Seasonal Variation of Fibrinogen in Dipper and Nondipper Hypertensive Patients

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Background—A seasonal variation with higher values in winter has been previously reported in plasma fibrinogen, a recognized marker of the potential risk of myocardial infarction and stroke. The lack of nocturnal decline in blood pressure has also been associated with an increase in cardiovascular events. Accordingly, we have compared the yearly variation of plasma fibrinogen in dipper and nondipper hypertensive patients.

Methods and Results—We studied 1006 stage 1 to 2 hypertensive patients (482 men and 524 women, 53.0±13.4 years of age). Blood pressure was measured every 20 minutes during the day and every 30 minutes at night for 48 consecutive hours. Physical activity was simultaneously evaluated at 1-minute intervals with a wrist actigraph. A blood sample was collected on the same day before starting blood pressure monitoring. The circannual variation of fibrinogen was established for all patients as well as for subgroups of dippers and nondippers (n=513; nocturnal blood pressure decline <10%) by multiple-component analysis. For the whole group of patients, fibrinogen was characterized by a highly significant seasonal variation (P<0.001) with a mean value of 318 mg/dL, double circannual amplitude (extent of predictable change along the year) of 40 mg/dL, and time of peak value in February. Throughout the year, the nondippers showed higher plasma fibrinogen levels than did the dippers (P<0.001).

Conclusions—The elevated plasma fibrinogen levels in nondipper patients appear to be directly related to their increased risk in vascular events, which are more prominent during the late winter months. (Circulation. 2003;108:1101-1106.)

Key Words: fibrinogen  ■  blood pressure  ■  seasons

Clinical trials and epidemiological observations have indicated that elevated plasma fibrinogen levels are strongly correlated with an increased frequency of vascular events. As such, fibrinogen is recognized as a significant parameter for assessing the potential risk of acute myocardial infarction and stroke. Moreover, a seasonal variation has been previously reported in plasma fibrinogen for small groups of mainly elderly subjects, with higher values occurring in the coldest months of the year.2–4 Several investigators have reported a seasonal variation in the incidence of onset of acute myocardial infarction with a peak in winter.5 Colder weather has been shown to alter hemodynamic (blood pressure [BP], sympathetic tone) and hematological (platelet count, fibrinogen) factors favoring arterial thrombosis.3,6,7 Thus, independent studies seem to conclude that the seasonal variation in plasma fibrinogen is related in time with the reported seasonal variation in coronary events.5

Fibrinogen is also characterized by a highly significant circadian variation, correlated in time with that of BP.8 Relevant studies have also shown statistically significant circadian rhythms in thromboxane and prostacyclin production, circulating platelets, platelet aggregation, and clotting and fibrinolytic inhibitors.9 Circadian rhythms of cardiac morbidity due to coronary occlusion seem to be related to these rhythms in clotting and fibrinolytic inhibitors: in the morning, an increase in platelet aggregability, blood viscosity, and concentration of coagulant factors, and a decrease in fibrinolytic activity lead to a state of relative hypercoagulability. Moreover, there is significant correlation between fibrinogen levels and age, whereas aging is also characterized by a progressive reduction in the nocturnal decline of BP.8

The lack of nocturnal decline in BP (nondipping9) has also been related to an increase in end-organ damage and cardiovascular events.10–13 The association between nondipping and target organ damage, however, is still somehow controversial. Thus, some authors have concluded that the lack of nocturnal fall in BP is not associated with an increase in left ventricular mass or in arterial disease independently of age.14 Contradictory results could be partly due to the inability to properly reproduce over time the classification of patients into dippers and nondippers.15 Along these lines, advantages of 48-hour
sampling instead of the most common 24-hour monitoring span in terms of reproducibility of results have been previously documented.15–17 The proper estimation of mean values in more dependent on monitoring span than on sampling rate. Therefore, a better definition of nondipping (by comparing nocturnal and diurnal means) can be obtained by sampling for 48 hours, even if data are obtained at a lower rate, than for just 24 hours.17

Taking into account that fibrinogen is an independent marker of cardiovascular risk, we have quantified its seasonal variation in a serially independent sample of hypertensive patients classified according to their circadian patterns of BP variation determined by ambulatory BP monitoring (ABPM) over 48 consecutive hours.

### Methods

#### Subjects

We studied 1006 patients (482 men and 524 women), 53.0±13.4 years of age (range 19 to 87), with diagnosis of stage 1 or 2 essential hypertension, according to the definition provided by the VI Joint National Committee.18 Among those, 407 patients (40.5%) were not receiving any treatment at the time of their evaluation. All patients received medical care at the Hypertension and Vascular Risk Unit, Hospital Clínico Universitario, Santiago de Compostela, Spain. The sample in this study corresponds to all consecutive patients sent for evaluation at the Ambulatory Monitoring Service of the Unit for 2.5 consecutive years (January 2000 to July 2002), who fulfilled all required criteria for this trial (see following sections). In all cases a complete clinical evaluation was performed following the standardized protocol at the Unit. Shift-workers and patients with either white-coat hypertension according to the definition provided by the VI Joint National Committee,18 stage 3 (severe) arterial hypertension, secondary arterial hypertension, or cardiovascular disorders other than essential hypertension were excluded from analysis.

#### Plasma Fibrinogen

Blood samples were obtained from an antecubital vein in the early morning hours (8 AM to 9 AM) after nocturnal fasting, on the same day before starting ABPM and transported immediately to the laboratory for analysis. Plasma fibrinogen (mg/dL) was determined by the Clauss method,19 with an intraserial analytical coefficient of variation of 4%.

#### BP Assessment

The systolic (SBP) and diastolic BP (DBP) of each patient were automatically measured every 20 minutes during the day (7 AM to 11 PM) and every 30 minutes at night for 48 consecutive hours with a validated SpaceLabs 90207 device (SpaceLabs Inc). Subjects were assessed during their usual day activity (8 AM to 11 PM for most) and sleep at night. They were instructed to go about their usual activities with minimal restrictions but to follow a similar schedule during the 2 days of ABPM. No person was hospitalized during monitoring. BP series were eliminated from analysis when they did not contain at least 70% of valid measurements, and when the patients showed an irregular rest-activity schedule during the 2 days of sampling, an odd sampling with spans of >3 hour without BP measurement, or a night resting span <6 hour or >12 hour. The BP cuff was worn on the nondominant arm with cuff size determined by upper arm circumference at each study visit. ABPM always began between 10 AM and noon.

#### Actigraphy

The patients wore a MiniMotionLogger actigraph (Ambulatory Monitoring Inc) on the dominant wrist to monitor physical activity every min at the time of ABPM. This compact (about half the size of a wrist watch) device functions as an accelerometer. The clock time of the actigraph and the ABPM device were always synchronized through their respective interfaces with the same computer. The information from the actigraph was used to determine diurnal and nocturnal means of BP for each patient according to individual resting time.

### Statistical Methods

BP values were edited according to commonly used criteria for the removal of outliers and measurement errors.20 Patients were classified as nondippers if they had <10% decline in sleep mean compared with awake mean in SBP using all data sampled for 48 consecutive hours. The seasonal variation in plasma fibrinogen was established for all patients as well as for the groups of dippers (n=493) and nondippers (n=513) by multiple components analysis of longitudinal time series,21 a method applicable to nonsinusoidal-shaped time series consisting of values distributed at equal or unequal intervals.

The method produces estimates of the rhythm-adjusted time series mean or MESOR (midline estimating statistic of rhythm, average value of the rhythmic function fitted to the data), as well as the amplitude (one-half the extent of change explainable by rhythmicity) and acrophase (crest time expressed as a lag in time from a designated reference) for every fitted component. When the shape of the rhythm is best approximated by a complex model composed of two or more cosine curves that are harmonics of the fundamental period (here 1 year), the method of multiple components provides three additional summary parameters: the overall amplitude (one-half the difference between the maximum and the minimum values of the best fitted curve), and the orthophase and bathyphase (peak and trough times, respectively, expressed as a lag from January 1).21 Circannual parameters were subsequently compared between groups dippers and nondippers with a nonparametric test developed to compare parameters obtained from multiple components analysis.21 Demographic characteristics provided in the Table were compared among dippers and nondippers by nonparametric testing (qualitative variables) and ANOVA (quantitative variables).

### Results

Results from the Table indicate similar physical characteristics, including body mass index (BMI), among dippers and nondippers. The percentage of regular smokers was slightly lower among nondippers. The percentages of patients who exercised regularly at least once a week and who consumed at least 1 alcoholic beverage weekly were statistically equivalent among dippers and nondippers. Conventional BP values determined at the hospital just before starting ABPM were also similar among these two groups of hypertensive patients.

For the whole group of patients, plasma fibrinogen is characterized by a highly significant seasonal variation (P<0.001) with a mean value of 318 mg/dL, double circannual amplitude (extent of predictable change along the year) of 40 mg/dL, and orthophase (time of peak value) in February. This circannual variation can be best represented by a model that includes components with periods of 12 and 6 months. Figure 1 shows this yearly variation in fibrinogen as well as bimonthly means and standard errors. The nonsinusoidal shaped curve shown in the graph is the best-fitting model determined by multiple-component analysis applied to all original values. The arrow descending from the top horizontal axis denotes the circannual orthophase for fibrinogen (peak time, expressed in months from January 1). The parameters of the seasonal rhythm are given in the table below the graph, which also includes information on the number of patients analyzed (1006), the percent rhythm (PR, percentage of the total variability in the data accounted for by the model fitted to the data), and the probability value from the actigraph.
These results confirm the stable and highly predictable record in February and lower values in September-October. The null-amplitude test. Comparison of the monthly means of plasma fibrinogen by ANOVA further corroborates the significant seasonal variation ($P < 0.001$). We did not find any significant difference in plasma fibrinogen levels among treated and untreated patients. Accordingly, the whole database was analyzed jointly and only divided according to dipping status. The same model of two components with periods of 12 and 6 months also characterizes dippers as well as nondippers analyzed separately (Figure 2). Nondippers (dash line) showed higher plasma fibrinogen throughout the whole year as compared with dippers ($P < 0.001$ from the test for comparison of circannual MESOR among both groups). Apart from this difference, the pattern of predictable circannual variability in fibrinogen is similar for dippers and nondippers. Thus, the circannual orthophase is comparable for dippers and nondippers ($P = 0.470$), indicating that, irrespective of the extent of the nocturnal dip in BP, the highest values of fibrinogen are consistently found in the middle of the winter season. Moreover, comparison of rhythm parameters also indicates the similarity of circannual amplitude among both groups of hypertensive patients ($P = 0.772$), indicating an equivalent extent of seasonal variation of plasma fibrinogen of about 42 mg/dL (Figure 2). Results from ANOVA indicate the statistically significant increase in fibrinogen for nondippers ($P = 0.003$), after correcting for potential confounding factors including physical exercise, alcohol consumption, smoking status, use of antihypertensive medication, and gender.

### Discussion

Fibrinogen levels in the population studied in this investigation show a significant seasonal variation, with higher levels recorded in February and lower values in September-October. These results confirm the stable and highly predictable circannual rhythm in fibrinogen previously reported, among others, in two independent studies on elderly subjects investigated for one year,\(^2,3\) in a cross-sectional study on subjects aged 55 years and over,\(^4\) and in a study on serially independent sampling of hospitalized military veterans covering 7 years of data.\(^22\) The seasonal predictable variation in fibrinogen levels in our study (40 mg/dL) is somehow smaller than that reported by Stout and Crawford (71 mg/dL) in elderly subjects,\(^2\) although higher than that observed by Woodhouse et al (13 mg/dL).\(^3\) and by van der Bom et al (31 mg/dL).\(^4\) Differences in the study populations and environmental situations may somehow explain these differences. In the Northwick Park Heart Study, an increase in plasma fibrinogen concentration above one standard deviation (59 mg/dL) increased the risk of death from ischemic heart disease in the first 5 years of follow-up by 67%.\(^23\) From this estimate, the seasonal difference seen in our study (40 mg/dL) would be associated with a 45% increase in risk during the winter. Although extrapolation of results from one study to another must be cautious, these figures show how the seasonal variation in a cardiovascular-disease risk factor could have a marked effect in mortality.

Apart from the seasonal variation shown in many other parameters related to cardiovascular risk, previous studies have shown that the seasonal variation in fibrinogen is timely correlated with the reported yearly variation in the incidence of onset of acute myocardial infarction and coronary artery disease deaths.\(^5\) The mechanisms underlying the seasonal variation in plasma fibrinogen still remain unknown. Woodhouse et al\(^11\) suggested that this variation might be induced by winter respiratory infections via activation of the acute phase response. However, recent studies on the seasonal variation of several responses to infection (including C-reactive protein, white cell count, interleukin-6, factor VII, soluble P-selectin, and plasminogen activator inhibitor) have concluded that there is no evidence that winter infections are

<table>
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<tr>
<th>Variable</th>
<th>All</th>
<th>Dippers</th>
<th>Nondippers</th>
<th>$P^*$</th>
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<td>N</td>
<td>1006</td>
<td>493</td>
<td>513</td>
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<td>Age, y</td>
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<td>44.0</td>
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<td>Height, cm</td>
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<td>162.8±9.8</td>
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<td>Weight, kg</td>
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<td>Waist, cm</td>
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<td>107.5±9.6</td>
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<td>SBP, mm Hg†</td>
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<td>152.0±22.0</td>
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<td>DBP, mm Hg†</td>
<td>86.6±10.9</td>
<td>86.4±11.5</td>
<td>86.8±10.2</td>
<td>0.536</td>
</tr>
</tbody>
</table>

Values are given as mean±SD or percent of patients. Drinkers: at least 1 alcoholic beverage once a week.

* $P$ for comparison, dippers vs nondippers.
†Values provided correspond to the average of 6 conventional BP measurements obtained at the clinic for each patient before starting ABPM.
responsible for the seasonal variation in fibrinogen or C-reactive protein.\textsuperscript{24,25}

Results from our study are based on a population of sequential hypertensive patients evaluated along the span of 2.5 consecutive years. The advantage of this cross-sectional design, as demonstrated in previous studies,\textsuperscript{4,22} is that all measurements are independent from each other. An inherent disadvantage of this design is that some characteristics of the study population may differ during the year. However, most potential confounders that could affect fibrinogen (age, gender, BMI, tobacco and alcohol consumption) can be easily and accurately measured, and as previously reported\textsuperscript{3} and also corroborated in our study, they do not present a seasonal rhythm. On the other hand, the seasonal variation in environmental temperature at our setting is rather small (11°C) as compared with that reported in other studies. This small change in temperature could somehow explain the lack of seasonal variation in ambulatory-monitored BP from this study, corroborating early reports from subjects studied at the same geographic location.\textsuperscript{26} In any event, previous results from highly controlled studies could not establish any clear relationship between the seasonal variation in fibrinogen and changes in either body or environmental temperature.\textsuperscript{3}

While corroborating the seasonal variation of high amplitude that characterizes plasma fibrinogen, this study presents first evidence on the significant elevation of fibrinogen in hypertensive nondipper patients as compared with dippers (Figure 2). Although the mechanism(s) involved in the lack of nocturnal dip is still unclear,\textsuperscript{27} O’Brien et al\textsuperscript{10} reported that nondipper hypertensive subjects had a significantly larger risk to suffer a stroke than dippers. Verdecchia et al\textsuperscript{11} showed that, after an average follow-up period of 3.2 years, hypertensive patients who were characterized as nondippers had nearly 3 times as many adverse events as the hypertensive dippers. More recently, Staessen et al\textsuperscript{12} presented results from a subgroup of 808 patients who underwent ABPM at study baseline and were included in the Syst-Eur trial. Patients who lacked a nocturnal decline in SBP had a greater incidence of stroke and myocardial infarction than patients who had a normal dipping pattern. The last evaluation of the data from the Ohasama study has indicated, after an average follow-up of 9.2 years, that a 5% decrease in the decline in nocturnal BP was associated with a 31% greater risk of cardiovascular mortality in hypertensive patients.\textsuperscript{13} What is even more relevant, dipper hypertensives had a relative hazard of car-

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Figure 1. Seasonal variation of plasma fibrinogen in patients with mild-moderate essential hypertension. †Months from January 1st.
diovascular mortality (2.37) similar to that of nondipper normotensives (2.16). These results indicate that cardiovascular risk could be influenced not just by BP elevation, but also by an abnormal circadian BP variability. Thus, to clarify how to treat nondippers has become an issue of marked relevancy.

The potential reduction in cardiovascular risk associated with a normalization in the circadian variability of BP (changing a nondipper pattern into dipper) has not yet been clearly established. However, recent results from the HOPE substudy where patients were evaluated by ABPM indicated a significant BP reduction mainly during nocturnal resting hours. The authors suggested that the beneficial effects on cardiovascular morbidity and mortality seen in the HOPE study may be related to the 8% increase in the night/day ratio of BP seen after ramipril was administered at bedtime. Another relevant study where the nondipper BP profile in patients with chronic renal failure was normalized after evening but not after morning dosing of isradipine did not provide any evaluation of potential changes in cardiovascular risk, mainly due to the short period of active treatment (4 weeks).

Our results are based on monitoring for 48 consecutive hours, in contrast with the most common 24-hour ABPM. As a compromise with practicability, monitoring over at least 48 hours has been shown to present advantages in the analysis of BP variability, diagnosis of disease, and evaluation of a patient’s response to treatment. Previous findings suggested that ABPM done only for 24 hour may be too short to characterize accurately the features of the day/night variation in BP, including the precise period of that variation. Along these lines, it has been recently demonstrated that, in patients evaluated by ABPM for the first time, there is a highly significant reduction during the second day of monitoring as compared with the first in the diurnal mean of BP. This “ABPM effect” increases SBP and DBP, on the average, by 7 and 5 mm Hg, respectively, during the first 4 hours of measurement, and it remains as statistically significant for at
least the first 9 hours of sampling. As a consequence of the decrease in BP during diurnal activity but not during nocturnal resting hours, 35% of the patients characterized as dippers during the first day of ABPM became nondippers in the second day of measurement. This result justifies in part the lack of reproducibility in the classification of patients according to their dipping status, as well as the underestimation of the true percentage of nondippers in many previous studies based on ABPM for 24 hour.

In summary, results indicate a highly significant seasonal variation in plasma fibrinogen, with higher values during the winter as compared with the rest of the year. Moreover, plasma fibrinogen is significantly elevated in all seasons in nondipper hypertensive patients as compared with dippers. These results could support the association between the lack of nocturnal decline in BP with an increase in cardiovascular events, taking also into account that the circannual variation in fibrinogen is timely correlated with the reported yearly variation in coronary events. Considering the high prevalence of nondippers in this trial (51%), this study provides further evidence on the need to identify an altered circadian pattern of BP variability, and to establish proper therapeutic strategies to treat nondipper hypertensive patients.

References
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