Low-intensity exercise training delays onset of decompensated heart failure in spontaneously hypertensive heart failure rats

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Emter, Craig A., Sylvia A. McCune, Genevieve C. Sparagna, M. Judith Radin, and Russell L. Moore. Low-intensity exercise training delays onset of decompensated heart failure in spontaneously hypertensive heart failure rats. Am J Physiol Heart Circ Physiol 289: H2030–H2038, 2005. First published July 1, 2005; doi:10.1152/ajpheart.00526.2005.— Data regarding the effectiveness of chronic exercise training in improving survival in patients with congestive heart failure (CHF) are inconclusive. Therefore, we conducted a study to determine the effect of exercise training on survival in a well-defined animal model of heart failure (HF), using the lean male spontaneously hypertensive HF (SHHF) rat. In this model, animals typically present with decompensated, dilated HF between ~18 and 23 mo of age. SHHF rats were assigned to sedentary or exercise-trained groups at 9 and 16 mo of age. Exercise training consisted of 6 mo of low-intensity treadmill running. Exercise training delayed the onset of overt HF and improved survival (P < 0.01), independent of any effects on the hypertensive status of the rats. Training delayed the myosin heavy chain (MyHC) isoform shift from α- to β-MyHC that was seen in sedentary animals that developed HF. Exercise was associated with a concurrent increase in cardiomyocyte length (~6%), width, and area and prevented the increase in the length-to-width ratio seen in sedentary animals in HF. The increases in proteinuria, plasma atrial natriuretic peptide, and serum leptin levels observed in rats with HF were suppressed by low-intensity exercise training. No significant alterations in sarco(endo)plasmic reticulum Ca2+ ATPase, phospholamban, or Na+/Ca2+ exchanger protein expression were found in response to training. Our results indicate that 6 mo of low-intensity exercise training delays the onset of decompensated HF and improves survival in the male SHHF rat. Similarly, exercise intervention prevented or suppressed alterations in several key variables that normally occur with the development of overt CHF. These data support the idea that exercise may be a useful and inexpensive intervention in the treatment of HF.

myosin heavy chain; proteinuria; cardiomyocyte morphology; atrial natriuretic peptide; leptin

HEART DISEASE is a leading cause of death among men and women in Western industrialized societies. Cardiovascular disease (CVD) currently affects over 70 million Americans, 65 million of which are estimated to have high blood pressure and nearly 5 million are diagnosed with congestive heart failure (CHF) (2). With the diagnosis of CHF, life expectancy decreases dramatically and chances of survival decrease with 70–80% of patients dying within 8 yr (2). Although chronic exercise training has clearly been shown to be beneficial in attenuating risk factors for CVD, including high blood pressure and cholesterol levels, insulin resistance, and obesity (9, 14, 53, 58), the utility of exercise as a clinical intervention for patients with developing CHF is less clear. Currently, there is a lack of clarity regarding the efficacy of chronic exercise training as a treatment and in reducing mortality for patients with CHF (47, 59). Among the important considerations is whether or not exercise has the potential to improve the prognosis of patients with CHF or whether exercise places a further excessive demand on an already overstressed myocardium.

The purpose of this study was to assess the effects of and characterize chronic low-intensity exercise training in an animal model of spontaneously developing heart failure (HF). The spontaneously hypertensive HF (SHHF) rat was the genetic model of HF chosen for this study. The true lean male SHHF rat develops hypertension at 3–4 mo of age and spontaneously develops terminal overt HF at 18–23 mo of age 100% of the time. The onset of hypertension preceding CHF in this model is particularly important because 75% of human CHF cases have antecedent hypertension (2). Our experiments were designed to test the hypotheses that in SHHF rats, chronic low-intensity exercise training would 1) delay the onset of overt CHF and improve survivability and 2) prevent alterations in the expression of calcium handling proteins, myosin heavy chain (MyHC) isoforms, whole heart and cardiomyocyte morphology, plasma atrial natriuretic peptide (ANP), and proteinuria that are typically seen during the progression toward advanced HF. Briefly, we found that low-intensity exercise training markedly delayed the onset of overt CHF without a reduction in antecedent hypertension. Additionally, we found that some, but not all, of the classic cellular and systemic physiological alterations normally associated with the development of overt CHF were attenuated with exercise training.

METHODS

Animal model. Male SHHF rats (age 9 or 16 mo), obtained from the colony at the University of Colorado at Boulder, were matched for body weight and assigned to a chronic low-intensity exercise training group (TR) or sedentary group (SED). Five groups of rats were used in this study: 9-mo control (9C, n = 5; used specifically for β-MyHC comparison), 15-mo trained (15TR, n = 9), 22-mo trained (22TR, n = 9), 15-mo sedentary (15SED, n = 10), and 22-mo HF/SEDentary (<22HF, n = 9). Exercise training consisted of treadmill running 3 days/wk, 45 min/day, for 6 mo starting at 9 and 16 mo of age with sedentary age-matched counterparts. During the first month of training, running speed was gradually increased from 10 m/min to 17.5 m/min. However, three animals in the 15TR group (i.e., at ~11 mo of age) experienced sudden death while exercising on the treadmill. Interestingly, no animals in the 22TR group were affected by the higher treadmill speed. As a result of the deaths observed in the
younger rats, running speed was reduced to 14 m/min for all groups for the remainder of the training protocol. No animals were lost during exercise after we reduced the treadmill speed. All animals were provided standard rat chow and water ad libitum on a 12:12-h light-dark cycle and housed in the same facility. At the end of training, animals in the TR group along with their age-matched SED counterparts (except the <22HF) were euthanized. One of the rats in the <22HF group died of CHF, so only necropsy data could be obtained, and the rest of the group was taken at the onset of terminal (<2–3 days before death) uncompensated HF as determined by the presentation of symptoms typically seen with the onset of terminal HF (33). These symptoms include labored breathing, subcutaneous edema, lethargy, piloerection, cold tail, cyanosis, and orthopnea. Dissection of vital tissues and removal of the plantar muscles for analysis of citrate synthase activity (60) occurred at the time of death. This study received prior approval from the Institutional Animal Care and Use Committee at the University of Colorado at Boulder and was conducted under the guidelines accepted by the American Physiological Society.

MyHC separation. α- and β-MyHC content of the left ventricle (LV) was analyzed by the methods of Warren and Greaser (69). Briefly, LV tissue was homogenized in buffer containing (in mmol/l) 137 NaCl, 20 Tris-HCl (pH 7.4), 0.2 EDTA (pH 8.0), 0.5 EGTA, 1 PMSF, 20 NaF, 1 Na3VO4, and 1 sodium pyrophosphate with 10% glycerol and 1% Triton X-100 and 4 mM CaCl2, 1 M leupeptin, 4 g/ml aprotinin, 4 g/ml leupeptin, 4 µg/ml pepstatin, 7.2 µg/ml TPCK, and 7.2 µg/ml tosyl-lysyl-chloromethylketone included. The LV homogenate was diluted 1:100 in urea-thioureac buffer containing 8 M urea, 2 M thiourea, 3% SDS (wt/vol), 75 mM EDTA, DTT, 0.03% bromophenol blue, and 50 mM Tris-HCl (pH 6.8), and 2 samples of varying volume (5 µl and 15 µl) were loaded onto a 6% SDS-PAGE gel (16 × 18 cm; 0.75 mm gel spacers). The gel was run at 8°C for 4.5 h at 16 mA/gel. Gels were subsequently silver stained (Bio-Rad, Hercules, CA), and the percent α- and β-MyHC protein was determined by densitometry using ImageJ software (42).

Cardiomyocyte isolation. Cardiomyocytes were obtained from the LV myocardium (LV + septum) of SHHF rat hearts using modifications to methods previously described (8, 37). The key modifications were as follows. In the original method (8), hearts were initially perfused for 15 min with a Krebs-Henseleit (KH) buffer containing 1.25 mM free Ca2+ and then perfused with a second solution (buffer B), which was a nominal Ca2+ (no added CaCl2) KH buffer. In this study, we dispensed with the first KH perfusate and directly perfused hearts with buffer B for 5 min. This nominal Ca2+ perfusion was then followed by heart perfusion (recirculating) for 5 to 15 min with 100 ml buffer C, which was a modified version of buffer B containing 104 µg/ml dialyzed heart albumin, 55 µM CaCl2, 182.4 U/ml collagenase (Worthington), and 1.3 mg/ml hyaluronidase; the collagenase concentration used in this study was lower than previously described (8). After dispersion and isolation (8), the myocytes were washed twice in medium in 199 and finally suspended in Spring Horn media (pH 7.7 before atmospheric gas equilibration with 5% CO2-95% O2) where myocytes were plated onto laminin-coated coverslips and incubated for 2–8 h at 37°C in a 5% CO2-20% O2-75% N2 atmosphere. All chemicals were obtained from Sigma Chemical (St. Louis, MO) except where noted.

Cardiomyocyte morphology. Cardiac myocytes obtained from the isolation procedure were used to obtain cell dimension measurements. Briefly, two-dimensional video images of individual myocytes were taken in normal Tyrode solution containing (in mmol/l) 140 NaCl, 6 KCl, 1 MgCl2, 2 CaCl2, 10 glucose, 2 pyruvate, and 5 HEPES (pH 7.4) and the presence of 25 mmol/l 2,3-butanedione monoxime (BDM). Cell length and width measures were calculated by using ImageJ software (42).

Western blot analysis for SERCA2, PLB, CSQ, and NCX1. Analysis of sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA2), phospholamban (PLB), and Na+/Ca2+ exchanger (NCX1) protein amounts was performed by using previously described methods (34, 62). A portion of the LV was removed, and tissue was homogenized in an ice-cold buffer containing (in mmol/l) 50 Tris (pH 8.0), 150 NaCl, 1 Na3VO4, 1 PMSF, 100 NaF, 1 EDTA, and 1 EGTA and 0.5% Nonidet P-40, 10 µg/ml leupeptin, and 10 µg/ml aprotinin. The homogenate was then centrifuged at 2000 g for 3 min, the supernatant fraction was retained, and protein concentrations were determined by using the bicinchoninic acid assay (Pierce; Rockford, IL). Homogenates were then stored at −80°C for subsequent Western blot analysis. Samples were placed in a sample buffer containing either 10 mM N-ethylmaleimide (for NCX1), 5% 2-β-mercaptoethanol (for SERCA2), or 2% SDS, 5% 2-β-mercaptoethanol, 10% glycerol, and 65% Laemmli sample buffer (Bio-Rad) (for PLB). Lysis was performed at 100°C for 10 min for NCX1 and SERCA2 samples and 20 min for PLB samples (to isolate 10 kDa form). Western blot analyses were performed by using SDS-PAGE in 7.5% (SERCA2 and NCX1) and 15% (PLB) polyacrylamide gels. Homogenate protein concentrations per sample were as follows (in µg): 30 NCX1, 0.935 SERCA2, and 0.475 PLB. For SERCA2 detection, a polyclonal rabbit anti-SERCA2 (1:10,000 dilution; Bethyl; Montgomery, TX) was used with goat anti-rabbit antibody (1:7,500 dilution; Santa Cruz Biotechnology; Santa Cruz, CA) as the secondary antibody. NCX1 was detected by using mouse anti-NCX1 as a primary antibody (1:500 dilution; Swant; Bellinzona, Switzerland) with goat anti-mouse (1:5,000 dilution; Santa Cruz Biotechnology) as the secondary antibody. PLB detection was accomplished by using a monoclonal mouse anti-PLB antibody (1:100,000; Affinity BioReagents; Golden, CO) with a goat anti-mouse (1:7,500 dilution; Santa Cruz Biotechnology) as the secondary antibody. Blots were developed on film after exposure to a chemiluminescent reagent (Western Lighting; Perkin-Elmer Life Sciences; Boston, MA), then stripped and reprobed for calnexitrin (CSQ) (primary antibody; rabbit anti-CSQ, 1:2,500 dilution, Affinity BioReagents; secondary antibody: goat anti-rabbit, 1:5,000 dilution, Santa Cruz Biotechnology) using standard methods. All blots were scanned into a computer and analyzed by using ImageJ software (42).

Proteinuria. Urine samples were collected from rats in metabolic cages with free access to water but with no food (for a period of 24 h) at five time points during the training protocol (start of the protocol and 7, 11, 14, 21, and ~26 wk). Samples were collected 48 h after exercise to eliminate any effects of acute exercise. Urine protein concentrations were determined by using the bicinchoninic acid assay (Pierce).

ANP and leptin. Serum and plasma samples were taken at the beginning and end of the 6-mo protocol (when the rats were removed from the metabolic cages) from rats placed on a 24-h fast. Samples were collected 48 h after exercise to eliminate any effects of acute exercise. Plasma ANP levels were determined by using a peptide enzyme immunoassay (Peninsula; San Carlos, CA) with modifications previously described (51). Serum leptin was determined via radioimmunoassay (Linco Research; St. Charles, MO).

Tail-cuff blood pressures. Tail-cuff blood pressure measurements were made by using the Gilson Duographs system (7, 33). This system uses pressure transducers and photoelectric sensors to determine blood pressure.

Statistical analysis. All data analyses were performed by using SPSS version 10.1 (SPSS; Chicago, IL). All comparisons between groups were made by using a 2 (age) × 2 (training) ANOVA. Specific age or training differences between groups were revealed by ANOVA simple effects. For the MyHC data, the ANOVA design did not include data from the additional 9C group. Urine proteinuria and ANP levels were analyzed by using a repeated measures ANOVA. All data are means ± SE, and significance is reported at P < 0.05, P < 0.01, and P < 0.001 levels (10, 70).

RESULTS

Gross morphology and citrate synthase activity. Morphological data from TR and SED animals are presented in Table 1.
“Aging” main effects were present for heart weight (P < 0.05), right adrenal (P < 0.01), left adrenal (P < 0.10), and retroperitoneal (RP) fat (P < 0.05). Exercise training decreased RP (TR main effect, P < 0.05), gonadal (Gon) (TR main effect, P < 0.10), and RP + Gon fat (TR main effect, P < 0.05). The 22TR animals had significantly less RP fat than 15TR (P < 0.10) and <22HF (P < 0.10) animals. The 22TR animals also showed less RP + Gon fat (P < 0.05) than age-matched SED (<22HF) animals.

No significant differences were found in citrate synthase activity in plantaris muscle between groups (15 ± 1, 16 ± 4, 15 ± 1, and 13 ± 2 μmol·g wet wt muscle⁻¹·min⁻¹ for 15TR, 15SED, 22TR, and <22HF, respectively).

Survival characteristics. Figure 1 shows the Kaplan-Meier survival curve for 22TR- and <22HF-aged animals. Exercise training improved survivability in SHHF rats (P < 0.01). All nine exercise trained animals lived through the 6-mo training protocol (started at 16 mo of age) and were euthanized at 22 mo of age for tissue analysis. Comparatively, in the 10 age-matched sedentary controls, 1 died of HF and 8 of the other (<22HF) animals presented with terminal overt decompensated CHF (starting at ≈19.5 mo of age) before the end of the training protocol. Once rats in the <22HF group presented with terminal, overt, and decompensated CHF, they were euthanized and their tissues were harvested for analysis.

Cardiac α-MyHC isoform expression. LV samples from 9C animals (Fig. 2A) displayed a significantly larger percentage of α-MyHC protein compared with all other groups (P < 0.01 vs. 15SED, <22HF, and 22TR; P < 0.05 vs. 15TR). Exercise training was associated with a significantly larger percentage of α-MyHC protein (P = 0.056, TR main effect) and delayed the isoform shift to β-MyHC that was seen in the SED animals (Fig. 2B). Hearts from rats in the <22HF group contained significantly less α-MyHC as a percentage of total myosin than the 15SED group (P < 0.01 vs. 15SED); α-MyHC protein expression in 22TR was not significantly different from the 15TR groups. Figure 2C shows representative gels.

Myocyte cell morphology. Myocytes isolated from TR animals were significantly longer (P < 0.10, TR main effect) than the SED animals. Myocytes from the 22TR group were significantly longer than those in the 15TR group (Fig. 3A, simple effect, P < 0.05) and the <22HF group (Fig. 3A, simple effect, P < 0.10). With the aging of rats, an increase in length was also apparent (P = 0.10, age main effect). Myocytes from 22TR hearts were significantly wider than their age-matched counterparts (P < 0.05 for <22HF). Width was significantly maintained during the course of training for the trained animals (Fig. 3B, P < 0.05, age x TR interaction).

Myocyte length-to-width ratios (LWR) are presented in Fig. 3C. Sedentary HF animals (<22HF) displayed a significantly larger LWR compared with 15SED animals (P < 0.05). The TR groups also exhibited maintenance of the LWR, whereas their SED counterparts displayed an increase in LWR (Fig. 3C, P < 0.05, age x TR interaction).

SERCA2, PLB, and NCX1 protein expression. Calcium-handling protein levels analyzed by Western blot analysis are shown in Fig. 4. No significant group differences were found in SERCA2 protein expression (Fig. 4A). An increase in SERCA2 protein levels with age was found (Fig. 4A, P < 0.10, age main effect). PLB protein expression (expressed as normalized to CSQ) in 22TR animals was significantly greater in 15TR animals (Fig. 4B, P < 0.05). The SERCA2-to-PLB ratio (0.7 ± 0.1, 0.8 ± 0.1, 1.8 ± 0.2, and 1.4 ± 0.3 for 15SED, 15TR, <22HF, and 22TR, respectively) significantly increased in both aged groups (P < 0.01, age main effect), and <22HF

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### Table 1. SHHF gross morphology

<table>
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<td>34±3</td>
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<td>2.9±0.5</td>
<td>2.0±0.2e</td>
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<td>2.7±0.2</td>
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Values are means ± SE. SHHF, spontaneously hypertensive heart failure (HF); RP, retroperitoneal; Gon, gonadal; 22TR, 22-mo trained rat group. Results of 2 aging main affect (AGE) × 2 training main affect (TR) ANOVA are indicated at *P = 0.01; †P ≤ 0.05; ‡P = 0.10. Simple effects are indicated at *P < 0.05, difference from 15-mo sedentary (SED) rat group (15SED); †P < 0.10, difference from 15-mo trained rat group (15TR) and <22-mo HF/SED rat group (<22HF); ‡P < 0.05, difference from <22HF.
animals displayed a significantly larger ratio than 15SED animals ($P < 0.01$). Figure 4C displays the results of NCX1 protein levels. A significant increase in expression with aging (Fig. 4C, $P < 0.10$, age main effect) was observed.

**Proteinuria.** Repeated measures ANOVA revealed a significant increase over time in proteinuria in 22-mo animals (Fig. 5, $P < 0.05$, age main effect). The <22HF animals exhibited higher proteinuria at 21 wk than both 22TR ($P < 0.10$) and 15SED ($P < 0.01$) animals (see Fig. 5).

**ANP and leptin.** Plasma ANP was lower (Fig. 6A) in 15-mo versus 22-mo animals over time (repeated measures ANOVA, $P < 0.01$, age main effect). Exercise training also maintained lower ANP levels over time (repeated measures ANOVA, $P < 0.05$, TR main effect). Exercise training resulted in decreased leptin levels ($P < 0.10$, TR main effect), and the <22HF animals demonstrated a significant increase in leptin compared with their age-matched counterparts (Fig. 6B, $P < 0.05$ vs. 22TR).

**Blood pressure.** Systolic blood pressures (SBPs) by group are displayed in Table 2. Exercise training maintained SBP ($P < 0.01$, TR main effect), whereas a decrease was seen with aging ($P < 0.01$, age main effect). Exercise training caused SBP to decrease less rapidly over time compared with their sedentary counterparts ($P < 0.05$, age × TR interaction). SBP decreased significantly with the onset of decompensated HF (<22HF, $P < 0.01$ vs. 15SED and 22TR).

**DISCUSSION**

It is widely recognized that exercise is effective in reducing a number of cardiovascular disease risk factors and that regular physical activity is generally useful in the prevention and treatment of developing cardiovascular disease (9, 14, 53, 58). There is less clarity with respect to the clinical efficacy of exercise training in patients with CHF. Emerging evidence suggests that carefully applied programs of exercise in patients with CHF are generally safe and may improve exercise tolerance, vascular endothelial function, central cardiac function, and overall quality of life (4, 28, 47, 59). The issue of whether exercise training improves survivability in patients with CHF is less clear. Although there are encouraging data that suggest such a positive exercise-induced benefit (59), it is generally acknowledged that a clear resolution of this issue will require more work (2, 59). This is due in part to the complexities associated with the study of patient populations that are often quite heterogeneous with respect to the etiology and severity of the disease that they suffer. In this context, we conducted a study specifically designed to determine the effect of a low-intensity exercise-training program on survival in a well-defined rat model of HF.

**Exercise improves survival in CHF.** The key finding of this study was that low-intensity exercise training markedly delayed the onset of decompensated HF and improved survival in the SHHF rat model of CHF (Fig. 1). This experimental outcome is significant for several reasons. First, our data clearly demonstrate that exercise training can reduce mortality in a rat model of HF that shares many common features with human HF. Second, improved survival occurred independent of any significant effect of exercise on the hypertensive status of the SHHF rats. Not surprisingly, pharmacological reduction of blood pressure in the SHHF model has been shown to prevent and/or improve a number of common morphological, biochemical, and humoral correlates of CHF and generally improves morbidity and mortality (7, 24, 64). Our results demonstrate that even in the face of a sustained basal hypertension, low-intensity exercise training is capable of delaying the onset of LV dilation and end-stage HF. Third, our results underscore the importance of using low-intensity exercise at a tolerable intensity. As noted in METHODS, early on in the 6-mo training protocol, several younger (~11 mo) SHHF rats experienced sudden death during exercise when the running speed was increased to 17.5 m/min. However, after reduction of running speed to 14 m/min for the duration of the 6-mo training period, no more animals died. This final training intensity was
quite low, as evidenced by the absence of a training-induced increase in skeletal muscle citrate synthase activity or a loss in body weight (Table 1). In light of the results of our study, the concept of “tolerable intensity” may have potential clinical utility. As indicated in a recently published American Heart Association Scientific Statement on Exercise and Heart Failure (47), precise exercise intensity guidelines for patients with stable CHF are lacking and an individualized approach is

Fig. 3. Cardiomyocyte length, width, and length-to-width ratio (LWR) under influence of 2,3-butanedione monoxime. A: myocyte length (*P < 0.05 vs. 15TR; †P < 0.10 vs. <22HF). B: width (*P < 0.05 vs. <22HF). C: LWR (*P < 0.05 vs. 15SED).

Fig. 4. Sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA2), phospholamban (PLB), and Na\(^+\)/Ca\(^{2+}\) exchanger (NCX1) protein expression. A: SERCA2 group expression. B: PLB protein expression normalized to calsequestrin (CSQ) (*P < 0.05 vs. 15TR). C: NCX1 group expression. All membranes were stripped and reprobed for CSQ to demonstrate even sample loading.
suggested. The positive outcomes occurring in response to a low-intensity stimulus in this study are encouraging in that they suggest that patients in the early stages of HF may be able to exercise at tolerable, albeit low, levels while still enjoying the cardiovascular benefits that would not be normally observable in normal or other patient populations.

General animal model characteristics: CHF and exercise. Increases in proteinuria and circulating levels of ANP have been positively correlated with the severity of HF in human HF (1, 5, 6, 25) and in this model (32, 33) of HF. Importantly, our exercise intervention slowed the age-related increase in plasma ANP as indicated by our observation that TR animals displayed significantly less ANP over time than their sedentary counterparts after 6 mo of training. This finding supports the idea that training delayed the development of advanced HF. We also found that training suppressed the elevation in proteinuria in the older group of SHHF rats throughout most of the training protocol. One point of interest is that the reduced proteinuria in the <22HF rats at the final collection point was likely an artifact of animal attrition secondary to the development of decompensated HF. As a result, only the healthier rats among the aged sedentary animals remained at the 22-mo sampling end point.

The fact that exercise training suppressed proteinuria in the older rats is interesting for several reasons. Exercise has often been associated with increased proteinuria, but this is generally associated with exhaustive bouts of exercise (19, 20, 48, 49). Our data clearly indicate that our exercise protocol was not sufficiently intense enough to elicit such an increase. Moreover, our training protocol prevented the increased proteinuria that typically occurs during the development of decompensated HF (1, 25, 32, 33). One interpretation of our data is that aberrant protein catabolism normally associated with CHF was prevented by exercise training. This interpretation fits well with our body composition data. Specifically, our exercise protocol elicited decreases in RP and Gon fat stores indepen-

Fig. 5. Group proteinuria over course of training protocol. Aging increases proteinuria over time (repeated measures ANOVA, \( P < 0.05 \), age main effect). <22HF animals exhibited higher proteinuria at 21 wk than both 22TR (simple effect, \( *P < 0.10 \)) and 15SED (simple effect, \( †P < 0.01 \)) animals.

Fig. 6. Plasma atrial natriuretic peptide (ANP) and serum leptin levels. A: group plasma ANP levels at beginning and end of 6-mo protocol. B: group serum leptin. <22HF animals had significantly more leptin than trained counterparts (\( *P < 0.05 \) vs. 22TR). C and D: body weights over training protocol for 15-mo (C) and 22-mo (D) animals.
dient of any statistical differences in body weight between the <22HF and 22TR groups. These data suggest that training fostered the preservation of lean body mass in the older rats. Finally, our observation that circulating leptin levels were lower in 22TR than in <22HF rats is consistent with our observation that training elicited a reduction in RP and Gon fat stores. The relationship between circulating leptin levels and HF is not altogether clear. Although there are reports that leptin levels increase with the development of advanced HF (12, 16) and severe exercise intolerance (54) in humans, there are inconsistencies in regard to the relationship between leptin levels and the severity of CHF. No changes in leptin levels in HF have been found (67), and findings of decreased leptin in disease states associated with CHF, such as cachexia, further cloud the issue (15). Discrepancies also exist in the literature in regard to the effects of exercise training on leptin levels (27). Several studies have shown a decrease in leptin after chronic exercise training. However, it is unclear whether these findings are a result of exercise per se (45, 52) or decreases in body fat indirectly related to the energy imbalances caused by exercise (26, 43, 46, 66). Despite these ambiguities regarding leptin, our results demonstrated that exercise training elicited a significant decrease in RP and Gon fat stores and a parallel decrease in serum leptin, both of which were associated with a lesser diseased state in our model.

**Cellular responses to CHF and exercise training.** In dilated cardiomyopathy, the elongation of cardiomyocytes resulting from the series addition of sarcomeres occurs in the absence of further increases in myocyte cross-sectional area (17, 18, 63). A morphological hallmark of dilated HF is, therefore, a marked increase in cardiomyocyte LWR (17, 18, 44, 63). Such an increase in LWR was observed in the <22HF group and is consistent with the gross morphological alterations in LV chamber dimension that occur in dilated HF (17) where an increase in LV chamber radius and a decrease in wall thickness combine to pathologically increase ventricular wall stress. In this study, we demonstrated that training prevented the marked increase in LWR that normally accompanies dilated HF. We found that training alone elicited a small (∼6%) but significant increase in cell length. This type of adaptation is similar to that previously described in younger healthy rat models of training (31, 36). The fact that LWR did not increase with training indicates that the longitudinal cardiocyte growth was also accompanied by cross-sectional myocyte growth. These simple training-induced dimensional adaptations are significant in that they culminated in physiological cardiogrowth that superseded the pathological cardiocyte growth that occurred in the older sedentary (<22HF) rats.

It has been previously shown that shifting of MyHC protein and mRNA from the α- to the β-isoform occurs in pathological hypertrophy and HF (13, 32, 33, 35, 41). The precise functional consequences of these isoform shifts have yet to be elucidated. However, Tardiff et al. (65) found a 15% decrease in cardiac contractility in a transgenic mouse model overexpressing β-MyHC, whereas another study (23) showed decreased power output in skinned cardiac myocytes overexpressing β-MyHC. It is generally thought that a greater percentage of α-MyHC protein is associated with a healthier state of the myocardium in rats and possibly in humans (35). In the current study, we found a significantly higher expression of α-MyHC protein in the exercise-trained animals at all ages. Our results demonstrate that exercise suppresses the MyHC isoform shift from α to β, which is typically seen with the development of CHF. Although it is tempting to speculate that a delay in the shift to the β-MyHC isoform commonly seen in HF may improve cardiac performance and contribute to improved survivability, no hard evidence currently exists to substantiate this hypothesis.

Despite the fact that there is significant literature on calcium-handling protein expression in HF, there is considerable variability with respect to the findings in the field. For example, NCX1 protein expression has been reported to decrease (71), increase (22, 61, 68), or remain unchanged (22, 57) in HF. Although it is generally thought that SERCA2 mRNA levels decrease in CHF (3, 11, 29, 55, 61, 68), no consensus exists in regard to protein levels, with some studies finding no change (29, 38–40, 55, 56) and others a decrease (11, 21, 34, 61, 68) in expression. The same ambiguity is true with respect to the SERCA2 regulatory protein PLB. Although there is substantial evidence that PLB mRNA expression decreases in CHF (3, 11, 29, 55), it appears that PLB protein expression is not significantly affected in numerous models of HF (11, 29, 34, 38–40, 55, 56). In our study, we found no evidence of significant alterations in SERCA2, PLB, or NCX1 protein expression with the development of HF in the SHHF rat. The effect of exercise training on calcium handling protein expression and/or function is not clear due to the fact that relatively few studies have addressed this issue and that the models of training and HF used have been widely variable. Lu et al. (30) found that exercise training normalized decreases in SERCA2 and increases in NCX1 mRNA and protein expression in a canine model of pacing-induced HF. In a rat model of myocardial infarction, Zhang et al. (71) found that NCX1 protein expression was reduced but could be normalized by a program of high-intensity sprint training. In our model of HF, we found evidence of only subtle increases in PLB and NCX1 protein expression with exercise training. These types of alterations might be expected to alter the relative contributions of the sarcoplasmic reticulum and sarcolemma to cellular Ca^{2+} regulation, but in the absence of direct measurements of sarcoplasmic reticulum function and sodium-calcium exchange, this is purely speculative.

**Limitations.** Improved survivability with exercise training was a major finding of this study. From the perspective of clinical relevance, one issue worthy of consideration is whether or not the exercise intervention at ∼16 mo of age occurred when the rats had already entered into the early stages of HF. Our ANP and proteinurea data are consistent with the idea that the rats were already entering into the early stages of HF. The ANP and proteinurea values reported in this study were ∼4 to 6 and ∼6 to 11 times higher, respectively, than those normally seen in similarly aged Wistar-Furth rats, the generally accepted

**Table 2. Systolic blood pressure by group before and after 6-mo training protocol**

<table>
<thead>
<tr>
<th>9-Mo Baseline</th>
<th>Intervention</th>
<th>15-Mo</th>
<th>22 Mo HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>196±5</td>
<td>TR</td>
<td>205±2</td>
<td>186±5†</td>
</tr>
<tr>
<td>194±6</td>
<td>SED</td>
<td>201±6</td>
<td>161±4†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Simple effects are indicated at *P < 0.01 vs. 15SED and 22TR; †P < 0.01 vs. 15TR.
nonpathological SHHF control (32). In addition, Radin et al. (50) previously demonstrated that between 12 and 15 mo of age, LV fractional shortening shows clear signs of deterioration. Collectively, these data provide indirect evidence in support of the idea that our exercise intervention was capable of improving mortality in rats that had already entered early decompensation and HF. We do concede, however, that absent of direct evidence of central cardiac function deterioration in this study, we cannot definitively conclude that exercise training imposed after the onset of early HF will improve mortality.

In summary and in conclusion, we demonstrated that 6 mo of low-intensity exercise training delayed the onset of decompensated HF and improved survival in the male SHHF rat. Furthermore, the exercise intervention prevented or suppressed alterations in MyHC isoform expression, cell morphology, proteinuria, plasma ANP, and body composition that normally occur with the development of overt HF. Our findings lend support to the idea that in a setting of developing HF, exercise training can positively influence both morbidity and mortality. Given the poor prognosis for those individuals diagnosed with CHF (2), low-intensity exercise training may be a viable low-cost therapy for improving life expectancy and the quality of life after diagnosis. Furthermore, the model of exercise training and HF described in this work may ultimately be of value in dissecting out the specific cellular and molecular processes that are influenced by training and that are required for improved survival in a setting of developing HF.

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REFERENCES


EXERCISE DELAYS HEART FAILURE IN SHHF RATS


