Relationships Between New Risk Factors and Circadian Blood Pressure Variation in Untreated Subjects With Essential Hypertension

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Recently a growing amount of interest has been focused on new risk factors for cardiovascular disease, such as insulin, leptin, homocysteine, and urinary albumin excretion (UAE). Furthermore, the absence of a nocturnal blood pressure (BP) decrease is emerging as an index for future target organ damage. In the present study we aimed to determine the relationship between these risk factors and circadian BP variations in essential hypertensive subjects.

One hundred six patients, aged 54 ± 7 years, with stage I–II untreated hypertension were classified as dippers and nondippers according to the diurnal variation of 10% between mean daytime and nighttime systolic BP (SBP) and diastolic BP (DBP) in 24-h noninvasive ambulatory BP monitoring. Venous blood samples were drawn for determination of insulin, leptin, and homocysteine plasma levels, whereas UAE was evaluated in three consecutive 24-h urine samples. Nondippers compared to dippers had significantly greater hemodynamic load and higher UAE (by 17 mg/24 h, P < .05). The two groups did not differ regarding serum insulin, plasma leptin, and homocysteine levels. In the entire population, leptin was positively correlated with age, body mass index, 24-h DBP, fasting serum insulin, and plasma homocysteine levels, whereas homocysteine levels were significantly related to 24-h SBP and DBP values. Multiple linear regression analyses revealed that only UAE was significantly related with nocturnal SBP and DBP decrease (P < .05 for both). These findings suggest that the increased UAE observed in nondipper hypertensive subjects possibly represents a useful indicator for future target organ damage.

Key Words: Circadian blood pressure variations, new risk factors, hypertension.

Ambulatory blood pressure (BP) monitoring, the gold standard method to reveal increased hemodynamic loads and abnormal circadian BP variations, has been proved to be better correlated with target organ damage (TOD) than office BP readings. Apart from the hemodynamic load, interest has been focused during the past decade on risk factors that have been considered to affect adversely the cardiovascular system. In particular, a slightly increased urinary albumin excretion (UAE) rate, termed microalbuminuria (MA), has been identified to predict all-cause mortality (especially cardiovascular) in the general population and in patients with diabetes mellitus. Furthermore, cross-sectional studies have implicated MA as a manifestation of vascular damage, which is not confined to the renal arterial bed alone. Besides being a marker of concomitant cardiovascular damage, MA has been associated with a worse pattern of older and newer atherosclerotic risk factors. High blood homocysteine concentrations have been associated with cardiovascular disease independently of other risk factors. Furthermore, obese subjects, among whom prevalence of essential hypertension (EH) is higher than in the lean population, have been found to be hyperinsulinemic and hyperleptinemic. However, in spite of an increased interest in the significance of these risk factors, several aspects of the hypertensive increased UAE, increased plasma leptin and homocysteine concentrations, and increased serum insulin levels, still remain to be established. Thus, in our study we have made an attempt to clarify the relationship between these risk factors and the circadian BP variation in untreated newly diagnosed EH subjects.

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Methods

Study Population

Seven hundred forty-eight hypertensive, nondiabetic, patients aged between 30 and 70 years who were referred to the outpatient hypertension unit of our institution between January 2000 and January 2001, were considered eligible for inclusion in the study. Patients were included if they presented with mild-to-moderate EH diagnosed within the previous 2 years, and have never received drug treatment. Office BP was measured according to the recommendation of the Sixth Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. All subjects underwent the usual clinical and laboratory work-up to rule out secondary hypertension. An oral glucose test was performed in all patients and they had fasting blood glucose <120 mg/dL, preserved glucose tolerance (plasma glucose <140 mg/dL 2h after a 75-g oral glucose test), and glycosylated hemoglobin A1c <6%. We excluded subjects with overt proteinuria detectable by urine dip strip test, family history of diabetes mellitus or obesity (body mass index [BMI] >30 kg/m²), history of cardiac or cerebrovascular disease, familia hypercholesterolemia, or other systemic disease. Women taking oral contraceptives or long-term estrogen replacement therapy were also excluded. One hundred six subjects who fulfilled these criteria were finally selected for participation in the study. The study protocol included anthropometric and metabolic determinations, echocardiographic examination, and ambulatory BP monitoring. All subjects gave written informed consent for participation and the ethics committee of our institution approved the study protocol.

Anthropometric Determinations

Weight and height were measured by standard techniques and BMI was calculated as body weight divided by height squared. Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest (normally the umbilical level) and hip circumference was measured at the trochanter level. Both circumferences were measured to the nearest 0.5 cm with plastic tape and the ratio between them provided the waist-to-hip ratio.

Ambulatory BP Monitoring

The procedure for ambulatory BP monitoring by using the automatic SpaceLabs unit 90207 (Space Labs Inc., Redmond, WA) has been previously described in detail. In keeping with current practice, daytime and nighttime were defined using short fixed-clock intervals, which ranged from 10 AM to 8 PM and from midnight to 6 AM, respectively. Reexamination was performed in five patients who complained of sleep disturbance during ambulatory BP monitoring.

According to the criterion of Verdecchia et al., hypertensive patients with a nocturnal reduction in average daytime systolic BP (SBP) and diastolic BP (DBP) of less than 10% were considered nondippers. The remaining subjects were classified as dippers. Because the reproducibility of circadian BP variation is limited, we included in the final analysis 106 subjects who were either dippers (66 patients) or nondippers (40 patients) in both ambulatory BP monitoring evaluation, whereas we excluded the remaining subjects (n = 56) who showed fluctuation between the two.

Cardiac Ultrasonography

The cardiac ultrasonographic studies were performed by a senior echocardiographer with a Hewlett-Packard Sonos 2500 ultrasound imager equipped with a 2.25-5-MHz transducer (HP Co., Medical Products Group, Palo Alto, CA) according to the recommendations of the American Society of Echocardiography. We estimated the relative wall thickness (RWT), the left ventricular (LV) mass by using the Penn convention, and the LV mass index (LVMI) by dividing LV mass by the body surface area.

Metabolic Determinations

For the determination of UAE, the study patients were asked to collect three consecutive 24-h urine samples (from 8 AM to 8 AM) as has been previously described. Urinary albumin concentrations were determined by immunonephelometric technique with a limit of detection of 0.4 mg/dL and an interassay variation of 0.035. The microalbuminuric patients had a UAE between 20 and 200 mg/24 h and the normoalbuminuric patients had a UAE <20 mg/24 h.

Venous blood samples were taken between 7:30 AM and 9:30 AM after a 12-h fast and 15 min of supine rest. Plasma leptin was determined by two-site immunoradiometric assay with an intra-assay and an interassay coefficient of variation of 2.6% to 3.7% and 3.7% to 6.1%, respectively, and insulin was assayed by microparticle enzyme immunoassay (Abbot Imx, Chicago, IL) with an intra-assay and interassay coefficient of variation of 4.1% and 2.9%, respectively. Homocysteine was measured with the method of fluorescence polarization immunoassay, with an intra-assay and interassay coefficient of variation of 1.4% to 2.2% and 3.7% to 5.2%, respectively.

Statistical Analysis

Data are expressed as mean ± SD. To approximate normal distribution, plasma leptin, homocysteine concentrations, and serum insulin concentration, and 24-h UAE, were logarithmically transformed (used in all calculations) and then back-transformed (used in the presentation of the results). Significant differences between the dippers and nondippers were determined using the Student independent samples t test, or the χ² test where appropriate. Multiple linear regression analysis was used to evaluate the relation of demographic characteristics, echocardiographic measurements, and laboratory variables with nocturnal BP decrease. Spearman correlation
was performed to determine correlation between any of the parameters. All tests were considered to be significant at the level of $P < .05$.

**Results**

Dippers and nondippers did not differ regarding age, sex, BMI, waist-to-hip ratio, smoking status, and duration of EH (Table 1). A significant difference between dippers and nondippers was observed in office SBP (142 ± 149 mm Hg, $P < .05$). Regarding ambulatory BP monitoring data (Table 2) nondippers compared to dippers had significantly greater 24-h SBP by 6.6 mm Hg ($P < .005$), 24-h DBP by 7.6 mm Hg ($P < .005$), 24-h diastolic load by 17% ($P < .005$), daytime DBP by 6 mm Hg ($P < .05$), and diastolic load by 16% ($P < .05$). By the definition, nondippers exhibited significantly greater values of nighttime SBP and DBP and loads, than dippers. The 24-h daytime as well as nighttime heart rate did not differ between the groups. Left ventricular mass index was within normal values for the entire population (110 ± 25 g/m²), whereas the RWT was increased (0.47 ± 0.3). Also, LVMI and RWT values were similar for the dippers and nondippers (107 ± 18 vs. 113 ± 31 g/m² and 0.46 ± 0.3 vs. 0.47 ± 0.3, respectively, $P = NS$ for both groups). Nondippers compared to dippers had significantly increased values of total cholesterol and LDL cholesterol ($P < .05$ for both groups) (Table 3). Also, 24-h UAE was significantly higher in nondipper compared to dipper hypertensives (by 17 mg/24 h, $P < .05$).

**Correlations**

In the entire population, the plasma leptin concentration was positively related with age ($r = 0.27$, $P = .014$), BMI ($r = 0.21$, $P = .04$), fasting serum insulin levels ($r = 0.34$, $P < .005$), homocysteine levels ($r = 0.26$, $P < .05$), 24-h DBP ($r = 0.30$, $P < .005$), and 24-h heart rate ($r = 0.33$, $P < .001$). The homocysteine concentration is significantly related to 24-h SBP and DBP values ($r = 0.44$ and $r = 0.47$, respectively, $P < .005$). Furthermore, UAE was positively related to RWT ($r = 0.31$, $P = .04$). In contrast, homocysteine concentration, serum insulin levels, and UAE did not relate significantly with age, BMI, and LVMI.

In the entire population, nocturnal SBP and DBP decrease did not relate with plasma levels of homocysteine, insulin, and leptin, whereas it negatively related with UAE ($r = -0.35$ and $r = -0.30$, $P < .005$). We applied multiple linear regression models in which age, BMI, LVMI, RWT, plasma levels of leptin and homocysteine, serum level of insulin, and UAE were used as independent variables.
variables and (daytime–nighttime) SBP and DBP decrease as dependent variables. This analysis revealed that only UAE was significantly associated with nocturnal SBP and DBP decrease ($P < .05$).

**Discussion**

The main finding of our study was that in subjects with mild-to-moderate EH, among the risk factors, only UAE rate was significantly related with the absence of the normal circadian BP variation, whereas plasma concentrations of insulin, homocysteine, and leptin do not appear to be influenced by BP.

At present, there are not definite data whether the unfavorable outcome in non-dipper hypertensive subjects is caused by the absence of nocturnal BP decrease or by the accompanied greater hemodynamic load in this setting. Several reports have shown that in these subjects, the pathologic BP circadian variation is associated with LV hypertrophy, coronary heart disease, and cerebrovascular manifestations. In the present study we tried to determine the relation between the absence of nocturnal BP decrease and risk factors such as UAE rate, plasma concentrations of leptin and homocysteine, and serum insulin levels in hypertensive subjects.

Microalbuminuria, a well-recognized marker for adverse cardiovascular outcomes in population-based studies, has been associated with other TOD in hypertensive subjects. In agreement, we found that UAE was positively related with RWT. Furthermore, if we postulate that increased pressure load is among the pathophysiologic factors leading to the early vascular damage of the kidneys, the elevated nocturnal BP may explain the greater incidence of MA in the nondipping hypertensive population. In our study, as well in previous studies, the magnitude of the blunted nighttime BP decrease is also closely related to the subclinical early phase of renal damage. More specifically, the established negative relation between nocturnal BP decrease and UAE means that in the early stages of EH this status probably represents those hypertensive patients who are at greater risk for cardiovascular adverse effects. Also, the finding that the nondipping population of our study exhibited MA but no other subclinical markers of TOD such as increased LVMI or RWT, means that MA could possibly be used as an early indicator of cardiovascular complications in this specific (nondippers) group of hypertensives. However, another concept, implying the primacy of metabolic over hemodynamic factors as the determinants of MA, has also been reported. The MA has been found to correlate with an adverse lipid profile, elevated serum leptin levels, and increased levels of insulin. It was hypothesized that circulating insulin might promote albuminuria by increasing endothelial dysfunction. In elderly non-diabetic subjects, simultaneous occurrence of hyperinsulinemia and MA could identify subjects with an increased risk for coronary artery disease. However, in our study, as well as in other studies, a lack of association between UAE and plasma insulin levels has been demonstrated.

Adipose tissue-derived peptide hormone, termed leptin, has been found to be increased in insulin-resistant states such as obesity and EH. Thus, we found that plasma leptin levels were significantly related with serum levels of insulin. However, the absence of LVH in our study may account, at least partially, for the absence of a significant relation of leptin levels with LVMI or RWT, as a previous study has shown.

Elevated plasma levels of homocysteine have been associated with clinical and subclinical states of atherosclerosis and increased BP. The latter is in accordance with the positive relation observed in our study between homocysteine concentration and 24-h SBP and DBP values.

Except for the independent adverse cardiovascular effects associated with these risk factors, in many cases these factors coexist, interact between them, and synergistically cause hypertension and TOD. In our study, plasma levels of insulin, leptin, and homocysteine were within normal limits and similar for dippers and nondippers, as well as in our hypertensive subjects, newly diagnosed with mild-to-moderate EH. The combination of these parameters perhaps explains the reasons why our patients had normal values of LVMI, RWT, and generally did not exhibit major adverse cardiovascular prognostic indicators, but only minor ones, more specifically

<table>
<thead>
<tr>
<th>Table 3. Laboratory data</th>
<th>Total Population ($n = 106$)</th>
<th>Dippers ($n = 66$)</th>
<th>Nondippers ($n = 40$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>$228 \pm 34$</td>
<td>$213 \pm 24$</td>
<td>$231 \pm 38$</td>
<td>.031</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>$56 \pm 11$</td>
<td>$60 \pm 11$</td>
<td>$55 \pm 12$</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>$148 \pm 29$</td>
<td>$135 \pm 19$</td>
<td>$151 \pm 31$</td>
<td>.024</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>$114 \pm 6.7$</td>
<td>$139 \pm 80$</td>
<td>$139 \pm 80$</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma homocysteine (µmol/L)</td>
<td>$12.3 \pm 6.3$</td>
<td>$13.92 \pm 6$</td>
<td>$14.72 \pm 7$</td>
<td>NS</td>
</tr>
<tr>
<td>Serum insulin (µmol/L)</td>
<td>$13.7 \pm 9$</td>
<td>$12.4 \pm 7$</td>
<td>$15.2 \pm 11$</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma Leptin (ng/mL)</td>
<td>$31.1 \pm 22$</td>
<td>$33.5 \pm 23$</td>
<td>$30.1 \pm 21$</td>
<td>NS</td>
</tr>
<tr>
<td>UAE (mg/24 h)</td>
<td>$22 \pm 14$</td>
<td>$14 \pm 11$</td>
<td>$31 \pm 24$</td>
<td>.03</td>
</tr>
</tbody>
</table>

HDL = high-density lipoprotein; LDL = low-density lipoprotein; UAE = urinary albumin excretion rate; other abbreviation as in Tables 1 and 2.
MA, which furthermore was confined only to the nondipping group. Thus, it is not possible to exclude the chance that, in more advanced stages of EH, some of these risk factors begin to act synergistically and thus accelerate the appearance of TOD.

In conclusion, our study shows that in untreated stage I–II EH patients, with an absence of the normal nocturnal BP decrease, a slight elevation of UAE was detected, but plasma concentrations of insulin, leptin, and homocysteine were within normal limits. Perhaps our findings suggest that MA represents a more specific marker for primary subclinical forms of TOD in the early stages of the nondipping status of hypertension. Further studies are needed to clarify the contribution of these findings in the future evolution and deterioration of adverse cardiovascular effects in this setting.

References

