B-TYPE NATRIURETIC PEPTIDE CAN DETECT SILENT MYOCARDIAL ISCHAEMIA IN ASYMPTOMATIC TYPE 2 DIABETES

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Exercise tolerance testing
Abstract
Objective: B-Type Natriuretic Peptide (BNP) has been shown to predict future cardiovascular events independent of left ventricular function. This is partly because BNP has now been shown to detect silent myocardial ischaemia in suspected cases of angina pectoris. Our aim was therefore to find out if BNP detects silent myocardial ischaemia in patients with type 2 diabetes, since these subjects have a high frequency of silent ischaemia leading to unexpected cardiac deaths.
Design: Prospective cross sectional study with consecutive recruitment of patients.
Setting: Outpatient, single centre.
Patients: 219 patients with type 2 diabetes. Patients were excluded if they had history or evidence of cardiac failure.
Outcome measures: BNP, echocardiography and exercise tolerance testing ETT. BNP was compared to the ETT result in all patients and specifically in those who had no apparent ischaemic heart disease (IHD).
Results: 121 subjects had no prior history of IHD or cardiac failure and of these patients 85 had a clearly positive or negative ETT result. BNP was higher in those with a positive compared to a negative ETT (p<0.001). In univariate analysis BNP was an independent predictor of a positive ETT (p<0.001). In multivariate analysis BNP remained an independent predictor of the ETT result. A BNP result over 20pg/ml detected a positive ETT result with a sensitivity of 87% and specificity of 37%, while a BNP result over 40pg/ml had a sensitivity of 63% but a specificity of 81%.
Conclusion: BNP is of value in predicting silent ischaemia on exercise testing in asymptomatic patients with type 2 diabetes.
INTRODUCTION
50% of all sudden cardiac deaths occur in individuals with no prior history of cardiac disease [1]. In fact, sudden cardiac death in people without prior cardiac disease accounts for nearly 10% of all adult deaths. This may be particularly pertinent to patients with diabetes in whom risk of coronary artery disease is high and this coronary artery disease is often silent. In order to prevent such events the first necessary step would be to develop a simple test (e.g. a blood test) which is able to identify those patients with diabetes with silent coronary artery disease. Recent studies have led us to hypothesise that B-type natriuretic peptide (BNP) might be able to do this. This is in addition to the well known ability of BNP to identify left ventricular systolic dysfunction [2 3].

Our hypothesis is based on three main observations. Firstly, Goetz et al [4] recently showed that ischaemic myocardial tissue expresses more BNP than non ischaemic tissue. Secondly, in vitro cardiomyocytes degranulate and release BNP when they are made to be hypoxic [5]. Thirdly, in symptomatic angina BNP is able to identify the presence of coronary artery disease and myocardial ischaemia [6 7 8 9 10]. However, no study has yet addressed the key population of asymptomatic patients who are nevertheless at high risk of cardiac events, such as patients with type 2 diabetes.

Our aim was to see if BNP is able to identify silent myocardial ischaemia identified on ETT in asymptomatic patients with type 2 diabetes.

METHODS

DESIGN & SETTING
Between 1999-2001, 219 patients with type 2 diabetes diagnosed between 3-6 years earlier, were consecutively recruited into this pilot study from the Diabetes Centre outpatient department at Ninewells Hospital. All patients successfully recruited underwent a single three hour assessment in the department of Clinical Pharmacology, Ninewells Hospital, Dundee. The Tayside Committee on Medical Research Ethics gave ethical approval, and written consent was obtained from each patient. The study was conducted in accordance with the declaration of Helsinki.

A comprehensive cardiac history and examination were performed, followed by a 12 lead ECG, transthoracic echocardiography and treadmill exercise testing. Patients were excluded if a history or clinical examination revealed evidence of heart failure or significant valvular disease. They were also excluded if they were unable to exercise, had intercurrent illness, rest pain, severe hypertension and significant brady- or tachy arrhythmias.

BNP SAMPLING
Venous blood samples were taken to measure B-type natriuretic peptide levels prior to exercise and after the patient had been lying supine for 30 minutes. The samples were measured in a single batch by an experienced technician. BNP was measured by a standard commercially available radioimmunoassay kit (Peninsula, UK). A BNP level of <100pg/ml is used to exclude cardiac failure.

RESTING ELECTROCARDIOGRAM (ECG)
Resting ECG’s were classified as abnormal if there were pathological Q waves present, left ventricular hypertrophy on voltage criteria, or ST/T wave abnormalities present. As the purpose of this study was to determine the usefulness of BNP as a screening test in those
with no known ischaemic heart disease, those with significant ECG abnormalities were excluded.

EXERCISE TREADMILL TESTING
A graded symptom limited maximal exercise test was performed using the Bruce protocol [11]. ECG monitoring, heart rate and blood pressure responses were assessed prior to and during exercise and for at least 5 minutes into the recovery period. The duration of the test was noted. The ECG tracings were analysed for the presence of $\geq 1$mm horizontal or downsloping ST segment depression measured at 0.08seconds after the J point. The exercise test (ETT) was classified as normal or abnormal based on these criteria. Cardiac symptoms experienced during the test and after were carefully recorded. Reasons for termination of the test included limiting symptoms experienced by the patient (e.g. fatigue, dyspnoea, chest pain, dizziness, palpitations), maximum predicted heart rate (220-age) or stage 4 of the protocol reached, significant ST depression ($\geq 3$mm), appearance of ventricular arrhythmias or conduction abnormalities, and a fall in heart rate or systolic blood pressure.

We also calculated the Duke treadmill score [12], a composite index designed to provide survival estimates based on results from the exercise test. It has been shown to provide diagnostic and prognostic predictive accuracy of coronary artery disease in symptomatic patients [13]. The Duke score is calculated as follows, [exercise time, in minutes]-[5 x maximum ST segment deviation, in mm]-[4 x treadmill angina index (0=no angina, 1=no limiting angina, 2=exercise limiting angina)]. A treadmill score of 5 or more is considered low risk, -10 to 4 intermediate risk, and less than –10, high risk of significant coronary artery disease. A value of less than 5 was used to define a test as positive, where necessary in the calculations.

LEFT VENTRICULAR FUNCTION ASSESSMENT
Transthoracic echocardiographic assessment was performed by a single experienced physician blinded to the rest of the test results, using a Hewlett-Packard Sonos 2000 scanner with a 2.5MHz transducer. Measurements were taken in accordance with the recommendations of the American Society of Echocardiography. Intraobserver variability was 9%. The operator was blind to the clinical details and results of the other investigations in each patient.

Quantitative assessment of LV function was possible in 155 patients. The remainder of patients had LV function subjectively assessed. Left ventricular ejection fraction [LVEF] was calculated using modified Simpson’s rule. Left ventricular systolic dysfunction was defined as an LVEF <45%, which complies with the local guidelines for defining left ventricular dysfunction. We chose a deliberately conservative cut off to ensure that only patients with no LV dysfunction were included in the analysis.

Left ventricular mass index (LVMI) was calculated using the formula of Devereux [14]. Left ventricular hypertrophy (LVH) was defined as a LVMI greater than 110gm$^{-2}$ in women and greater than 135gm$^{-2}$ in men.
STATISTICAL ANALYSIS
Statistical analysis of the data was performed using SPSS (version 10.1) for windows. BNP values were log transformed prior to analysis to achieve normal distribution. To determine difference between variables independent samples t test was used for continuous data and Chi-squared ($\chi^2$) for discrete data. Forward, stepwise logistic regression analyses were performed to assess whether variables were independent predictors of outcome (ETT or DUKE score). Receiver Operating Characteristic (ROC) curves were used to assess the clinical usefulness of predicting outcome. Sensitivities and specificities were also calculated.
BNP values are presented as median and interquartile range (IQR).

RESULTS
Of the 219 patients who were screened, BNP results were unavailable in 34 patients. 10 samples were mislaid, 15 samples were of insufficient volume and in 9 samples the results were not reproducible even when done in triplicate. Therefore 185 patients (85%) had BNP samples that were suitable for analysis. Of the 185 patients who had BNP samples suitable for analysis, 121 (65%) had no previous history or evidence of ischaemic heart disease (IHD) or congestive cardiac failure. Of these 121 patients 85 (70%) had an ETT result that could be classed definitively as positive or negative, rather than equivocal (20 patients were unable to complete the ETT). See flow chart. Characteristics of these 85 patients who had no evidence of IHD or congestive cardiac failure, and had results for both BNP and ETT are presented in table 1.

Baseline characteristics of the 185 patients are summarised in table 2. 19% of patients had evidence of microalbuminuria, 49% were on Metformin, 41% were on a sulphonylurea, 12% were on insulin and 9% were controlled on diet therapy alone. With respect to cardiovascular medication, 27% were on an ACE inhibitor, 25% were on a calcium channel blocker, 14% were on a $\beta$-blocker, 25% were on aspirin and 12% were on diuretics.

Median BNP (IQR) concentration for the group as a whole was 32.0pg/ml (30.6pg/ml). This is comparable with previous studies in patients with diabetes without known cardiac disease [15 16].

Is BNP elevated in those with an abnormal exercise test?
The median (IQR) of BNP defined by a normal or abnormal test result are shown in table 3. BNP was significantly elevated in subjects with an abnormal compared to a normal exercise test (p<0.001).

When considering only those patients with no evidence of IHD or cardiac failure, BNP remained significantly greater in those with an abnormal ETT compared to those whose ETT was normal; median (IQR) BNP values for subjects with a normal and abnormal ETT were 24.4 (15.7)pg/ml and 58.2 (46.3)pg/ml, respectively (p for difference < 0.001). When known cardiovascular risk factors and confounders of BNP level (history of hypertension, gender, age, smoking history - as dichotomous variables, plus microalbuminuria, cholesterol, body mass index (bmi), HbA1C, LVMI and LVEF - as continuous variables) were included in a multivariate logistic regression analysis along with BNP, BNP remained
an independent predictor of a positive ETT (Table 4). Indeed, it was the strongest independent predictor.

**Is BNP elevated in those with an abnormal Duke score?**

Duke score was employed as a second index of myocardial ischaemia. A total of 181 subjects had a Duke score calculated, of which 99 had no history of IHD or cardiac failure. More subjects have a result for Duke score than for ETT as it is possible to ascribe a Duke score even when the ETT result is clinically equivocal.

In the whole group BNP was significantly greater in those with a Duke score that indicated ischaemia than in those whose Duke score did not indicate ischaemia (p=0.003). BNP levels correlated inversely with Duke score on exercise testing, i.e. the more abnormal the Duke score (increasingly negative score) the higher the BNP level; correlation coefficient – 0.184, p = 0.013. When patients with high BNP values (>100pg/ml) were excluded from analysis BNP quartiles still inversely correlated with Duke score (r = -0.26, p=0.002). Figure 1.

When subjects with no evidence of IHD or cardiac failure were analysed alone BNP was still higher in the subjects with a positive compared to those with a negative Duke score; median (IQR) BNP values for those with a positive and negative Duke score were 33.4 (31.2)pg/ml and 24.7 (16.9)pg/ml, respectively (p for difference = 0.05). There was also a significant inverse correlation between BNP and Duke score (as a continuous variable); r=-0.23, p=0.01. However, when known cardiovascular risk factors and confounders of BNP level (history of hypertension, gender, age, smoking history - as dichotomous variables, plus microalbuminuria, cholesterol, body mass index (bmi), HbA1C, LVMI and LVEF - as continuous variables) were included in a multivariate analysis along with BNP, BNP was no longer a significant predictor of duke score.

**Would BNP be a useful screening test to identify those likely to have a positive exercise test?**

To determine BNP’s usefulness as a screening test for prediction of a positive exercise test in patients with no prior history of cardiovascular disease, we constructed a receiver operating characteristic (ROC) curve (figure 2). BNP was sensitive in detecting those with an abnormal exercise test but it lacked specificity. For a BNP value above 20pg/ml the sensitivity and specificity for detection of a positive ETT are 87% and 37%, respectively (table 5).

**DISCUSSION**

The results from the present study suggest that BNP can identify an abnormal exercise test in asymptomatic patients with type 2 diabetes when patients with apparent IHD have been excluded. The AUC in this study (0.76) that is similar to that seen by Weber et al [6] in symptomatic angina patients.

This data may partly explain recent data showing that B-type natriuretic peptide (BNP) predicts future cardiovascular events, even in patients who do not have heart failure [17 18 19 20]. More pertinent to this current study are recent publications showing BNP predicts mortality in diabetic clinic attendees [15 21 22].
Although a BNP level of 20pg/ml is sensitive it lacks specificity to identify those with an abnormal ETT. This means that a normal BNP is good at ruling out ischaemia, which would mean that it is unlikely that a high-risk patient would be missed.

The question that naturally arises is: what therapeutic options might be useful in patients in whom silent ischaemia is identified? Firstly, those younger patients at higher risk may be eligible for invasive intervention (e.g.: angioplasty). There have not been any large scale trials of intervention in silent ischaemia, but small studies have found angioplasty to be safe, and give a better outcome than either medical therapy or no therapy [23 24 25]. The ACIP trial also found that CABG was of value in patients with silent ischaemia [25]. A second possible outcome arises from the EUROASPİRE data showing how poor risk factor control currently is in practice. [26]. This can be exemplified here by the fact that the average systolic BP was 142mmHg despite the target systolic BP for a patient with diabetes being <130mmHg. It is possible that a high BNP level would spur the consulting doctor on to achieve better risk factor control than the EUROASPİRE average : such an approach has in general been shown to work [27]. A third potential outcome might be that those with silent ischaemia will be targeted to receive more aggressive treatment of BP or cholesterol or glucose to lower than current target levels. Such an intensive risk reduction strategy when applied generally in diabetes has recently been shown to reduce cardiovascular and microvascular events by almost 50% [28].

Potential weaknesses of our study.
We are assuming that an abnormal ETT indicates silent myocardial ischaemia. Although the ETT has been shown to be useful in patients with diabetes [29] the gold standard test for coronary disease remains angiography. But as our patients were all asymptomatic, at the time of the study it was deemed unethical for most of them to proceed to angiography. In addition, exercise testing is by far the commonest investigation used routinely in the UK to investigate for a possible diagnosis of coronary disease. Also, whether or not further imaging demonstrates coronary artery lesions the ETT result is independently predictive of outcome in patients with diabetes [30].
In this current study, coronary artery disease was indeed seen in all five of the patients who were selected for coronary angiography because their exercise test was positive at exceptionally low workloads.

Another limitation is that we did not formally assess diastolic function in this study. This was because, firstly, at the time of the study there was no consensus on parameters to diagnose diastolic dysfunction and secondly, there remain no treatments which significantly reduce cardiovascular events even in symptomatic diastolic dysfunction.

Patients attended for assessment throughout the day and there is some evidence to suggest that BNP levels fluctuate throughout the day [31]. However, it is highly unlikely that all patients with a positive (or negative) ETT result were randomly studied at a particular time of day.
In conclusion these results suggest that BNP might be useful as a screening test for silent myocardial ischaemia in asymptomatic patients with type 2 diabetes. Thus BNP pre-
screening followed by an ETT in selected patients might become a useful strategy in asymptomatic patients with diabetes with the aim ultimately of reducing cardiovascular events by better targeting of cardioprotective therapies. Future research in larger numbers is now required to give more accurate figures on the precise diagnostic accuracy of BNP and to see if different cutoffs for BNP might perform better in different subgroups of patients.

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References


Figure legends:

Figure 1: ROC curve describing BNP’s ability to detect a positive ETT in subjects with no prior history of IHD.

Figure 2: BNP quartiles within the normal range compared to Duke score (mean and 95% CI).
Flow chart demonstrating number of patients with results for analysis

Completed assessment

219

↓

BNP results available

185

↓

No history or evidence of IHD or Cardiac failure

121

↓

Definitive ETT result available

85

IHD is ischaemic heart disease, ETT is exercise tolerance test.
Table 1. Characteristics of the 85 patients with definitive ETT result, who had both BNP sample results and no evidence or history of IHD or cardiac failure. Results are classified by ETT positive or negative.

<table>
<thead>
<tr>
<th></th>
<th>ETT negative</th>
<th>ETT positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age years</td>
<td>54 (11)</td>
<td>60 (7)*</td>
</tr>
<tr>
<td>Gender</td>
<td>53 (M), 17 (F)</td>
<td>10 (M), 5 (F)</td>
</tr>
<tr>
<td>Smoking N(Y)</td>
<td>48 (22)</td>
<td>14 (1)*</td>
</tr>
<tr>
<td>Hypertension N(Y)</td>
<td>41 (29)</td>
<td>6 (9)</td>
</tr>
<tr>
<td>Hyperlipidaemia N(Y)</td>
<td>56 (14)</td>
<td>9 (6)</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>7.6 (1.4)</td>
<td>7.8 (1.9)</td>
</tr>
<tr>
<td>Cholesterol (mMol/l)</td>
<td>5.3 (1.6)</td>
<td>5.5 (1.3)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>142 (17)</td>
<td>151 (21)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>82 (12)</td>
<td>83 (11)</td>
</tr>
<tr>
<td>BMI</td>
<td>30 (5.7)</td>
<td>30 (6.3)</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>24.4 (15.7)</td>
<td>58.2 (46.3)**</td>
</tr>
</tbody>
</table>

Data is described as patient number for gender, smoking, hypertension and hyperlipidaemia history, median and IQR for BNP, and as mean and sd for other variables. BP is blood pressure (S, systolic and D, diastolic). BMI is body mass index. ** and * indicate significant differences between groups at the p<0.001 and p≤0.05 levels, respectively.
Table 2. Characteristics of the 185 patients with BNP samples.

<table>
<thead>
<tr>
<th></th>
<th>All patients (185)</th>
<th>Patients with no IHD or cardiac failure (121)</th>
<th>Patients with IHD or cardiac failure (64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58.6 (10)</td>
<td>57 (10)</td>
<td>65(7)**</td>
</tr>
<tr>
<td>Gender</td>
<td>109 (M), 76 (F)</td>
<td>74 (M), 47 (F)</td>
<td>35 (M), 29 (F)</td>
</tr>
<tr>
<td>Smoking N(Y)</td>
<td>147 (38)</td>
<td>93 (28)</td>
<td>54 (10)</td>
</tr>
<tr>
<td>Hypertension N(Y)</td>
<td>100 (85)</td>
<td>58 (63)</td>
<td>42 (22)</td>
</tr>
<tr>
<td>Hyperlipidaemia N(Y)</td>
<td>132 (53)</td>
<td>90 (31)</td>
<td>42 (22)</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>7.79 (1.5)</td>
<td>7.6 (1.5)</td>
<td>7.8 (1.3)</td>
</tr>
<tr>
<td>Cholesterol (mMol/l)</td>
<td>5.3 (1.3)</td>
<td>5.3 (1.5)</td>
<td>5.3 (0.9)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>142 (21)</td>
<td>142 (21)</td>
<td>145 (15)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>82 (11)</td>
<td>81 (10)</td>
<td>79 (10)</td>
</tr>
<tr>
<td>BMI</td>
<td>30.1 (5.98)</td>
<td>31 (5.9)</td>
<td>30 (5.1)</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>30.0 (30.6)</td>
<td>29.8 (25.7)</td>
<td>44.7 (38.0)</td>
</tr>
</tbody>
</table>

Data are presented for all patients and also for those with and without evidence of IHD or cardiac failure. It is described as patient number for gender, smoking, hypertension and hyperlipidaemia history, median and IQR for BNP, and as mean and sd for other variables. BP is blood pressure (S, systolic and D, diastolic). BMI is body mass index. ** indicates significant difference at the p<0.001 level between the “IHD or cardiac failure” and the “no IHD or cardiac failure” groups.
Table 3. BNP levels defined by normal and abnormal test results- all patients

<table>
<thead>
<tr>
<th>Test (number of subjects who had a valid test result)</th>
<th>BNP (pg/ml)</th>
<th>Normal test result</th>
<th>Abnormal test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETT (104)</td>
<td></td>
<td>26.1 (18.7)</td>
<td>59.2 (44.9) **</td>
</tr>
<tr>
<td>Duke Score (181)</td>
<td></td>
<td>25.9 (19.1)</td>
<td>33.9 (31.3) *</td>
</tr>
</tbody>
</table>

Table shows BNP median (IQR) for each normal or abnormal test result, for all patients. * and ** indicate difference between normal and abnormal group at the p<0.05 and p<0.001 levels.

Table 4. Independent predictors of a positive ETT in multivariate analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>B value</th>
<th>CI of B value</th>
<th>P value</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>0.23</td>
<td>0.04-0.42</td>
<td>0.01</td>
<td>0.97</td>
</tr>
<tr>
<td>HbA1C</td>
<td>1.54</td>
<td>0.23-2.85</td>
<td>0.02</td>
<td>0.67</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.07</td>
<td>0.13-2.01</td>
<td>0.03</td>
<td>0.48</td>
</tr>
<tr>
<td>LnBNP</td>
<td>11.15</td>
<td>3.67-18.6</td>
<td>0.003</td>
<td>3.81</td>
</tr>
</tbody>
</table>

LnBNP is log BNP
Table 5. Sensitivity and specificity of BNP in detecting a positive ETT in patients with no prior history of ischaemic heart disease.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNP &gt; 20</td>
<td>87</td>
<td>37</td>
<td>21</td>
<td>90</td>
</tr>
<tr>
<td>BNP &gt; 35</td>
<td>69</td>
<td>72</td>
<td>34</td>
<td>91</td>
</tr>
<tr>
<td>BNP &gt; 40</td>
<td>63</td>
<td>81</td>
<td>42</td>
<td>90</td>
</tr>
</tbody>
</table>
Figure 1

P for linear trend=0.002
Figure 2

AUC = 0.76, p=0.001