Chronic Dynamic Exercise Improves a Functional Abnormality of the G Stimulatory Protein in Cardiomyopathic BIO 53.58 Syrian Hamsters

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Background. The effects of chronic exercise training on myocardial contractility and β-adrenergic signal transduction in hearts with left ventricular dysfunction have not been determined.

Methods and Results. Fourteen-week-old cardiomyopathic BIO 53.58 and normal F1B Syrian hamsters underwent 10 weeks of treadmill training and were compared with 24-week-old BIO 53.58 and F1B untrained controls. Left ventricular isovolumic maximum positive dP/dt and peak developed pressure were significantly lower in BIO 53.58 than in F1B controls. Exercise training improved left ventricular contractile indices in BIO 53.58 but not F1B hamsters. The left ventricular β-adrenergic receptor number (Bmax) was similar in BIO 53.58 and F1B controls. Basal adenylate cyclase activity (ACA) and ACAs stimulated by isoproterenol, 5'-guanylylimidodiphosphate (GppNHp), sodium fluoride, and forskolin were significantly lower in BIO 53.58 than in F1B controls. The functional activity of stimulatory guanine nucleotide-binding protein (G), as determined by reconstitution with S49 lymphoma cyclophosphamide cell membranes, was significantly lower in BIO 53.58 controls. After 10 weeks of exercise training, Bmax and basal and isoproterenol-stimulated ACAs were unchanged in either BIO 53.58 or F1B hamsters compared with controls. However, in F1B hamsters, training decreased ACAs stimulated by GppNHp, sodium fluoride, and forskolin, with a reduced functional activity of G. In contrast, these ACAs increased significantly in association with an enhanced G activity in cardiomyopathic BIO 53.58 hamsters after training.

Conclusions. Chronic exercise training does not change receptor-mediated β-adrenergic responsiveness in either F1B or BIO 53.58 hamsters. However, exercise training reduces G activity in normal F1B hamsters and improves the functional abnormality of G in cardiomyopathic BIO 53.58 hamsters. This improvement may potentially contribute to augmented left ventricular contractility in BIO 53.58 after training.

Key Words: proteins • exercise

Chronic exercise training provides several cardiovascular adaptations in human healthy subjects and normal animals. It leads to an increase in peak exercise oxygen consumption (V\textsubscript{O2}), which is achieved by an increase in cardiac output, an improvement in the effectiveness of the peripheral circulation, or both. These central or peripheral adaptations to exercise training would be potentially beneficial in the presence of chronic heart failure. Indeed, considerable attention has been focused on the ability of exercise training to increase exercise tolerance in patients with congestive heart failure. However, the physiological and biochemical effects of exercise training on the myocardium have not been well defined in heart failure. The purpose of the present study was to assess the effects of chronic exercise training on left ventricular myocardial contractility and the β-adrenergic receptor-stimulatory G protein/adenylate cyclase system in the heart with left ventricular dysfunction. For this physiological and biochemical analysis, we used the BIO 53.58 golden Syrian hamster as an animal model of the diseased heart and F1B hamsters as normal controls.

We found that exercise training augmented depressed left ventricular contractility and improved impaired β-adrenergic signal transduction in cardiomyopathic hamsters. In addition, these improvements were accompanied by substantial elevations of plasma catecholamine levels at rest. To determine whether these exercise-induced physiological and biochemical changes relate to the elevated plasma catecholamine levels, exercise training was also performed in cardiomyopathic animals under blockade of β-adrenergic receptors.

Methods

Experimental Animals

Male 14-week-old cardiomyopathic BIO 53.58 Syrian hamsters and age-matched BIO F1B Syrian hamsters were obtained from BIO Breeders (Fitchburg, Mass). The hamsters were allowed free access to food and water. They were randomly assigned to two groups each: control (n=15 for F1B hamsters and n=18 for BIO 53.58 hamsters) and trained (n=16 for F1B hamsters and n=16 for BIO 53.58 hamsters) groups. Training was done by having the hamsters run on a motor-driven treadmill (model TK-175, Unicom Instrument) set at a 20° incline. The animals were exercised twice a day, 3 hours apart, 6 days a week for 10 weeks in a progressive fashion. F1B hamsters initially ran for 30 minutes at 15 m/min for 1 week, then at 20 m/min for 2 weeks, and finally at 25
m/min for 7 weeks. To avoid a fall-off in performance, the speed was 5 m/min slower in each step of the training protocol for cardiomyopathic hamsters than for normal hamsters. Control hamsters were hand manipulated in the same fashion as the trained animals with each exercise session. The hamsters were studied 24 hours after the last session of a 10-week training period. The timing was chosen to dissociate, as much as possible, a training effect from a postexercise event. After deep anesthesia was induced with intraperitoneal pentobarbital (60 mg/kg), a tracheotomy was performed, and a tracheal cannula was inserted. The animals were mechanically ventilated with 100% oxygen using a volume-controlled respirator (model SN485-5, Shinano Apparatus, Tokyo). A thoracotomy was performed carefully, and the chest was retracted to expose the heart.

In some animals of each of the control (n=7 for F1B hamsters and n=7 for BIO 53.58 hamsters) and trained (n=7 for F1B hamsters and n=7 for BIO 53.58 hamsters) groups, blood was sampled for plasma catecholamine measurements, and the hearts were rapidly excised, washed in saline solution, weighed, frozen in liquid nitrogen, and stored at −80°C for biochemical studies.

In other animals of each of the control (n=8 for F1B hamsters and n=11 for BIO 53.58 hamsters) and trained (n=9 for F1B hamsters and n=9 for BIO 53.58 hamsters) groups, after exposure of the heart, the ascending aorta was surgically isolated, a 2-0 silk thread was passed around the aorta with a small curved mosquito, and the ends of the silk thread were threaded to make a snare. Left ventricular pressure measurements were made by inserting a fluid-filled 22-gauge needle into the left ventricle through the apex. The needle was connected directly to a transducer (Statham P-23Db, Gould, Cleveland, Ohio). Left ventricular pressure development (dp/dt) was obtained from a differential amplifier in the recorder with the high-frequency filter cutoff set at 70 Hz. Heart rate was determined from the systemic pressure tracing. Hamsters were allowed at least 10 minutes after surgical preparation to reach steady state. The peak left ventricular pressure and dp/dt were recorded at a paper speed of 100 mm/s (model R-60, TEAC Co, Musashino, Tokyo). All values obtained from simultaneous left ventricular pressure were calculated as a mean of at least five cardiac cycles. After baseline left ventricular pressure and dp/dt were recorded, the aorta was occluded for 5 to 6 seconds to produce isovolumic contraction. The peak left ventricular pressure and dp/dt were recorded, and the values of two occlusions were averaged. These studies conformed to the guiding principles of the American Physiological Society and were approved by the institutional animal care committee.

In other groups of BIO 53.58 hamsters, the effects of the nonselective β-receptor–blocking agent arotinolol (Sumitomo Pharmaceutical Products, Osaka, Japan) on baseline and exercise-induced alterations in myocardial contractility and β-adrenergic responsiveness were investigated. Arotinolol (40 mg/kg) was given orally via a tube inserted into the esophagus 5 to 10 minutes after the end of exercise in 15 cardiomyopathic hamsters. The dosage was determined in pilot experiments and was selected because administration for 1 week produced a significant reduction of heart rate at rest. The exercise protocol for these animals was the same as the one for BIO 53.58 hamsters without arotinolol treatment. In 15 others, arotinolol was given without exercise training, and these animals served as controls for the trained group. Physiological studies (n=8 for each) and biochemical analysis (n=7 for each) for these two arotinolol-treated control and trained groups were done as described above.

Membrane Preparation

Cardiac membranes were isolated and prepared by the method described by Feldman et al with a minor modification. Ventricular myocardium from one heart was minced with scissors and homogenized with polytron PT10 in 10 mmol/L Tris-Cl (pH 7.8)/1 mmol/L EGTA buffer, and the resulting homogenate was added to an equal volume of 1 mol/L KCl and stirred for 15 minutes at 4°C. After centrifugation at 49 000 g for 15 minutes at 4°C, the resulting pellet was resuspended in 50 mmol/L Tris-Cl (pH 7.4) containing 250 mmol/L sucrose and 1 mmol/L EGTA and was stored at −80°C.

β-Adrenergic Receptor Binding Studies

β-Adrenergic receptor number was measured by [125I]-iodocyanopindolol (ICYP) binding. Fifty to 150 μg of cardiac membranes were incubated with increasing concentrations (10 to 640 mmol/L) of ICYP in 20 mmol/L Tris (pH 7.5) containing 150 mmol/L NaCl and 1 mmol/L ascorbate. Preliminary experiments revealed that the range of ICYP concentrations used was sufficient to demonstrate saturation in radioligand binding isotherms and that binding of ICYP to cardiac membranes reached equilibrium within 30 minutes and remained stable thereafter for at least 120 minutes at 37°C. Therefore, the membranes were incubated for 60 minutes at 37°C during the course of this study. At the end of the incubation, binding was stopped by the addition of 5 mL of incubation buffer and immediate filtration through glass-fiber filters (GF/C, Whatman, Maidstone, UK). Each filter was washed three times with 5 mL of buffer, and the bound radioactivity was determined by use of a gamma counter at 75% efficiency. Specific binding was defined as the difference between total binding and binding inhibited by 1 mmol/L (±)-propranolol. Maximal binding capacity (Bmax) and dissociation constant (Kd) were determined by nonlinear curve fitting with the LIGAND binding analysis program.

Adenylate Cyclase Activity

Adenylate cyclase activity was assayed by the method of Salomon. The final reaction mixture (100 μL) contained 25 mmol/L Tris-HCl, 10 mmol/L MgCl2, 0.1 mg/mL bovine serum albumin, 1 mmol/L dithiothreitol, 1 mmol/L EDTA, 1 mmol/L cAMP, 1 mmol/L ATP, 0.1 mmol/L GTP, 10 mmol/L creatine phosphate, 10 μM creatine phosphokinase, 4 to 6×10⁶ cpm [α-32P]ATP, and various agonists as indicated. Maximum adenylate cyclase activity was measured by measuring cAMP production in the presence of 10⁻⁵ mol/L (−)-isoproterenol, 100 μmol/L 5'-guanylylimidodiphosphate (Gpp(NH)p), 10 mmol/L sodium fluoride, and 100 μmol/L forskolin. The reaction mixtures were prepared at 4°C, and the reaction was initiated at 37°C after the addition of cardiac membranes (30 to 50 μg). The reaction was terminated at 15 minutes by addition of 150 μL cold stopping solution containing 0.5% sodium dodecyl sulfate (SDS), 1.5 mmol/L ATP, 0.5 mmol/L cAMP, and 10 000 cpm of [³H]cAMP as a recovery marker and heating for 3 minutes at 90°C. cAMP was separated from ATP by Dowex-alumina chromatography. All reactions were performed in triplicate, and recovery of cAMP was >70%.

Gq Activity

The activity of Gq was assessed by reconstitution of fluoride-stimulated Mg ATP–dependent adenylate cyclase activity in membranes of S49 cyc− lymphoma cells. S49 cyc− lymphoma cells were propagated in Dulbecco’s modified Eagle’s medium containing 10% (vol/vol) heat-inactivated horse serum. Plasma membranes were prepared as described by Sternweis and Gilman, with sucrose density-gradient separation to purify the membranes. The cyc− membranes were suspended at a concentration of 2.5 mg/mL in buffer containing 20 mmol/L HEPES (pH 8.0), 10 mmol/L MgCl2, and 1 mmol/L EDTA. Samples were then frozen and stored at −80°C.

In the present study, the reconstitution assay was performed by a modification of the method described by Feldman et al and Longabaugh et al. Cardiac membranes were incubated at 37°C for 30 minutes and then solubilized in 0.2% Lubrol-PX in a buffer of 10 mmol/L Tris-Cl (pH 7.5), 0.1 mmol/L EDTA, 10 mmol/L MgCl2, and 1 mmol/L dithiothreitol for 60 minutes.
captoethanol and electrophoresed on a 10% gel using SDS-polyacrylamide gel electrophoresis.26 The resolved proteins were electrophoretically transferred to Immobilon P膜 membranes (Millipore Co, Bedford, Mass) using 25 mmol/L Tris/192 mmol/L glycine (pH 8.3) in 10% (vol/vol) methanol.27 The transfer membranes were then incubated for 1 hour at 37°C in 50 mmol/L Tris-Cl (pH 8.0) containing 2 mmol/L CaCl2, 80 mmol/L NaCl, 5% (wt/vol) nonfat dry milk, and 0.02% (wt/vol) sodium azide (immunoblotting buffer) to which 0.2% (vol/vol) Nonidet P-40 and 0.2% SDS. After three washings (10 minutes) with immunoblotting buffer containing detergent, the blots were incubated with goat anti-rabbit 

Materials

[α-32P]ATP and [125I]ICYP were from DuPont Co (Wilmington, Del); culture medium was from Gibco Corp (Grand Island, NY). All other reagents were the highest grade commercially available.

Data Analysis

The concentrations of plasma catecholamines were determined by the radioenzymatic assay of Peuler and Johnson.28 Protein concentration was measured by the method of Lowry et al.29 Values in figures and tables are expressed as mean±SEM. The statistical significance of differences among the three animal groups was determined by one-way ANOVA and multiple comparisons with Tukey's procedure. Comparisons between controls and trained animal groups were analyzed using an unpaired t test. The differences in the dose-response curves between the control and trained groups were analyzed by two-way ANOVA with repeated measures. Statistical significance was considered achieved at P<.05.

Results

Effects of Exercise Training on Body Parameters

Table 1 demonstrates the effect of endurance training on the body weights and heart weights in each of six animal groups. The mean body weights of exercised groups were lower than the controls for both F1B and BIO 53.58 hamsters. The heart weight was higher in trained F1B hamsters compared with F1B controls, whereas the difference between trained and control BIO 53.58 is not significant. The ratio of heart weight to body weight was significantly higher in the trained

Quantification of $G_i$

The relative amount of the α-subunit of $G_i$ ($\alpha G_i$) in cardiac membranes from normal and cardiomyopathic hamsters was measured immunochemically.12 The preparation and characterization of the antisera to the synthetic peptides corresponding to the carboxyl-terminal decapeptides of $\alpha G_i$ have been described.16,20 For immunoprecipitation, the antibody obtained from commercially available anti-$\alpha G_i$ antisera (RM/I, DuPont Co, Boston, Mass) was used. The anti-$\alpha G_i$ antisera were directed against a synthetic peptide deduced from the nucleotide sequence at the carboxyl-terminal end of $\alpha G_i$ (RMHLRQYELL) in several species.21-24 The amino acid sequence at the carboxyl terminus of Syrian hamsters $\alpha G_i$ deduced from a cardiac cDNA clone is identical to this sequence.25 Cardiac membranes (40 μg) were suspended in electrophoresis buffer (50 μL) containing 62 mmol/L Tris-Cl (pH 6.8), 2% (wt/vol) SDS, 10% (wt/vol) glycerol, and 0.7 mol/L 2-mer-
groups compared with control groups for both F1B and BIO 53.58 hamsters. Lung weight was significantly lower in trained BIO 53.58 hamsters than in control hamsters.

**Left Ventricular Hemodynamics**

At baseline, heart rate, left ventricular peak systolic pressure, and maximum dP/dt were lower in BIO 53.58 controls than in F1B controls (Table 2). End-diastolic pressure did not differ between the two 24-week-old control groups. Isovolumic left ventricular peak systolic pressure was lower in BIO 53.58 controls compared to F1B controls, but end-diastolic pressure did not differ between the two animal groups. As a result, developed peak systolic pressure was significantly lower in association with a decelerated maximum positive dP/dt in BIO 53.58 controls than in F1B controls. These observations indicate that left ventricular contractile state was relatively depressed in 24-week-old cardiomyopathic hamsters compared with age-matched normal hamsters.

Ten weeks of exercise training did not change left ventricular hemodynamics in F1B hamsters either under baseline conditions or under aortic constriction. In contrast, exercise training augmented the left ventricular contractile state in BIO 53.58. Maximum positive dP/dt was significantly faster in trained BIO 53.58 than in BIO 53.58 controls both in baseline conditions and under aortic constriction. In addition, left ventricular peak systolic developed pressure was significantly greater in the trained BIO 53.58 hamsters.

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**Table 1. Effect of Exercise Training on Body Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>Heart Weight, g</th>
<th>Heart Weight/Body Weight, mg/g</th>
<th>Lung Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F1B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=15)</td>
<td>132±3</td>
<td>0.364±.009</td>
<td>2.77±.07</td>
<td>0.583±.017</td>
</tr>
<tr>
<td>Trained (n=16)</td>
<td>121±5</td>
<td>0.404±.010</td>
<td>3.35±.08</td>
<td>0.56±.008</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt;.01</td>
<td>&lt;.05</td>
<td>&lt;.01</td>
<td>NS</td>
</tr>
<tr>
<td><strong>BIO 53.58</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=18)</td>
<td>112±3*</td>
<td>0.311±.010*</td>
<td>2.78±.05</td>
<td>0.567±.042</td>
</tr>
<tr>
<td>Trained (n=16)</td>
<td>101±4†</td>
<td>0.309±.011†</td>
<td>3.05±.04</td>
<td>0.439±.019†</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt;.05</td>
<td>NS</td>
<td>&lt;.01</td>
<td>&lt;.05</td>
</tr>
<tr>
<td><strong>BIO 53.58+arotinolol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=19)</td>
<td>108±2*</td>
<td>0.286±.008*</td>
<td>2.65±.06</td>
<td>0.506±.026</td>
</tr>
<tr>
<td>Trained (n=17)</td>
<td>94±2†</td>
<td>0.293±.009†</td>
<td>3.12±.16</td>
<td>0.439±.013†</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt;.01</td>
<td>NS</td>
<td>&lt;.01</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

Values are mean±SEM. _P_ value represents a significance in comparison between each control and trained animal group.

*P<.01 vs control F1B; †P<.01 vs trained F1B.

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**Table 2. Effect of Exercise Training on Left Ventricular Hemodynamics**

<table>
<thead>
<tr>
<th></th>
<th>Baseline Condition</th>
<th>Aortic Constriction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR, bpm</td>
<td>LVP, mm Hg</td>
</tr>
<tr>
<td><strong>F1B</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>344±8</td>
<td>143±9</td>
</tr>
<tr>
<td>Trained (n=9)</td>
<td>345±18</td>
<td>139±5</td>
</tr>
<tr>
<td><em>P</em></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>BIO 53.58</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=11)</td>
<td>301±9*</td>
<td>95±5*</td>
</tr>
<tr>
<td>Trained (n=9)</td>
<td>319±7</td>
<td>100±5†</td>
</tr>
<tr>
<td><em>P</em></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>BIO 53.58+arotinolol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>276±8*</td>
<td>100±5*</td>
</tr>
<tr>
<td>Trained (n=8)</td>
<td>286±13†</td>
<td>111±7†</td>
</tr>
<tr>
<td><em>P</em></td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

HR indicates heart rate; bpm, beats per minute; LVP, left ventricular peak systolic pressure; EDP, left ventricular end-diastolic pressure; max+dP/dt, maximum positive dP/dt; and dLVP, developed LVP. Values are mean±SEM. _P_ value represents a significance in comparison between each control and trained animal group.

*P<.01 vs control F1B; †P<.05 vs trained F1B.
Clotrimazole and Miconazole are effective in treating Candida albicans infections in the skin and nails. They penetrate well into these tissues and can be used topically for localized infections. Clotrimazole is also available in a vaginal cream for treating candidiasis of the vagina. Miconazole is effective in treating oral thrush and other oral candidiasis, as well as fungal infections of the skin and nails.

In terms of side effects, both clotrimazole and miconazole are generally well tolerated. The most common side effect associated with clotrimazole is skin irritation. Miconazole may cause skin irritation or sensitivity in some individuals. It's important to wash and dry the affected area thoroughly before applying the medication to avoid unnecessary skin irritation. In the case of miconazole, it is used for oral thrush, so the mouth should be rinsed with water after use to prevent irritation.

For clotrimazole, the duration of treatment depends on the specific condition being treated. For fungal infections of the skin and nails, a course of treatment lasting 1 to 4 weeks is typical. For oral thrush, a course of treatment lasting 2 weeks may be sufficient. It's important to follow the instructions provided by your healthcare provider or pharmacist carefully.

In conclusion, clotrimazole and miconazole are effective antifungal medications used to treat various fungal infections. However, it's essential to consult a healthcare professional to determine the appropriate medication and dosage for your specific condition. Regular follow-up appointments may be necessary to monitor your condition and adjust your treatment as needed.
TABLE 5. Effects of Exercise Training on Adenylate Cyclase Activities

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Isoproterenol 10^{-4} mol/L</th>
<th>GppNHp 0.1 mmol/L</th>
<th>NaF 10 mmol/L</th>
<th>Forskolin 0.1 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=7)</td>
<td>12±1</td>
<td>30±2</td>
<td>40±2</td>
<td>109±5</td>
<td>323±19</td>
</tr>
<tr>
<td>Trained (n=7)</td>
<td>10±1</td>
<td>28±1</td>
<td>32±2</td>
<td>83±4</td>
<td>239±7</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>&lt;.05</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>BIO 53.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=7)</td>
<td>9±1</td>
<td>18±1*</td>
<td>15±1†</td>
<td>39±4†</td>
<td>105±10†</td>
</tr>
<tr>
<td>Trained (n=7)</td>
<td>9±1</td>
<td>19±1</td>
<td>22±1</td>
<td>57±6§</td>
<td>158±17§</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>&lt;.01</td>
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<td>BIO 53.58+arotinol</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>10±1</td>
<td>18±1*</td>
<td>17±2†</td>
<td>44±3†</td>
<td>100±13†</td>
</tr>
<tr>
<td>Trained (n=8)</td>
<td>10±1</td>
<td>19±2§</td>
<td>21±2§</td>
<td>63±4‡</td>
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<td>NS</td>
<td>NS</td>
<td>&lt;.05</td>
<td>&lt;.01</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

GppNHp indicates 5'-guanylylimidodiphosphate. Values are mean±SEM. Activity is expressed as cAMP pmol·min^{-1}·mg protein^{-1}. P value represents a significance in comparison between each control and trained animal group.

G, or heat-inactivated membrane extracts. The bioactivity of G, expressed as the slope of the line was significantly less in cardiac membranes from BIO 53.56 control hamsters than in those from F1B control animals (2.7±0.2 versus 3.9±0.1 fmol·min^{-1}·µg^{-1} cAMP, P<.01). Exercise training decreased reconstituted adenylate cyclase activity significantly in F1B trained hamsters (3.2±0.1 fmol·min^{-1}·µg^{-1} cAMP, P<.01) compared with its controls, whereas it increased activity in BIO 53.58 trained animals (3.7±0.3 fmol·min^{-1}·µg^{-1} cAMP, P<.05) (Fig 4 and Table 6).

Arotinolol treatment did not change the functional activity of G, in BIO 53.58 compared with nontreated controls. In addition, it did not influence the exercise training–induced improvement in the functional abnormality of G, in BIO 53.58 animals (2.6±0.2 fmol·min^{-1}·µg^{-1} cAMP in the nontrained animals versus 3.9±0.2 fmol·min^{-1}·µg^{-1} cAMP in trained animals, P<.01).

Quantification of G,  
The αG, antisera (RM/1) directed against the carboxyl-terminal end of αG, revealed two distinct bands of peptides (M, 45 000 and M, 52 000 kD) on immunoblots from cardiac membranes. There was a linear relation between the amount of protein applied (10 to 80 µg) to the immunoblot and each band density on the autoradiographs (Table 7).

In contrast to the findings obtained on the functional bioactivity of αG, in this study, the immunologial amount of αG, in BIO 53.58 controls was similar to that in the F1B controls. Ten weeks of treadmill running did not alter the levels of the M, 52 000 peptides, but it decreased those of the M, 45 000 peptides in F1B hamsters. By contrast, there was a slight but statistically significant increase in the amount of the M, 52 000 peptides in BIO 53.58 hamsters after exercise training, whereas the training did not change the levels of the M, 45 000 peptides.

Arotinolol treatment did not alter the levels of either M, 52 000 or M, 45 000 peptides in the BIO 53.58 control hamsters. However, exercise training significantly increased the levels of the M, 52 000 peptides but not of the M, 45 000 peptides during the treatment.

Discussion  
Cardiac Hemodynamics and Biochemical Characteristics in BIO 53.58 Hamsters  
The BIO 53.58 golden Syrian hamster arose from a spontaneous mutation of the BIO 14.6 strain. Both of these hamsters ultimately develop congestive heart failure. However, in contrast to the BIO 14.6 hamster, the BIO 53.58 hamster does not develop cardiac hypertrophy before dilatation. In 24-week-old untrained BIO 53.58 hamsters, isovolumic left ventricular maximum positive dP/dt and peak developed pressure under aortic constriction, determined as indices of myocardial contractility, were significantly lower in the cardiomyopathic hamsters than those in age-matched F1B controls. This depressed myocardial contractility was accompanied by diminished cAMP production in membranes prepared from left ventricles. Basal adenylate cyclase activity and the activities in the presence of isoproterenol, GppNHp, sodium fluoride, and forskolin were all significantly reduced in BIO 53.58 compared with F1B controls. In addition, these reductions in β-adrenergic responsiveness were associated with a functional abnormality of G, rather than downregulation of β-adrenergic receptors. These physiological and biochemical characteristics observed in 24-week-old BIO 53.58 hamsters are similar to those recently reported by Feldman et al in 100-day-old (14-week-old) hamsters. The studies by these authors and ours demonstrate that the defect of bioactivity of G, is not associated with a reduction of immunologic levels of the αG.
relationship to $\beta$-adrenergic responsiveness were studied in normal F1B hamsters and cardiomyopathic BIO 53.58 hamsters. Fourteen-week-old BIO 53.58 and age-matched F1B hamsters underwent a 10-week exercise training program. Exercise training did not alter isovolumic left ventricular positive maximum dP/dt or peak developed pressure in F1B hamsters compared with their controls, whereas it increased these contractile indices in BIO 53.58 hamsters. The key finding of the present study is that chronic treadmill running provided no significant change in the receptor-mediated $\beta$-adrenergic responsiveness, but it did alter the responsiveness at the level distal to the receptor in both normal and cardiomyopathic hamsters. Exercise training did not change membrane-bound $\beta$-adrenergic receptor number in either F1B or BIO 53.58 trained hamsters compared with controls. In addition, it did not induce significant effects on basal or isoproterenol-stimulated adenylate cyclase activity in either normal or cardiomyopathic hamsters. Importantly, however, Gpp(NH)p-
stimulated, sodium fluoride–stimulated, and forskolin-stimulated adenylate cyclase activities decreased in association with a reduced functional activity of G, in F1B trained hamsters compared with F1B controls. In contrast, these adenylate cyclase activities increased in association with an improved functional abnormality of G, in BIO 53.58 trained hamsters compared with their controls. GppNHp, fluoride ion, and forskolin require αG, for maximal stimulation of the catalytic unit of adenylate cyclase.32,33 In our study, despite an increase or decrease in the functional G, activity, the training did not change the isoproterenol-stimulated adenylate cyclase activity in either F1B or BIO 53.58 hamsters. This suggests that the coupling of G, with β-adrenergic receptor was also altered by exercise training. In addition, these functional changes in G, after exercise training were associated with quantitative alterations in its α-subunit. Exercise training decreased the density of the autoradiographic band to be identical to that of the M, 45 000 peptides in F1B hamsters, whereas it increased the density corresponding with the M, 52 000 peptides in BIO 53.58 hamsters. Previous studies have demonstrated that the M, 45 000 and 52 000 αG, proteins are formed by alternative splicing of a single gene34,35 and that the physiological functions of these two protein products are identical.36 By contrast, the results of this study suggest the possibility that these two proteins could be independently altered by a given intervention. However, although there was a statistical significance, the exercise-induced increase in the amount of immunodetectable αG, is small in BIO 53.58 hamsters in the present study. Thus, it is not certain that improved functional activity of G, after exercise training actually reflects an increase in αG,. Conversely, the observations of this study support the concept of noncoordinate regulation of cardiac β-adrenergic receptors and G, protein as recently proposed by Hammond et al.,37 who studied the effect of chronic dynamic exercise in the normal pig heart. Previous studies of exercise training–induced changes in myocardial contractility and receptor-mediated β-adrenergic responsiveness of cardiac membranes in the normal heart have reported conflicting results.38-45 In contrast to these studies, our finding that exercise training alters β-adrenergic responsiveness at a level distal to the receptor in normal and diseased hearts is unique.

In the present study, exercise training substantially elevated the plasma catecholamine concentrations at rest, especially in BIO 53.58 hamsters. It is possible that this indirect effect of exercise training might result in improved β-adrenergic responsiveness distal to the receptor, ie, at the level of the stimulatory G protein or catalytic unit of adenylate cyclase. Therefore, we assessed the effects of β-adrenergic receptor blockade on the exercise-induced improvement of G, function, adenylate cyclase activity, and augmentation of depressed myocardial contractility. Heart rate was slightly but not significantly lower in the arotinolol-treated BIO 53.58 hamster compared with nontreated hamsters in each of the control and trained groups. In addition, plasma epinephrine and norepinephrine levels were significantly reduced in the arotinolol-treated BIO 53.58 hamster compared with nontreated hamsters, although the mechanism of this reduction is not known. These observations indicate that oral administration of arotinolol effectively blocked the circulatory β-adrenergic stimuli on the receptors. Despite this blockade, exercise training augmented left ventricular contractility and improved adenylate cyclase activity as well as the functional abnormality of G,. Accordingly, the exercise-induced physiological and biochemical improvements observed in cardiomyopathic hamsters are not caused by β-adrenergic stimuli resulting from elevated plasma catecholamine levels.

**Clinical Implications and Study Limitations**

BIO 53.58 hamsters with left ventricular systolic dysfunction were able to accomplish a 10-week treadmill running program without encountering any obvious deleterious effects. Left ventricular end-diastolic pressure both in baseline conditions and under aortic constriction remained normal in BIO 53.58 trained hamsters, indicating
that left ventricular diastolic dysfunction is not induced by this exercise protocol. However, these benefits cannot be simply applied to the rehabilitation program in the diseased human heart. There are several differences between physiological responses to endurance training in this animal model and those previously reported in patients with impaired left ventricular function.5,10 First, both normal and cardiomyopathic hamsters had substantially elevated plasma catecholamine concentrations at rest after exercise training. This elevation of plasma catecholamines is similar to that in some previous animal studies.6,41 However, this observation is not identical to the diseased human heart with exercise training.48 Continuous and prolonged elevation in circulating catecholamines might potentially cause myocardial cell injury by an induction of intracellular calcium overload. Elevation of plasma catecholamines might also lead to an increase in peripheral vascular resistance. Both of these potential effects can result in worsening congestive heart failure. Second, the present study showed that 10 weeks of treadmill running did not induce bradycardia at rest in either normal F1B or cardiomyopathic BIO 53.58 hamsters. Although this finding is similar to the study reported by Sembrowich et al49 in hamsters, exercise training is usually associated with bradycardia at rest50-51 or at any given exercise work load51-53 in the hearts of most other species. Third, the relation between improved functional abnormality of Gβ and augmented left ventricular contractile function in trained cardiomyopathic hamsters is not clear in the present study. Recently, it has become increasingly evident that, in addition to its action as a transducer of the β-adrenergic system, Gβ protein can positively regulate calcium channels and may inhibit sodium channels by direct interactions.54,55 The potential role of these actions of Gβ in affecting myocardial function requires further investigation.

In conclusion, chronic dynamic exercise did not change receptor-mediated β-adrenergic responsiveness. However, it augmented depressed myocardial contractility in association with an improved functional abnormality of Gβ in the BIO 53.58 hamster, an animal model of human dilated cardiomyopathy. Increased β-adrenergic responsiveness at the level distal to the receptor of cardiac ventricular membranes may play a role in the central adaptation of the diseased heart to chronic dynamic exercise training.

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References

Exercise and β-Adrenergic Signal Transduction


Chronic dynamic exercise improves a functional abnormality of the G stimulatory protein
in cardiomyopathic BIO 53.58 Syrian hamsters.
T Tomita, T Murakami, T Iwase, K Nagai, J Fujita and S Sasayama

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