

Effects of exercise training on functional capacity, quality of life, cytokine and brain natriuretic peptide levels in heart failure patients.

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Abstract

Purpose: To establish whether exercise training (ExT) might modify functional capacity, quality of life (QOL), brain natriuretic peptides (BNP) and pro-inflammatory cytokines in heart failure (CHF) patients. **Methods:** 22 CHF patients, 20 men, 64.7±8.3 years, weight 83±13.5(kg), left ventricular ejection fraction (LVEF) 28.6±8(%) completed 16 weeks ExT. Cytokines, BNP, VO₂ and QOL were measured before and after ExT. **Results:** After ExT peak VO₂ increased 20% from 11.6±4.7 (ml/kg/min) to 13.9±4.6 ml/kg/min (p=0.003), while TNF-alpha, IL-1, IL-6 and BNP were unaltered. **Conclusion:** Functional capacity and QOL were improved, however pro-inflammatory cytokines and BNP were unaltered by 16 weeks ExT.

Introduction

Inflammatory activation with increased serum cytokine levels has been described as an important factor in the progression of chronic heart failure (CHF) (5, 13, 24). In multi-factorial analyses, elevated levels of tumour necrosis factor-alpha (TNF-alpha) and interleukin (IL)-6 were identified as prognostic heart failure markers (10, 25, 30). Patho-physiologically, cytokines act as catabolic factors involved in the pathogenesis of muscle wasting and cardiac cachexia (4, 5). Increased levels of serum TNF-alpha have been found in patients with reduced skeletal muscle cross-sectional area and peripheral muscle strength (24). There also exists an association between elevated serum cytokine levels (especially TNF-alpha) and subjective New York Heart Association functional class as well as exercise intolerance (13). Adverse effects of inflammatory cytokines in chronic heart failure may alter skeletal muscle function. Mechanisms responsible for this are reduced skeletal muscle blood flow and cachexia. Inflammatory cytokines may also have an effect on cardiac function via left ventricular remodelling and depression of cardiac contractility. Inflammatory cytokines may also cause vascular dysfunction through progression of atherosclerosis, oxidative stress, NO impairment, vasoconstriction, endothelial cell apoptosis and adverse vascular remodelling (5). Brain Natriuretic Peptide (BNP) has widely been studied as an invaluable tool in the diagnosis of heart failure (18). BNP is used to diagnose, manage and treat heart failure patients. In addition BNP is used as a marker of cardiac function, to evaluate treatment and to assess prognosis (18).

Exercise training and quality of life (QOL). Continuous exercise training requires a sustained exercise effort of 30 or more minutes in the case of heart failure patients. Quality of life (QOL) in this work refers to the physical and mental well-being, which has shown to be reduced in heart failure patients compared to healthy populations (21). Exercise training has been shown to be a beneficial therapy for CHF patients, improving QOL (22), functional capacity and in some cases cardiac function (12, 22, 33).

Changes in inflammatory cytokines following exercise training. Several small studies have documented effects of exercise training on circulating cytokine levels of heart failure patients (20, 21, 35). Changes in skeletal muscle, but not systemic (serum) expression of TNF-alpha, IL-1-beta and IL-6 were reported in heart failure patients undertaking a strict regimen of 10 minutes cycling, 4-6 times daily for 6 months (13). Dubach's (13) in-patient exercise program resulted in large changes in maximal oxygen consumption (29%), nearly twice the mean increment (17%) shown from our review of 81 heart failure exercise training studies (33). Dubach's (13) study suggested the existence of a cytokine cascade where levels may be changed at altered rates in different tissues.

Brain natriuretic peptide and heart failure BNP has recently emerged as a powerful tool in the diagnosis of heart failure on acute presentation [18]. While there exists some information on the effects of exercise training on BNP levels in heart failure patients, evidence is equivocal (3, 17, 18, 23, 28, 36). This study therefore sought to establish whether an outpatient exercise training program of shorter duration was able to modulate systemic cytokine and/or BNP levels in heart failure patients.

Methods

Ethical approval and informed consent. All patients completed a written informed consent after ethics approval was obtained from the relevant University of Queensland and Princess Alexandra Hospital committees. **Subject Selection** Thirty seven CHF patients with at least two minor and one major Framingham heart failure criteria were selected. Echocardiographic data on this cohort have been previously published [33]. Patients with left ventricular ejection fraction (LVEF) greater than 35% were excluded as were those with primary valvular heart disease, unstable angina or inability to exercise. Four patients withdrew before 8 weeks, for non health-related reasons. Eleven blood samples were inadequately handled so the final study group involved 22 patients in whom follow-up data and samples of TNF-alpha, IL-1, IL-6 and BNP were gathered. All 22 had been on stable medical management for at least one month prior to baseline testing. The clinical characteristics of the patients, including Framingham heart failure criteria, co-morbid diseases and drug therapy can be seen in the results section. Most patients were in New York Heart Association functional class III.

Blood sampling and analysis. Plasma or serum samples were obtained by venipuncture, within 24-72 hours after the last exercise training session. Within one hour of venipuncture, samples were centrifuged at 4°C, 1500-2000 RPM for 10 minutes and separated into aliquots and stored at -75-80°C. Concentrations of TNF-alpha, IL-6 and IL-1 were measured by commercially available enzyme-linked immunosorbent assays (ELISA) (R&D systems Minneapolis, Minnesota). The procedure is described below and one plate was prepared for both TNF-alpha, IL-1 and IL-6 using manufacturer recommended reagents. Each sample, standard, blank and control samples were assayed in duplicate. To each standard 100 µl of appropriate standard diluent was added. Diluent was added to wells B1, B2, C1, C2, D1, D2, E1, E2, F1, F2. Standard (200 µl) was pipetted into wells A1 and A2. From A1 and A2 100µl was transferred to B1 and B2 wells. The contents were mixed by repeated aspirations and ejections. This procedure was repeated from the wells B1, B2 to wells C1, C2 and from wells C1, C2 to D1, D2 and so on creating two parallel rows of standard dilutions ranging from 200 to 6.25 pg/ml. From wells F1 and F2 100 µl was discarded and 100 µl of appropriate standard diluent was added to the blank wells (G1-G2). To control wells, H1 and H2, 100 µl of standard was added and 100 µl of control. To all wells was added 50 µl of diluted reagent. The plate was covered and incubated for 1 hour at room temperature (18°C - 25°C). The plate was aspirated with 0.3 ml of washing solution in each well, this process was twice repeated. The HRP solution was prepared and 100 µl of HRP solution was dispensed into all wells, including the blanks. The microwell strips were incubated at room temperature for 30 minutes. The plate cover was removed and the wells emptied. Into all wells 100 µl of ready-to-use TMB substrate solution was added, including blank wells. This solution was incubated in the dark for 12-15 minutes at room temperature. The enzyme-substrate reaction was stopped by pipetting 100 µl of 1.8 N sulfuric acid into each well, including the blank wells, to completely and uniformly inactivate the enzyme. Results were read immediately after the addition of sulfuric acid on a spectrophotometer using 450 nm as the primary wavelength. Standard curves were plotted and analysed. The intra- and inter-assay coefficient of variation were <10% for all assays. BNP levels were measured by immediate analysis of whole blood by fluorescent immunoassay (Biosite Triage BNP, San Diego, California).

Exercise testing. All subjects underwent metabolic exercise testing on a cycle ergometer using a 10 Watt per minute stepped protocol. As delays in the return of the ST- segment to the iso-electric line may be indicative of cardiac ischemia, the electrocardiogram was continuously monitored for ST-segment changes and arrhythmias. Blood pressure and 12-lead electrocardiograms were recorded before exercise, every 2 minutes during the test and during the post-exercise recovery period. Tests were symptom-limited, with the usual end-points being dyspnea and leg fatigue. Peak heart rate and workload were recorded

immediately upon cessation of the exercise test, these values were used to generate the initial training workload. Peak VO_2 (ml/kg/min) was obtained by breath-by-breath analysis of expired gas (V29C SensorMedics, Yorba Linda, CA), averaged over 20 second intervals. Every three sequential measurements were averaged and peak VO_2 was defined as the greatest mean value during exercise.

Exercise echocardiography. Before and immediately after the exercise VO_2 test, 2D echocardiography was recorded with commercially available equipment (System FiVe, General Electric-Vingmed, Milwaukee, WI) with the patient in the supine left lateral decubitus position. Images were obtained using a 3.5-MHz transducer at 16-cm depth in five standard views at rest and after exercise. Left atrial size, resting and peak LV volumes were calculated using Simpson's biplane method (29). Contractile reserve was defined as the increment between resting and peak exercise left ventricular ejection fraction (LVEF).

Transmitral and myocardial Doppler analysis. Digital images were acquired with a commercially-available ultrasound system (Vivid 7, General Electric-Vingmed, Milwaukee, WI), at a depth of 16cm in the three standard apical views. Color Doppler frame rates varied from 99 to 265 frames/sec depending on the sector width, with pulse repetition frequencies between 500 and 1000 Hz, resulting in aliasing velocities between 16 and 32 cm/sec. Three cardiac cycles from each 2-D echo view, triggered to the QRS complex of the electrocardiogram, were saved to magneto-optical disk in cine-loop format for off-line analysis using standard software (Echopac, General Electric-Vingmed), by readers blinded to visual wall motion analysis.

Peak long-axis systolic velocities within each segment (26) were obtained by locating the sample volume in the middle of each segment at rest. To overcome regional variations, the lateral systolic velocity (V_{sl}) and septal systolic velocity (V_{ss}) segments were analyzed separately and early diastolic velocities were also measured at the septal and lateral aspects of the mitral annulus (V_{es} and V_{el}). The mean of systolic and early diastolic velocities was also analyzed. Although not site-specific, tissue Doppler is robust and reproducible, and is predictive of outcome (8, 14).

Myocardial strain-rate analysis was derived by analysis of tissue Doppler data using standard software (Echopac Version 6.2; General Electric-Vingmed). Strain rate data was measured as the slope of the regression line based on all the velocity estimates between two points in the myocardium separated by distance r (in this study, 12mm), corresponding to the region that is used to estimate local contraction (16). The mid-segment from the septal and inferior walls were assessed, avoiding the anterior and lateral walls, which are often poorly visualized and have unfavorable insonation angles ($>30^\circ$). Transmitral flow was obtained with pulsed wave Doppler at the leaflet tips; E and A wave velocities, E/A ratio, D_T and E/E' ratio (surrogate of diastolic filling pressure) were also measured.

Quality of Life measures. Three previously validated questionnaires assessing quality of life (QOL) were completed by all patients at baseline and 16 weeks. The Minnesota Living with Heart Failure Questionnaire (31) is specific to heart failure; its 21 questions give a total score and also a physical and emotional dimension score. The Hare-Davis Cardiac Depression Scale (15) is a general tool administered to the cardiac patient population; as with the Minnesota questionnaire, lower scores indicate that patients perceive their health to be improving. Finally, we followed the 8 dimensions of the SF-36 General Health Questionnaire (7).

All patients undertook 16 weeks of supervised cycle ergometer exercise training at 60 RPM, at a frequency of three, 30 minute sessions weekly, and at a workload corresponding to an initial intensity of 60-70% peak oxygen consumption from the metabolic exercise test. In addition, during weeks 8-16 patients also performed a series of five strength exercises including wall push ups, alternating leg lunges, tricep dips, bicep curls and sit to stand from a chair. Exercise intensity was up-titrated by 2-5 Watts per week, provided that patients were tolerating the cycle training. In patients in paced rhythm or experiencing frequent ectopy, rate of perceived exertion (RPE) was also used to guide exercise intensity, using a target RPE 3-5 (moderate to hard) on the modified Borg scale (11). In patients who were most limited by shortness of breath, a respiratory rate <30 /min was used to monitor exercise intensity.

Statistical analysis. Results are reported as mean \pm SD. Baseline and 16 week data were compared using paired student t-tests. Correlations with change in peak VO_2 were performed with clinical variables, resting and peak LVEF, contractile reserve, hemodynamic responses to stress, peak systolic and early diastolic tissue velocities and other measures of cardiac function. Statistical analyses were performed with SPSS (version 10.1).

Results

Baseline clinical patient characteristics and pharmacology. Mean age was 64.6 ± 8.3 years, LVEF $28.6 \pm 8\%$ and functional capacity 11.6 ± 4.7 ml/kg/min, body weight 83 ± 13.5 kg. Many patients exhibited clinical characteristics of heart failure; hypertension (84%), diabetes (28%), ischemic cardio-myopathy (64%), shortness of breath (96%) and (80%) showed signs of edema. Patients were well medicated, (96%) were taking ACE-inhibitors, (92%) beta blockers, (68%) aspirin and (76%) were on lipid lowering medication.

Circulating levels of cytokines and BNP at baseline and 16 weeks. None of the pro-inflammatory cytokines TNF-alpha ($p=0.08$), IL-1 ($p=0.19$) or IL-6 ($p=0.20$) were significantly altered after exercise training. The same was true of BNP levels ($p=0.15$) table 1.

Table 1. Circulating levels of cytokines and BNP at baseline and 16 weeks.

	Baseline	16 Weeks	% Change
TNF-alpha pg/ml	1.08 ± 0.89	2.51 ± 4	232
IL-1 pg/ml	20 ± 67	27 ± 77	35
IL-6 pg/ml	9.3 ± 14	27 ± 67	290
BNP pg/ml	152 ± 187	121 ± 161	-21

Table 2. Quality of Life measures at baseline and 16 weeks.

Questionnaire	Baseline	16 Weeks	% Change
Minnesota Total	46 ± 16	34 ± 17	-26
Minnesota Physical	15 ± 7	11 ± 7	-27
Minnesota Emotional	10 ± 5	6 ± 4	-40
Hare-Davis	97 ± 13	82 ± 19	-15

Quality of life measures at baseline and 16 weeks. All three dimensions of the Minnesota Living with Heart Failure (MLWHF) questionnaire were all significantly improved (reduced) after exercise training. Total score ($p=0.001$), physical dimension ($p=0.004$) and emotional dimension ($p=0.001$) of the MLWHF questionnaire can be seen in table 2. Hare-Davis cardiac depression scale scores were also significantly improved (reduced) ($p<0.001$) at 16 weeks (table 2). Five of the eight dimensions of the SF-36 questionnaire showed improvement (increase) at 16 weeks, bodily pain ($p=0.04$), physical role ($p=0.04$), vitality ($p=0.01$), social functioning ($p=0.01$) and emotional role ($p=0.03$) figure 1.

Figure 1. SF-36 Dimensions at baseline and 16 weeks.

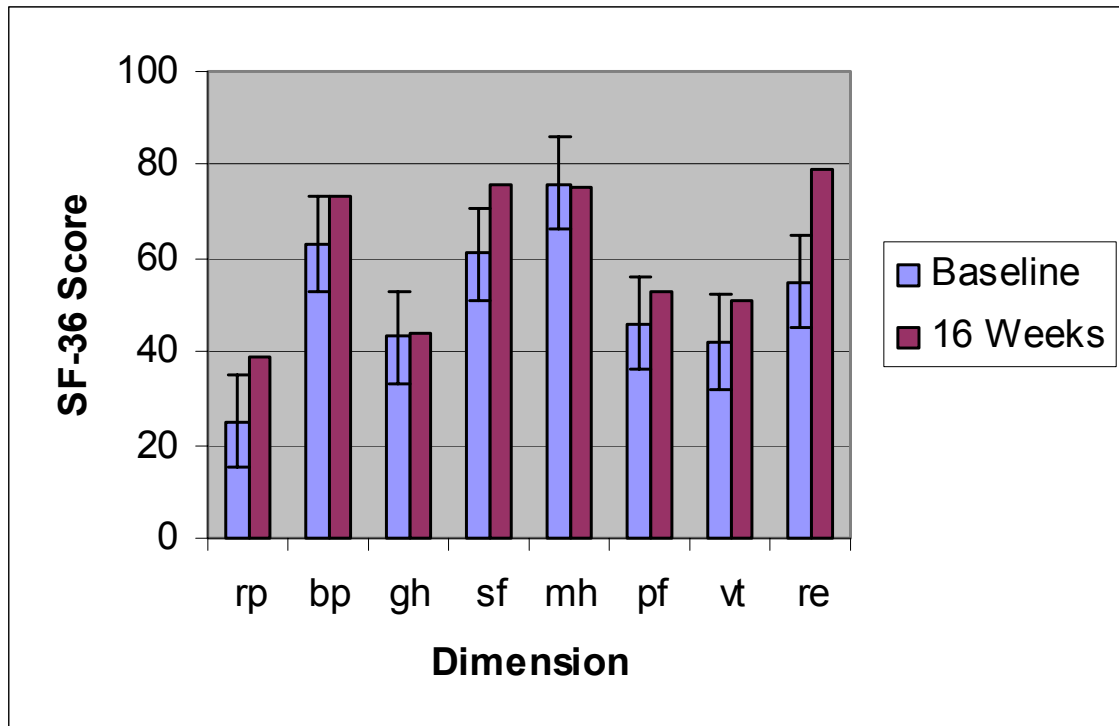


Figure 1 Key

RP = physical role BP = bodily pain GH= general health
 SF = social function MH = mental health PF = physical functioning
 VT = vitality RE = emotional role

Metabolic Exercise Test Variables at baseline and 16 weeks. Table 3 shows metabolic exercise testing values. Peak VO_2 ($p=0.003$), ventilatory threshold ($p<0.001$), peak heart rate ($p=0.001$) and respiratory exchange ratio (RER) ($p=0.006$) were all significantly increased at 16 weeks.

Echocardiography and tissue Doppler values at baseline and 16 weeks. Systolic and diastolic left ventricular volumes, resting and peak ejection fraction and contractile reserve all remained unchanged after 16 weeks exercise training.

Correlates of change in cytokine levels Change in contractile reserve showed an inverse correlation with change in TNF-alpha ($r=-0.45$, $p=0.04$). Change in IL-1 was negatively correlated with change in both TNF-alpha ($r=-0.92$, $p<0.001$) and IL-6 levels ($r=-0.93$, $p<0.001$). Change in TNF-alpha was also correlated with IL-6 ($r=-0.95$, $p<0.001$).

Discussion

Despite expected improvements in functional capacity and quality of life, the results of this study demonstrate that four months of exercise training did not significantly alter cytokine and brain-natriuretic peptide expression in a group of heart failure patients. Nevertheless change in TNF-alpha was inversely related to change in contractile reserve. Expression of cytokines IL-6, IL-1 and TNF-alpha were interrelated.

Baseline BNP levels Baseline BNP levels reported in this study are lower than previously published results [22, 26]. This discrepancy may be explained by several factors. First patients in this study were stabilised and treated optimally for at least one month prior to

starting exercise training. Secondly, our patients were usually recruited after their initial, rather than subsequent, heart failure diagnosis. Third, the two factors mentioned above limited the number of eligible participants, this in turn made the study group small.

Table 3. Metabolic Exercise Test Variables at baseline and 16 weeks.

Variable	Baseline	16 weeks	% Change
Peak VO ₂ (ml/kg/min)	11.6±4.7	13.9±4.6	20
Ventilatory T'hold (ml/kg/min)	7.3±1.6	9.9±2.8	36
VE/VCO ₂ Slope	35±6.4	31±5.8	-11.4
Peak Heart Rate (BPM)	97±26	108±28	11
Rate Pressure Product (x 1000)	146±70	157±54	7.5
Respiratory Exchange Ratio	1.08±0.06	1.10±0.08	2

Effect of exercise on cytokine expression Our study suggests that while 90 minutes per week of exercise training over a 4 month period improves functional capacity it may be insufficient to lower cytokine expression. Increasing neuro-hormonal expression is a normal consequence of heart failure disease progression. Previous exercise training studies in heart failure patients have shown mixed results in the alteration of cytokine expression. Several studies demonstrated exercise training to have a favorable effect on circulating cytokine levels in heart failure patients (1, 2, 9, 21), while other work has showed no change (35). One study showed an increase in IL-6 and TNF-alpha, but not IL-1, cytokine expression in response to an acute bout of exercise training (19). The large standard deviation observed in both cytokine and BNP levels may be due to the time range (24-72 hours) of blood collection between patients. This may also explain the 2-3 fold increases in TNF and IL-6 levels. A limitation of our work was not confirming ELISA data with immuno-blotting. Another confounding factor was not all patients were taking aspirin and other anti-inflammatory drugs, this coupled with small experimental numbers meant sub-analyses of these effects were insignificant.

Effect of exercise on brain natriuretic peptide (BNP) expression. Recent exercise training studies in heart failure patients also showed mixed results in alterations of post-training levels of brain natriuretic peptide expression. Two pieces of work showed decreased BNP expression post-exercise training (23, 28), while another study reported lowered BNP levels after a combination of cardiac surgery and six months exercise training (3). Other work, like this study demonstrated unchanged BNP expression following exercise training (17).

Previous work has demonstrated that peak exercise may induce significant increases in plasma levels of ANP and BNP which are more closely related to left ventricular end-diastolic and end-systolic diameter than their resting values (36). However resting BNP may be a better independent predictor of LV dimensions and systolic function in patients with heart failure (36).

Mechanisms for exercise training induced changes in cytokine or BNP expression in heart failure patients. A handful of exercise training studies have demonstrated improved systolic (12, 34) and diastolic (6) function in heart failure patients in response to exercise

training. BNP is cleaved from N-pro BNP upon ventricular myocyte stretch. As exercise may reduce ventricular filling pressure, BNP levels may be reduced as filling pressures are improved (32). The work of Adamopoulos (1) suggests that reductions in expression of certain cytokines may be related to improvements in functional status of heart failure patients. Our work suggested that cytokine expression may be inversely related to changes in contractile reserve after exercise training. While no exercise training studies have reported such a correlation, this finding may be similar to changes after drug therapy (27). Great variability of exercise training prescriptions is exhibited in studies of cytokine expression in heart failure patients. It may be that certain prescriptions e.g. aerobic or strength training may be more effective in managing cytokine over-expression. Limitations of this work are the small study size and the absence of an age-matched control group. These factors prevent normal trends in cytokine and BNP expression of heart failure patients from being established. Another limitation is that cytokine data from the two females may be affected by hormonal cycle, although both of these women were post-menopausal and sub-analysis was unfeasible with such small numbers. Finally blood tests were not performed before daily medication had been taken and not conducted at standardized periods after completion of exercise training.

Conclusions

This work highlights that established functional capacity and quality of life changes following exercise training may not be concurrent with alterations in cytokine expression.

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