N-Terminal Pro-B-Type Natriuretic Peptide as an Indicator of Possible Cardiovascular Disease in Severely Obese Individuals: Comparison with Patients in Different Stages of Heart Failure

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Background: Mild stages of heart failure might be difficult to diagnose in severely obese individuals with a body mass index (BMI) >40 kg/m². Measurement of the N-terminal fragment of pro-B-type natriuretic peptide (NT-proBNP) is feasible for detecting cardiac impairment. The aims of our study were to measure NT-proBNP in plasma of severely obese patients and to compare the results with results for patients in different stages of manifest cardiac dysfunction.

Methods: In 61 severely obese individuals (median BMI, 43.2 kg/m²) and 96 nonobese patients with existing heart failure [classified into New York Heart Association (NYHA) classes I–IV], NT-proBNP was measured in the fasting condition. A medical history, physical examination, electrocardiography, blood chemistry, and chest x-ray were performed in the obese group. In addition, echocardiography was performed in the NYHA group.

Results: In obese individuals, NT-proBNP was increased to a median of 356 (interquartile range, 221–458) pmol/L [854 (530–1099) ng/L] and was comparable (P >0.05) to the median value for NYHA I patients {289 (258–451) pmol/L [694 (619–1082) ng/L]}, but was significantly lower than in the other NYHA groups (P <0.001 for each).

Conclusion: The prognostic relevance of increased NT-proBNP for risk of developing cardiac insufficiency in severely obese patients needs to be further evaluated.

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It is well established that obesity influences cardiovascular morbidity and mortality (1–3). Heart failure (HF) on its own is an increasingly common disorder (4) that is characterized by poor prognosis and reduced quality of life for patients (5), and it is responsible for high healthcare costs. Dyspnea is a common symptom in HF as well as in obesity, making the early diagnosis of cardiac impairment difficult in obese patients (6).

Early changes in obesity include left ventricular hypertrophy and left ventricular dysfunction, which are both associated with higher mortality rates (7, 8). Poor prognosis has been demonstrated to be improved by adequate therapy (e.g., angiotensin-converting enzyme antagonists and beta-blockers) in a study population with diabetes or metabolic syndrome, which are closely linked to or include obesity (9). Echocardiography generally has poor precision in severely obese individuals because it is limited by excessive pericardial adipose tissue mass. Transesophageal echocardiography would therefore be the method of choice, but it is not routinely performed for screening purposes when there is no evidence of cardiac impairment (10). Improving the reliability of diagnosis of...
early HF, especially in severely obese individuals, is essential because determining the stage of HF leads to different management choices. These treatments improve symptoms, quality of life, and prognosis of disease and reduce healthcare use and costs (11–13).

Recently it was demonstrated that B-type natriuretic peptide (BNP) and immunoreactive amino-terminal proBNP (NT-proBNP) are increased in patients with chronic HF and are correlated with New York Heart Association (NYHA) classification. Both peptides are co-secreted predominantly in the left cardiac ventricle as a result of capacity overload and cardiac stretch (14). This condition is often present in obese individuals because of volume overload leading to left ventricular hypertrophy and left ventricular dysfunction (15). Although we have demonstrated increased NT-proBNP concentrations in severely obese patients (16), the importance of these findings has not been ascertained. To address this issue, we compared NT-proBNP concentrations in severely obese patients with those in patients with manifest HF.

**Patients and Methods**

**STUDY DESIGN AND PATIENTS**

This cross-sectional study took place between January 2002 and January 2003. Obese patients were enrolled in the obesity outpatient clinic in the Department of Internal Medicine V, Wilhelminenspital (Vienna, Austria). Patients with HF were enrolled in the Department of Internal Medicine IV, Hospital Lainz (Vienna, Austria). All patients gave informed consent, and the study had approval from the local ethics committee.

**METHODS AND INCLUSION CRITERIA**

**Obese patients.** Sixty-one patients had been referred to the outpatient obesity clinic for disease-specific therapy, including bariatric surgery. None of them had a history or clinical signs of acute or chronic heart disease or left ventricular hypertrophy in the electrocardiograms. Echocardiography was not performed in any of the obese patients. All of them had normal renal and hepatic function. Patients suffering from any other severe disease and pregnant females were excluded from the study. Thirty-four patients of this study population (5 men and 29 women) with ages ranging from 23 to 62 years [mean (SD), 40.7 (9.9) years] have already been published to demonstrate the change in NT-proBNP concentrations in severely obese patients with a body mass index (BMI) >40 kg/m² before and after weight loss (16).

**Patients with HF.** Ninety-six consecutive patients with HF were investigated. Clinical examinations included observation, palpation, and auscultation. Dyspnea was defined from the patients history, whereas peripheral edema was defined from patients history and/or clinical examination. Additionally, 12-lead electrocardiography was performed. HF was classified on the basis of the NYHA classification and European guideline criteria (17); therefore, echocardiography, including Doppler studies, were carried out in all patients. In addition to the exclusion criteria for the obese group (except heart disease by definition), patients with acute events such as incipient myocardial infarction or angina pectoris were not included into the study.

Blood pressure was measured twice on the right arm with the patient in a supine position after at least a 10-min rest, using an appropriate cuff size with a width of at least 40% of the circumference of the arm. The mean of the two measurements was used to determine the blood pressure. All patients were weighed, their height was measured, and the BMI was determined by dividing the weight by the height in meters squared. After overnight fasting for 12 h and supine rest for 5 min, venous blood samples for biochemical evaluation were gathered.

**MEASUREMENT OF NT-proBNP**

Blood for the peptide assay was drawn into 5-mL potassium EDTA tubes and then kept at room temperature. Within 3 h, the tubes were centrifuged, and plasma was removed before being processed. If samples could not be evaluated on the same day, they were kept at −70 °C. Before analysis, every tube was inverted several times to ensure homogeneity. Samples were then analyzed in duplicate. The test is a high-sensitivity competitive immunnoassay (Biomedica) using an immunoaffinity-purified sheep antibody specific for NT-proBNP (amino acids 8–29) immobilized to the surface of microtiter plate wells, with horseradish peroxidase as tracer. We perform the ELISA on an automated system (MINI-BOS; Biomedica). Comparison with the frequently used Roche NT-proBNP assay (F. Hoffmann-La Roche), used in the United States is not fully possible because the Roche assay is a noncompetitive two-site (sandwich) enzyme immunoassay incorporating a biotinylated polyclonal NT-proBNP-specific antibody (amino acids 1–21) and another polyclonal antibody labeled with a ruthenium complex (amino acids 39–50). Because different epitopes are detected by both assays, there is considerable lack of agreement (18).

**REFERENCE VALUES**

Samples with NT-proBNP concentrations <250 pmol/L (600 ng/L) were regarded as negative, those with concentrations 250–350 pmol/L (600–840 ng/L) were considered borderline, and those with concentrations >350 pmol/L (840 ng/L) were considered as positive. The reference values stated for the used NT-proBNP assay (Biomedica) were reassessed in our laboratory by use of blood from 100 healthy donors with a median (5th–95th percentiles) of 208 (142–301) pmol/L [499 (340–722.4) ng/L]. All samples were <250 pmol/L (600 ng/L), which is comparable to the stated values of Biomedica. The dynamic range of the assay covers 1000 pmol/L (2400 ng/L). The assay detection limit was 5 pmol/L (12 ng/L).
PRECISION OF NT-proBNP MEASUREMENTS

As stated by the manufacturer, the intraassay imprecision (CV) is 6.5% at 320 pmol/L (768 ng/L) and is 4.0% at 676 pmol/L (1622 ng/L), and the interassay imprecision was 4.4% at 320 pmol/L (768 ng/L) and 3.8% at 676 pmol/L (1601 ng/L).

STATISTICAL ANALYSIS

Because data were not gaussian distributed (Shapiro-Wilk test) they are presented as the median (interquartile range). Differences between groups were calculated with a Kruskal-Wallis and a Dunn post hoc test using Statistica 5.5 (Statsoft, Inc.; www.statsoft.com) and Instat 3.05 (GraphPad; www.graphpad.com). A two-tailed α of 0.05 was chosen as the threshold P value.

Independent risk factors for increased NT-proBNP were calculated with multiple regression analysis, which in general allows the investigation of the influence of multiple risk factors (independent variables) on one factor of interest (dependent variable). There are two major possibilities, clinical judgment or statistical calculation (correlation analysis), to define which independent variable should be included in the model. NT-proBNP values were log-transformed to achieve a near-gaussian distribution (Kolmogorov-Smirnov test, P > 0.05) to be used as the dependent variable for the regression analysis. For each population, a Spearman rank-order correlation analysis was performed to identify bivariately correlated risk factors for increased NT-proBNP. The following variables were correlated to the log-transformed NT-proBNP at P ≤0.1:

NYHA groups. The variables correlated to the log-transformed NT-proBNP were age (Spearman r = 0.23; P = 0.03), fasting blood glucose (r = 0.17; P = 0.1), creatine kinase (r = −0.19; P = 0.07), C-reactive protein (r = 0.17; P = 0.11), glycohemoglobin (r = 0.31; P = 0.003), weight (r = −0.21; P = 0.05), BMI (r = −0.17; P = 0.1), and dyspnea (r = 0.42; P < 0.0001).

Obese group. The variables correlated to the log-transformed NT-proBNP were fasting blood glucose (r = −0.31; P = 0.02), serum cholesterol (r = 0.99; P < 0.00001), serum triglycerides (r = 0.99; P < 0.00001), HDL-cholesterol (r = 0.99; P < 0.0001), LDL-cholesterol (r = 0.99; P < 0.0001), systolic blood pressure (r = 0.55; P < 0.0001), and diastolic blood pressure (r = 0.21; P = 0.01).

After excluding variables that were clearly not independent from each other (BMI from the pair BMI/weight of NYHA groups and HDL- and LDL-cholesterol from the triple (HDL-/LDL-/serum cholesterol), we calculated the multiple regression with a standard model at first and then with a stepwise backward approach.

Results

We included 61 obese individuals (52 females and 9 males) in the study. The median age was 41.5 years (interquartile range, 33.5–49.5 years; minimum–maximum, 23–62 years), the median BMI was 43.2 kg/m² (interquartile range, 40.6–48.0 kg/m²; minimum–maximum, 38–68.4 kg/m²), and the median weight was 123.4 kg (interquartile range, 113–138 kg; minimum–maximum, 98–181 kg). Of these patients 52% presented as having dyspnea after a short walking distance; 16% (n = 10) suffered from diabetes; 13% (n = 8) were on antidiabetic drugs; 1 patient was taking insulin, and 1 was on a diet only. Median systolic blood pressure 140 mmHg (interquartile range, 130–150 mmHg; minimum–maximum, 105–200 mmHg), and diastolic blood pressure was 87 mmHg (interquartile range, 80–95 mmHg; minimum–maximum, 70–120 mmHg).

All of these patients had a regular electrocardiogram and a normal chest x-ray. They had normal renal and thyroid function and no evidence of cardiovascular disease in their medical histories.

In total, 96 NYHA group I–IV patients (38 females and 58 males) with HF were examined: Thirty-one patients (32%) presented with NYHA stage I, 21 (22%) with NYHA II, 27 (28%) with NYHA III, and 17 (18%) with NYHA IV. Patients with HF were significantly older than the obese group, with a median (interquartile range) age of 69.5 (61.6–77.8) years vs 41.5 (33.5–49.5) years (P < 0.001). The median BMI in the NYHA class I group was 25.6 (23.0–34.9) kg/m² and was 27.8 (24.7–36.4) kg/m² in NYHA class II, 25.6 (23.3–30.5) kg/m² in NYHA class III, and 25.4 (22.9–29.5) kg/m² in NYHA class IV individuals. Nearly all patients (98%) were on antihypertensive drugs, and 36% had diabetes. BMI was not statistically different between single NYHA groups but was significantly (P < 0.001) lower than that of obese patients (Table 1).

There was a significant relationship between NT-proBNP and cardiac function from NYHA class I [median (interquartile range), 289 (258–451) pmol/L [694 (619–1082) ng/L], to NYHA class II [587 (515–730) pmol/L [1409 (1236–1752) ng/L], to NYHA class III [816 (567–1140) pmol/L [1958 (1351–2736) ng/L], to NYHA class IV [1837 (1386–3399) pmol/L [4409 (3326–8158) ng/L]], with a P < 0.0001 between single groups. In the obese group, we found NT-proBNP concentrations statistically comparable to those in NYHA I patients [356 vs 289 pmol/L (854 vs 694 ng/L); P > 0.05; Fig. 1].

The following bivariately correlated variables were included in the standard model for the regression analysis in patients with HF: age, fasting blood glucose, creatine kinase, C-reactive protein, glycohemoglobin, weight, and dyspnea. The adjusted R² of this model was 0.33 (P < 0.00001). Only creatine kinase (β = −0.18; P = 0.05) and dyspnea (β = 0.36; P = 0.0003) were significantly correlated to the log-transformed concentrations of NT-proBNP. In the backward stepwise model (adjusted R² = 0.20; P < 0.00001), only dyspnea was correlated (β = 0.46; P = 0.00001) with NT-proBNP. In obese patients, fasting blood glucose, serum cholesterol, serum triglycerides, and systolic and diastolic blood pressure were included in the
standard model (adjusted $R^2 = 0.75; P < 0.00001$), yielding cholesterol ($\beta = 1.18; P = 0.000002$) and triglycerides ($\beta = -0.50; P = 0.02$) as multiple significantly correlated variables. In the stepwise backward model (adjusted $R^2 = 0.72; P > 0.00001$), cholesterol ($\beta = 0.65; P < 0.00001$) and systolic blood pressure ($\beta = 0.36; P = 0.00002$) remained as significantly correlated variables.

**Discussion**

To date, several studies on the use of cardiac peptides (CPs) for detecting left ventricular dysfunction in selected patient groups have been conducted (19–21), and other studies have shown a relationship between CP concentrations and the severity of HF (22, 23). The value of CPs has already been recognized by their inclusion in the recent European guidelines for the diagnosis of chronic HF (17). There is clear evidence that a simple assay that measures either NT-proBNP or BNP enables the diagnosis of cardiac dysfunction (22, 24).

NT-proBNP is a stable and sensitive marker of cardiac function even in early cardiac decompensation, whereas BNP seems more suitable for detection of acute events (14). In addition, CP measurement is safe because of its high negative predictive value (25). On the other hand, increased CP concentrations are powerful predictors of

**Table 1. Characteristics and clinical data for patients with severe obesity or cardiac insufficiency (by NYHA stages).**

<table>
<thead>
<tr>
<th>NYHA class</th>
<th>Obese (n = 61)</th>
<th>P*</th>
<th>I–IV (n = 96)</th>
<th>I (n = 31)</th>
<th>II (n = 21)</th>
<th>III (n = 27)</th>
<th>IV (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/Male, %</td>
<td>85/14</td>
<td>&lt;0.001</td>
<td>41/59</td>
<td>42/58</td>
<td>52/48</td>
<td>33/67</td>
<td>35/65</td>
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<tr>
<td>Age, years</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Median</td>
<td>41.5</td>
<td></td>
<td>69.5</td>
<td>67.5</td>
<td>67.9</td>
<td>73.6</td>
<td>75.9</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>33.5–49.5</td>
<td>&lt;0.001</td>
<td>61.6–77.8</td>
<td>57.0–77.0</td>
<td>63.3–74.0</td>
<td>61.8–79.2</td>
<td>62.9–78.7</td>
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<tr>
<td>BMI, kg/m²</td>
<td>43.2</td>
<td></td>
<td>26.3</td>
<td>25.6</td>
<td>27.8</td>
<td>25.6</td>
<td>25.4</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>40.6–48.0</td>
<td>&lt;0.001</td>
<td>23.2–31.4</td>
<td>23.0–35.0</td>
<td>24.7–36.4</td>
<td>23.3–30.5</td>
<td>22.9–29.5</td>
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<tr>
<td>Diabetes mellitus, %</td>
<td>16</td>
<td></td>
<td>36</td>
<td>19</td>
<td>38</td>
<td>41</td>
<td>47</td>
</tr>
<tr>
<td>Antidiabetic therapy, %</td>
<td>13</td>
<td>0.06</td>
<td>29</td>
<td>13</td>
<td>24</td>
<td>41</td>
<td>41</td>
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<tr>
<td>Hemoglobin, %</td>
<td>5.5</td>
<td></td>
<td>5.9</td>
<td>5.7</td>
<td>5.9</td>
<td>6.0</td>
<td>6.8</td>
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<tr>
<td>Interquartile range</td>
<td>5.3–5.9</td>
<td></td>
<td>5.2–7.3</td>
<td>5.1–6.2</td>
<td>5.4–6.8</td>
<td>5.2–7.9</td>
<td>5.3–8.5</td>
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<tr>
<td>Blood pressure, mmHg</td>
<td></td>
<td>0.12</td>
<td></td>
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<td>Systolic</td>
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<td>130–150</td>
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<td>130–145</td>
<td>140</td>
<td>130–150</td>
<td>140</td>
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<tr>
<td>Diastolic</td>
<td>130–150</td>
<td>&lt;0.001</td>
<td>140–150</td>
<td>135–165</td>
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<tr>
<td>Interquartile range</td>
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<td>80–85</td>
<td>75–86</td>
<td>70–80</td>
<td>70–90</td>
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<td>Creatine kinase, U/L</td>
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<tr>
<td>Median</td>
<td>33</td>
<td>40</td>
<td>26</td>
<td>25</td>
<td>36</td>
<td></td>
<td></td>
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<td>22–46</td>
<td>17–47</td>
<td>19–46</td>
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<td>C-Reactive protein, mg/L</td>
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<tr>
<td>Median</td>
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<td>5</td>
<td>8.5</td>
<td>10</td>
<td>13</td>
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<td>5–18</td>
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<td>Antihypertensive therapy, %</td>
<td>98</td>
<td>84</td>
<td>95</td>
<td>93</td>
<td>100</td>
<td></td>
<td></td>
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<tr>
<td>Dyspnea, %</td>
<td>62</td>
<td>29</td>
<td>62</td>
<td>70</td>
<td>94</td>
<td></td>
<td></td>
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<tr>
<td>Left ventricular dysfunction, %</td>
<td>0</td>
<td>0</td>
<td>81</td>
<td>45</td>
<td>91</td>
<td>89</td>
<td>100</td>
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</tbody>
</table>

* P for obese vs all NYHA patients, Kruskal–Wallis and Dunn post hoc test or Fisher exact test.

b HbA1c, glycohemoglobin.
cardiac condition, as described recently. Increased NT-proBNP was found to be closely related to cardiac structure and function and to be a strong independent indicator for long-term outcome in HF patients (23). In 104 patients, NT-proBNP reflected left ventricular ejection fraction and NYHA class more sensitively than did BNP (14). A contemporary study showed that NT-proBNP is a useful and specific marker to predict major adverse cardiac events (26).

We intended to use NT-proBNP testing, which can basically be done during routine blood sampling, as a safe and fast means of risk stratification for mild forms of HF in obesity. Obviously increased NT-proBNP could be an indicator for further and more intense diagnostic procedures. Because even in nonobese patients with an abnormal electrocardiogram, Nielsen et al. (27) considered the probability of left ventricular systolic dysfunction based on the combined use of diastolic blood pressure and NT-proBNP concentration without echocardiography. This protocol was accurate enough to identify patients who should be referred to echocardiography (27).

Discordant findings to our study have been reported in a study involving 3389 offspring of the Framingham study (28). BNP and N-type A-type natriuretic peptide were measured and related to BMI. Obese individuals were found to have lower circulating concentrations of these CPs than healthy controls, which, as concluded by the authors, may contribute to their susceptibility to hypertension and related disorders (28). Similarly, Mehra et al. (29) reported decreased BNP in obese individuals with HF. However, we could not confirm these results in our survey. Conversely, we found higher NT-proBNP concentrations in our obese patients. Our findings underlie the results from a different study in which equivalent concentrations of increased A-type natriuretic peptide and BNP were found in obese normo- and hypertensive and in lean hypertensive individuals (30). The authors of yet another report found a trend toward increasing BNP with progressively higher BMIs (31).

As reported here, the chemical stability of NT-proBNP is better in circulating blood than that of BNP, possibly making NT-proBNP a better marker for detection of cardiac dysfunction (14). In a recent letter, the authors reported excellent stability of NT-proBNP even after multiple freeze-thaw cycles (32). BNP, on the other hand, may be degraded by proteolytic enzymes in the circulation. Even different protease inhibitors give different results for slowing the rate of degradation in samples stored at 2–8 °C for 10 days (33). Another study demonstrated that the presence of aprotinin prevents BNP degradation in samples preserved for 1 month at −20 °C before assay (34). The stability of N-type A-type natriuretic peptide in samples stored at room temperature for 2–3 days was excellent in both the presence or absence of aprotinin (35).

We must note that this still does not explain why considerably lower CP concentrations were found in obese and in overweight individuals in contrast to individuals with normal BMI (28). However, as we, in contrast to the reports mentioned above (28, 29), are reporting on severely obese patients with a BMI >40 kg/m², which is often characterized by prevalent visceral fat accumulation, it should be considered that because of the tremendous cardiac burden, CP secretion could be massively increased in higher BMI ranges.

We therefore conclude that because of the different settings and CPs measured in the contradictory reports, they may not be fully comparable. Important limitations to our study should be mentioned. Echocardiography was not performed the obese patients. Further studies with sufficient numbers of obese individuals will have to be performed to verify our findings on whether obese patients with increased NT-proBNP have left ventricular dysfunction, as diagnosed by echocardiography.

Methodologic studies on the stabilities and recovery rates of the different CPs in different settings of frozen storage could aid in interpreting the contradictory results presented here. In addition, the relevance of increased NT-proBNP for cardiac function in moderately obese patients should be investigated in more detail, especially in patients with dyspnea, which was an independent predictor of increased NT-proBNP in the obese group. Because of the controversial findings, the use of NT-proBNP as a screening tool in severely obese patients remains unclear at this time and awaits further investigations based on larger study groups.


