Can Exercise-Induced Changes in B-Type Natriuretic Peptides Be Used to Detect Cardiac Ischemia?

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Abstract

Background: We reviewed the current medical literature that pertained to the question of whether myocardial ischemia triggers the release of B-type natriuretic peptides (BNPs) and, in particular, whether transient exercise-induced ischemia can be detected by the measurement of changes in these biomarkers. BNPs are well-established as markers of left ventricular dysfunction, particularly heart failure. There is accumulating evidence that various conditions with the common denominator of myocardial ischemia are also associated with increased circulating levels of these peptides.

Methods and Results: Recently published methods and results, which includes our published and unpublished data, were reviewed.

Conclusion: The results show that exercise-induced ischemia or its associated regional wall-motion abnormalities trigger the release of BNPs and that the measurement of plasma levels of N-terminal pro brain natriuretic peptide and BNP before and immediately after symptom-limited exercise can distinguish patients with and without ischemia with a high degree of accuracy.

Keywords: Natriuretic peptide, cardiac ischemia, exercise stress testing.
than angiographically demonstrated coronary stenosis, because stenosis may be present in the absence of ischemia and ischemia may be present in the absence of the stenosis. In addition, it is ischemia, not coronary stenosis per se, that is presumed to produce reversible abnormalities on the exercise electrocardiogram. Thus, in summary, there is accumulating evidence that the sensitivity of the exercise electrocardiogram for the detection of ischemia is considerably lower than has generally been believed.

The natriuretic peptides are a family of chemically related neurohormones that are produced predominantly in the heart. Biochemical details of these compounds have been described elsewhere. It has been well-established that levels of these peptides are elevated in circumstances of volume or pressure overload of the ventricle (for example, congestive heart failure) \textsuperscript{6-8}; the triggering mechanism is believed to be an increase in wall stress with accompanying myocyte stretch that leads to BNP gene transcription by way of a p38 mitogen-activated protein kinase mechanism.\textsuperscript{9}

**B-Type Natriuretic Peptides (BNPs) as Biomarkers of Ischemia**

**Experimental and In Vitro Evidence**

In 1994, Toth et al\textsuperscript{10} demonstrated that hypoxia increases BNP secretion in isolated rat myocardium, which provided the first evidence that stimuli other than increased wall stress might trigger BNP release. More recently, D'Souza et al,\textsuperscript{11} who used isolated perfused rat hearts, found that BNP concentrations in coronary effluent during reperfusion correlated with the duration of induced myocardial ischemia. Of particular interest was the finding that short periods of ischemia (2–5 minutes) were not associated with rises in end-diastolic pressure, which suggests that ischemia per se, rather than increases in wall stress as the result of ischemia, triggers BNP release. This study also confirmed the very rapid release of BNP from ischemic myocardium. Goetze et al\textsuperscript{12-13} have demonstrated subsequently in a porcine model that the induction of myocardial ischemia in hearts with normal ventricular function results in a rapid and significant rise in BNP gene expression in the affected tissues and in isolated perfused ventricular myocytes that are incubated in a hypoxic medium.

**Clinical Evidence**

In the clinical setting, various investigations have now shown elevations of BNP and N-terminal pro-brain natriuretic peptide (NT-proBNP) in a number of other pathologic conditions in the absence of overt left ventricular failure; among these conditions are myocardial infarction,\textsuperscript{14-15} unstable angina,\textsuperscript{16-18} diastolic dysfunction,\textsuperscript{19-20} and percutaneous coronary angioplasty.\textsuperscript{21-22} Of note, Tateishi et al\textsuperscript{22} observed that coronary angiography alone, without balloon angioplasty, did not result in elevations of BNP; a plausible explanation for this observation is that vessel occlusion by balloon inflation during angioplasty produces transient tissue ischemia, whereas angiography alone does not.

Few studies have examined the effect of exercise on cardiac markers in plasma. Four studies examined the effect of single episodes of exercise on BNP levels \textsuperscript{23-26}; of these, 2 studies included patients with coronary artery disease (CAD) and data on nuclear perfusion imaging.\textsuperscript{25-26} Although there was a trend toward increases in BNP in patients compared with normal control subjects, the studies were limited by small sample sizes, unmatched control subjects, submaximal work loads and peak heart rates, and a lack of documentation of ischemia.

Our laboratory recently performed a study to test the hypothesis that transient, exercise-induced ischemia would trigger the release of BNP and NT-proBNP, either directly or through the induction of regional wall motion abnormalities, and that increases in these peptides after exercise might be a marker of ischemia.\textsuperscript{27} We studied 21 healthy volunteers and 74 patients with known CAD (most of whom were diagnosed by coronary angiography), normal left ventricular function, and normal resting levels of NT-proBNP and BNP who were referred for exercise testing with radionuclide imaging. Resting and post-exercise blood samples (<1, 10, 30, and 60 minutes after exercise) were analyzed in batches for NT-proBNP, with the Roche electrochemiluminescent immunoassay on an Elecsys 1010 autoanalyzer, the Shionogi radioimmunoassay, and the Biosite fluorescent point-of-care BNP immunoassay. The following data were coefficients of variation for the assays: NT-proBNP, 1.3%–2.4%; BNP, 11.2%–14.6%.\textsuperscript{28}

All patients and volunteers underwent symptom-limited exercise testing; the CAD patient group also underwent concurrent radionuclide single photon emission computed tomography (SPECT) imaging. Patients with CAD were divided into 2 groups, ischemic (n = 40) and nonischemic (n = 34), on the basis of the presence or absence of inducible ischemia on radionuclide imaging.

Volunteer blood was analyzed for NT-proBNP only, and pre-exercise (baseline) levels were within normal limits for all subjects. In the ischemic and nonischemic patient groups, although baseline
levels of NT-proBNP and BNP were within normal limits, median levels of both peptides were significantly higher in the ischemic group (NT-proBNP, 120.5 pg/mL vs 53.5 pg/mL; \( P < .0001 \); BNP, 40.5 pg/mL vs 16.5 pg/mL; \( P < .001 \)). Interquartile ranges showed no overlap in NT-proBNP values and only modest overlap in BNP values. Resting NT-proBNP values were lower in the healthy volunteers (median, 25 pg/mL) than in the CAD patient groups (\( P = .0053 \) vs nonischemic healthy), which is consistent with their much younger age.27

A comparison of the incremental rise of NT-proBNP (\( \Delta \)NT-proBNP) between patients with and without reversible ischemia at 1, 10, 30, and 60 minutes after exercise showed that maximal \( \Delta \)NT-proBNP was already observed at 1 minute after exercise (Fig. 1; unpublished data). This difference in \( \Delta \)NT-proBNP is statistically significant. To address the contribution of previous myocardial infarction to the exercise-induced increases in the peptides, a subset analysis of the ischemic patients without fixed defects on nuclear images was conducted and found that neither resting nor post-exercise levels differed significantly from the ischemic group as a whole.

\[ \Delta \text{NT-proBNP} = \text{post-exercise value} - \text{resting value} \]

Fig. 1. Kinetics of \( \Delta \)NT-proBNP in patients with (circles) and without (squares) reversible ischemia.

At approximately 1 minute after exercise, the median \( \Delta \)NT-proBNP in the healthy volunteers and nonischemic patients was not statistically significant (5 pg/mL vs 4 pg/mL; \( P = \) not significant; Fig. 2).27 However, the \( \Delta \)NT-proBNP and \( \Delta \)BNP in the ischemic patient group were significantly higher than in the nonischemic patient group (\( \Delta \)NT-proBNP, 14.5 pg/mL vs 4 pg/mL [\( P < .0001 \)]; \( \Delta \)BNP, 36.5 pg/mL vs 7.5 pg/mL [\( P < .0001 \)]; Fig. 2).27 As with resting levels, there was no overlap in the interquartile ranges for NT-proBNP and modest overlap for BNP.

Fig. 2. (A) Changes in NT proBNP and (B) BNP in nonischemic and ischemic subjects. Ranges, medians and 25th–75th percentiles are shown. Reproduced from reference 27 with permission from American College of Cardiology Foundation.

To evaluate the ability of \( \Delta \)NT-proBNP and \( \Delta \)BNP levels to predict the presence or absence of ischemia in individual patients, we constructed receiver operator characteristic curves for each peptide (Fig. 3; unpublished data) for a subset of 72 patients. The area under the receiver operator characteristic curve assesses the overall accuracy of each assay’s ability to detect ischemia; for \( \Delta \)NT-proBNP, it was 0.806 (95% CI, 0.705–0.907); for Biosite, the \( \Delta \)BNP was 0.685 (95% CI, 0.559–0.810), and for
Shionogi radioimmunoassay, the ΔBNP was 0.654 (95% CI, 0.525–0.783). Although there was a trend towards higher area under the receiver operator characteristic curve for ΔNT-proBNP compared with ΔABNP for the detection of ischemia, the differences were not statistically significant in this subset of 72 patients.

An analysis of the complete series of 74 patients demonstrated that at equivalent specificity of 58.8%, ΔNT-proBNP >5 pg/mL showed 2.4-fold higher sensitivity, and ΔABNP >10 pg/mL showed 2.1-fold higher sensitivity, when compared with ≥1 mm ST depression on electrocardiography. Thus, a ΔNT-proBNP of >5 pg/mL or ΔABNP of >10 pg/mL is associated with an increased positive likelihood ratio of approximately 2.0 for ischemia in patients who undergo exercise-stress testing (Table 1).

**TABLE 1.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Predictive Value (%)</th>
<th>Likelihood Ratio</th>
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<td>Predictive Value (%)</td>
<td></td>
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<td>Positive</td>
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<tr>
<td>Predictive Value (%)</td>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
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<td>ΔNT-proBNP &gt;5 pg/mL</td>
<td>90.0</td>
<td>58.8</td>
<td>72.0</td>
<td>83.3</td>
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<tr>
<td>ΔABNP &gt;10 pg/mL</td>
<td>80.0</td>
<td>58.8</td>
<td>69.6</td>
<td>71.4</td>
</tr>
<tr>
<td>≥1-mm ST depression on electrocardiogram</td>
<td>37.5</td>
<td>58.8</td>
<td>51.7</td>
<td>44.4</td>
</tr>
</tbody>
</table>

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* Change after exercise in N-terminal fragment of brain natriuretic peptide pro-hormone.
† Change after exercise in brain natriuretic peptide (carboxy-terminal active fragment).
To assess the association of ΔNT-proBNP and ΔBNP with the extent and severity of ischemia, we examined the relationship of the delta peptide levels with sum difference scores (SDS), the computer analysis of perfusion images that assesses both size and severity of reversible defects. We found a strong correlation between the delta peptide levels and SDS scores (Pearson r = 0.33; P = .004), which suggests that the greater the extent and severity of ischemia, the greater the rise in BNP levels. Results of this analysis are shown in Figure 4 (unpublished data).

Our findings suggested that exercise-induced ischemia results in detectable increases in levels of B-type natriuretic peptides. Although we cannot exclude the possibility that the immediate proximate trigger is ischemia-induced regional wall motion abnormalities, all patients in our study had normal resting left-ventricular function, and other evidence cited earlier, particularly the in vitro studies of isolated myocardial cells, suggests that the trigger may be ischemia per se. Of particular note is that the median absolute ΔNT-proBNP in the nonischemic patient group was almost identical to that of the healthy volunteers, despite marked differences in age, exercise capacity, maximal heart rates, and resting NT-proBNP levels in the nonischemic patients that were more than twice as high as those of the volunteers. On the other hand, an unexpected finding revealed that median levels of both NT-proBNP (120.5 vs 53.5 pg/mL) and BNP (40.5 vs 16.5 pg/mL) were significantly higher at rest in the group of patients with inducible ischemia than in the group without. If, as our findings suggest, ischemia triggers the release of these natriuretic peptides, patients with ischemia on scans may have higher baseline levels of NT-proBNP and BNP because of chronic or recurrent episodes of ischemia before testing.

**Corroborative Studies**

Since completion of our study, several other investigators have found similar associations between levels of NT-proBNP and/or BNP and inducible ischemia. Bibbins-Domingo et al. measured resting levels of BNP in 355 patients with stable CAD who underwent subsequent exercise treadmill testing with exercise echocardiography. Of this group, 113 patients (32%) had inducible ischemia. Those patients in the highest quartile of resting levels (≥105 pg/mL) had double the risk of having ischemia...
(relative risk, 2.0; 95% CI, 1.20–2.6; \( P = .008 \)) compared with those in the lowest quartile (\( \leq 16.4 \) pg/mL). Of interest was that the association between resting BNP and inducible ischemia in this study group held only for patients with a history of previous myocardial infarction. Asada et al\(^\text{30}\) studied 317 patients who were undergoing dobutamine stress echocardiography and measured BNP levels before and immediately after dobutamine stress. Thirty-one of their patients (10%) had ischemia and the risk of ischemia that was correlated with increasing tertiles of resting BNP levels (4%, 9%, and 16%, respectively; chi-squared test for trend = 8; \( P = .0059 \)); interestingly, changes in BNP with dobutamine stress were not associated with ischemia. Palumbo et al\(^\text{31}\) measured BNP levels at rest in 4 groups: (group A) patients with stable angina and normal 201Thallium SPECT stress imaging, (group B) patients with exertional angina and reversible defects on imaging, (group C) patients with fixed defects on imaging, and (group D) normal volunteers. Left ventricular ejection fractions were not different among the 4 groups. Compared with the normal volunteers, BNP levels increased progressively in groups A, B, and C, with the highest levels in group C, which suggests that both ischemia and infarction are associated with elevations in resting BNP levels. Weber et al\(^\text{32}\) measured NT-proBNP at rest and at 15 minutes after exercise in patients who were undergoing 201Thallium SPECT stress imaging and subsequent coronary angiography. They found significantly increased NT-proBNP levels in patients with either reversible defects on imaging or with significant coronary stenosis on angiography. Furthermore, there was a significant correlation (Spearman \( r = 0.418; \ P < .01 \)) between the peptide levels and the extent of ischemia on imaging, which was measured as a percentage of total myocardium. In addition, resting NT-proBNP levels were linked strongly to the severity of coronary disease: Median levels were 148 pg/mL, 269 pg/mL, and 624 pg/mL in patients with no CAD or 1/2- or 3-vessel CAD (\( P < .001 \)). They found no significant differences between groups in the change in BNP at 15 minutes after exercise compared with the resting level. Interestingly, the kinetics that we observed in our study (Fig. 1) would suggest that a sample drawn at this time interval might fail to detect the increase in peptide levels that are seen immediately after exercise. Sabatine et al,\(^\text{33}\) in a study similar to ours, looked at natriuretic peptide levels in 112 patients who were undergoing exercise testing with nuclear perfusion imaging. BNP and NT-proBNP were measured before, immediately after, and 4 hours after exercise. These investigators found, as did we, that baseline level and after exercise level increases in both peptides were associated strongly with the presence of ischemia on nuclear perfusion imaging. They further subdivided patients into groups with no ischemia, mild to moderate ischemia, and severe ischemia and found baseline levels of 43, 62, and 101 pg/mL, respectively, for BNP and 109, 158, and 302 pg/mL, respectively, for NT-proBNP for the 3 groups. All results were statistically significant. Examining immediate post-exercise samples, they found that the incremental rise also was associated strongly with ischemia and, like baseline levels, was correlated with the severity of ischemia on nuclear imaging. Interestingly, they found that the increases in BNP were relatively larger than the increases in NT-proBNP, a finding which may be related to the different expression, storage, release, and half-lives of the 2 compounds. These relationships held true when patients with reduced left ventricular function were excluded.

**Conclusions and Implications for Further Study**

In summary, both experimental and clinical studies have provided considerable evidence to date that myocardial ischemia, even for brief periods such as those induced by exercise, causes the increased expression and secretion of the BNPs by ventricular myocytes. This increase appears to be independent of global left ventricular function. Our findings and those of a number of other investigators, who used the model of exercise-induced ischemia, suggest that ischemia triggers the release of BNPs and that the measurement of plasma levels of NT-proBNP and BNP before and immediately after symptom-limited exercise can distinguish patients with and without ischemia with a high degree of accuracy. Studies are underway currently to elucidate further the nature and magnitude of these changes in different populations, and to assess their usefulness as an aid to the diagnosis of CAD.

If the hypothesis that ischemia triggers BNP and NT-proBNP release is substantiated, these compounds then become, by definition, biomarkers of ischemia. The implications of this may be quite profound. As is well-known, serum markers for CAD currently are limited to markers of risk (eg, serum lipids and C-reactive protein) or markers of myocardial injury (eg, cardiac troponins I and T). Neither of these groups are true biomarkers of ischemia per se and thus do not assist, for example, in the differential diagnosis of patients with chest pain, unless myocardial injury began to occur at least 2 to 6 hours before clinical evaluation. On the other hand, ischemic biomarkers, the BNPs have several attributes that may enhance their usefulness as clinical tools: (1) They appear to be released in the presence of ischemia, even if it is insufficient to cause injury; (2) the release kinetics are very rapid, as demonstrated by the increases seen immediately after exercise in patients with ischemia; (3) they can
be measured accurately and quickly by assays with low coefficients of variation; and (4) these assays can be run either at the bedside or on autoanalyzers and are relatively inexpensive. Currently, there is no other biomarker of ischemia with these desirable characteristics.

Further research is needed to assess the role of these peptides in the evaluation of patients with known or potential cardiac disease. At present, the intraindividual biologic variability of these compounds is characterized incompletely, as is the variability of normal levels across age, gender, and different degrees of renal function. In addition, it is clear that levels rise in the setting of other forms of cardiac pathologic conditions, notably left ventricular dysfunction, and in other non-cardiac conditions (eg, subarachnoid hemorrhage); thus, sorting out the contribution of ischemia to elevated levels in many settings will be challenging. Despite these limitations, the establishment of a link between myocardial ischemia per se and increases in the levels of the BNPs offer new opportunities to improve our ability to recognize CAD in both its acute and chronic stages.

Acknowledgment

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References


31 B. Palumbo, D. Siepi and G. Lupattelli et al., Usefulness of brain natriuretic peptide levels to discriminate patients with stable angina pectoris without and with electrocardiographic myocardial ischemia and patients with healed myocardial infarction, Am J Cardiol 94 (2004), pp. 780–783.
