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Incremental Importance of Peak-Exercise Plasma Levels of Endothelin-1 and Natriuretic Peptides in Chronic Heart Failure

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Summary: Chronic heart failure (CHF) studies investigating the clinical, hemodynamic, and therapeutic importance of endothelin-1 (ET-1), atrial natriuretic peptide (ANP), and brain natriuretic peptide (BNP) are largely based on resting plasma levels, which may vary with prior exertion and postprandial status. This study investigated the importance of peak-exercise plasma levels of ET-1, ANP, and BNP in the assessment of left ventricular (LV) systolic function. Thirty-six male-patients ages 58 ± 10 (mean ± SD) with NYHA class I–IV CHF due to coronary artery disease or idiopathic dilated cardiomyopathy were enrolled. LV systolic function was assessed by echocardiography and radionuclide ventriculography. Resting and peak cardiopulmonary exercise venous blood sampling and treadmill exercise testing were performed in the fasting state. Resting plasma levels of ET-1, ANP, and BNP were elevated compared with reference laboratory normal values. Exercise induced significant ($p < 0.0001$) increase in plasma levels of ET-1, ANP, and BNP. On univariate analysis peak-exercise plasma levels of ET-1, ANP, and BNP were more closely related to echocardiographically determined LV end-diastolic diameter and end-systolic diameter than their resting values. Multiple stepwise regression models identified resting and peak-exercise plasma levels of ET-1 and ANP but only the resting BNP as independent predictors of LV dimensions and systolic function. Peak exercise plasma levels of ANP and ET-1 are potentially more reliable and important than their resting levels as markers of LV systolic dysfunction and LV dimensions in patients with heart failure. **Key Words:** Chronic heart failure—Natriuretic peptides—Endothelin-1—Exercise.

The conventional pathophysiologic view of chronic heart failure (CHF) has shifted from a simple model of pump failure to a more complex one including neurohumoral activation, which contributes to the pathogenesis and progression of the disease (1–5). Pharmacologic strategies of CHF management, which reduce mortality and improve functional status, are associated with a reduction in neurohumoral activation, underlining the

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therapeutic potential of neurohumoral interventions (6,7). Novel therapeutic strategies are targeting atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and endothelin-1 (ET-1) after the success achieved with angiotensin-converting-enzyme inhibitors and beta-blockers for the treatment of CHF. Elevated resting plasma levels of ANP, BNP, and ET-1 in patients with CHF are proportional to hemodynamic and clinical severity of the disease (8–10). Studies investigating clinical, hemodynamic, and therapeutic importance were largely based on resting plasma levels of ET-1 and natriuretic peptides. Given the variability over time in plasma levels of these peptides, a single measurement may not be entirely reliable for this purpose. Dynamic exercise is known to augment circulating plasma natriuretic peptides and ET-1 levels in patients with CHF (11,12). The additional value of peak-exercise plasma levels of these peptides has not been investigated in such patients. Therefore, the purpose of this study is to investigate the importance of peak-exercise plasma levels of ET-1, ANP, and BNP in assessing left ventricular (LV) systolic function on the basis that this may give a more standardized setting for their evaluation. This may have potential future implication in predicting the response to pharmacologic agents or follow-up for clinical progress.

METHODS

Study design

Thirty-six male-patients (ages 58 ± 10 years; mean ± SD) with CHF due to coronary artery disease (n = 13) and idiopathic dilated cardiomyopathy with angiographically normal coronary arteries (n = 23) were enrolled in the study. Patients were ambulatory and clinically stable and had experienced no cardiac events within the preceding 3 months. New York Heart Association (NYHA) functional class was I (n = 4), II (n = 20), and III–IV (n = 12). Before enrollment all patients were receiving a stable combination of diuretics and angiotensin-converting-enzyme inhibitors. Patients with ischemic cardiomyopathy were also taking aspirin. Study patients were recruited from the specialist heart failure clinic at our tertiary referral center. LV systolic function was assessed by echocardiography and radionuclide ventriculography. Cardiopulmonary exercise testing was performed and blood samples were obtained in the fasting state between 9:00 A.M. and 11:00 A.M. The study was approved by the Ethics Committee of the National Heart and Lung Institute and Royal Brompton and Harefield Hospital.

Echocardiography

Echocardiographic examination was performed by an experienced cardiologist using a Hewlett–Packard SONOS 5500 system (Hewlett–Packard, Andover, MA, U.S.A.). A multifrequency ultrasound probe was used to obtain images. Two-dimensional guided M-mode recordings of LV minor axis were obtained from the conventional parasternal view with the patient in a left semilateral position. All records were obtained photographically on a paper speed of 100 mm/s with a superimposed electrocardiogram and phonocardiogram. LV end-diastolic diameter (EDD) was taken at the onset of q wave and end-systolic diameter (ESD) at the first high-frequency vibration of the second heart sound of the phonocardiogram using advanced methodology. LV fractional shortening (FS) was estimated as the percentage fall of LV dimension in systole with respect to that in diastole ([EDD − ESD] ÷ EDD × 100).

Radionuclide ejection fraction

Multigated acquisition scanning was performed to determine LV ejection fraction (LVEF) as a marker of LV systolic function.

Blood sampling

A 16-gauge polyethylene catheter was inserted percutaneously into the antecubital vein for blood sampling. Pre-exercise blood samples were drawn after 30 min of supine rest. Postexercise samples were taken at peak exercise. Both blood samples were promptly centrifuged at −4°C to separate the plasma, which was stored at −80°C until analysis.

Cardiopulmonary exercise testing

Maximal cardiopulmonary exercise testing was performed using the modified Bruce treadmill protocol. Respiratory gases were analyzed breath by breath with a spectrometer (AMIS 2000, Innovision, Odense, Denmark) to determine peak oxygen consumption and anaerobic threshold. Continuous 12-lead electrocardiogram was recorded (Marquette Electronics, GE Medical System, Milwaukee, WI, U.S.A.). Before enrollment, all patients underwent one or more exercise tests as part of a comprehensive heart failure evaluation in our heart failure unit.

Neurohumoral assay

Plasma ANP and BNP levels were measured directly without prior extraction by immunometric radioimmunoassay with I^{125} as a tracer (Shionoria ANP, Shionoria BNP, Shionogi Ltd., Osaka, Japan). Plasma ET-1 levels

were measured using enzyme-linked immunosorbent assay (ELISA) system (Biotra Cellular Communication Assays, Amersham International, Amersham Place, U.K.). We previously measured resting plasma levels of ANP (4.37 ± 2.23 p*M*), BNP (1.08 ± 1.40 p*M*), and ET-1 (1.60 ± 0.52 p*M*) in 10 healthy control subjects ages 44 ± 13 years, who had no history or clinical evidence heart or other diseases.

Statistical analysis

Continuous variables were described as mean ± SD. Logarithmic transformation was performed to achieve normal distribution when the data were skewed. Distribution of variables was considered normal if the W value (Shapiro–Wilk W test) was >0.95. Univariate regression was used to elucidate the relation between systolic function and individual circulating humoral factors. To identify the independent markers of systolic function, multiple step-wise regression models were used. A probability value of <0.05 was taken as the level of statistical significance. The statistical programs STATA 6.0 and Stat-View for Windows were used to analyze the data.

RESULTS

Baseline clinical and laboratory characteristics are presented in Table 1. Results of cardiopulmonary exercise testing, plasma levels of ET-1 and natriuretic peptides, and echocardiographic and radionuclide measurements in the patient population as a whole and in subgroups according to etiology are shown in Table 2. No significant difference was observed in exercise parameters, plasma levels of the peptides, echocardiographic measurements and LVEF between patients with idiopathic dilated and ischemic cardiomyopathy. Plasma levels of ANP, BNP, and ET-1 were all elevated at rest in the study population compared with laboratory normal values as described above. Exercise induced an increase in plasma levels of ET-1 (from 2.4 ± 0.9 to 3.4 ± 1.2 p*M*, $p < 0.0001$), ANP (from 19.8 ± 16.7 to 30.7 ± 26.8 p*M*, $p < 0.0001$), and BNP (from 32.3 ± 42.3 to 39.6 ± 50.4 p*M*, $p < 0.0001$). None of the measured plasma peptide was correlated with VO_2.

TABLE 1.
Baseline characteristics

	Total (n = 36)	DCM (n = 23)	ICM (n = 13)	p value
Age (years)	58 ± 10	55 ± 9	63 ± 10	0.11
Body mass index	24.6 ± 8.8	23.2 ± 2.0	26.4 ± 2.5	0.42
NYHA class	2.1 ± 0.5	2.1 ± 0.4	2.2 ± 0.3	0.58
Resting heart rate (beats/min)	71 ± 11	71 ± 10	70 ± 16	0.94
Resting mean blood pressure (mm Hg)	95 ± 16	99 ± 18	90 ± 11	0.64
Serum sodium (m*M*)	136.5 ± 1.9	136.0 ± 2.5	137.0 ± 1.3	0.54
Serum potassium (m*M*)	4.2 ± 0.2	4.2 ± 0.3	4.1 ± 0.1	0.64
Urea (m*M*)	8.0 ± 1.5	8.6 ± 1.6	5.6 ± 0.7	0.23
Serum creatinine	120 ± 25	109 ± 19	128 ± 28	0.27

DCM, idiopathic dilated cardiomyopathy; ICM, ischemic cardiomyopathy; NYHA, New York Heart Association class.

Echocardiographic measurements

Table 3 displays the results from univariate regression model analysis. Regression coefficient, coefficient of determination (R^2), and p value were incorporated to display the relation between plasma variables and LV dimensions and systolic functional indices. R^2 is an indicator of how well the model fits the data (e.g., R^2 close to 1.0 indicates that we have accounted for almost all variability with the variables specified in the models).

Resting plasma levels of ET-1 ($p = 0.002$) and BNP ($p < 0.0001$), but not ANP ($p = 0.08$), were closely correlated with EDD. In contrast to resting levels, peak-exercise plasma levels of all these peptides were closely correlated with EDD ($p = 0.01$ to < 0.0001). Resting and peak-exercise plasma levels of ET-1, ANP, and BNP were all significantly correlated with ESD ($p = 0.02$ to < 0.0001). Resting and peak-exercise plasma levels of all three peptides were negatively correlated with LVFS ($p = 0.015$ to 0.003). Plasma levels of resting and peak-exercise ET-1 and BNP were negatively correlated with LVEF ($p = 0.02$ to 0.006), but neither resting ($p = 0.07$) nor peak-exercise ($p = 0.27$) plasma ANP was correlated with LVEF.

Among the six plasma variables, resting ANP, resting BNP, and peak-exercise ET-1 were identified as independent predictors of EDD (R^2: 0.67; $p = 0.001$, 0.005, 0.01) and ESD (R^2: 0.64, $p = 0.002$, 0.05, 0.048). However, with respect to FS, only resting plasma BNP was identified as an independent predictor (R^2: 0.30, $p = 0.003$). This regression model identified resting ET-1 ($p = 0.01$) and BNP ($p = 0.03$) and peak ANP ($p = 0.04$) as independent predictors of LVEF (R^2: 0.36)

DISCUSSION

There are three main findings of this study. First, there is a significant correlation between circulating ET-1 and

TABLE 2.
Results

Measured variables	Total population (n = 36)	Subgroup analysis DCM (n = 23)	Subgroup analysis ICM (n = 13)	p value
Cardiopulmonary exercise parameters				
Peak heart rate (beats/min)	139 ± 30	144 ± 27	124 ± 15	0.24
Peak systolic blood pressure (mm Hg)	142 ± 18	145 ± 18	136 ± 19	0.29
Exercise duration (seconds)	499 ± 199	449 ± 232	448 ± 134	0.80
Exchange ratio	1.08 ± 0.11	1.08 ± 0.1	1.09 ± 0.1	0.95
Anaerobic threshold (ml/kg/min)	11.2 ± 4.1	11.1 ± 4.0	10.3 ± 3.2	0.71
Peak oxygen consumption (mg/kg/min)	19.9 ± 16.7	21.3 ± 8.9	16.3 ± 4.6	0.06
Plasma neurohumoral factors (p*M*)				
Resting atrial natriuretic peptide	19.8 ± 16.7	21.0 ± 16.3	18.2 ± 18.1	0.33
Peak atrial natriuretic peptide	30.7 ± 26.8	33.8 ± 30.8	24.2 ± 18.9	0.72
Resting brain natriuretic peptide	32.4 ± 42.3	33.2 ± 40.6	26.3 ± 30.9	0.73
Peak brain natriuretic peptide	39.6 ± 50.4	39.9 ± 55.2	37.5 ± 42.0	0.13
Resting E1-1	2.4 ± 0.9	2.3 ± 0.8	2.4 ± 0.9	0.23
Peak E1-1	3.4 ± 1.2	3.2 ± 1.1	3.6 ± 1.3	0.06
Echocardiographic parameters				
EDD (cm)	6.9 ± 1.2	7.1 ± 1.3	6.9 ± 1.0	0.71
ESD (cm)	5.7 ± 1.4	5.9 ± 1.6	5.7 ± 1.6	0.78
FS (%)	18.5 ± 8.5	17.6 ± 9.0	18.7 ± 9.3	0.80
LVEF (%)	27 ± 14	25 ± 13	32 ± 16	0.23

DCM, idiopathic dilated cardiomyopathy; ICM, ischemic cardiomyopathy; EDD, end-diastolic diameter; ESD, end-systolic diameter; FS, fractional shortening; LVEF, left ventricular ejection fraction; ET-1, endothelin-1.

natriuretic peptides (except resting ANP) at rest and during exercise and LV functional indices on univariate analysis. Second, resting ANP and BNP but peak-exercise ET-1 were the independent markers of LV dimensions. Resting BNP is the only independent predictor of LVFS. And third, resting ET-1 and BNP but peak-exercise ANP were identified as independent predictors of LVEF. Plasma levels of these three peptides were elevated at rest compared with laboratory reference normal values. Exercise resulted in increased circulating plasma ET-1 and natriuretic peptides in agreement with the previously published reports (11–14).

Systolic function and natriuretic peptides and endothelin-1

There are conflicting reports with respect to clinical and diagnostic significance of plasma natriuretic peptides and ET-1 in patients with CHF (15,16). Plasma concentrations of natriuretic peptides were found to correlate poorly with LV systolic dysfunction in survivors of acute myocardial infarction in a community-based study (17). Likewise, plasma levels of natriuretic peptides and ET-1 were found to be poor indicators of the severity of heart failure in subjects with NYHA class I and II (18). Conversely, other clinical studies and epide-

TABLE 3.
Univariate regression models

Plasma variables	EDD Coef	EDD R^2	EDD p value	ESD Coef	ESD R^2	ESD p value	FS Coef	FS R^2	FS p value	LVEF Coef	LVEF R^2	LVEF p value
Resting ET-1	0.57	0.30	0.002	0.61	0.36	0.001	–0.54	0.25	0.005	–0.63	0.20	0.006
Peak-exercise ET-1	0.7	0.45	<0.0001	0.70	0.46	<0.0001	–0.55	0.25	0.008	–0.61	0.19	0.010
Resting ANP	0.36	0.1	0.08	0.45	0.19	0.02	–0.45	0.2	0.015	–0.28	0.1	0.07
Peak-exercise ANP	0.48	0.21	0.01	0.57	0.3	0.002	–0.54	0.28	0.005	–0.20	0.04	0.27
Resting BNP	0.67	0.40	<0.0001	0.71	0.47	<0.0001	–0.55	0.30	0.003	–0.38	0.15	0.02
Peak-exercise BNP	0.70	0.46	<0.0001	0.72	0.51	<0.0001	–0.54	0.28	0.005	–0.42	0.18	0.013

Coef, regression coefficient; R^2, coefficient of determination; EDD, end-diastolic diameter; ESD, end-systolic diameter; FS, fractional shortening; LVEF, left ventricular ejection fraction; ET-1, endothelin-1; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide.

miologic surveys have demonstrated that these peptides may be useful in identifying systolic ventricular dysfunction and assessing hemodynamic and clinical severity of the disease (16,19–23). Studies investigating the relative importance of resting plasma ANP and BNP have yielded contradictory results. In particular, resting plasma ANP has been shown to correlate with atrial size and pressure, LV geometry and end-diastolic pressure, LV filling pattern, echo-derived LVEF, LVEDD, and LVESD in CHF studies (20,24). Other studies have demonstrated superiority of circulating BNP over ANP as a humoral marker of altered hemodynamics in CHF (25).

In clinical studies evaluating potential use of peak-exercise neurohormones, de Groote et al. (26) reported that ANP at peak exercise is an independent marker of cardiovascular death, whereas Madsen et al. (13) observed no prognostic importance of this peptide in patients with CHF. This is the first observation of an additional importance of peak-exercise plasma peptides as markers of LV dimensions and systolic function. In this study we have demonstrated peak-exercise ANP rather than resting ANP as an independent predictor of LVEF.

Circulating ET-1 is also known to correlate with pulmonary artery pressure and prognosis in patients with heart failure. Endothelin receptor blockade results in reduction in pulmonary vascular resistance and improvement in cardiac function (27,28), thus underlining the role of ET-1 in pulmonary artery pressure and ventricular remodeling in heart failure. However, the literature lacks evidence for any relation between ET-1 and LV systolic function. This study has clearly demonstrated the potential importance of plasma ET-1 as a marker of LV dimensions and systolic function. Further, we observed a superiority of peak-exercise level over resting ET-1 as an independent humoral marker of LV dimensions.

Clinical implications

There is no simple and readily available indicator of LV systolic function or method for assessing the therapeutic response in patients with heart failure. Measurements of LV systolic function by echocardiography or radionuclide imaging are relatively insensitive in demonstrating early changes in heart failure pathophysiology. Unlike LV ejection fraction and peak oxygen consumption, which are known for their practical limitations, circulating ET-1 and natriuretic peptides are potentially useful markers of LV function, as well as early therapeutic response in patients with CHF (29,30). In addition to resting levels, peak-exercise ET-1 and natriuretic peptides may provide additional hemodynamic and clinical information that may help optimize heart failure treatment.

Limitations

This study incorporated a small number of well-treated stable heart failure patients who were tracked regularly in a specialist heart failure clinic in a tertiary referral center. The study population is younger than general heart failure patients in the community. The results of this study should be cautiously translated to CHF patients in general, who are mostly treated with less intensive investigations at district general hospitals. We have not evaluated several other factors that might determine prognosis and clinical status in CHF, in particular, cytokines, catecholamines, and nitric oxide, which may offer additional information in selected patients.

CONCLUSION

In conclusion, for the evaluation of patients with CHF, peak-exercise plasma levels of ANP and ET-1 are potentially more important than their resting levels as predictors of LV dimensions and systolic function. Long-term studies involving larger samples of patient with repeated measurements are needed to confirm the accuracy of these peptides in the follow-up of heart failure patients in routine clinical practice.

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