

Diagnostic potential of serum N-terminal pro-B-type brain natriuretic peptide level in detection of cardiac wall stress in women with polycystic ovary syndrome: a cross-sectional comparison study*

Onder Celik^{1,6}, Ibrahim Sahin², Nilufer Celik³, Seyma Hascalik¹, Lezzan Keskin², Hamdi Ozcan⁴, Ahmet Uckan⁵ and Feridun Kosar⁵

¹Department of Obstetrics and Gynecology, Medical Faculty, Turgut Ozal Medical Center, Inonu University, Malatya 44069, Turkey; ²Department of Endocrinology, Medical Faculty, Inonu University, Malatya, Turkey; ³Beydagi State Hospital Biochemistry, Malatya, Turkey; ⁴Department of Dermatology, Medical Faculty, Inonu University, Malatya, Turkey; ⁵Department of Cardiology, Medical Faculty, Inonu University, Malatya, Turkey

⁶Correspondence address. Tel: +90-422-341-0660; Fax: +90-422-341-0728; E-mail: oncelik@inonu.edu.tr

BACKGROUND: In addition to the negative effect on fertility, polycystic ovary syndrome (PCOS) has been associated with cardiac pathology. Brain natriuretic peptide (BNP) is a possible marker for cardiac risk, therefore we investigated whether N-terminal pro-B-type BNP (NT-proBNP) increases in women with PCOS compared with healthy women of comparable age and body mass index. **METHODS:** Thirty women with PCOS and 30 healthy women not suffering from overt cardiac disease were involved in the study. Fasting insulin and serum NT-proBNP levels were measured, and M-Mode echocardiography was performed. Insulin resistance was calculated using the homeostasis model assessment insulin resistance index (HOMA-IR). **RESULTS:** PCOS subjects had higher NT-proBNP levels than the control subjects ($P < 0.001$). Abnormal echocardiography indices were detected in 14 of the PCOS subjects (but none of the controls), including valvular heart disease in nine, diastolic dysfunction in two, right ventricular enlargement in one, right atrial enlargement in one and pulmonary hypertension in one. PCOS subjects ($n = 30$) showed an increased left ventricular mass (LVM) ($P < 0.001$) and left ventricular posterior wall thickness (LVPWT) ($P = 0.006$). In addition, NT-proBNP concentration was positively correlated with LVM ($r = 0.587$, $P = 0.001$) and negatively correlated with sex-hormone-binding globulin ($r = -0.528$, $P = 0.003$). There was a positive correlation between LVM and HOMA-IR ($r = 0.295$, $P = 0.03$) while LVPWT was positively correlated with fasting insulin and HOMA-IR ($r = 0.335$, $P = 0.031$ and $r = 0.346$, $P = 0.045$, respectively) in PCOS subjects ($n = 30$). **CONCLUSION:** The present study demonstrated that the level of NT-proBNP was increased in PCOS subjects with asymptomatic heart disease.

Keywords: N-terminal pro-B-type brain natriuretic peptide; polycystic ovary syndrome; echocardiography; cardiac disease; insulin

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age (Knochenhauer *et al.*, 1998; Carmina and Lobo, 1999; The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). PCOS not only has a negative effect on fertility, but also has been (Scarpitta and Sinagra, 2000) associated with cardiac pathology (Franks, 2001). In various studies, structural and functional abnormalities, which increase the risk of coronary artery disease, in women with PCOS have been reported

(Guzick, 1996; Tiras *et al.*, 1999; Cibula *et al.*, 2000; Talbott *et al.*, 2000). Evidence incorporating biochemical and clinical cardiovascular risk factors reveals that the risk of myocardial infarction in PCOS subjects is seven times greater than the normal population (Dahlgren *et al.*, 1992).

Identifying novel serum markers of cardiovascular risk in PCOS is now challenging (Bickerton *et al.*, 2005). Brain natriuretic peptide (BNP), produced in ventricles of the heart with a sequence homologous to atrial natriuretic peptide, is a newly identified marker of cardiac risk (Mokuyama *et al.*, 1991; Suga *et al.*, 1992). The human BNP gene is associated with chromosome 1 and encodes the 108 amino acid prohormone BNP. The biologically active 32 amino acid BNP

*Presented at the 7th International Congress of the Turkish German Gynecological Association, May 16–20, 2007, Antalya, Turkey.

hormone is separated from the N-terminal part of the prohormone (NT-proBNP) in the circulation (Sawada *et al.*, 1997). Therefore, both biologically active BNP and NT-proBNP (76 amino acids) hormones can be measured through immunoassay in human blood.

It has been reported that NT-proBNP is more sensitive and specific in being a marker of ventricular dysfunction than the biologically active BNP (Yu *et al.*, 1996; Birdsall *et al.*, 1997; Hunt *et al.*, 1997). The main stimulus for BNP synthesis and secretion is cardiac wall stress (Magga *et al.*, 1994). Takase *et al.* (2007) have suggested the BNP measurements for detecting asymptomatic cardiac abnormalities in healthy populations. Since the increase of cardiac wall stress is a common denominator of women with PCOS (Tiras *et al.*, 1999), measuring NT-proBNP may be useful in identifying cardiac abnormalities in PCOS subjects. Thus far, there has been no study linking NT-proBNP level to cardiac abnormalities in women with PCOS. In this setting, the primary aim of this study was to determine whether women with PCOS have an increased concentration of NT-proBNP, compared with a healthy woman population having a similar age and body mass index (BMI). The secondary aim was to determine whether PCOS subjects with an increased NT-proBNP level have evidences of cardiac disease using echocardiography.

Materials and Methods

Thirty women with PCOS and 30 healthy women, as the control subjects, having similar age and BMI were enrolled in this study. The PCOS subjects were selected from a group of PCOS patients who were seeking treatment for acne, hirsutism and infertility at Turgut Ozal Medical Center, Inonu University.

PCOS was documented when at least two of the following three features were present after the exclusion of other etiologies (Rotterdam criteria): oligo/amenorrhoea (fewer than six menstrual periods in the preceding year); clinical (Ferriman–Gallwey score >8) and/or biochemical signs of hyperandrogenism and ultrasonographic findings (Ferriman and Gallwey, 1962). The ultrasound criteria used for diagnosis of PCOS were the presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increase of ovarian volume (>10 ml).

The clinical and biochemical diagnostic features of the examined PCOS subjects are shown in Table 1. Clinical hyperandrogenism was quantified by Ferriman–Gallwey scoring system. Hirsutism scores on each body area were made by two experienced physicians. Agreement analysis has demonstrated that two physicians' scores were quite concordant. The mean kappa value for nine body areas was 0.71, which is similar to the values reported by Api *et al.* (2007).

All patients had a normal renal, hepatic and thyroid function. They were not suffering from anemia, pregnancy, adrenal disorder including congenital adrenal hyperplasia, diabetes mellitus, hypertension, myocardial infarction, stroke and peripheral vascular disease. They were not taking antiandrogen drugs, antidiabetics, lipid lowering medication, glucocorticoids or other hormonal drugs. Hence, the chosen PCOS subjects were considered as not having cardiovascular risk factors.

The control group had normal biochemical and hormonal profiles, and were menstruating regularly and not suffering from PCOS. For each subject, the height, weight, BMI, waist circumference, heart rate and systolic (SBP) and diastolic blood pressure (DBP) were

Table 1: Clinical and biochemical diagnostic features of the 30 PCOS subjects

Characteristic	n (%)
Oligo/amenorrhea	30 (100)
Hirsutism ^a	30 (100)
Acne	16 (53.3)
Seborrhea	5 (16.6)
Androgenetic alopecia	8 (26.6)
Acanthosis nigricans	1 (3.3)
Testosterone >2 nmol/l	12 (40)
DHEAS >10 µmol/l	1 (3.3)
Polycystic ovary at USG	30 (100)

^aAs evaluated by Ferriman–Gallwey score. DHEAS, dehydroepiandrosterone sulfate; USG, ultrasonography.

evaluated by standard methods. BMI was measured as the ratio of the weight to the square of the height.

Waist circumference of subjects was measured in standing position by placing a soft tape measure midway between the lowest rib and the iliac crest. All other measurements were performed when the patients were in a standing position with feet together, relaxed abdomen and arms at their sides (Yanovski, 1993). Blood pressure was measured on the right arm, with the subjects in a sitting position and relaxed. The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and was approved by the local ethics committee. Informed consent was obtained from all participants.

Measurement of NT-proBNP serum levels

Blood samples for NT-proBNP analysis were taken on Days 2–5 of a spontaneous or progestin-induced menstrual cycle, and the serum was separated and frozen at –50°C until assayed. NT-proBNP levels were measured by a fully automated commercial modulator analytics E170 using electrochemiluminescence sandwich immunoassay (proBNP, Roche Diagnostics GmbH, D-68298 Mannheim). In this immunoassay, the coefficient of variability was 0.8–3.0% and the minimum detection limit was 0.6 pmol/l.

Biochemical study

In both PCOS and control subjects, fasting glucose, triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), very-low-density lipoprotein cholesterol (VLDL-C) and high-density lipoprotein cholesterol (HDL-C) concentrations were measured by standard procedures in the morning following an overnight fast during early follicular phase (Day 2–5) of the spontaneous or progesterone-induced withdrawal bleeding. The measurements were performed using an Olympus AU 2700 autoanalyzer (Olympus Optical Co. Ltd., Japan) and commercially available kits (Olympus Diagnostica GmbH, WendenstraBe, Hamburg, Germany). Fasting insulin levels in all subjects were also measured to estimate the insulin sensitivity. Insulin resistance (IR) was calculated using the homeostasis model assessment insulin resistance index (HOMA-IR) (Matthews *et al.*, 1985), given as:

$$\text{HOMA-IR} = \text{Fasting serum insulin (mU/ml)} \\ \times \text{Fasting plasma glucose (mg/dl)}$$

serum LDL-C was calculated using Friedewald formula (Friedewald *et al.*, 1972):

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - \text{TG}/5$$

where the respective inter- and intraassay coefficients of variation were 3.4 and 3.0% for fasting glucose, 2.7 and 2.4% for TC, 11.6 and 8.1% for HDL-C and 3.2 and 2.7% for TGs.

In all subjects, serum FSH, LH, estradiol (E₂), total testosterone, sex-hormone-binding globulin (SHBG), insulin and dehydroepiandrosterone sulfate (DHEAS) levels were measured using competitive chemiluminescent enzyme immunoassay (Immulite 2000 Analyzer, Diagnostic Products Corporation; DPC, Los Angeles, CA, USA) with a lower analytical sensitivity of 0.1, 0.05 mIU/ml, 15 pg/ml (55 pmol/l), 15 ng/dl (0.5 nmol/l), 2 µIU/ml, 0.02 nmol/l and 3 µg/dl, respectively. The respective inter- and intraassay coefficients of variation were 7.3 and 5.5% for FSH, 7.6 and 5.0% for LH, 6.6 and 5.1% for E₂, 8.3 and 6.2% for testosterone, 7.0 and 5.2% for SHBG, 5.7 and 4.3% for insulin and 5.3 and 3.9% for DHEAS.

Cardiac structure and function

The cardiac parameters: interventricular septum thickness (IVST), left ventricular posterior wall thickness (LVPWT), isovolumetric relaxation time (IVRT), left atrium size (LAS), deceleration time (DT), left ventricular end-diastolic and end-systolic diameters were measured on Days 2–5 of a spontaneous or progestin-induced menstrual cycle using ultrasound (ATL system HDI 5000 ultrasound) with a 2.5-MHz transducer. M-Mode, two-dimensional and pulsed Doppler echocardiography were employed. The left ventricular ejection fraction (LVEF) was then estimated according to the modified Simpson method (Quinones *et al.*, 1981). The LVEF was considered as normal when it was above 50%. The measurements were made over a minimum of three consecutive heart cycles and evaluated by an experienced reader who was blind to participants' clinical data, NT-proBNP levels and menstrual cycle phase. All patients were studied in the left lateral position after 5-min resting period according to the recommendations of the American Society of Echocardiography (Feigenbaum, 1979).

Doppler study has provided indices of ventricular filling that were derived from the mitral flow velocity curves; maximal early diastolic flow velocity (E; centimeters per second), maximal late diastolic flow velocity (A; centimeters per second) and the ratio between E and A curves (E/A; normal, >1). The left ventricular mass (LVM) was calculated by Devereux's formula (Devereux *et al.*, 1984) according to Penn's convention with the regression-corrected cube formula given as:

$$\text{LVM} = 0.8 \times 1.04[(\text{LVID} + \text{PWT} + \text{VST})^3 - \text{LVID}^3] + 0.6$$

where LVID is left ventricular internal diameter; PWT the posterior wall thickness and VST the ventricular septal thickness.

Intrareading reliability for the echocardiographer involved in the study was determined by repeating measurement sessions on ten unidentified subjects, and the variation coefficients for IVST, LVPWT, and LVM were determined as 3.8, 5.3 and 5.5%, respectively. The limits of agreement were generally small (e.g. 0.4–1.1 mm for wall thicknesses, 1.1–2 mm for cavity dimensions and –20 to 25 g for LVM) and agreed with those already stated for reproducibility within the cardiological literature (Bland and Altman, 1986; de Leonardis and Cinelli, 1986).

Statistical analysis

The Statistical Package for Social Sciences, version 11.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Normality of continuous variables in groups was tested by the Shapiro–Wilk test. Since the variables did not show a normal distribution, Mann–Whitney *U*-test was used for comparison. The data were presented as median ± interquartile range (IQR). Categorical variables were tested using

Pearson chi-square test, where appropriate. Spearman's rank order correlation coefficients were used to assess associations between NT-proBNP level and biochemical and echocardiographical indices. A subgroup analysis of PCOS subjects with abnormal echocardiographic indices was performed to detect possible relationship between NT-proBNP and metabolic variables. For all comparisons, statistical significance was defined by $P < 0.05$.

Results

The clinical characteristics and hormone profiles of patients with PCOS and those of the controls are shown in Table 2. There was no significant difference between the two groups regarding to their age, height, BMI, waist circumference, SBP, DBP, heart rate, FSH and DHEAS. In contrast, there were significant differences in NT-proBNP (Fig. 1), total testosterone, E₂, SHBG, LH levels and the Ferriman–Gallwey hirsutism score between the groups.

The results for biochemical markers of cardiovascular risks are shown in Table 3. There was no significant difference in fasting glucose, C-peptide, TC, LDL-C, VLDL-C, HDL-C and TG concentrations of the groups. On the contrary, significant difference in HOMA-IR and fasting insulin levels were detected between the groups. While the serum NT-proBNP concentration negatively correlated with SHBG level (Fig. 2, $r = -0.528$, $P = 0.003$), it did not show any correlation with age, fasting glucose, BMI, waist circumference, HOMA-IR, fasting insulin, SBP and DBP. Nonetheless, subgroup analysis accomplished on PCOS subjects with abnormal echocardiographic indices revealed a positive correlation between fasting insulin and NT-proBNP levels (Fig. 3).

The echocardiographical parameters obtained for PCOS ($n = 30$) and control ($n = 30$) subjects are shown in Table 4. From the cardiac point of view, while LVPWT and LVM in PCOS subjects increased compared with the controls, LVEF, IVST, IVRT, DT, LAS and E/A ratio (early to late mitral flow velocity) were found to be within the normal range in both groups. Serum NT-proBNP concentration was positively correlated with LVM (Fig. 4, $r = 0.587$, $P = 0.001$), but it did not show any correlation with IVST, IVRT, DT, LAS, LVPWT and LVEF (Table 5). In addition, there was positive correlation between LVM and HOMA-IR ($r = 0.295$, $P = 0.03$), while LVPWT was positively correlated with fasting insulin and HOMA-IR ($r = 0.335$, $P = 0.031$ and $r = 0.346$, $P = 0.045$, respectively) in PCOS subjects ($n = 30$).

Among the PCOS subjects it was found that NT-proBNP level was higher in the subjects with abnormal echocardiographic indices than in those having normal indices [96.53 ± 93.9 (range, 50.3–243.9) versus 37.0 ± 23.9 (range, 6.68–49.33) pg/ml, $P < 0.000$]. Abnormal echocardiograph findings were detected in 14 of the 30 PCOS subjects, 9 of which were valvular heart disease, 2 of which were diastolic dysfunction and the other 3 were independent right ventricular enlargement, right atrial enlargement and pulmonary hypertension. The nine cases with valvular heart diseases included mitral regurgitation ($n = 4$), mitral valve prolapsus ($n = 3$) and tricuspid regurgitation ($n = 2$).

Table 2: Descriptive variables in PCOS ($n = 30$) and control subjects ($n = 30$) (median \pm IQR) (minimum–maximum)

Characteristic	PCOS ($n = 30$)	Control ($n = 30$)	P -value*
Age (years)	21.00 \pm 4.75 (17.0–29.0)	23.00 \pm 2.00 (20.0–33.0)	0.318
Height (cm)	163.00 \pm 7.00 (150.0–180.0)	163.00 \pm 5.00 (155.0–169.0)	0.628
Weight (kg)	59.00 \pm 14.5 (43.0–90.0)	54.50 \pm 10.6 (44.0–62.0)	0.076
Body mass index (kg/m ²)	21.00 \pm 5.20 (16.9–39.6)	20.00 \pm 4.38 (17.6–23.0)	0.505
Waist circumference (cm)	70.00 \pm 11.0 (60.0–108.0)	67.50 \pm 6.75 (62.0–80.0)	0.411
FSH (mIU/ml)	5.99 \pm 2.69 (2.21–8.80)	5.77 \pm 1.71 (3.06–10.56)	0.692
LH (mIU/ml)	7.27 \pm 8.26 (1.43–17.30)	5.58 \pm 5.1 (1.51–13.19)	0.021
DHEAS (μ g/dl)	207.0 \pm 142.0 (102.0–372.0)	265.35 \pm 117.35 (122.0–483.7)	0.141
Total testosterone (ng/dl)	60.55 \pm 31.5 (24.0–158.0)	50.00 \pm 18.62 (13.40–97.0)	0.021
Estradiol (pg/ml)	33.50 \pm 15.8 (20.0–70.1)	91.59 \pm 66.64 (43.87–436.8)	0.000
SBP (mmHg)	110.0 \pm 20.0 (90.0–130.0)	110.0 \pm 20.0 (90.0–130.0)	0.728
DBP (mmHg)	70.00 \pm 20.0 (60.0–90.0)	70.00 \pm 18.75 (60.0–90.0)	0.898
Heart rate (beats/min)	74.00 \pm 10.00 (68.0–85.0)	74.00 \pm 10.0 (64.0–90.0)	0.394
SHBG (nmol/ml)	29.50 \pm 14.0 (14.76–180.0)	48.60 \pm 26.98 (32.90–88.0)	0.001
M-Ferriman–Gallwey score	15.0 \pm 7.00 (8.0–29.0)	8.00 \pm 5.50 (6.0–14.0)	<0.001

* $P < 0.05$ considered significant. DBP, diastolic blood pressure; SBP, systolic blood pressure; SHBG, sex-hormone-binding globulin.

Discussion

Even though some evidence showing cardiovascular risks in patients with PCOS has been collected (Birdsall *et al.*, 1997; Tiras *et al.*, 1999; Orio *et al.*, 2004), substantial numbers of PCOS patients may consult gynecologists or endocrinologists rather than cardiologists, leading to underestimation of genuine cardiovascular disease prevalence. The information in this direction is limited to echocardiography probably due to the lack of cost-effective methods. Our primary aim was to investigate whether NT-proBNP level could be a preliminary screening tool for cardiac disease in patients with PCOS before undertaking echocardiography.

The echocardiography of PCOS subjects showed an increased LVM and LVPWT. LVPWT was not associated

with NT-proBNP level, but LVM had a positive correlation with NT-proBNP and HOMA-IR. This correlation may be clinically important. Left ventricular hypertrophy (LVH) is one of several metabolic and cardiovascular risk factors which have been associated with IR (Vetta *et al.*, 1998; McFarlane *et al.*, 2001). Also previous studies support the hypothesis that IR may contribute to myocardial dysfunction in PCOS (Tiras *et al.*, 1999; Orio *et al.*, 2004). Therefore, PCOS might be considered as an aggravating factor in increasing LVM and consequently the occurrence of LVH. Despite the fact that no significant correlation between HOMA-IR and NT-proBNP level was detected in individual subjects, a positive correlation between fasting insulin and NT-proBNP level was found through subgroup analysis, conducted on the 14 PCOS subjects with abnormal echocardiographic indices. This finding may encourage one to think that the high HOMA-IR may be the reason for the increase in LVM and consequently the elevation of NT-proBNP level.

Low level of SHBG has been proposed as a surrogate marker of metabolic syndrome in PCOS subjects (Chen *et al.*, 2006). Insulin is one of the modulators of SHBG. By inhibiting SHBG production (Plymate *et al.*, 1988; Nestler *et al.*, 1991; Botwood *et al.*, 1995) a greater serum-free androgen level can be produced. The low level of SHBG has also been associated with the increase of cardiovascular risk in both pre- and post-menopausal women (Rexrode *et al.*, 2003; Sutton-Tyrrell *et al.*, 2005). In Women's Health Study, SHBG has been inversely related to C-reactive protein in post-menopausal women who had manifested clinical cardiovascular disease (Joffe *et al.*, 2006). Our results did not show a definitive relationship between SHBG and androgen levels, but showed a negative correlation between SHBG and NT-proBNP levels. Regarding the cardioprotective effects of NT-proBNP, including natriuresis, diuresis, antiproliferative and vasodilatory effects as well as antagonism of the renin-angiotensin-aldosterone system (Saito *et al.*, 1989; Yoshimura *et al.*, 1991; Molina *et al.*, 1998; de Lemos *et al.*, 2003; Chang *et al.*, 2007), it may be speculated that stimulation of high levels of NT-proBNP could protect against ventricular hypertrophy in PCOS subjects with cardiovascular risk associated with low

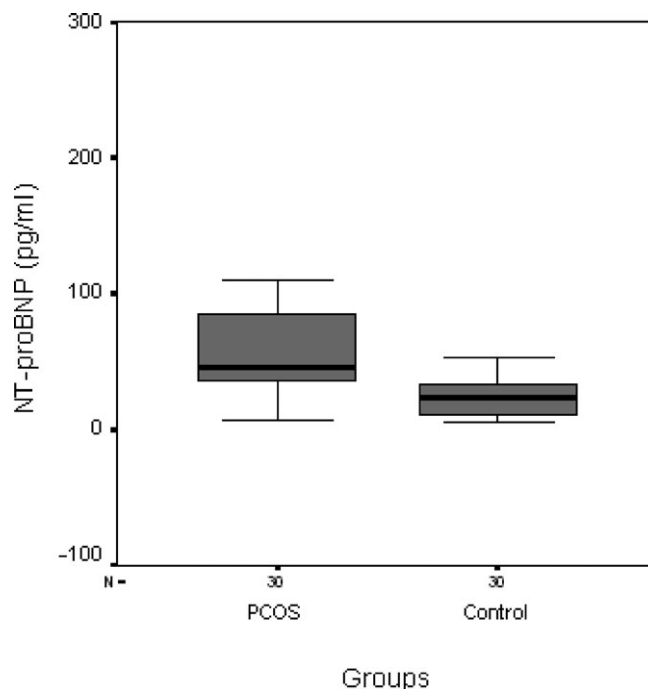


Figure 1: Comparison of NT-proBNP level in PCOS ($n = 30$) and control subjects ($n = 30$). Dark line = median, Box shows 1QR, lines maximum and minimum

Table 3: Metabolic profile and cardiovascular risk factors of PCOS (*n* = 30) and control subjects (*n* = 30) (median ± IQR) (minimum–maximum)

Characteristic	PCOS (<i>n</i> = 30)	Control (<i>n</i> = 30)	<i>P</i> -value*
Fasting glucose (mg/dl)	89.00 ± 15.00 (78.0–124.0)	91.00 ± 27.75 (71.0–122.0)	0.554
Fasting insulin (mU/ml)	19.83 ± 31.15 (4.81–49.59)	11.85 ± 11.86 (3.95–27.10)	0.005
C-peptide (ng/ml)	2.40 ± 1.44 (1.22–5.0)	2.17 ± 1.62 (0.90–5.20)	0.846
HOMA-IR	4.44 ± 6.49 (1.10–15.0)	2.25 ± 1.67 (.95–6.07)	0.008
TC (mg/dl)	151.00 ± 41.75 (109.0–240.0)	173.00 ± 47.75 (110.0–227.0)	0.091
VLDL-C (mg/dl)	19.40 ± 15.6 (6.60–59.0)	22.10 ± 9.35 (8.40–39.0)	0.109
LDL-C (mg/dl)	82.00 ± 21.0 (58.0–171.0)	96.50 ± 30.28 (65.8–155.0)	0.100
HDL-C (mg/dl)	46.00 ± 17.0 (12.4–76.0)	59.00 ± 18.75 (34.0–79.0)	0.379
TG (mg/dl)	96.00 ± 74.0 (33.0–289.0)	105.0 ± 55.75 (42.0–198.0)	0.105
NT-proBNP (pg/ml)	46.29 ± 46.52 (6.68–174.60)	27.48 ± 22.59 (5.0–68.87)	<0.001

**P* < 0.05 considered significant. HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistance index; LDL-C, low-density lipoprotein cholesterol; NT-proBNP, N-terminal pro-B-type brain natriuretic peptide; TC, total cholesterol; TG, triglycerides; VLDL-C, very-low-density lipoprotein cholesterol.

SHBG level. However, because many women with PCOS exhibit IR (Arcaro *et al.*, 2002), it is not clear enough whether NT-proBNP level is secondary to IR or hyperandrogenism itself contributes to IR and increases the NT-proBNP level.

Our results have shown that approximately one-half (46.6%) of PCOS subjects with a high NT-proBNP level have cardiac abnormalities. The NT-proBNP level in 7 of 14 asymptomatic PCOS subjects with cardiac abnormalities was above 103 pg/ml and this level is in accordance with the well-known report of New York Heart Association class II (NYHA II) (Wu *et al.*, 2003). The NT-proBNP level for the remaining seven subjects was below 100 pg/ml, which is in agreement with NYHA I (Wu *et al.*, 2003; Takase *et al.*, 2007).

On the other hand, 16 of the 30 PCOS subjects having high NT-proBNP levels did not show any abnormal indices through echocardiography. Hence, the occurrence of high levels of NT-proBNP in PCOS subjects with normal echocardiography

is limiting us, at this stage, regarding speculation about the use of NT-proBNP concentration for screening of PCOS subjects for cardiac abnormalities.

This study, unfortunately, has some other inherent shortcomings: (i) a single measurement and a small population of women with PCOS, (ii) a long-term monitoring such as ambulatory electrocardiogram was not performed to be able to demonstrate any relation of arrhythmia to NT-proBNP level, (iii) the NT-proBNP level has not been compared in lean and obese PCOS subjects and (iv) not implementing an invasive test or tissue Doppler echocardiography might have caused us to miss some patients with subclinical cardiac abnormalities in PCOS subjects with high NT-proBNP concentrations. In spite of these limitations, as a first clinical trial, this study was able to detect a difference in the NT-proBNP levels,

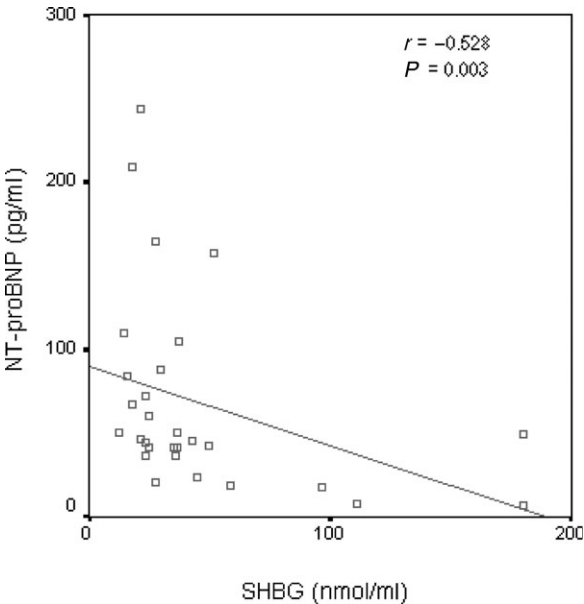


Figure 2: Correlation between serum NT-proBNP and SHBG levels in women with PCOS (*n* = 30)

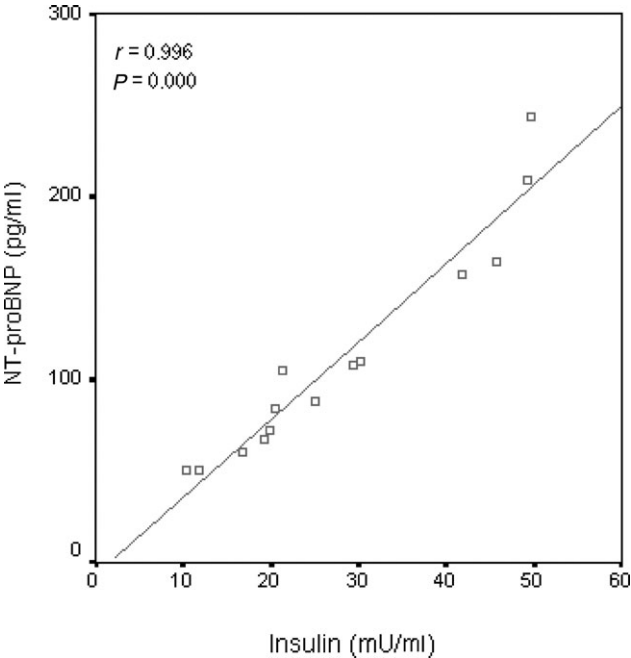
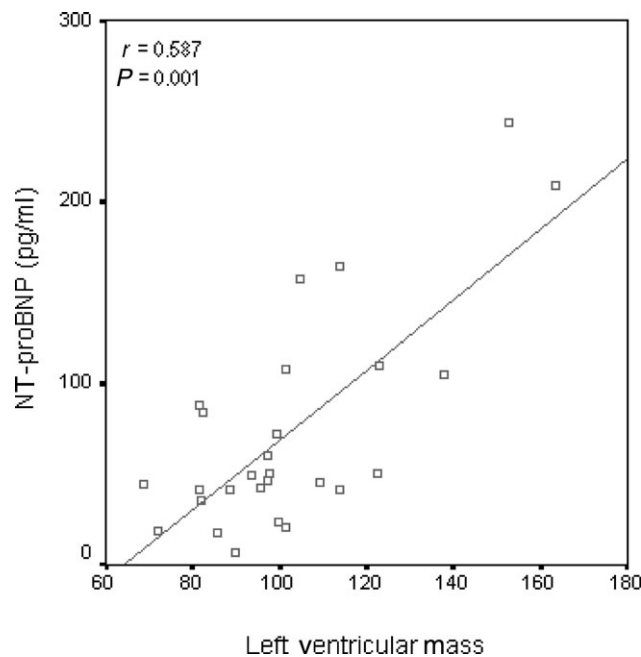


Figure 3: Correlation between serum NT-proBNP and fasting insulin levels in PCOS subjects (*n* = 14) with abnormal echocardiographic indices

Table 4: Echocardiographic findings in PCOS ($n = 30$) and control subjects ($n = 30$) (median \pm IQR) (minimum–maximum)

Characteristic	PCOS ($n = 30$)	Control ($n = 30$)	P -value*
IVST (mm)	7.0 \pm 0.1 (7.0–8.0)	7.5 \pm 0.1 (7.0–11.0)	0.256
IVRT (msn)	70.0 \pm 7.5 (65.0–110.0)	70.0 \pm 10.0 (60.0–90.0)	0.216
DT (msn)	170.0 \pm 17.5 (140.0–225.0)	160.0 \pm 20.0 (130.0–185.0)	0.106
LAS (mm)	30.0 \pm 0.5 (25.0–34.0)	29.0 \pm 0.6 (24.0–35.0)	0.194
LVPWT (mm)	6.04 \pm 2.7 (4.7–8.4)	5.20 \pm 0.5 (4.7–5.6)	0.006
LVM (g)	97.34 \pm 29.43 (68.82–163.38)	78.80 \pm 15.15 (62.81–95.46)	<0.001
LVEF (%)	65.0 \pm 5.0 (60.0–70.0)	65.0 \pm 8.0 (60.0–70.0)	0.674
Early to late mitral flow velocity (E/A)			
E/A; normal, >1	29 (96.7%)	30 (100%)	E; centimeters per second
E/A; abnormal, >2	1 (3.3%)	0	A; centimeters per second

* $P < 0.05$ considered significant. DT, deceleration time; IVRT, isovolumetric relaxation time; IVST, interventricular septum thickness; LAS, left atrium size; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; LVPWT, left ventricular posterior wall thickness.

**Figure 4:** Correlation between serum NT-proBNP level and left ventricular mass in PCOS ($n = 30$) subjects

being higher in PCOS than in the control subjects, which may indicate a positive correlation with cardiovascular risk. So, the evaluation of NT-proBNP together with insulin and SHBG levels may be a potential marker to select patients with PCOS for echocardiographical referral.

The findings from this cross-sectional analytic study need to be confirmed. A larger, prospective and controlled study and long-term follow-up are needed to help determine sensitivity, specificity and predictive value of this promising marker of cardiac dysfunction in women with PCOS. The cost-effectiveness of such a strategy also need to be investigated.

Acknowledgements

We thank Associate Professor Saim Yologlu for assistance in the statistical analysis of the manuscript.

Table 5: Spearman correlation coefficients (r) between serum NT-proBNP level and measured parameters in PCOS subjects ($n = 30$)

	NT-proBNP	
	r	P -value*
Fasting insulin ^a	−0.024	0.901
Fasting glucose	−0.054	0.786
HOMA-IR ^b	−0.043	0.830
Testosterone	−0.339	0.083
SHBG	−0.528	0.003
TC	−0.335	0.059
FSH	0.025	0.899
LH	0.325	0.085
IVST	−0.179	0.372
LVM	0.587	0.001
IVRT	−0.139	0.490
DT	−0.284	0.152
LAS	0.236	0.236
LVPWT ^c	−0.203	0.309
LVEF	−0.044	0.826

* $P < 0.05$ considered significant. ^aThere was positive correlation between fasting insulin and Nt-proBNP levels in the subset of PCOS subjects ($n = 14$) with abnormal echocardiographic recording ($r = 0.996$, $P = 0.000$) (Fig. 3); ^bthere was positive correlation between LVM and HOMA-IR ($r = 0.295$, $P = 0.03$) in PCOS ($n = 30$) subjects; ^cthere was positive correlation between LVPWT and fasting insulin level and HOMA-IR ($r = 0.335$, $P = 0.031$ and $r = 0.346$, $P = 0.045$, respectively) in PCOS ($n = 30$) subjects.

References

- Api M, Akca A, Bodoglu B, Gorgen H, Cetin A. Interobserver variability of Modified Ferriman Gallwey Hirsutism scores on each body area in unselected Turkish population. *Arch Gynecol Obstet* 2007; **275**(Suppl 1):29.
- Arcaro G, Cretti A, Balzano S, Lechi A, Muggeo M, Bonora E, Bonadonna RC. Insulin causes endothelial dysfunction in humans: sites and mechanisms. *Circulation* 2002; **105**:576–582.
- Bickerton AS, Clark N, Meeking D, Shaw KM, Crook M, Lumb P, Turner C, Cummings MH. Cardiovascular risk in women with polycystic ovarian syndrome (PCOS). *J Clin Pathol* 2005; **58**:151–154.
- Birdsall MA, Farquhar CM, White HD. Association between polycystic ovaries and extent of coronary artery disease in women having cardiac catheterization. *Ann Intern Med* 1997; **126**:32–35.
- Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; **1**:307–310.
- Botwood N, Hamilton-Fairley D, Kiddy D, Robinson S, Franks S. Sex hormone-binding globulin and female reproductive function. *J Steroid Biochem Mol Biol* 1995; **53**:529–531.

- Carmina E, Lobo RA. Polycystic ovary syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. *J Clin Endocrinol Metab* 1999;**84**:1897–1899.
- Chang AY, Abdullah SM, Jain T, Stanek HG, Das SR, McGuire DK, Auchus RJ, de Lemos JA. Associations among androgens, estrogens, and natriuretic peptides in young women: observations from the Dallas Heart Study. *J Am Coll Cardiol* 2007;**49**:109–116.
- Chen MJ, Yang WS, Yang JH, Hsiao CK, Yang YS, Ho HN. Low sex hormone-binding globulin is associated with low high-density lipoprotein cholesterol and metabolic syndrome in women with PCOS. *Hum Reprod* 2006;**21**:2266–2271.
- Cibula D, Cifkova R, Fanta M, Poledne R, Zivny J, Skibova J. Increased risk of non-insulin dependent diabetes mellitus, arterial hypertension and coronary artery disease in perimenopausal women with a history of the polycystic ovary syndrome. *Hum Reprod* 2000;**15**:785–789.
- Dahlgren E, Janson PO, Johansson S, Lapidus L, Oden A. Polycystic ovary syndrome and risk for myocardial infarction. Evaluated from a risk factor model based on a prospective population study of women. *Acta Obstet Gynecol Scand* 1992;**71**:599–604.
- de Lemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. *Lancet* 2003;**362**:316–322.
- de Leonardi V, Cinelli P. Evidence of no interobserver variability in M-mode echocardiography. *Clin Cardiol* 1986;**9**:324–326.
- Devereux RB, Lutas EM, Casale PN, Kligfield P, Eisenberg RR, Hammond IW, Miller DH, Reis G, Alderman MH, Laragh JH. Standardization of M mode echocardiographic left ventricular anatomic measurements. *J Am Coll Cardiol* 1984;**4**:1222–1230.
- Feigenbaum H. *Echocardiography*, 2nd edn. Philadelphia: Lea and Febiger, 1979.
- Ferriman D, Gallwey JD. Clinical assessment of body hair growth in women. *J Clin Endocrinol Metab* 1962;**21**:1440–1447.
- Franks S. Are women with polycystic ovary syndrome at increased risk of cardiovascular disease? Too early to be sure, but not too early to act!. *Am J Med* 2001;**111**:665–666.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;**18**:499–502.
- Guzick DS. Cardiovascular risk in women with polycystic ovarian syndrome. *Semin Reprod Endocrinol* 1996;**14**:45–49.
- Hunt PJ, Richards AM, Nicholls MG, Yandle TG, Doughty RN, Espiner EA. Immunoreactive amino-terminal pro-brain natriuretic peptide (NTPROBNP): a new marker of cardiac impairment. *Clin Endocrinol (Oxf)* 1997;**47**:287–296.
- Joffe HV, Ridker PM, Manson JE, Cook NR, Buring JE, Rexrode KM. Sex hormone-binding globulin and serum testosterone are inversely associated with C-reactive protein levels in postmenopausal women at high risk for cardiovascular disease. *Ann Epidemiol* 2006;**16**:105–112.
- Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. *J Clin Endocrinol Metab* 1998;**83**:3078–3082.
- Maggia J, Marttila M, Mäntymaa P, Vuolteenaho O, Ruskoaho H. Brain natriuretic peptide in plasma, atria, and ventricles of vasopressin- and phenylephrine-infused conscious rats. *Endocrinology* 1994;**134**:2505–2515.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;**28**:412–419.
- McFarlane SI, Banerji M, Sowers JR. Insulin resistance and cardiovascular disease. *J Clin Endocrinol Metab* 2001;**86**:713–718.
- Mokuyama M, Nakao K, Nosada K. Brain natriuretic peptide as a novel cardiac hormone in humans: evidence for an exquisite dual natriuretic peptide system, ANP and BNP. *J Clin Invest* 1991;**87**:1402–1412.
- Molina CR, Fowler MB, McCrory S. Hemodynamic, renal and endocrine effects of atrial natriuretic peptide infusion in severe heart failure. *J Am Coll Cardiol* 1988;**12**:175–186.
- Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Rittmaster RS, Clore JN, Blackard WG. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. *J Clin Endocrinol Metab* 1991;**72**:83–89.
- Orio F Jr, Palomba S, Spinelli L, Cascella T, Tauchmanova L, Zullo F, Lombardi G, Colao A. The cardiovascular risk of young women with polycystic ovary syndrome: an observational, analytical, prospective case-control study. *J Clin Endocrinol Metab* 2004;**89**:3696–3701.
- Plymate SR, Matej LA, Jones RE, Friedl KE. Inhibition of sex hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin. *J Clin Endocrinol Metab* 1988;**67**:460–464.
- Quinones MA, Waggoner AD, Reduto LA, Nelson JG, Young JB, Winters WL Jr, Ribeiro LG, Miller RR. A new, simplified and accurate method for determining ejection fraction with two-dimensional echocardiography. *Circulation* 1981;**64**:744–753.
- Rexrode KM, Manson JE, Lee IM, Ridker PM, Sluss PM, Cook NR, Buring JE. Sex hormone levels and risk of cardiovascular events in postmenopausal women. *Circulation* 2003;**108**:1688–1693.
- Saito Y, Nakao K, Itoh H. Brain natriuretic peptide is a novel cardiac hormone. *Biochem Biophys Res Commun* 1989;**158**:360–368.
- Sawada Y, Suda M, Yokoyama H, Kanda T, Sakamaki T, Tanaka S, Nagai R, Abe S, Takeuchi T. Stretch-induced hypertrophic growth of cardiocytes and processing of brain-type natriuretic peptide are controlled by proprotein-processing endoprotease furin. *J Biol Chem* 1997;**272**:20545–20554.
- Scarpitta AM, Sinagra D. Polycystic ovary syndrome: an endocrine and metabolic disease. *Gynecol Endocrinol* 2000;**14**:392–395.
- Suga S, Nakao K, Hosoda K, Mukoyama M, Ogawa Y, Shirakami G, Arai H, Saito Y, Kambayashi Y, Inouye K. Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. *Endocrinology* 1992;**130**:229–239.
- Sutton-Tyrrell K, Wildman RP, Matthews KA, Chae C, Lasley BL, Brockwell S, Pasternak RC, Lloyd-Jones D, Sowers MF, Torrens JJ, SWAN Investigators. Sex-hormone-binding globulin and the free androgen index are related to cardiovascular risk factors in multiethnic premenopausal and perimenopausal women enrolled in the Study of Women Across the Nation (SWAN). *Circulation* 2005;**111**:1242–1249.
- Takase H, Toriyama T, Sugiura T, Takahashi A, Sugiyama M, Ohashi M, Ueda R, Dohi Y. Brain natriuretic peptide detects cardiac abnormalities in mass screening. *Eur J Clin Invest* 2007;**37**:257–262.
- Talbott EO, Guzik DS, Sutton-Tyrrell K, McHugh-Pemu KP, Zborowski JV, Remsberg KE, Kuller LH. Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. *Arterioscler Thromb Vasc Biol* 2000;**20**:2414–2421.
- The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus Statement. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004;**81**:19–25.
- Tiras MB, Yalcin R, Noyan V, Maral I, Yildirim M, Dortlemmez O, Daya S. Alterations in cardiac flow parameters in patients with polycystic ovarian syndrome. *Hum Reprod* 1999;**14**:1949–1952.
- Vetta F, Cicconetti P, Ronzoni S, Rizzo V, Palleschi L, Canarile G, Lupattelli MR, Migliori M, Morelli S, Marigliano V. Hyperinsulinaemia, regional adipose tissue distribution and left ventricular mass in normotensive, elderly, obese subjects. *Eur Heart J* 1998;**19**:326–331.
- Wu AH, Smith A, Wiecek S, Mather JF, Duncan B, White CM, McGill C, Katten D, Heller G. Biological variation for N-terminal pro- and B-type natriuretic peptides and implications for therapeutic monitoring of patients with congestive heart failure. *Am J Cardiol* 2003;**92**:628–631.
- Yanovski SZ. A practical approach to treatment of the obese patient. *Arch Fam Med* 1993;**2**:309–316.
- Yoshimura M, Yasue H, Morita E. Hemodynamic, renal and hormonal responses to brain natriuretic peptide infusion in patients with congestive heart failure. *Circulation* 1991;**84**:1581–1588.
- Yu CM, Sanderson JE, Shum IO, Chan S, Yeung LY, Hung YT, Cockram CS, Woo KS. Diastolic dysfunction and natriuretic peptides in systolic heart failure. Higher ANP and BNP levels are associated with the restrictive filling pattern. *Eur Heart J* 1996;**17**:1694–1702.

Submitted on March 29, 2007; resubmitted on July 31, 2007; accepted on August 13, 2007