

# Aminoterminal pro-brain natriuretic peptide (NT-proBNP) and sleep-disordered breathing in morbidly obese females: a cross-sectional study

JUAN YBARRA, FRANCESC PLANAS, JOSE M POU

## Abstract

**S**leep-disordered breathing (SDB) is often encountered in morbid obesity (MO) in conjunction with insulin resistance (IR). Aminoterminal pro-brain natriuretic peptide (NT-proBNP) is a promising marker for left ventricular dysfunction (LVD) in MO. We sought to explore the factors that may influence the relationships of SDB and IR with NT-proBNP in MO women.

We performed a cross-sectional pilot study involving 110 asymptomatic MO ( $44.5 \pm 0.7$  kg/m<sup>2</sup>) young women. SDB risk was assessed using a modified version of the Berlin Questionnaire (BQ). IR was assessed using the homeostasis model assessment (HOMA) index and adiponectin levels. LVD was assessed using NT-proBNP and echocardiograms.

In this study, NT-proBNP levels and LVD increased significantly along the BQ strata. Multiple regression analysis identified BQ and log-transformed HOMA as the independent variables predicting as much as 48.0% of the variability of logNT-proBNP.

In conclusion, NT-pro-BNP levels are independently predicted by SDB and IR in asymptomatic MO women. Larger prospective studies are warranted.

*Diabetes Vasc Dis Res* 2008;5:19–24  
doi:10.3132/dvdr.2008.004

**Key words:** Berlin Questionnaire, diastolic dysfunction, HOMA index, morbid obesity, NT-proBNP.

## Introduction

The prevalence of obesity has reached unprecedented pro-

portions. Nearly 40 million Americans are obese, and 300,000 deaths are attributed to obesity annually in the US.<sup>1</sup> Severe obesity is often accompanied by a wide array of comorbidities,<sup>2</sup> including serious pulmonary diseases such as sleep-disordered breathing (SDB), obstructive sleep apnoea (OSA) and obesity-hypoventilation syndrome.<sup>3–6</sup> A marked excess in abdominal fat mass can interfere mechanically with lung function because of the increased weight on the chest wall and thoracic cage.

Obesity has been reported to be a strong risk factor for left ventricular hypertrophy (LVH), left ventricular (LV) dysfunction and heart failure.<sup>7</sup> The heart failure has been basically attributed to LV mechanical overload due to the increased intravascular volume and cardiac output necessary to supply the increased metabolic demands of excessive body weight and/or from pressure overload of obesity-related systemic hypertension, neurohumoral activation and increased cytokine production.<sup>8</sup> Other factors and co-morbid conditions that frequently accompany obesity may also play a pathogenetic role, leading to progression of LV remodelling; these include conditions such as diabetes, glucose intolerance, insulin resistance and SDB, though their contribution to LVH is still controversial.<sup>9–11</sup>

Aminoterminal pro-brain natriuretic peptide (NT-proBNP), a neurohormone secreted by myocytes in the ventricular wall in response to increased wall stress, is a promising marker for heart failure diagnosis, prognosis and treatment.<sup>12–14</sup> However, for reasons that remain unexplained, plasma levels of NT-proBNP appear to be inversely associated with body mass index (BMI) in subjects with and without heart failure,<sup>15–18</sup> possibly owing to suppression of synthesis or release of natriuretic peptides from cardiomyocytes in obese subjects.<sup>19,20</sup>

We designed this cross-sectional pilot study to detect the factors that may influence the relationships between obesity and heart structure and function, as assessed by echocardiography and NT-proBNP levels, in a group of asymptomatic morbidly obese women. We were particularly interested in the role of SDB and insulin resistance.

## Methods

Participants were recruited consecutively from the pool of individuals attending an Obesity Outpatient Clinic (ICAMED) at Centro Médico Teknon in Barcelona between

Instituto de Cardiología y Medicina Avanzada (ICAMED), Centro Médico Teknon, C/ Vilana 12, 08021 Barcelona, Spain.

**Juan Ybarra**, Senior Consultant in Endocrinology

**Francesc Planas**, Senior Consultant in Cardiology

Servicio de Endocrinología y Nutrición, Hospital de Sant Pau, UAB, Barcelona, Spain.

**Jose M Pou**, Senior Consultant in Endocrinology

**Correspondence to:** Dr Juan Ybarra

Instituto de Cardiología y Medicina Avanzada, Centro Médico Teknon, C/ Vilana 12, 08021 Barcelona, Spain.

Tel: 00 34 93 2906459; Fax: 00 34 93 2906458

E-mail: juanybarra@hotmail.com

January 1st 2006 and December 31st 2006. A total of 240 patients (64 male and 176 female) were screened at our Institution (ICAMED/Teknon) during that period.

Exclusion criteria for the study were: male gender, coronary artery disease (and/or prior angina or myocardial infarction), cardiovascular co-morbidity (prior cerebrovascular accidents and/or peripheral vascular disease), vasoactive treatment, statins, fibrates, current/prior alcohol consumption averaging > 60 g/day, current/active smoking, creatinine > 2 mg/dL, presence of neoplasia and/or systemic disease and a suboptimal echocardiographic window.

Thus, the 64 males were excluded from the study. Of the 176 female candidates only 110 were finally entered into the study. The 66 other female patients were excluded due to: suboptimal echocardiographic windows (n=20), subclinical hypothyroidism (i.e. thyroid-stimulating hormone [TSH] > 10 with normal free thyroxine and tri-iodo-thyronine serum values; n=18), psychiatric disorder (n=20) and suspicion of active coronary heart disease (i.e. abnormal ECG and/or echocardiogram; n=4). One hundred and ten asymptomatic morbidly obese female patients without apparent underlying cardiac disease were included in the study, which was approved by the locally appointed Ethics Committee. Written informed consent was obtained from all the participants. The study was carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000. All patients underwent a detailed clinical, biochemical and echocardiography study.

Subjective sleep quality and the risk for sleep apnoea were assessed using a well validated sleep survey, the Berlin Questionnaire (BQ),<sup>21</sup> which all participants filled out on the day of their recruitment. It includes questions about snoring behaviour (category 1), chronic daytime sleepiness (category 2) and the presence of hypertension and/or BMI exceeding 30 kg/m<sup>2</sup> (category 3). This instrument predicts high risk for sleep apnoea with a sensitivity of 0.86, a specificity of 0.77, a positive predictive value of 0.83 and a likelihood ratio of 3.2. Since obesity was present in our entire patient cohort and hypertension was present in a substantial number of participants, we used a modified BQ score excluding responses in category 3, as reported previously.<sup>22</sup>

Briefly, each of the other categories was assigned a score of either 0 (or no symptoms), 1 for frequent symptoms (< 3–4 times a week), or 2 for persistent symptoms (≥ 3–4 times a week). To be considered as high risk for sleep apnoea, a patient had to have a cumulative score of 2 or higher.

Patients were considered to be hypertensive on the basis of two separate blood pressure readings ≥ 140 / ≥ 90 mmHg using appropriate blood pressure cuffs or when given a prior diagnosis of hypertension or taking antihypertensive treatment.

#### *Echocardiographic studies*

Standard two-dimensional (2D) echocardiography (Aloka SSD-4000, Aloka Co., Ltd., Wallingford, Connecticut, US), was used to measure LV dimensions and ejection fraction (EF), cardiac output (CO), interventricular wall thickness (IVWTh), left ventricular mass (LVM) and left atrial size (LA). Left ventricular hypertrophy (LVH) was defined by wall thick-

ness criteria (IVWTh ≥ 12 mm). Overall ventricular diastolic function was assessed by measuring early (E) and late (A) peak Echo-Doppler mitral flow velocities.<sup>23</sup> In addition, we measured peak E, A and systolic tissue Doppler velocities (Em, Am and Sm) by sampling of the mitral annulus. The ratio of the early mitral inflow velocity, sampled by pulsed Doppler, to the early mitral annulus velocity, sampled by TDI ratio (E/Em), was obtained as an index of LV filling pressures.<sup>24,25</sup> Intra-observer variability was 0.0–1.0 mm and 5% for wall thickness assessment and LV diameters, respectively.

Blood samples were drawn after an overnight fast (10–12 hours), one week pre-operatively. Insulin was measured using an immunochemoluminometric assay (IMMULITE Diagnostic Products Corporation, Los Angeles, CA, US). The intra- and inter-assay imprecision was 3% and 7%, respectively. Cross-reactivity with proinsulin was less than 0.01%. Insulin resistance was calculated using the homeostasis model assessment (HOMA) index<sup>26</sup> and adiponectin serum concentrations. Plasma adiponectin concentrations were measured using a commercial human adiponectin radioimmunoassay kit (Linco, Labodia SA, Yens, Switzerland) with a detection limit and intra- and inter-assay coefficient of variation of 2.0 ng/ml, 5.0 and 5.0%, respectively. Diabetic patients were excluded for the purposes of calculating HOMA index or expressing adiponectin results.

Diabetes mellitus was defined according to the American Diabetes Association 1997 criteria.

Serum NT-proBNP were measured using an immunochemoluminometric assay (Elecys®, Roche Diagnostics, Indianapolis, IN, US).<sup>27</sup> The calibration curve covers a NT-proBNP concentration range from 0 pmol/L up to 600 pmol/L. The analytical detection limit of the assay was estimated to be 2.7 pmol/L (3 SD). The intra-assay coefficient of variation is 5.7% (at 50 pmol/L) and 6.1% (at 250 pmol/L), while the inter-assay CVs are 15.8% (50 pmol/L) and 8.2% (250 pmol/L).

#### *Statistical analysis*

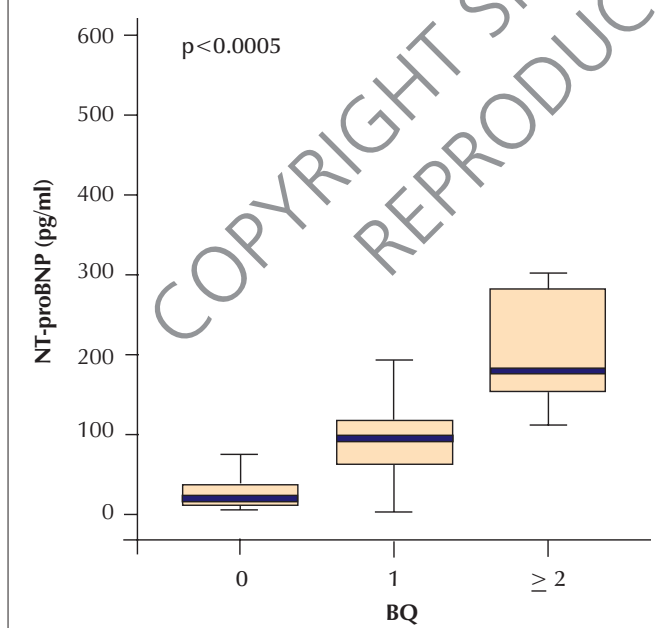
Before statistical analysis, normal distribution and homogeneity of the data were tested. Parameters that did not fulfil these criteria (HOMA index, NT-proBNP) were log transformed. Qualitative variables are expressed as sample size (number of cases) and percentage (%), and quantitative variables are expressed as mean and standard error of the mean (SEM). Categorical variables included hypertension, diabetes mellitus and modified BQ score. The relationship between two qualitative variables (i.e. IVWTh values) was assessed using the Chi-squared test, with a continuity correction whenever necessary. The relationship between two quantitative variables was assessed using Pearson's correlation coefficient. Logistic regression analysis was used for assessing the relationship between categorical variables. Stepwise multiple regression analysis was used to determine the dependence of logNT-proBNP over several other independent variables (BQ, HOMA index, age, LVM, IVWTh, BMI, hypertension). Level of statistical significance was set at p<0.05. Data were analysed and figures constructed using the SPSS 12.0 statistical package (SPSS Inc., Chicago, Illinois, US).

**Table 1. Demographic, anthropomorphic and biochemical characteristics of the patients**

n=110 (female)	X ± SD	Median	Range
Age (years)	37.8±10.9	37.0	24.0
Weight (kg)	124.0±22.3	122	121
BMI (kg/m <sup>2</sup> )	44.5±6.7	44.7	41.0
Modified Berlin Questionnaire			
Category 0	n=70		
Category 1	n=28		
Category ≥ 2	n=12		
Hypertension prevalence	34.5%		
T2DM prevalence	4.5%		
Basal plasma glucose (mg/dL)	85.0±14.5	83	14.5
Basal insulin (μUI/L)	18.3±11.8	15.9	67
HOMA	4.1±3.3	3.25	19.2
Adiponectin (ng/dL)	6.6±3.9	5.9	18.3
Creatinine (mg/L)	1.1±0.4	0.95	0.25
TSH (mUI/L)	1.9±1.2	1.68	1.2
NTpro-BNP (pg/ml)	47.1±66.9	31	560.5

**Key:** BMI = body mass index; T2DM = type 2 diabetes mellitus; HOMA = homeostasis model assessment; TSH = thyroid-stimulating hormone; NT-proBNP = aminoterminal pro-brain natriuretic peptide

**Figure 1. The modified Berlin Questionnaire (BQ) scores (x axis) plotted against NT-proBNP plasma values (y axis). The plots represent median, 25th and 75th percentiles as vertical boxes with error bars (10th and 90th percentiles). Kruskal-Wallis one-way analysis of variance (p<0.0005)**



## Results

Table 1 depicts the demographic, anthropomorphic and biochemical characteristics of the patients. As shown, the patients were relatively young, asymptomatic, morbidly

**Table 2. Main echocardiographic parameters stratified according to BQ scores**

	Modified Berlin Questionnaire score		
	0 n=70	1 n=28	2 n=12
BMI (kg/m <sup>2</sup> )	41.1±10.3	47.5±6.6*	49.3±11.0*
HTN (%)	15	45##	73##
LVH (%)	8.7	31##	40##
IVWTh (mm)	10.6±1.5	11.6±1.8*	12.4±1.5*
LVM (g)	209.0±66	257±77**	277±77**
LVM/BSA (g/m <sup>2</sup> )	94.8±24.0	107.0±32.0	110±45.0
LVM/BMI (g/kg/m <sup>2</sup> )	4.8±1.5	5.2±1.7	5.6±2.6
LA (mm)	37.0±4.6	39.9±5.2**	41.0±7.2**
LVEF (%)	62.2±7.2	60.2±9.1	61.6±7.0
CO (L/min)	5.04±1.43	5.97±1.5*	5.96±0.34*
Em/Am	1.18±0.46	1.07±0.45*	0.82±0.26*
E/Em	7.4±3.26	8.0±2.4*	8.0±1.7*

**Key:** BMI = body mass index; HTN = hypertension; LVH = left ventricular hypertrophy; LVM/BSA = left ventricular mass adjusted for body surface area; LVM/BMI = left ventricular mass adjusted for body mass index; IVWTh = inter ventricular wall thickness; LVM = left ventricular mass; LA = left atrial; LVEF = left ventricular ejection fraction; CO = cardiac output (continuous method); Em/Am = the ratio of the early mitral annulus velocity to the late mitral annulus velocity, sampled by tissue Doppler imaging (TDI); E/Em = the ratio of the early mitral inflow velocity, sampled by pulsed Doppler, to the early mitral annulus velocity, sampled by TDI ratio.

\*\* p<0.0005 (analysis of variance [ANOVA] with Bonferroni post-hoc correction: BQ=0 vs. BQ=1 vs. BQ=2). \* p<0.001 (ANOVA: BQ=2 & BQ=1 vs. BQ=0). ## p<0.0005 (chi-squared: BQ=2 vs. BQ=0 vs. BQ=1)

obese women. Most participants (n=70) did not show any suspicion of SDB (i.e. had a BQ score of 0), a smaller group (n=28) scored 1 (BQ=1; frequent SDB symptoms) while the smallest group (n=12) revealed a BQ ≥ 2 (persistent SDB symptoms). In total, 40 out of the 110 participants had altered modified BQ scores. Hypertension was present in 34.5% while type 2 diabetes mellitus was recorded in 4.5% of the patient cohort. All patients had normal serum creatinine and TSH values and liver function tests. Mean NT-proBNP plasma levels were found to be normal.

Figure 1 illustrates a boxplot between modified BQ scores (on the x axis) and plasma NT-proBNP levels (on the y axis). As shown, there is a step-by-step significant increment in plasma NT-proBNP levels with BQ scores. Participants with BQ=0 displayed the lowest NT-proBNP levels; those with BQ=1 displayed significantly higher and intermediate NT-proBNP levels; the highest NT-proBNP levels were displayed by participants with a BQ ≥ 2.

The main echocardiographic parameters appear in table 2, stratified according to the modified BQ scores. Of note, there was a stepwise significant rise in BMI, the percentage of hypertensive patients, LVM, the percentage of participants disclosing LVH, IVWTh, left atrial diameters and cardiac output with higher BQ score. Conversely,





