Effects of exercise training on LV performance and mortality in a murine model of dilated cardiomyopathy

KIRK T. SPENCER, KEITH COLLINS, CLAUDIA KORCARZ, RICHARD FENTZKE, ROBERTO M. LANG, AND JEFFREY M. LEIDEN

Departments of Medicine and Pathology, The University of Chicago, Chicago, Illinois 60637

Received 28 June 1999; accepted in final form 3 January 2000.

Spencer, Kirk T., Keith Collins, Claudia Korcarz, Richard Fentzke, Roberto M. Lang, and Jeffrey M. Leiden. Effects of exercise training on LV performance and mortality in a murine model of dilated cardiomyopathy. Am J Physiol Heart Circ Physiol 279: H210–H215, 2000.—Dilated cardiomyopathy (DC) is a leading cause of cardiovascular morbidity, and nonpharmacological therapies, such as exercise training, have been suggested. The effects of exercise on left ventricular (LV) function and mortality remain controversial. Using a recently described murine model of DC, which involves a dominant-negative form of the cAMP response element binding protein (CREB) transcription factor (CREBA133) under the control of the cardiac myocyte-specific α-myosin heavy chain promoter, we sought to assess the effects of moderate-intensity exercise training on LV performance and mortality. Thirty-two transgenic mice were subjected to exercise training and compared with sedentary controls. There was progressive enlargement in LV dimensions in both the sedentary and exercise-trained mice. LV performance was progressively impaired, and exercise training did not prevent this decline. The sedentary CREBA133 mice displayed a significantly increased rate of death, and exercise training did not prevent or delay this excess mortality. The CREBA133 murine model of inherited DC demonstrated progressive ventricular dilatation and dysfunction with increased mortality, which was not altered with 12 wk of moderate-intensity exercise training.

DILATED CARDIOMYOPATHY (DC) is a leading cause of cardiovascular morbidity and mortality and is increasing in prevalence. Despite recent advances in the pharmacotherapy of DC, the 5-yr mortality of this disease remains high. Nonpharmacological therapies, such as exercise training, may also play a role in the management of patients with DC. Recently, exercise training has been recommended for patients with compensated dilated cardiomyopathies. This recommendation is based on studies that have demonstrated improvement in patient exercise tolerance and quality of life associated with participation in exercise rehabilitation programs (2, 4, 6, 24, 31).

Despite these apparent benefits, the effects of exercise on left ventricular (LV) function and size remain controversial. In addition, serial evaluations of the effects of exercise throughout the natural history of DC have been limited. Finally, the studies performed to date have not assessed the effects of exercise on mortality. Additional limitations to most of these prior human studies include the small number of patients in each study, the fact that most studies have only included subjects with ischemic cardiomyopathies, the wide variety of pharmacological regimens used to treat study subjects, and variability in patient compliance.

Our laboratory has recently described a murine model of DC (9). These mice, which express a dominant-negative form of the cAMP response element binding protein (CREB) transcription factor (CREBA133) under the control of the cardiac myocyte-specific α-myosin heavy chain (MHC) promoter, develop a four-chamber DC that displays the clinical features of human cardiomyopathy, including substantially increased mortality (9). Histologically, the hearts of these mice demonstrate fibrosis and extensive myocyte loss. The development of miniaturized instrumentation by our laboratory and others (10, 14, 20) has allowed us to both invasively and noninvasively characterize the cardiovascular physiology in these mice (10). Using these tools, we have established that CREBA133 mice have increased chamber dimensions, severely reduced LV systolic function, and depressed contractile reserve (10).

The CREBA133 mice represent an ideal model for studying the effects of therapeutic interventions on the natural history of DC because 1) all of the mice share a common molecular etiology of DC, 2) the mice can be studied throughout the time course of disease progression, 3) the use of a murine model allows us to control multiple confounding variables, such as medications, comorbidities, and compliance, and 4) the relatively short time course of the disease allows the experiments to be carried out conveniently and relatively inexpensively.

In the studies described in this report, we sought to assess the effects of moderate-intensity exercise training on LV performance and mortality in the CREBA133 murine model of DC. Specifically, we assessed the
effects of exercise training on 1) LV size, 2) LV systolic performance, and 3) mortality.

METHODS

Exercise protocol. Sixty-two transgenic mice of both sexes expressing a dominant-negative form of CREB<sub>133</sub> under the control of the cardiac-specific α-MHC promoter were used in this study (9). CREB<sub>133</sub> mice were confirmed through PCR analysis of tail DNA. The anatomic, hemodynamic, and clinical features of DC in these mice have been described previously (10). Mice were randomly divided into a sedentary (n = 30) or exercise training group (n = 32). All mice had free access to food and water. Starting at 9 wk of age, the mice in the exercise training group were subjected to scheduled exercise at a frequency of five times per week for 16 wk. Exercise was performed on an eight-lane rodent treadmill (Exer-8M; Columbus Instruments, Columbus, OH). There was a 3-wk graded increase in exercise duration and speed as follows: week 1, 10 min at 10 m/min; week 2, 20 min at 10 m/min; week 3, 30 min at 12 m/min; and weeks 4–16, 40 min at 14 m/min. At weeks 0, 6, and 12 of exercise training, mice underwent a two-dimensional echocardiographic examination. All procedures were performed in accordance with the guidelines established by the American Physiological Society and were approved by the Animal Care and Use Committee at the University of Chicago.

Echocardiographic imaging. Immediately before performing the echocardiographic study, animals were anesthetized by administering halothane in a closed chamber at 5% (Ohmeda Fluotec 3; Matrix Medical, Orchard Park, NY). Subsequently, mice received 0.5–2% halothane through a nose cone as needed to maintain sedation while spontaneously breathing. Once anesthetized, mice were secured to a custom-made water bed in a shallow left lateral decubitus position to facilitate ultrasound imaging. The bed was connected to warm circulating water to prevent hypothermia. Electrocardiographic monitoring was performed continuously during the cardiac imaging procedure. The anterior chest was shaved to facilitate ultrasound imaging. At the completion of the echocardiographic study, the anesthetic was discontinued, and mice were returned to their cages for recovery. Animal weights and heart rates were recorded at each imaging session.

Two-dimensional imaging was performed with a 15-MHz linear array transducer (Hewlett-Packard, Andover, MA). When imaging, particular attention was paid to avoid applying excessive pressure on the chest wall. Two-dimensionally targeted M-mode echocardiographic recordings of the LV were obtained at the midpapillary muscle level (Fig. 1). All echocardiographic data were recorded to a magneto optical disk for off-line analysis. The LV septal (SWT) and posterior wall thickness (PWT) as well as end-systolic (ESD) and end-diastolic (EDD) dimension were measured in a blinded fashion, and the average of three measurements was used. Fractional shortening was computed as \( \frac{(EDD) - (ESD)}{EDD} \times 100 \). LV mass was estimated using the cube formula 1.04 \( \times (SWT + PWT + EDD)^{3} - (EDD)^{3} \). In mice with sufficient-quality M-mode recordings, continuous endocardial borders were traced, and the maximum rate of diastolic chamber enlargement was computed \(( -dD/dt)\).

Continuous-wave aortic Doppler recordings were obtained, with two-dimensional guidance, from a right supraclavicular view using a 12-MHz phased-array transducer (Hewlett Packard). Peak aortic velocity and velocity time integral were determined (Fig. 2). The average of three determinations was used. LV stroke volume was calculated as the product of the velocity time integral of the aortic Doppler recording and the aortic cross-sectional area. The aortic diameter \((D)\) was measured using M-mode echocardiography at the level of the proximal ascending aorta, and aortic cross-sectional area was computed as \( (D/2)^{2} \times \pi \). Cardiac output was calculated as stroke volume times heart rate.

Statistical analysis. All echocardiographic parameters are presented as the means ± SD. Intergroup comparisons were made using an unpaired Student’s t-test. Changes in the echocardiographic parameters over time were assessed using repeated-measures ANOVA. Survival was assessed with actuarial analysis. A P value < 0.05 was considered statistically significant.

RESULTS

At the initial evaluation (9 wk of age), no intergroup differences were noted in total body weights or resting heart rates. During the exercise protocol, mice in both
the sedentary and exercise groups demonstrated the phenotypic characteristics of a severe DC with heart failure, including lethargy, dyspnea, and anasarca. At the end of the exercise protocol, surviving mice in both sedentary and exercise groups were killed. Hearts from both groups of animals demonstrated marked four-chamber cardiac enlargement (Fig. 3). Representative M-mode and Doppler recordings are shown in Fig. 4.

As shown in Table 1, M-mode echocardiographic data were similar for LV chamber dimension and wall thickness for both groups at the beginning of the exercise protocol (9 wk of age). The values for EDD and ESD are similar to those previously reported in our hemodynamic characterization of the CREBA133 myopathic mice (9) and are significantly larger than those previously reported by our laboratory for normal mice of the same age, strain, and weight (EDD 3.2 ± 0.2 mm and ESD 2.0 ± 0.2 mm; see Ref. 10). Similarly, fractional shortening in both the exercise and sedentary cohorts was markedly reduced compared with the normal values established by our laboratory (20 vs. 38%; see Ref. 10). LV PWT was 0.6–0.7 mm, and LV mass was calculated at 125 and 117 mg in the sedentary and exercise groups, respectively (P = not significant).

As shown in Fig. 5, there was a progressive enlargement in LV EDD in both the sedentary mice and in the animals undergoing exercise training. The increase in ESD was larger than that of the EDD, leading to a significant reduction in shortening fraction in the sedentary group. As shown in Fig. 6, exercise training did not prevent this decline in LV performance. Although no change in PWT was noted over time, the significant increase in LV EDD led to a significant increase in LV mass in both groups of mice. Diastolic function as assessed by the maximum rate of diastolic cavity expansion, was not different between the two groups and was not affected by exercise training (sedentary 4.4 ± 1.7 to 3.1 ± 1.3, exercise 3.8 ± 1.8 to 3.0 ± 1.2).

Doppler echocardiography also documented a progressive decline in LV systolic performance as manifested by a serial reduction in peak aortic systolic velocity from 86 to 66 cm/s in the sedentary group and from 82 to 61 cm/s in the exercise cohort. There was a reduction in stroke volume in both sedentary and ex-
Table 1. M-mode and Doppler echocardiographic data for sedentary and exercise CREB mice

<table>
<thead>
<tr>
<th>Weeks of Exercise</th>
<th>Sedentary</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (9)</td>
<td>6 (15)</td>
</tr>
<tr>
<td></td>
<td>0 (9)</td>
<td>6 (15)</td>
</tr>
<tr>
<td>Weight, g</td>
<td>31 ± 5</td>
<td>36 ± 6*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>424 ± 124</td>
<td>504 ± 54*</td>
</tr>
<tr>
<td>EDD, mm</td>
<td>4.7 ± 0.4</td>
<td>5.0 ± 0.4*</td>
</tr>
<tr>
<td>ESD, mm</td>
<td>3.7 ± 0.6</td>
<td>4.3 ± 0.6*</td>
</tr>
<tr>
<td>SF, %</td>
<td>21 ± 9</td>
<td>14 ± 6*</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>125 ± 29</td>
<td>125 ± 31</td>
</tr>
<tr>
<td>Ao V(_{max}), cm/s</td>
<td>86 ± 17</td>
<td>74 ± 17*</td>
</tr>
<tr>
<td>SV, (\mu l)</td>
<td>49 ± 13</td>
<td>39 ± 13*</td>
</tr>
<tr>
<td>CO, ml/min</td>
<td>20 ± 6</td>
<td>20 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SD. Animal ages (wk) in parentheses. EDD, end-diastolic dimension; ESD, end-systolic dimension; SF, shortening fraction; PWT, posterior wall thickness; LV, left ventricular; Ao V\(_{max}\), maximum systolic aortic velocity; SV, stroke volume; CO, cardiac output. CREB, cAMP response element binding protein. *P < 0.05 vs. week 0.

DISCUSSION

DC is a common clinical condition that is associated with substantial morbidity and mortality and a significant consumption of health care dollars. Although meaningful reductions in morbidity and mortality have recently been observed with the use of newer pharmacological therapies, patients with DC continue to have a poor prognosis. Although initially thought to be contraindicated, exercise therapy has been increasingly prescribed for patients with compensated DC. This recommendation is based on several studies that have demonstrated improvements in exercise tolerance and quality of life with exercise training. (2–6, 8, 16, 17, 21, 24, 31). In this study, we evaluated the effects of exercise on LV size and function as well as mortality in a murine model of inherited DC.

Murine cardiomyopathy. Studies on the effects of exercise on heart failure progression in humans have had major limitations. Most of these studies have included small numbers of patients who often represent different stages of the natural history of this disease. Studies in humans are also intrinsically confounded by a variety of comorbidities and therapies as well as by patient motivation and compliance. In addition, the majority of exercise training trials performed in humans have included predominately subjects with ischemic cardiomyopathy. The effects of exercise conditioning on LV function and mortality in patients with nonischemic cardiomyopathies remain to be studied.

The CREB\(_{A133}\) murine model of DC circumvents many of the limitations of human exercise training trials by providing a more uniform time course of the disease and by controlling for comorbidities, medications, and compliance. In addition, the 6-mo duration and relatively uniform natural history of DC in these mice facilitates studies of disease progression and mortality. We have previously shown, using invasive and noninvasive techniques, that these mice have physiological disturbances similar to human idiopathic DC, including increased chamber dimensions, reduced systolic function, and depressed contractile reserve (9). However, despite these similarities to human DC, it is not certain that this model will duplicate the therapeutic responses of the human disease. In particular, because CREB may lie downstream of the \(\beta\)-adrenergic signal transduction pathway in cardiomyocytes, it is possible that the CREB\(_{A133}\) mice will not respond to therapeutic interventions that modulate the hyperadrenergic state.
Effects of exercise on LV size in DC. Previous studies of exercise training in cardiomyopathic subjects have demonstrated variable effects on LV size. Several studies have shown no change in LV chamber dimension in subjects with LV dysfunction participating in regular exercise (8, 21, 23, 31). Giannuzzi et al. (17) demonstrated that exercise training attenuated the LV enlargement that occurred in the control sedentary subjects with ischemic cardiomyopathy. In contrast, a number of human studies have demonstrated either no effect or a deleterious effect of exercise training on LV dilatation in patients with DC. The Exercise in Anterior Myocardial Infarction trial evaluated 31 subjects with LV dysfunction after an anterior Q wave myocardial infarction before and after 6 mo of exercise training (16). This trial demonstrated progressive LV dilatation in the sedentary subjects that was not influenced by exercise training. Jugdutt et al. (22) showed that exercise training after an anterior myocardial infarction was associated with excess LV enlargement in a small group of patients with LV dysfunction subjected to 12 wk of exercise. Consistent with these results, prior animal experiments have shown that exercise training, in a postinfarction model of LV dysfunction, resulted in significant increases in LV size compared with sedentary controls (15, 26).

It is worth noting that all of these prior clinical and animal studies demonstrating excessive ventricular dilatation with exercise training utilized postmyocardial infarction models that may have a different ventricular remodeling response to exercise compared with nonischemic DC (16, 15, 22, 26). To our knowledge, the present study is the first to assess the effects of moderate exercise on the progression of LV size in a non-ischemic model of DC.

Effects of exercise training on LV systolic performance in DC. Most, but not all, prior studies in cardiomyopathic patients have demonstrated no change in LV systolic function with exercise training (3, 8, 16, 21, 23, 24, 31). However, the Exercise in Left Ventricular Dysfunction trial demonstrated an improvement in LV function with exercise (17). This study randomized 77 patients with an ejection fraction <40% after a first Q wave myocardial infarction to a 6-mo exercise training program or usual care. The average echocardiographic ejection fraction was 34% in the control group and remained unchanged, whereas the ejection fraction in the exercise group increased from 34 to 38%. In contrast, Jugdutt et al. (22) have shown a deterioration in LV ejection fraction (43–30%) in a small subgroup of patients with LV dysfunction who underwent 12 wk of exercise training after myocardial infarction. Our study demonstrated a progressive deterioration in LV systolic function over time in mice with inherited DC. Moderate-intensity exercise training failed to prevent or attenuate the deterioration in LV systolic performance. The lack of improvement in systolic function, despite prior data suggesting improved functional status with exercise training, is consistent with the known poor relationship between LV systolic function and functional class (13, 29). The previously reported improvement in functional state observed with exercise training may represent the beneficial effects of exercise on the peripheral circulation and skeletal muscles rather than improvement in LV systolic function (16, 8, 25, 28, 31). Because it is not possible to measure hemodynamic function in exercising mice, our study would have failed to detect improvements in contractile reserve or the peripheral vascular responses to exercise in the exercised CREB A133 mice.

Effects of exercise training on mortality in DC. There are no prior reports evaluating the effect of exercise training on mortality in human subjects. Such studies have been difficult due to the large number of subjects and the prolonged duration of follow-up required. Evaluation of the effect of exercise on the mortality of subjects with LV dysfunction is essential, as therapies that demonstrate beneficial effects on exercise tolerance and quality of life do not ensure mortality improvement. Indeed, several therapies for DC, such as oral inotropic agents, have shown improvements in exercise tolerance that are associated with excess mortality (18). Potential improvements in mortality with exercise in cardiomyopathic patients might be expected, since reductions in resting catecholamine levels, improved sympathovagal balance, and enhanced heart rate variability (4, 7, 27) have been demonstrated with exercise training, all of which are associated with improved patient outcome.

Animal studies have demonstrated a decrease in overall survival with endurance training in cardiomyopathic rats (15, 19). However, these studies utilized postinfarction models of DC. Our study demonstrated that myopathic mice have considerably higher mortality than normal mice and that this increased mortality could not be prevented with exercise training. It is unlikely that the lack of exercise effect was due to an inadequate exercise regimen, as the treadmill routine used in this study represents a moderate exercise level for mice (~80% maximum oxygen consumption (V O2); see Refs. 11 and 12). This level of exercise is consistent with the recommendations for exercise training in human subjects with cardiovascular disease of moderate training intensity (40–85% maximum VO2) for 20–60 min, 3–5 times/wk (30). It is possible that the effects of exercise training on

Fig. 7. Survival curves for normal, sedentary CREB A133, and exercised CREB A133 mice. No statistically significant difference in survival was noted with exercise training between the two groups of CREB A133 mice.
mortality would have been different if the animals had been simultaneously treated with angiotensin-converting enzyme inhibitors or β-blockers.

Limitations. We investigated a single model of DC. Mice with DC due to other causes might display different responses to exercise. Because CREB may lie downstream in the β-adrenergic signaling pathway, the CREBΔ133 mice may not respond to therapeutic interventions that alter the hyperadrenergic state that accompanies progressive heart failure. Last, the animals were under anesthesia when echocardiographic measurements were taken, which may have had a cardiovascular effect. Similarly, all measurements occurred in resting animals as it is not possible to detect beneficial alterations in exercise hemodynamics in mice.

The CREBΔ133 model of murine cardiomyopathy demonstrated the natural history of an untreated DC with progressive ventricular dilatation and dysfunction and increased mortality. The LV dilatation and dysfunction were not altered with 12 wk of exercise training. In addition, moderate-intensity regular exercise did not alter the substantial excess mortality of mice with nonischemic inherited DC.

Current address for J. M. Leiden: Harvard School of Public Health, Laboratory of Cardiovascular Biology, 677 Huntington Ave., Bldg. II, Rm. 117, Boston, MA 02115 (E-mail: Leiden@cvlab.harvard.edu).

REFERENCES


