Relationship Between Exercise Intolerance and Levels of Neurohormonal Factors and Proinflammatory Cytokines in Patients With Stable Chronic Heart Failure

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SUMMARY

We investigated the correlations between exercise intolerance and the plasma levels of neurohormonal factors and proinflammatory cytokines in chronic heart failure (CHF) patients.

Sixty-two CHF patients who underwent cardiopulmonary exercise testing (CPX) were enrolled in this study. Peak oxygen uptake (peak VO2) and the plasma concentrations of noradrenaline (NA), brain natriuretic peptide (BNP), and soluble tumor necrosis factor receptors I and II (TNFR-I and -II) were all measured during the CPX. The patients were divided into three groups according to their peak VO2; a severe exercise intolerance group (severe group; peak VO2 < 18 mL/min/kg), moderate exercise intolerance group (moderate group; 18 ≤ peak VO2 ≤ 24), and mild exercise intolerance group (mild group; peak VO2 > 24).

There were no significant differences in left ventricular ejection fraction (EF) among the three groups. NA and BNP both increased gradually in parallel with the worsening of exercise intolerance (NA, 211.5 ± 75.7 pg/mL, 331.8 ± 163.7, 441.9 ± 202.9, respectively; BNP, 37.9 ± 25.4 pg/mL, 148.9 ± 117.1, 247.9 ± 150.0, respectively). TNFR-I and II were significantly higher in the severe group than in the moderate group (1746.1 ± 950.7 versus 1085.2 ± 370.5 pg/mL and 2855.3 ± 1550.9 versus 2047.7 ± 648.7 pg/mL, respectively), while the values in the moderate group were not significantly different from those in the mild group. EF showed no significant correlations with NA, BNP, TNFR-I, or TNFR-II, whereas peak VO2 exhibited significant negative correlations with NA (r = −0.50, P < 0.0001), BNP (r = −0.53, P < 0.0001), TNFR-I (r = −0.50, P < 0.0001), and TNFR-II (r = −0.45, P < 0.0001).

It is concluded that NA and BNP rise in parallel with the degree of exercise intolerance, while TNFR-I and -II rise only when exercise intolerance reaches severe levels. (Int Heart J 2005; 46: 1049-1059)

Key words: Exercise, Heart failure, Tumor necrosis factor, Brain natriuretic peptide, Severity
CHRONIC heart failure (CHF) is a condition characterized by insufficient blood supply to peripheral organs resulting from low cardiac output due to chronic cardiac dysfunction. Patients with CHF often suffer reductions in their quality of life and exercise tolerance. Hemodynamic parameters such as left ventricular ejection fraction (EF) and cardiac output (CO) have been conventionally used to evaluate the severity of CHF, but newer parameters assessed by cardiopulmonary exercise testing (CPX), such as the anaerobic threshold (AT) and peak oxygen uptake ($\bar{V}O_2$), have now widely replaced them.\(^1\)\(^2\) Peak $\bar{V}O_2$ is also used as a criteria to select candidates for heart transplantation from among patients with severe CHF.\(^3\)\(^5\) Mounting evidence in recent years has suggested that atrophy and dysfunction of peripheral skeletal muscle strongly contribute to the mechanism of exercise intolerance in CHF patients, as well as to the degree of left ventricular dysfunction.\(^6\) On the other hand, investigators generally agree that proinflammatory cytokines disrupt the metabolism of the skeletal muscle by decreasing its blood flow and inducing alterations and atrophy. For this reason, the proinflammatory cytokines may play a primary role in the development of cardiac cachexia in CHF patients.\(^7\) Moreover, increases in proinflammatory cytokines such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and neurohormonal factors such as noradrenaline (NA) and brain natriuretic peptide (BNP) have been demonstrated to aggravate the condition of CHF patients\(^8\) and are consequently considered useful for evaluating CHF severity.\(^9\) Evaluation of BNP is especially useful for monitoring CHF patients on a daily basis, and the procedure is covered by medical insurance in Japan. Nonetheless, the relationships between exercise intolerance and neurohormonal factors and proinflammatory cytokines have yet to be fully elucidated. In this study we sought to shed more light on these relationships.

METHODS

Subjects: Sixty-two male outpatients with stable CHF were enrolled in this study (mean age, 55.6 ± 11.3 years). Our inclusion criteria were documented left ventricular systolic dysfunction with no admission or change of oral medication due to a worsening of CHF for at least three months before entry in the study. Patients with acute myocardial infarction, chronic respiratory disease, or malignancy were excluded. Forty patients had dilated cardiomyopathy (DCM), 17 had old myocardial infarction (OMI), 4 had hypertrophic cardiomyopathy (HCM), and 1 had valvular heart disease. Coronary artery disease was excluded by cardiac catheterization or radionuclide study in the patients with HCM and DCM. All OMI patients underwent coronary arteriography in the acute phase and revascularization by percutaneous coronary intervention or coronary artery bypass grafting.
We obtained the approval of the ethics committee of our institution before commencing this study. All of the subjects were informed of the details of the study, including possible adverse events, and gave their written consent.

**Cardiopulmonary exercise testing:** All subjects underwent cardiopulmonary exercise testing (CPX) using a bicycle ergometer with a ramp protocol. The testing consisted of an initial 3 minutes of rest on the ergometer, 3 minutes of warm-up (0 or 20 watts load), and full exercise with a linear increase in load by 1 watt every 6 seconds. Heart rate, ST-T changes, and arrhythmias were followed carefully on a 12-lead ECG, and blood pressure was measured and recorded every 1 minute by an automatic sphygmomanometer. The criteria for halting exercise testing in the study were based on the guidelines of the American College of Sports Medicine.\(^{10}\) Expired gas analysis was performed throughout testing on a breath-by-breath basis with an Aeromonitor AE-300S (Minato Medical Science, Osaka, Japan). The minute ventilation (\(\dot{V}E\)), oxygen uptake (\(\dot{V}O_2\)), and carbon dioxide output (\(\dot{V}CO_2\)) were derived from the CPX and used to calculate the anaerobic threshold (AT), peak \(\dot{V}O_2\), and slope of the ventilatory equivalent to \(\dot{V}CO_2\) (\(\dot{V}E / \dot{V}CO_2\) slope). The AT was usually determined by the V-slope method,\(^{11}\) though in some cases it was determined by comprehensively referring to the ventilatory equivalent of \(\dot{V}O_2\) (\(\dot{V}E / \dot{V}O_2\)) and the gas exchange ratio (\(R = \dot{V}CO_2 / \dot{V}O_2\)) due to failure of the V-slope method to produce clear results. The \(\dot{V}E / \dot{V}CO_2\) slope was calculated by linear regression analysis using the values of \(\dot{V}E\) and \(\dot{V}CO_2\). The criteria for halting exercise testing from expired gas analysis data was determined as \(R\) reached 1.25.

**Measurement of neurohormonal factors and proinflammatory cytokines:** A blood sample was taken after 15 minutes of rest in the supine position before CPX through a catheter inserted into a cubital vein for the measurement of NA, BNP, and the two soluble TNF receptors (TNFR-I, II). The blood samples were immediately immersed in an ice box and centrifuged (3000 rpm) for 10 minutes at 4°C. The plasma was stored at −70°C and used to measure NA by high-performance liquid chromatography, BNP by radioimmunoassay, and determine TNFR-I and R-II by enzyme-linked immunosorbent assay within 1 week.

**Classification of patients by exercise capacity:** The subjects were divided into three groups based on the peak \(\dot{V}O_2\) obtained during CPX: severe exercise intolerance group (severe group; < 18 mL/min/kg), moderate exercise intolerance group (moderate group; 18-24 mL/min/kg), and mild exercise intolerance group (mild group; > 24 mL/min/kg).

**Statistical analysis:** The data are expressed as mean ± standard deviation. Analysis of variance was used to compare data among groups and single regression analysis was used to examine correlations among groups. A \(P\) value of less than 0.05 was assumed to indicate statistical significance.
RESULTS

1. Patient characteristics (Table): The total study population had a mean EF of 41.7 ± 16.2% and mean peak \( \dot{V}O_2 \) of 19.8 ± 4.2 mL/min/kg. The mild, moderate, and severe groups consisted of 12, 31, and 19 patients, respectively. The Table shows the age, height, weight, EF, CPX parameters, neurohormonal factors, and proinflammatory cytokines in each group.

No significant differences in age, height, weight, or EF were found among the groups. None of the subjects experienced anginal chest pain, ischemic ECG changes, severe arrhythmia, or any other complications during the CPX.

2. Comparison of CPX parameters among the three groups (Table): Significant differences in the CPX parameters were obtained among the three groups. The patients in the mild group exhibited a higher AT and lower VE / VCO₂ slope than the other groups, and these parameters gradually worsened as their peak \( \dot{V}O_2 \) decreased (\( P < 0.0001 \) in each).

3. Comparison of neurohormonal factors and soluble TNF receptors among the three groups (Table, Figures 1 and 2): Both NA and BNP increased gradually as

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**Table. Patient Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>62</td>
<td>12</td>
<td>31</td>
<td>19</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.6 ± 11.3</td>
<td>52.1 ± 10.6</td>
<td>54.6 ± 11.7</td>
<td>59.4 ± 10.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.0 ± 6.0</td>
<td>166.2 ± 6.3</td>
<td>166.4 ± 6.6</td>
<td>168.4 ± 4.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.7 ± 11.1</td>
<td>70.8 ± 10.1</td>
<td>65.5 ± 11.8</td>
<td>69.2 ± 10.2</td>
</tr>
<tr>
<td>EF (%)</td>
<td>41.7 ± 16.2</td>
<td>45.6 ± 15.1</td>
<td>44.9 ± 15.5</td>
<td>34.2 ± 16.2</td>
</tr>
<tr>
<td>AT (mL/min/kg)</td>
<td>13.4 ± 2.5</td>
<td>16.1 ± 1.6</td>
<td>13.9 ± 1.7***</td>
<td>10.8 ± 1.5***</td>
</tr>
<tr>
<td>Peak ( \dot{V}O_2 ) (mL/min/kg)</td>
<td>19.8 ± 4.2</td>
<td>25.2 ± 1.0</td>
<td>21.1 ± 1.5***</td>
<td>14.6 ± 2.3***</td>
</tr>
<tr>
<td>VE / VCO₂ slope</td>
<td>32.4 ± 7.2</td>
<td>27.7 ± 2.8</td>
<td>30.5 ± 6.2*</td>
<td>38.5 ± 6.9***</td>
</tr>
<tr>
<td>NA (pg/mL)</td>
<td>342.3 ± 181.3</td>
<td>211.5 ± 75.7</td>
<td>331.8 ± 163.7***</td>
<td>441.9 ± 202.9***</td>
</tr>
<tr>
<td>BNP (pg/mL)</td>
<td>157.8 ± 137.4</td>
<td>37.9 ± 25.4</td>
<td>148.9 ± 117.1*</td>
<td>247.9 ± 150.0***</td>
</tr>
<tr>
<td>TNFR-I (pg/mL)</td>
<td>1272.7 ± 676.5</td>
<td>991.8 ± 293.6</td>
<td>1085.2 ± 370.5</td>
<td>1746.1 ± 950.7***</td>
</tr>
<tr>
<td>TNFR-II (pg/mL)</td>
<td>2239.2 ± 1081.4</td>
<td>1742.5 ± 555.2</td>
<td>2047.7 ± 648.7</td>
<td>2855.3 ± 1550.9***</td>
</tr>
<tr>
<td>Diuretic</td>
<td>50 (82%)</td>
<td>10 (83%)</td>
<td>23 (74%)</td>
<td>17 (89%)</td>
</tr>
<tr>
<td>( \beta )-Blocker</td>
<td>21 (34%)</td>
<td>6 (50%)</td>
<td>6 (19%)</td>
<td>9 (47%)</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>29 (47%)</td>
<td>8 (67%)</td>
<td>11 (35%)</td>
<td>10 (53%)</td>
</tr>
<tr>
<td>All receptor antagonist</td>
<td>19 (31%)</td>
<td>2 (17%)</td>
<td>7 (23%)</td>
<td>10 (53%)</td>
</tr>
<tr>
<td>Calcium antagonist</td>
<td>4 (64%)</td>
<td>0 (0%)</td>
<td>2 (6.0%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>40 (65%)</td>
<td>8 (66%)</td>
<td>24 (77%)</td>
<td>8 (42%)</td>
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<tr>
<td>Diabetes mellitus</td>
<td>9 (14%)</td>
<td>1 (8%)</td>
<td>6 (19%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Smoking (past)</td>
<td>39 (64%)</td>
<td>7 (59%)</td>
<td>21 (68%)</td>
<td>11 (62%)</td>
</tr>
</tbody>
</table>

EF indicates left ventricular ejection fraction; AT, anaerobic threshold; VE / VCO₂ slope, slope of minute ventilation to carbon dioxide output; NA, noradrenaline; BNP, brain natriuretic peptide; TNFR, soluble receptor of tumor necrosis factor; ACE, angiotensin-converting enzyme, and AII, angiotensin II.

*\( P < 0.05 \), **\( P < 0.001 \), ***\( P < 0.0001 \) versus mild group.
Figure 1. Comparisons of NA and BNP in each group. Both NA and BNP gradually increased in parallel with the progression of exercise intolerance.

Figure 2. Comparisons of TNFR-I and II in each group. The levels of TNFR-I and II in the severe group were significantly higher than those in the mild group, but they were not significantly different from those in the moderate group.
the exercise intolerance worsened (NA, 211.5 ± 75.7 pg/mL, 331.8 ± 163.7, 441.9 ± 202.9, respectively; NA, 37.9 ± 25.4 pg/mL, 148.9 ± 117.1, 247.9 ± 150.0, respectively). TNFR-I and TNFR-II were significantly higher in the severe group than in the moderate group (P < 0.0001, P < 0.01, respectively) and mild group (P < 0.0001, P < 0.01, respectively), but they did not significantly differ between the mild group and moderate group.

4. Correlations of EF and VO2 with neurohormonal factors and soluble TNF receptors (Figures 3 and 4): EF was not significantly correlated with NA, BNP, TNFR-I, or TNFR-II. However, peak VO2 showed significant negative correlations with NA (r = −0.50, P < 0.0001), BNP (r = −0.53, P < 0.0001), TNFR-I (r = −0.50, P < 0.0001), and TNFR-II (r = −0.45, P < 0.0001).
Correlation between exercise tolerance and cardiac function, neurohormonal factors, and proinflammatory cytokines: Peak VO$_2$ is not related to left ventricular function parameters such as EF, but it has been confirmed to be an independent predictor of CHF severity. Kinugawa, et al reported that neurohormonal factors and proinflammatory cytokines such as NA, BNP, atrial natriuretic peptide (ANP), TNF-$
abla$ and interleukin (IL)-6 were higher in CHF patients than in normal subjects. They also reported that NA and ANP were independent predictors of peak VO$_2$ obtained by CPX.

The significant correlations of NA, BNP, TNFR-I, and TNFR-II with peak VO$_2$ in the present study confirm the importance of these neurohormonal factors and proinflammatory cytokines as indices of CHF severity. Peak VO$_2$ has been
recognized to be more reliable than EF in evaluating CHF severity, and the parameter is now widely used to determine candidates for heart transplantation all over the world.\(^{16}\)

Elevations in various kinds of cytokines such as TNF-\(\alpha\), IL-6, and IL-1 have been reported in patients with CHF.\(^{17-19}\) TNFR-I and II, the soluble TNF receptors, reflect the biological activity of TNF-\(\alpha\). One group has reported that TNFR-II is more predictive of the prognosis of CHF than TNF-\(\alpha\) itself,\(^{20}\) and Rauchhaus, \textit{et al}\(^{18}\) has found that TNFR-I correlates more closely with mortality than TNF-\(\alpha\) in CHF patients. We chose to measure TNFR-I and II as alternatives to TNF-\(\alpha\) in the present study since the soluble TNF receptors have been confirmed to be much more sensitive than TNF-\(\alpha\) and to directly reflect the history of inflammation response in the past.\(^{18}\) The significant negative correlations of the TNF soluble receptors with peak VO\(_2\) in the present study convinced us that TNFR-I and II are as useful for assessing CHF severity as the neurohormonal factors.

While TNF-\(\alpha\) may lead to reductions in cardiac function, several findings rule it out as an independent factor. Our results revealed no correlation between the EF and the soluble TNF receptors, for example, and TNF-\(\alpha\) has been reported to exert a negative inotropic effect on cardiac muscle.\(^{20-22}\) TNF receptors may be influenced not only by low cardiac function, but also other peripheral factors such oxidative enzymes in skeletal muscle, vascular endothelial function, and vascular dilatation capacity.

**Influence of proinflammatory cytokines on exercise intolerance and its mechanism:** Peak VO\(_2\) and the VE / VCO\(_2\) slope obtained by CPX have been widely used as indices of exercise tolerance and abnormal ventilation in CHF patients. The worsening of these factors has been found to be strongly associated with peripheral factors such as blood flow and skeletal muscle atrophy, as well as central factors such as left ventricular function.\(^{6}\) Although the mechanism remains unclear, the significant negative correlations between peak VO\(_2\) and the soluble TNF receptors in this study suggest that proinflammatory cytokines such as TNF-\(\alpha\) might influence the reduction in the peak VO\(_2\). Given that the levels of proinflammatory cytokines did not significantly relate with left ventricular function in our study, we speculate that the cytokine levels may be related to peripheral factors that contribute to functional impairment. The decrease of blood flow in exercised skeletal muscle, change in muscle expenditure, and skeletal muscle atrophy resulting from the high proinflammatory cytokines in our subjects may have played a role in reducing the peak VO\(_2\). Anker, \textit{et al} found\(^{23}\) cardiac cachexia to be an independent and important predictor of mortality, and body weight loss has been strongly associated with high proinflammatory cytokine levels in patients with severe heart failure. We obtained no definitive data to support
hypotheses on muscle changes in our CHF group since the subjects maintained their body weights with no significant losses. For this reason, we suspect that the decreased blood flow in the skeletal muscle and altered quality of skeletal muscle in our patients both contributed to the deterioration of peak VO$_2$. If our study population had included more severe heart failure patients, the cytokine levels might have influenced the exercise capacity in the patients via effects on cardiac function and muscle volume.

Only a few studies have investigated how cytokines act on skeletal muscle in any depth. Tsutsui, et al noted increased active oxygen in skeletal muscle of a CHF animal model, but the chronic administration of antioxidant failed to attenuate the effect. Moreover, excessive active oxygen has been shown to induce apoptosis of cardiac muscle cells by producing oxidative stress and systolic cardiac dysfunction. The mitochondrial electron transfer system is another source of active oxygen production in skeletal muscle. Accordingly, we know that CHF patients are subjected to oxidative stress in skeletal muscle as well as cardiac muscle cells, and thereby develop dysfunction of the skeletal muscle. However, we are still unable to clearly determine the stages at which cytokine elevation, skeletal muscle volume loss, and impaired exercise tolerance occur. Ferrari, et al reported that TNF-α was significantly increased in only end-stage CHF in NYHA class IV patients. The severe patients in our study were the only patients to exhibit elevations in TNFR-I and II, and even these patients maintained stable skeletal muscle volume. For this reason, we conclude that the loss of skeletal muscle volume occurs only in parallel with long-term cytokine activation even in patients with high levels of TNFR-I and II.

Cicoira, et al reported that the TNF-α level had a direct relation to a loss of skeletal muscle in CHF patients. Dividing their population of CHF patients into four groups based on TNF-α level, they found that the peak VO$_2$ in the highest TNF-α quartile was significantly lower, while the VE/VCO$_2$ slope and catecholamine levels were significantly higher in the highest TNF-α quartile than in the other groups. Levine, et al reported that the highest TNF-α level among patients with the lowest exercise tolerance was almost equal to that in patients with cardiac cachexia. From these findings, we suspect that CHF patients with high TNF-α levels may develop cachexia in the future even if they presently exhibit no clinical signs of cardiac cachexia. We were unable to obtain the same result in our study in spite of our decision to measure TNFR-I and II rather than TNF-α.

Unlike BNP and NA, TNFR-I and II did not rise in the early phase of CHF, and they showed no potential as an early diagnostic tool in CHF. In other words, CHF may reach the severe stage before the first elevations of TNFR-I and II are observed. Patients with mild CHF have yet to develop sufficient cytokine mani-
festation, and their skeletal muscle and exercise tolerance are preserved. In severe cases, the cytokines are thought to be activated over the long term, to act on cardiac and skeletal muscle, and to decrease exercise tolerance.

**Study limitations:** The cutoff value for evaluation of severity by peak \( \text{VO}_2 \) was less than 18 mL/min/kg in the severe exercise intolerance group. This was because the total study population had a mean peak \( \text{VO}_2 \) of 19.7 mL/min/kg, which seemed to be a relatively high value for CHF. A few of the cases were also suspected of cardiac cachexia (loss of body weight and skeletal muscle volume), hence, we had to add more severe cases with apparent cardiac cachexia in order to reach a strict conclusion.

We did not investigate the time course changes in neurohormonal factors, cytokines, body weight, medication, or exercise tolerance. An investigation of these factors might have allowed us to predict the development of cardiac cachexia. Given that these cytokine levels are known to decrease with aerobic exercise, their observation over time would probably provide useful data.

**Conclusion:** The results of the present study suggest that NA and BNP rose in parallel with the degree of exercise intolerance, while TNFR-I and II rose only in the patients with severe exercise intolerance.

**REFERENCES**

7. Anker SD, Coats AJ. Cardiac cachexia: a syndrome with impaired survival and immune and neuroendocrine activation. Chest 1999; 115: 836-47. (Review)