Anemia as a Factor That Elevates Plasma Brain Natriuretic Peptide Concentration in Apparently Healthy Subjects

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SUMMARY

Plasma brain natriuretic peptide (BNP) is widely used as a biomarker of heart failure (HF); however, its concentration is often found to be high even in apparently healthy subjects and little is known about which factors contribute to physiological change in plasma BNP concentration in subjects without HF. We examined the effects of gender, age, and anemia on plasma BNP concentration in apparently healthy subjects. The study population consisted of 1036 healthy subjects who underwent an annual health examination at their company in 2005. There were 874 women, ranging in age from 30 to 63 years (mean, 41 years). Plasma BNP concentration was abnormal (> 18.4 pg/mL) in 292 subjects. The incidence was significantly higher in women than in men (31% versus 14%, P < 0.01). Mean plasma BNP concentration was higher in women than in men. The difference in plasma BNP concentration was associated with the difference in blood hemoglobin and age. Logarithmically transformed BNP concentration correlated inversely with blood hemoglobin (r = -0.30, P < 0.01 for all; r = -0.21, P < 0.01 for women; r = -0.20, P < 0.01 for men). By multiple regression analysis, logarithmically transformed BNP concentration correlated with hemoglobin, age, and gender. In conclusion, anemia is likely a critical determinant that elevates plasma BNP concentration in apparently healthy subjects. (Int Heart J 2008; 49: 577-586)

Key words: Anemia, Brain natriuretic peptide, Hemoglobin

BRAIN natriuretic peptide (BNP) is a cardiac neurohormone specifically secreted from the ventricles in response to increased transmural wall stress.1) BNP functions as a protective factor in such conditions, but its plasma concentration increases in proportion to the degree of LV dysfunction or heart failure (HF).2,3) Measurements of plasma BNP concentration are useful to detect asymp-
tomatic left ventricular (LV) dysfunction,\textsuperscript{2,4-6} to diagnose HF,\textsuperscript{7,8} and even as a guidance for the treatment of HF.\textsuperscript{9} Most recently, several studies have shown plasma BNP concentration increases in other cardiovascular disease states such as ischemia, arrhythmias, fibrosis, cardiac hypertrophy, and coronary endothelial dysfunction, suggesting the possibility that the plasma BNP concentration may be useful as a biomarker of other cardiovascular diseases or the prognosis for future cardiovascular events.\textsuperscript{10-12}

Clinical use of BNP as a biomarker in HF is expanding. However, the specificity of BNP for HF is not robust, suggesting that there are other mechanisms beyond simple increased transmural wall stress. A few studies have suggested that both aging and gender influence plasma BNP concentration,\textsuperscript{13-16} while another group has suggested that anemia may influence plasma BNP concentration in patients with diastolic HF.\textsuperscript{17} However, the role of anemia in the plasma BNP concentration has not been clarified in subjects without cardiovascular diseases. If plasma BNP concentration increases in the presence of anemia, its dependence on age and gender may be at least partially explained by the blood hemoglobin level.

Therefore, we assessed the influences of age, gender, and blood hemoglobin on plasma BNP concentration in healthy subjects without cardiovascular diseases. The data were analyzed to clarify whether the gender-related difference in plasma BNP concentration is explained by the difference in blood hemoglobin level between women and men.

\textbf{METHODS}

\textbf{Subjects:} The study population consisted of 1036 apparently healthy subjects who had undergone a company-sponsored annual health examination in 2005. There were 874 women and 162 men, ranging in age from 30 to 63 years (mean, 41 years). Eligible subjects were defined as those who did not have a history of HF, coronary artery disease, atrial fibrillation, or renal failure. Electrocardiograms and chest roentgenograms were obtained in all subjects, and no significant abnormal findings were evident in any. Height, weight, and casual blood pressure were measured in all subjects just prior to the blood sampling. Venous blood was collected at the time of the examination. We used creatinine clearance (\(C_{\text{Cr}}\)) calculated by the Cockroft-Gault equation for evaluating renal function because most subjects in our study were free from renal disease.\textsuperscript{18} The Cockroft-Gault equation was reported to be more accurate than the MDRD-prediction equation of GFR in subjects with a normal or increased GFR.\textsuperscript{19} All subjects gave informed consent in advance to their participation.

\textbf{Measurement of plasma BNP concentration:} Plasma BNP concentration was
measured using noncompetitive immunoradiometric assays for human BNP that are based on a 2-site sandwich antibody system as previously described (Shionogi Co. Ltd., Osaka, Japan).20)

**Echocardiography:** After all of the blood sampling data were obtained, conventional M-mode and 2-dimensional echocardiographic study with Doppler color flow imaging was recommended in subjects with an elevated plasma BNP concentration. All subjects with BNP > 18.4 pg/mL were free from symptoms and signs of HF in our study population. Since the capacity of the echocardiography laboratory in the clinic of the company was limited, 34 subjects whose plasma BNP concentration was > 50 pg/mL were selected for echocardiography. Three subjects declined so echocardiographic study was performed in 31 of the 34 subjects. Ejection fraction (EF) was measured in M-mode LV echograms as previously described.21) LV mass was calculated using the following equation: LV Mass = 0.8 \times [1.04 (LVIDD+IVST+PWT)^3 - (LVIDD)^3] + 0.6, where LVIDD represents LV end-diastolic internal dimension, and IVST and PWT indicate the end-diastolic thickness of the interventricular septum and LV posterior wall, respectively.22) LV mass index (g/m²) is the ratio of LV mass to body surface area. The pulsed Doppler mitral flow velocity pattern was recorded to measure the ratio of peak early diastolic filling velocity to peak filling velocity at atrial contraction (E/A ratio).

**Statistical analysis:** All statistical analyses were performed using a commercially available statistical software (STATVIEW version 5.0, SAS Institute Inc., Cary, North Carolina). Values are reported as the mean ± SD. Common log transformation was used in the regression analyses of plasma BNP concentration versus blood hemoglobin, age, systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), and C Cr. Multiple regression analysis was used to identify variables which correlated with logarithmically transformed BNP concentration. Hemoglobin, age, gender, SBP, DBP, BMI, and C Cr were included as independent variables. Differences between women and men were assessed using the Student t test. A P < 0.05 was considered to be statistically significant.

**RESULTS**

**Plasma BNP concentrations:** Plasma BNP concentration was elevated beyond the normal range (> 18.4 pg/mL) in 292 subjects, and > 50 pg/mL in 34 subjects. Absence of symptoms of HF was reconfirmed by cardiologists in the 34 subjects, 31 of whom (29 women, 2 men) agreed to be studied by echo Doppler. EF was normal (> 50%) without any wall motion abnormalities or LV dilatation (end diastolic LV dimension ≥ 55 mm) in all 31 subjects. Echo Doppler studies revealed mild aortic regurgitation in one subject, but no other abnormal findings
were evident such as changes in LV and left atrial chamber size, hypertrophy, valve diseases, and mitral flow velocity pattern.

**Comparison between women and men:** Mean age was older in men than in women. SBP, DBP, BMI, and \( C_{Cr} \) were higher in men than in women. Plasma BNP concentration was higher in women than in men. The incidence of elevated plasma BNP concentration (> 18.4 pg/mL) was also higher in women than in men. Blood hemoglobin and hematocrit were higher in men than in women (Table I). Plasma iron concentration was higher in men than in women. Twelve women had anemia. Anemia was defined as Hb < 12 g/dL in women and Hb < 13 g/dL in men according to the World Health Organization criteria.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Women</th>
<th>Men</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>874</td>
<td>162</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40 ± 3</td>
<td>47 ± 3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>21 ± 3</td>
<td>23 ± 3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>113 ± 36</td>
<td>122 ± 16</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>67 ± 11</td>
<td>78 ± 12</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.2 ± 1.3</td>
<td>15.5 ± 0.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>40.6 ± 3.3</td>
<td>47.0 ± 2.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Fe (µg/dL)</td>
<td>105.4 ± 47.6</td>
<td>128.6 ± 40.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Incidence of plasma BNP &gt; 18.4 pg/mL (%)</td>
<td>31</td>
<td>14</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Plasma BNP (pg/mL)</td>
<td>16.7 ± 15.4</td>
<td>10.0 ± 1.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>( C_{Cr} ) (mL/minute)</td>
<td>106.0 ± 24.6</td>
<td>113.5 ± 32.0</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Values are the mean ± SD. BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Fe, serum iron concentration; BNP, brain natriuretic peptide; and \( C_{Cr} \), creatinine clearance.
Effects of aging and anemia on plasma BNP concentration: Logarithmically transformed plasma BNP concentration correlated inversely with blood hemoglobin ($r = -0.30, P < 0.01$ for all; $r = -0.21, P < 0.01$ for women; $r = -0.20, P < 0.01$ for men) (Figure 1). Logarithmically transformed BNP concentration correlated with age ($r = 0.11, P < 0.01$ for all; $r = 0.13, P < 0.01$ for women; $r = 0.29, P < 0.01$ for men) (Table II, Figure 2). Logarithmically transformed BNP concentration did not correlate with SBP, DBP, BMI, or $C_Cr$ (Table II). To identify independent factors that determine plasma BNP concentration, multiple regression analysis was used. At first, we performed multiple regression analysis in the whole study population with logarithmically transformed BNP concentration as the dependent variable and hemoglobin, age, gender, SBP, DBP, BMI and $C_Cr$ as independent variables. Hemoglobin, age, and gender were factors associated with plasma BNP
concentration. Second, we performed separate multiple regression analyses for men and women respectively. Logarithmically transformed BNP concentration correlated with blood hemoglobin and age in both men and women (Table III).

**Correlation between echocardiographic parameter and plasma BNP concentration:** Echo and Doppler parameters were compared with logarithmically transformed BNP concentration in the 31 subjects in whom both data were obtained. Logarithmically transformed BNP concentration did not correlate with LV mass index \((r = 0.08, P = 0.66)\), EF \((r = 0.28, P = 0.13)\), LVDd \((r = 0.07, P = 0.71)\), or E/A ratio \((r = 0.02, P = 0.92)\).

**Effects of gender on relationships between anemia and plasma BNP concentration:** The simple regression lines of the relationships between plasma BNP concentration and blood hemoglobin were determined in men and women, respectively.
The female line was located slightly above but quite close to the male line (Figure 3).

**DISCUSSION**

In this study, plasma BNP concentration was higher in women than in men, and associated better with blood hemoglobin than with age or gender. There was an independent association between blood hemoglobin and plasma BNP concentrations in healthy subjects. These findings indicate anemia is a main contributor that elevates plasma BNP concentration in apparently healthy subjects, and the gender-related difference in plasma BNP concentration appeared to be at least partially explained by the difference in blood hemoglobin.

**Effects of anemia on BNP concentrations:** A previous study showed an inverse correlation between plasma BNP concentration and blood hemoglobin in patients with diastolic HF; however, the relation was unclear in subjects without cardiovascular diseases. In this study, we showed that logarithmically transformed plasma BNP concentration correlated inversely with blood hemoglobin in healthy subjects. Although earlier studies have shown that plasma BNP concentration increases with age and mean age was older in men than in women in this study, our study results suggested plasma BNP concentration associated more strongly with blood hemoglobin than with age. The exact mechanisms for the association cannot be explained from this study, thus, we can only speculate about the mechanisms. First, cardiac output is usually increased in patients with anemia to meet the oxygen demand of the peripheral circulation. The degree of anemia was not severe enough in our patients to support the idea that anemia initiated myocardial lactate production and hence myocardial ischemia. However, increased cardiac output may well increase transmural wall stress even in the absence of myocardial ischemia, and hence facilitating the synthesis and release of BNP in the ventricle. Alternatively, natriuretic peptides may be involved in the regulation of volume homeostasis. Anemia may modulate neurohumoral activation. This is a reason why anemia is likely a risk factor of HF. Anemia deteriorates cardiac function, because it causes cardiac stress through tachycardia and increased cardiac output. It may reduce renal blood flow and fluid retention, adding further stress to the heart. These are the reasons why the normalization of blood hemoglobin levels using erythropoietin and/or iron supplements improves the symptoms of HF, exercise tolerance, and quality of life in patients with HF. Plasma BNP concentration correlated more strongly with blood hemoglobin than with age in this study. Although one might think anemia is a stronger contributor to plasma BNP concentration than aging, the correlation coefficient may well depend on the population of the subjects. Thus, the data may
not be sufficient to conclude so, but we may safely conclude that anemia is at least as important as aging, as a contributor to plasma BNP concentration.

**Effects of gender on BNP concentration:** In healthy subjects, women had a higher plasma BNP concentration than men, which is consistent with previous findings reported by other investigators.\(^{16,24}\) Although mean age was older in men than in women, plasma BNP concentration was higher in women than in men in this study. Our data indicate that anemia contributes to the higher plasma BNP levels in women than in men, but gender itself is also an independent factor that determines plasma BNP levels. Plasma BNP concentration was shown to have increased in women who were taking hormone replacement therapy, suggesting a relationship between hormone replacement therapy and plasma BNP concentration.\(^{16}\) Female sex hormones may be another cause of elevated BNP levels in women because many women in our study population are premenopausal. However, we did not measure female sex hormones and therefore cannot comment on the relationship between female sex hormones and plasma BNP concentration. Further studies are thus necessary.

**Study limitations:** Four limitations of the study are noted. First, this study was confined to subjects 35 to 63 years of age, and elderly subjects were not included. This may at least partially account for the relatively low correlation between plasma BNP concentration and age. Second, most of the subjects were female (84% of all subjects). Third, the mean age and SBP were slightly higher in men than in women. However, the effects of these factors both work to elevate the plasma BNP concentration in men rather than in women. Thus, it is unlikely either factor contributes to the gender-related difference in plasma BNP concentration. Finally, echocardiographic data on LV function were not necessarily available in all subjects. Data were available in 31 subjects with a plasma BNP concentration of 50 pg/mL or greater, and were considered normal in all subjects. Thus, the lack of echocardiographic data in subjects with a plasma BNP concentration smaller than 50 pg/mL should hardly affect the conclusions of the study, although few, if any, subjects may have LV functional abnormalities.

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**REFERENCES**