of three readings centered on the lowest nighttime reading. Evening BP was defined as the average BP during the 2 h before going to bed (four BP readings) (*32*). Preawake BP was defined as the average BP during the 2 h just before wakeup time (four BP readings) (*32*). The morning BP surge (MBPS) was calculated as the morning SBP minus the nighttime lowest SBP (*32*). SBP were used for all these calculations.

### Statistical Analysis

The analysis was conducted for the 30 patients for whom the first and second ambulatory BP recordings were successfully obtained. The changes from the baseline values were analyzed statistically using the paired Wilcoxon-test for each subgroup. Two-sided Mann-Whitney *U*-tests and $\chi^2$-tests

**Table 1. Patient Characteristics**

| | Morning administration group ($n$=16) | Bedtime administration group ($n$=14) | Total group ($n$=30) |
|---|---|---|---|
| Age (years) | 68±9.0 | 66±13 | 67±11 |
| Men/women ($n$) | 7/9 | 10/4 | 17/13 |
| Body mass index (kg/m$^2$) | 25.2±5.1 | 23.8±3.3 | 24.6±4.3 |
| Other antihypertensives ($n$) | 7 | 6 | 13 |
| Calcium antagonist ($n$) | | | |
| Short-acting | 0 | 1 | 1 |
| Long-acting | 5 | 4 | 9 |
| $\alpha$-Blocker | 1 | 1 | 2 |
| $\beta$-Blocker | 1 | 0 | 1 |
| Dose of trandolapril (mg/day) | 1.4±0.5 | 1.2±0.5 | 1.3±0.5 |
| Clinic SBP (mmHg) | | | |
| Baseline | 158±8.7 | 161±12 | 160±10 |
| After trandolapril | 141±17 | 143±14 | 142±15 |
| Reduction | 17±16 | 19±16 | 18±16 |
| Clinic DBP (mmHg) | | | |
| Baseline | 93±9.6 | 92±15 | 92±12 |
| After trandolapril | 83±7.6 | 84±13 | 83±11 |
| Reduction | 10±7.9 | 8.3±6.1 | 9.3±7.1 |
| Sleep time (h) | | | |
| Baseline | 8.9±1.2 | 8.7±1.3 | 8.8±1.2 |
| After trandolapril | 8.8±1.4 | 8.9±1.1 | 8.9±1.2 |

BP, blood pressure; SBP, systolic BP; DBP, diastolic BP. Data are shown as the means±SD or the number of patients.

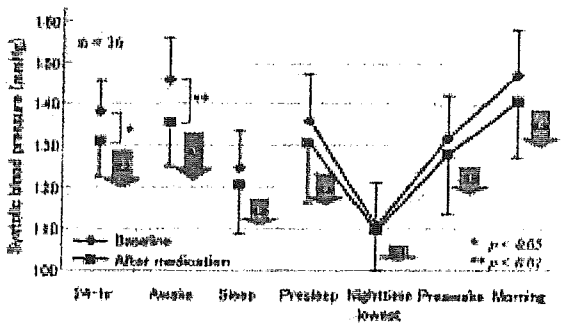

**Fig. 2.** *Change in ambulatory blood pressure levels in the morning-administration group.*

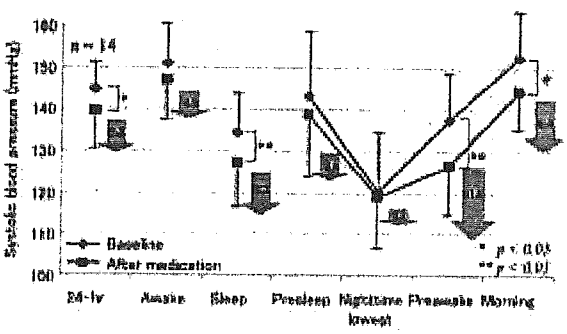

**Fig. 3.** *Change in ambulatory blood pressure levels in the bedtime-administration group.*

were used to test differences between the two groups in the mean values of continuous measures and prevalence rates, respectively. The criterion for determining statistical significance was $p<0.05$. The results are given as the mean±SD.

## Results

The baseline characteristics of the study subjects as well as the baseline BPs and BPs after trandolapril therapy were comparable between the morning- and bedtime-administration groups (Table1). In addition, there were no significant differences in the baseline ABPM-derived BP parameters between the 2 groups. The 24-h BPs were reduced significantly in both groups, and the degrees of the reductions were also comparable between the two groups (Figs. 2, 3). There were no significant differences in the BP-lowering effects on ABPM parameters between subjects taking and those not taking additional antihypertensive medications.

In the morning-administration group, awake BPs were significantly reduced, but the reduction of sleep BPs, morning BPs and preawake BPs did not reach statistical significance (Fig. 2). In the bedtime-administration group, the reduction

of morning BPs and preawake BPs was significant (Fig. 3). Sleep BPs were significantly reduced, but the nighttime lowest BPs were not further decreased in the bedtime-administration group. However, the differences in the reductions of ABP parameters, including preawake SBP ($p=0.186$), between the two groups were not statistically significant.

Although the morning BP surge was not significantly reduced in either group, the morning BP surge was reduced to less than 45 mmHg in all five hypertensives with exaggerated morning BP surge $\geq$45 mmHg (the highest tertile) in the bedtime-administration group. On the other hand, morning BP surge remained $\geq$45 mmHg in three of the five hypertensives with exaggerated morning BP surge in the morning-administration group.

## Discussion

The present results indicate that bedtime administration of the long-acting lipophilic ACE inhibitor trandolapril is a safe and effective means of controlling morning BP in hypertensive patients without an excessive fall in nocturnal BP.

Recently, we showed that the morning BP surge was significantly associated with clinical stroke risk in hypertensive patients (*32*). This association was independent of age, 24-h BP level, and silent cerebral infarct (*32*), which is a powerful predictor of clinical stroke events (*34*, *35*). In addition to the morning BP surge, the morning BP level is also an important predictor of stroke events in hypertensive patients (*32*). In this study, bedtime administration of trandolapril significantly reduced morning BP levels after waking. In addition, the prewaking BP was also significantly reduced by the bedtime administration. On the other hand, the morning administration of trandolapril significantly reduced awake BP, but the reduction of morning and preawake BPs was limited. Thus, the bedtime administration of trandolapril could be a specific treatment for reducing morning BP.

There have been several studies of antihypertensive medications specific for morning BP. Long-acting effects are the most important characteristic for sufficient morning BP control by once-daily use of antihypertensive medication (*29*, *36*, *37*). Antihypertensive therapy should provide the most effective protection at the time of the greatest risk, that is, in the morning hours. Pharmacokinetically, an extended-release form of verapamil was also reported to be highly effective for BP reduction (*38*). In addition, it may be possible to achieve more specific chronological treatment for morning BP surge by using an antihypertensive medication which reduces the pressor effect of neurohumoral factors potentiated in the morning, such as inhibitors of the sympathetic activity or the renin-angiotensin system. $\alpha$-Adrenergic blockers and $\alpha\beta$-blockers might be effective for reducing morning BP surge in hypertensive patients. Bedtime administration of $\alpha$-adrenergic blockers has the most marked BP-lowering effect in the morning (*39*). This study also demonstrated the potential benefit of bedtime ACE inhibitors for controlling morning BP in clinical practice for hypertensive patients.

Nighttime administration of the ACE inhibitor trandolapril appears to be more effective than morning administration for specifically controlling morning SBP. However, we cannot state this conclusively based on the present results. The differences in the reductions of ABP parameters, including preawake SBP, between the two groups did not reach the level of statistical significance. One of the limitations of this study was the lack of a control group. Thus, the reproducibility of each ABPM parameter was not sufficiently clear to render the results conclusive. Other, shorter-acting ACE inhibitors might increase the difference between the two groups due to the weaker BP-lowering effect of the morning administration. Because of its lipophilic nature, trandolapril has the longest-acting ACE inhibitory activity and BP-lowering effect of all the ACE inhibitors (*40*). In fact, the 24-h BP-lowering effects were comparable among the two groups. However, awake BP was not significantly reduced in the bedtime-administration group. A longer-acting BP-lowering effect is the most important effect for an antihypertensive medication, and in addition, for those with higher morning BP level, bedtime administration seems an alternative or additional antihypertensive strategy.

Recently, it has been demonstrated that, in addition to circulating factors, the tissue renin-angiotensin-aldosterone secretion of the cardiovascular system exhibits diurnal variation (*41*), possibly in relation to a clock gene (*42*, *43*). In addition to the reduction of the morning BP level, the morning activation of the tissue renin-angiotensin-aldosterone system might be effectively suppressed by bedtime administration of an ACE inhibitor, leading to more effective protection against hypertensive target organ damage and cardiovascular events in hypertensive patients.

The bedtime administration of antihypertensive medication has the potential hazard of ischemia of target organs because of excessive nocturnal BP reduction. Extreme-dippers with marked nocturnal BP falls have increased potential risk for ischemic cardiovascular events when treated with strict antihypertensive medication or specific medication just before going to bed (*10*, *44*). Since it has been reported that ACE inhibitor reduced normal BP levels further in white-coat hypertensive patients (*45*), we expected the nighttime lowest BP levels to be reduced further by bedtime administration of trandolapril. However, the nighttime lowest BP was not further decreased, although the average sleep BP decreased, particularly in the preawake period. Thus, bedtime administration of trandolapril can be considered safe with respect to ischemia of target organs during sleep.

In conclusion, bedtime administration of the long-acting ACE inhibitor trandolapril was effective for controlling morning BP and was suggested to be safe in the sense that it caused no marked nocturnal BP reduction in hypertensive patients.

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# Potential of free-form TFPI and PAI-1 to be useful markers of early atherosclerosis in a Japanese general population (the Suita Study): association with the intimal-medial thickness of carotid arteries

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**Abstract**

This study assessed markers of vascular endothelial cell dysfunction associated with early atherosclerosis in carotid arteries. We measured the plasma levels of free-form tissue factor pathway inhibitor (free TFPI), plasminogen activator inhibitor-1 (PAI-1), and von Willebrand factor (vWF) in 522 adults without cardiovascular disease enrolled in the Suita Study. For each sex, we analyzed the association of the degree of intimal-medial thickness (IMT) with hemostatic markers using logistic regression analysis considering potential confounding risk factors, including age, body mass index, lifestyle (current smoking and drinking), illness (diabetes mellitus and hyperlipidemia), systolic blood pressure, and antihypertensive drug use. The age-adjusted levels of free TFPI and PAI-1 were positively and independently associated with the degree of IMT for men. Even after adjustment for all confounding factors, the level of PAI-1 was positively associated with the degree of IMT. These results indicate that measurement of the levels of free TFPI and PAI-1 is a potentially useful tool for the detection of early atherosclerosis in men.


*Keywords:* Atherosclerosis; Endothelium-derived factors; Hypertension

## 1. Introduction

Measurement of the intimal-medial thickness (IMT) of carotid arteries has been used as a non-invasive endpoint in epidemiological studies and clinical trials to assess the progression and regression of atherosclerosis [1,2]. Furthermore, IMT has recently been used not only as a surrogate endpoint for atherosclerosis of the coronary artery but also as a good indicator of the presence and extent of coronary artery disease [3–6]. Case-reference studies in a general population have been performed in regard to the association between markers of vascular endothelial cell dysfunction and atherosclerosis by measuring IMT of the carotid artery [7–9]. However, to detect early atherosclerosis, it is essential to study the association between these markers and the extent of atherosclerosis, using a general population free from cardiovascular disease (CVD).

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In this study, we focused on the association between three markers of endothelial cell dysfunction, namely free-form tissue factor pathway inhibitor (free TFPI), plasminogen activator inhibitor-1 (PAI-1), and von Willebrand factor (vWF) and IMT of carotid arteries in a Japanese general population (the Suita Study). Plasma concentrations of vWF and PAI-1 have previously been used as surrogate markers of endothelial damage [10,11]. TFPI inhibits tissue factor-initiated coagulation by binding to factor Xa and tissue factor-activated factor VII complex [12,13]. Most TFPI is synthesized by vascular endothelial cells, and is distributed into at least four pools in vivo. The majority of TFPI synthesized by vascular endothelial cells is associated with endothelial cells, whereas other pools circulate in the blood as complexes with lipoproteins (Lp-TFPI) or as a free form (free TFPI). A minor pool of TFPI is present in platelets. It has been demonstrated that free TFPI strongly correlates with endothelial cell markers such as thrombomodulin, vWF, and tissue-type plasminogen activator, whereas total TFPI does not [14]. There is a strong, positive correlation between the free TFPI and endothelial cell-associated TFPI levels [15]. Therefore, we selected free TFPI as a marker of endothelial cell dysfunction, instead of Lp-TFPI or total TFPI.

Here, we have demonstrated the potential of free TFPI and PAI-1 to be useful markers of early atherosclerosis by studying their association with IMT in relation to conventional risk factors for CVD.

## 2. Methods

### 2.1. Study population

The study population was based on samples randomly selected from the residents of Suita, a city located in the second largest urban area in Japan (the Suita Study) [5]. The subjects have been visiting the National Cardiovascular Center every 2 years since 1989 for regular health checkups. Only subjects who provided written informed consent to have a blood examination were enrolled in this study. The subjects included 245 men and 277 women who were free of cardiovascular disease, aged from 34 to 91 years, and attended the National Cardiovascular Center from 5 August 1998 to 24 December 1998. Subjects were classified as smokers if they smoked at least one cigarette per day. Subjects were defined as hypertensive if their diastolic blood pressure was $\geq$95 mmHg, their systolic blood pressure was $\geq$160 mmHg, or they were taking antihypertensive medication. Subjects whose fasting blood glucose levels were $\geq$7.78 mmol/L, whose blood glucose levels were $\geq$11.11 mmol/L 2 h after a 75-g oral glucose loading, or who were taking antidiabetic medication were defined as diabetic. Subjects whose total serum cholesterol level was $\geq$5.68 mmol/L (220 mg/dl), or who were taking anti-hypercholesterolemic medication were defined as having hypercholesterolemia.

### 2.2. IMT measurements

The details of the ultrasonic carotid examination have previously been published [16]. We used a high-resolution B-mode ultrasonic machine with 7.5-MHz transducers, yielding an axial resolution of 0.2 mm. The regions between 30 mm proximal from the beginning of the dilation of the bifurcation bulb and 15 mm distal from the flow divider of both common carotid arteries were scanned. All measurements were made at the time of scanning using the instrument's electronic caliper and were recorded as photocopies. The IMT in common carotid arteries was measured on a longitudinal scan of the common carotid arteries at a point 10 mm proximal from the beginning of the dilation of the bifurcation bulb. We defined the IMT as mean IMT of the near and far walls at the point of measurement.

### 2.3. Blood collection and analysis

After a minimum 12-h fast and between 10 a.m. and 1 p.m., blood samples for hemostatic profile were collected into disposable, siliconized, evacuated glass tubes containing 0.1 vol. of 3.13% trisodium citrate, and blood collected in a second tube was used for the coagulation assay. The samples were centrifuged at 4600 $\times$ $g$ for 10 min at room temperature within 1 h of collection. The PAI-1 antigen level was immediately determined, and the remaining plasma was aliquoted in plastic tubes and stored at $-80$ °C until use. The thawed samples were used to measure free TFPI and vWF.

The antigen level of free TFPI was measured by a sandwich enzyme immunoassay method [17]. The coefficient of intra-assay variation for the assay was 2.7%. The antigen levels of PAI-1 and vWF were automatically measured by latex photometric immunoassay using an LPIA-tPAI kit (Mitsubishi Kagaku Medical) and STA liatest vWF kit (Diagnostica Stago), respectively. The coefficients of intra-assay variation of PAI-1 and vWF were 1.0 and 4.3%, respectively.

### 2.4. Statistical analysis

All statistical analyses were performed independently by sex. We first used Spearman correlation analysis to assess the association between the progression of IMT and the analyzed parameters (Tables 1 and 2). We then used ANCOVA to investigate whether plasma levels of free TFPI, PAI-1, and vWF are positively and independently associated with the degree of carotid intimal thickness or not (Table 3). We have performed two types of adjustments. First, adjustments were made for age only. Second, further adjustments were made for lifestyle (drinking and smoking), illness (diabetes, hypercholesterolemia), body mass index, systolic blood pressure, and antihypertensive drug use. Differences with a value of $P < 0.05$ for ANCOVA were

Table 1
Demographic characteristics and unadjusted hemostatic parameters according to rank of intimal-medial thickness (IMT) of the carotid artery in men

| | IMT-rank | | | | P |
|---|---|---|---|---|---|
| | Q1 ($n = 58$) | Q2 ($n = 70$) | Q3 ($n = 57$) | Q4 ($n = 60$) | |
| Median of IMT (mm) | 0.73 | 0.83 | 0.93 | 1.05 | |
| Age (year) | 47.4 ± 7.8 | 59.2 ± 9.2 | 69.2 ± 8.7 | 70.4 ± 8.0 | <0.0001 |
| Current drinking (%) | 79 | 73 | 67 | 62 | <0.0009 |
| Smoker (%) | 78 | 73 | 67 | 62 | <0.0007 |
| Body mass index (kg/m$^2$) | 23.2 ± 3.3 | 23.7 ± 3.2 | 23.4 ± 3.3 | 22.8 ± 3.1 | <0.4217 |
| Diabetes (%) | 0 | 3 | 5 | 10 | <0.0511 |
| Hypertension (%) | 7 | 29 | 32 | 45 | <0.0001 |
| Hypercholesterolemia (%) | 7 | 11 | 9 | 15 | <0.1685 |
| LDL-cholesterol (mg/dl) | 113.9 ± 27.5 | 124.8 ± 29.5 | 120.3 ± 26.7 | 131.0 ± 26.4 | <0.0060 |
| HDL-cholesterol (mg/dl) | 60.8 ± 17.2 | 56.1 ± 12.5 | 55.2 ± 16.1 | 55.2 ± 16.1 | <0.0255 |
| Free TFPI (ng/ml) | 15.7 ± 4.5 | 16.0 ± 3.8 | 17.2 ± 3.2 | 18.2 ± 4.6 | <0.0006 |
| PAI-1 (ng/ml) | 32.3 ± 25.2 | 32.9 ± 30.9 | 29.3 ± 19.7 | 33.0 ± 38.8 | <0.2059 |
| von Willebrand factor (%) | 115.0 ± 43.7 | 137.1 ± 45.9 | 151.7 ± 54.9 | 160.8 ± 63.9 | <0.0001 |

Values are mean ± S.D. or percent. *P*-values were calculated by simple linear regression analysis. TFPI; tissue factor pathway inhibitor, PAI-1; plasminogen activator inhibitor-1.

Table 2
Demographic characteristics and unadjusted hemostatic parameters according to rank of intimal-medial thickness (IMT) of the carotid artery in women

| | IMT-rank | | | | P |
|---|---|---|---|---|---|
| | Q1 ($n = 66$) | Q2 ($n = 73$) | Q3 ($n = 63$) | Q4 ($n = 75$) | |
| Median of IMT (mm) | 0.70 | 0.78 | 0.85 | 0.95 | |
| Age (year) | 45.8 ± 6.7 | 55.2 ± 7.6 | 62.2 ± 7.2 | 71.9 ± 7.8 | <0.0001 |
| Current drinking (%) | 45 | 33 | 35 | 25 | <0.0897 |
| Smoker (%) | 12 | 5 | 5 | 8 | <0.6107 |
| Body mass index (kg/m$^2$) | 21.3 ± 2.5 | 21.6 ± 2.9 | 23.2 ± 3.5 | 22.9 ± 3.6 | <0.0145 |
| Diabetes (%) | 2 | 0 | 5 | 4 | <0.6649 |
| Hypertension (%) | 2 | 10 | 19 | 40 | <0.0001 |
| Hypercholesterolemia (%) | 8 | 10 | 11 | 19 | <0.0913 |
| LDL-cholesterol (mg/dl) | 115.4 ± 30.5 | 126.4 ± 30.0 | 137.9 ± 25.7 | 137.0 ± 26.5 | <0.0001 |
| HDL-cholesterol (mg/dl) | 73.1 ± 17.9 | 69.2 ± 15.5 | 67.5 ± 16.7 | 62.1 ± 14.8 | <0.0002 |
| Free TFPI (ng/ml) | 11.5 ± 3.2 | 14.9 ± 5.0 | 16.3 ± 4.2 | 17.5 ± 4.8 | <0.0001 |
| PAI-1 (ng/ml) | 20.2 ± 18.3 | 19.8 ± 12.8 | 25.7 ± 19.1 | 26.2 ± 18.0 | <0.0707 |
| von Willebrand factor (%) | 107.8 ± 31.4 | 126.5 ± 41.8 | 136.5 ± 51.6 | 157.2 ± 59.2 | <0.0001 |

Values are mean ± S.D. or percent. *P*-values were calculated by simple linear regression analysis. TFPI; tissue factor pathway inhibitor, PAI-1; plasminogen activator inhibitor-1.

considered to be significant. All analyses were performed with SAS statistical software (release 8.2 SAS Institute Inc).

## 3. Results

### 3.1. Demographic characteristics and unadjusted parameters according to rank of IMT of carotid arteries

We measured IMT in a general population, divided it into four quartiles by sex, and analyzed the demographic characteristics and unadjusted parameters according to IMT rank, as shown in Tables 1 and 2. The IMT median of each quartile (*Q*1, *Q*2, *Q*3 and *Q*4) is shown in the first column of each table. In both sexes, the plasma levels of free TFPI, vWF, and LDL-cholesterol as well as age and hypertension increased in a stepwise manner from the first to the forth IMT quartile.

### 3.2. Multivariate analysis of free TFPI, PAI-1, and vWF levels according to IMT rank

As summarized in Table 3, we analyzed the plasma levels of free TFPI, PAI-1, and vWF according to IMT ranks after either adjusting for age only or adjusting for age, lifestyle (drinking and smoking), body mass index, systolic blood pressure, diabetes, hypercholesterolemia, and hypertensive drug use. Age adjusted free TFPI levels in men increased in a stepwise manner from the first to the fourth IMT quartile ($P = 0.003$, for trend) and the levels of free TFPI in the third and the fourth quartiles compared to the lowest IMT quartile remained statistically significant in the multivariate analysis. However, the statistically significant increases of free TFPI

Table 3
Adjusted mean levels of free TFPI, PAI-1, and von Willebrand factor according to rank of intimal-medial thickness (IMT) of the carotid artery

| | IMT-rank | | | | *P* for trend |
|---|---|---|---|---|---|
| | *Q*1 | *Q*2 | *Q*3 | *Q*4 | |
| Free TFPI (ng/ml) | | | | | |
| Men | | | | | |
| Age adjusted | 15.3 ± 0.7 | 15.9 ± 0.5 | 17.5 ± 0.5‡ | 18.4 ± 0.6‡ | 0.003 |
| All adjusted | 16.2 ± 0.7 | 15.9 ± 0.5 | 17.2 ± 0.6 | 17.9 ± 0.6 | 0.075 |
| Women | | | | | |
| Age adjusted | 14.3 ± 0.7 | 15.8 ± 0.5‡ | 15.6 ± 0.5 | 14.8 ± 0.6 | 0.410 |
| All adjusted | 14.9 ± 0.6 | 15.9 ± 0.5 | 15.2 ± 0.5 | 14.5 ± 0.6 | 0.250 |
| PAI-1 (ng/ml) | | | | | |
| Men | | | | | |
| Age adjusted | 21.9 ± 4.9 | 31.1 ± 3.5 | 35.1 ± 4.2 | 39.8 ± 4.3‡ | <0.001 |
| All adjusted | 24.1 ± 5.3 | 30.8 ± 3.5 | 34.7 ± 4.3 | 38.3 ± 4.5 | <0.001 |
| Women | | | | | |
| Age adjusted | 18.0 ± 2.9 | 19.1 ± 2.1 | 26.3 ± 2.2‡ | 28.4 ± 2.7‡ | 0.227 |
| All adjusted | 20.3 ± 2.6 | 20.8 ± 1.9 | 23.7 ± 2.0 | 26.9 ± 2.5 | 0.317 |
| von Willebrand factor (%) | | | | | |
| Men | | | | | |
| Age adjusted | 144.0 ± 8.3 | 142.1 ± 6.0 | 135.6 ± 7.2 | 143.0 ± 7.3 | 0.353 |
| All adjusted | 141.3 ± 8.9 | 142.1 ± 6.0 | 136.9 ± 7.3 | 144.4 ± 7.7 | 0.180 |
| Women | | | | | |
| Age adjusted | 133.7 ± 7.6 | 134.0 ± 5.5 | 130.8 ± 5.9 | 131.8 ± 7.1 | 0.042 |
| All adjusted | 133.5 ± 7.7 | 134.7 ± 5.7 | 129.9 ± 6.0 | 132.0 ± 7.4 | 0.049 |

Values are mean ± errors adjusted for age or adjusted for age, life style (current drinking and smoking), body mass index, present illness (diabetes, hypercholesterolemia), systolic blood pressure, and hypertensive drug use. (‡) $P < 0.05$ compared with Q1 subjects. TFPI; tissue factor pathway inhibitor, PAI-1; plasminogen activator inhibitor-1.

levels in men were not detected after adjustment for several possible confounding factors (all adjusted). The free TFPI levels in women demonstrated a mountain-shaped relationship with the degree of IMT in the multivariate analysis.

Age-adjusted PAI-1 levels in men increased with increasing IMT rank ($P < 0.001$) and the levels in the fourth quartile compared to the lowest IMT quartile remained statistically significant in the multivariate analysis. Age-adjusted PAI-1 levels in women also increased with increasing IMT rank and the levels in the third and fourth quartiles compared to the lowest quartile were statistically significant, although the $P$ value for trend was 0.227. The statistically significant increases of PAI-1 levels in men was detected after all adjustments.

In contrast, the age-adjusted vWF levels in both sexes did not show significant changes among IMT quartiles, although the $P$ values for trend in women after all adjustments were significant. These results indicate that measurement of the levels of free TFPI and PAI-1 is a potentially useful tool for the detection of early atherosclerosis in men.

## 4. Discussion

In this cross-sectional analysis, we have demonstrated that increased levels of free TFPI and PAI-1 in men without CVD were closely associated with the elevation of IMT in common carotid arteries as measured by B-mode ultrasonography. These findings suggest that free TFPI and PAI-1 may be sensitive markers reflecting early atherosclerosis in the carotid arteries.

It has been demonstrated that TFPI localizes with tissue factor within atherosclerotic plaques in human carotid and coronary arteries and modulates the thrombogenicity of the plaque by attenuating the tissue factor activity [18–20]. Enhancement of TFPI expression in the atherosclerotic plaque will cause an increase in the free TFPI concentration in the plasma of patients with cardiovascular disease. In fact, elevated free TFPI levels have been reported in the plasma of patients with ischemic heart disease [21,22]. These findings imply that an elevated level of free TFPI in the plasma is closely associated with hypercoagulable states in atherosclerotic diseases. However, the role of TFPI associated with subclinical or early atherosclerosis was rarely reported. Sakkinen et al. reported a significant positive relationship between the level of plasma TFPI activity and subclinical cardiovascular disease in a healthy elderly cohort study [23]. However, the relationship between age/gender and TFPI levels in a general population has not been examined in detail. In this study, we have extended the above results and demonstrated a direct link between the extent of carotid artery atherosclerosis and the plasma level of free TFPI antigen in men in a Japanese general population without CVD.