A Comparison of Two Exercise Training Programs on Cardiac Responsiveness to β-Stimulation in Obesity

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We demonstrated previously that exercise training did not restore normal cardiac β-adrenergic responsiveness in obese rabbits. This study tested the hypothesis that an increased training volume was required to attenuate obesity-related reductions in isolated heart responsiveness to isoproterenol. Female New Zealand White rabbits were divided into lean control, lean exercise-trained, obese control, and obese exercise-trained groups. For the exercise-trained groups, total treadmill work over 12 weeks was increased 27% when compared with lean and obese animals trained with lower total training volume. After 12 weeks, Langendorff isolated hearts were used to study developed pressure, $+dP/dt_{max}$, and $-dP/dt_{max}$ responses to isoproterenol ($10^{-9} - 3 \times 10^{-7}$ M). Concentration-response data were fit to a sigmoidal function using a four-parameter logistic equation. Controls were compared with animals trained under the low- and high-training volume programs using one-way analysis of variance and Tukey's post-hoc test; separate analyses were conducted for lean and obese rabbits. In both lean and obese groups trained under the high-training volume program, EC_{50} values for $+dP/day_{tmax}$ and $-dP/dt_{max}$ were higher compared with same-weight controls and animals trained under the low-training volume program, indicating that contractility and relaxation responsiveness to isoproterenol was reduced by the higher training volume. Therefore, these data indicate that increased training volume failed to attenuate obesity-related decrements in isolated heart responsiveness to β-adrenergic stimulation and caused reduced sensitivity to isoproterenol in both lean and obese animals. Exp Biol Med 230:180–188, 2005

Key words: rabbit model of obesity; cardiac contractility; cardiac relaxation; isoproterenol; systolic function; isolated heart

Introduction

Exercise training may benefit cardiac function as a result of systemic hemodynamic alterations, structural changes, and/or intrinsic functional changes. Because cardiac function during exercise is heavily dependent on sympathetic stimulation, training-induced alterations in intrinsic cardiac responsiveness to β-adrenergic stimulation are of interest. Of greater interest is whether exercise training can attenuate the reduced responsiveness to β-adrenergic stimulation seen in aging, hypertension, or obesity (1–3). In humans, this has been assessed using submaximal infusions of a β agonist. In younger (4) and older men (5), as well as in obese subjects (6), infusions of a submaximal dose of isoproterenol increased blood pressure and heart rate responses after training. However, *in vivo* studies are limited in their ability to identify changes in intrinsic cardiac β-adrenergic responsiveness because of hemodynamic, neural, or hormonal reflexes. *In vitro* studies of training-related changes in cardiac β-adrenergic responsiveness are rare (7, 8), and we have not been able to identify any *in vitro* studies involving training-related changes in obesity.

Previously, we demonstrated that 12 weeks of progressive treadmill exercise training in obese rabbits reduced blood pressure and increased citrate synthase activity, left ventricular chamber diameter, and resting stroke volume (9, 10). Yet obesity-related decreases in β-adrenergic responsiveness of the isolated heart were not altered (2, 11). In assessing the reason for this outcome, we considered the well-known finding that training-induced adaptations are dependent on intensity, frequency, and/or duration of the training program. Equally important is the fact that different physiological outcomes may vary in time course or intensity threshold for exhibiting training-induced adaptation (12–14) and the fact that training-induced adaptations are often directly related to exercise workload (15). Therefore, we hypothesized that a greater exercise stimulus was required to restore normal cardiac β-adrenergic responsiveness in obesity. We compared animals trained under a program in which total training volume over 12 weeks was increased 27% to those trained under a previous program (11). We

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also characterized other physiological adaptations to increased workload, including hormonal profile, body composition, citrate synthase activity, and cardiac β-receptor density and sensitivity.

Materials and Methods

Animals. Protocols were approved by the Animal Care and Use Committee of the University of North Texas Health Science Center. All programs were carried out according to the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication 86-23, revised 1985) and regulations of the Animal Welfare Act. Female New Zealand White rabbits were purchased when they were 15–17 weeks old and weighed 3.25–3.75 kg (Myrtle's Rabbitry, Thompson Station, TN). After 1 week of acclimation to the animal facilities, they were divided into four groups: lean sedentary control (n = 7), obese sedentary control (n = 12), lean exercise-trained (n = 7), and obese exercise-trained (n = 7). Lean groups ate a maintenance diet, while obese groups were given a high-fat diet *ad libitum*; this diet consisted of standard rabbit chow with 10% added fat. Experiments were performed after 12 weeks of diet/exercise.

Exercise Training. Exercise training consisted of 12 weeks (5 days/week) of progressive treadmill exercise. Because of the differing abilities of lean and obese animals, fixed weekly workloads were used to define the training stimulus instead of fixed treadmill speeds and grades. Treadmill speeds were 16.1–21.4 m/min with 2.5% incline. Daily exercise duration was adjusted based on running speed and body weight. Weekly workloads (kpm) were: Week 1, 160; Week 2, 230; Week 3, 290; Week 4, 350; Week 5, 410; Week 6, 460; Week 7, 500; Weeks 8–12, 530 (kpm is a unit of work [force × distance], where force (mass × acceleration) is measured in kiloponds (kp) and 1 kp = 1 kg undergoing unit acceleration). In comparison to the earlier protocol (11), the present protocol required 27% more total work over 12 weeks (Fig. 1). Because animals in the previous program were already running at the highest speed that could be maintained for the entire training period, the increase in training volume was due principally to increased daily duration.

Blood Pressure Measurement and Hormone Analyses. After 12 weeks, blood pressure in the conscious rabbit was measured from a central ear artery catheter for 60–90 mins, as described previously (11). Fasting arterial blood samples for measurement of plasma renin activity (PRA), aldosterone (ALDO), cortisol, atrial natriuretic peptide (ANP), epinephrine, and norepinephrine were taken and analyzed as described previously (16).

Isolated Heart Experiment. General anesthesia was induced with 4%–5% isoflurane with an oxygen flow of 1 L/min administered with a facemask. An endotracheal tube was positioned in the trachea for subsequent mechanical ventilation. A midline thoracotomy was performed, the superior and inferior vena cava identified and isolated, and silk sutures placed around these vessels. The heart was then subject to cardioplegic arrest by infusing cold (4°C) Euro-Collins solution retrograde into the aortic root. The heart was prepared for the Langendorff preparation by excising the mitral valve and placing a latex balloon containing a high-fidelity micromanometer in the left ventricle for pressure measurements. Responsiveness in the isolated heart was evaluated by measuring peak developed pressure, $+dP/dt_{max}$ and $-dP/dt_{max}$ to isoproterenol. Perfusion pressure was maintained at 70 mm Hg. Hearts were paced by an external pacemaker at a rate of 240 beats/min. Coronary flow was determined using an in-line flow transducer (Transonic Systems, Inc., Ithaca, NY) positioned immediately before the aortic cannula. Data were averaged every 10 secs using commercially available software (MicroMed, Louisville, KY). After initiating retrograde perfusion with a modified Krebs-Henseleit buffer, heart function was allowed to stabilize for approximately 30 mins. Volume was added to the left ventricular balloon to achieve an end diastolic pressure of approximately 5 mm Hg; control measurements of peak pressure, end diastolic pressure, $+dP/dt_{max}$, and $-dP/dt_{max}$ were then recorded. Isoproterenol was added to the perfusate through a side arm in the aortic cannula (final concentrations 10^{-9} to 3×10^{-7} M). Values recorded during the final minute of each 5-min perfusion were averaged. After completion of the experiment, the right ventricle was cut away from the left ventricle, and the ventricles were weighed separately. In a subset of hearts, portions of the left ventricle were analyzed for maximum density of β receptors (B_{max}) and receptor affinity for agonist (K_d), as previously described (17). Citrate synthase activity, a marker for muscle oxidative capacity, was determined in red portions of the gastrocnemius muscle and was used as a marker of improved oxidative capacity (18).

Body Composition. After sacrifice, the carcass was shaved and emptied of blood and gastrointestinal contents. The entire carcass (minus blood, gastrointestinal contents, and fur) was homogenized (Robot Coupe, Jackson, MS) and 2–3-g samples were analyzed for body composition components. To determine water composition, samples were frozen in liquid nitrogen and dried to a constant weight under vacuum (Savant Instruments, Farmingdale, NY). Using the same sample, fat was extracted with a micro-Soxhlet apparatus (Wheaton, Millville, NJ), using acetone and chloroform as solvents for a minimum of 4 hrs each. Samples were again dried to a constant weight and weighed to the nearest 0.1 mg. The remaining sample was heated in a furnace (Thermolyne, Dubuque, IA) at gradually increasing temperatures up to 650°C for a total of 7 hrs to determine solids and ash content. Values from triplicate samples were averaged for analysis.

Statistical Analyses. For isolated heart experiments, data from each animal were fit to a sigmoidal function using a four-parameter (minimum, maximum, EC_{50}, and slope) logistic equation (TableCurve 2D 5.0,

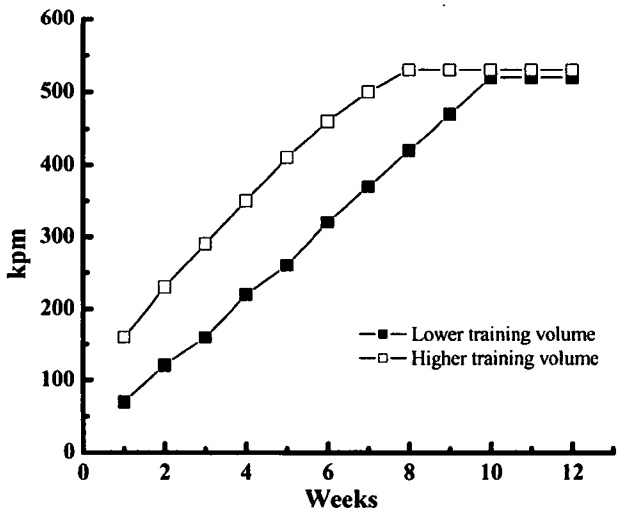

Figure 1. Comparison of weekly treadmill workloads in the high- and low-workload groups. Work (force × distance) is calculated in kpm, where force (kp) = mass (body weight in kg) × acceleration and distance = meters.

SPSS, Inc., Chicago, IL). To determine the effects of different training programs on cardiac responsiveness to isoproterenol, we compared data from the present study with that of our prior study (11). First, unpaired *t* tests were used to compare lean controls from the prior study ($n = 10$) with the present lean controls. The two lean control groups did not differ significantly in any measured variable. Similarly, unpaired *t* tests were used to compare obese controls from the prior study ($n = 9$) with the present obese controls. The present obese controls had greater maximum $-dP/dt_{max}$ and lower blood pressure than the prior obese controls, but they did not differ significantly in terms of any other variable. Therefore, controls from the two studies were combined to form a single lean control group ($n = 17$) and a single obese control group ($n = 21$) for further analyses. Control groups were compared using unpaired *t* tests.

Lean controls were then compared with lean animals trained under the low- and high-training volume programs using a one-way analysis of variance (ANOVA) and Tukey's post-hoc test. Separately, obese controls were compared with obese animals trained under the low- and high-training volume programs using a similar analysis.

To determine B_{max} and K_d, data were fit to a model of mass action binding using computer-assisted nonlinear least squares regression analysis. To understand possible effects of increased ventricular weight in obesity on receptor density, B_{max} was expressed as fmol/mg membrane protein and pmol/ventricle. Receptor binding data were not available from animals trained under the low-training volume program; therefore, only controls and animals trained under the high-training volume program were analyzed. Similarly, because of different assay procedures than were used in the previous study (10), hormone concentrations are presented for animals from the present study only. For hormone analyses, receptor binding data, body composition data, and citrate synthase data, lean and obese controls were compared with unpaired *t* tests. Controls and exercisers were then analyzed using a 2 × 2 ANOVA with interaction. Main effects were diet (lean vs. obese) and exercise status (trained vs. control). All data are expressed as mean ± SEM. Significance was accepted when $P \leq 0.05$.

Results

Animal Characteristics. Characteristics of lean and obese controls and lean and obese exercisers trained under the low- and high-volume training programs are shown in Table 1. Comparisons of lean and obese controls reinforce our previous findings (11) that obesity in the rabbit model results in higher body weight, blood pressure, heart rate, and right and left ventricular weights. Body weights did not differ among the three lean groups or among the three obese groups. Exercise training did not affect resting blood pressure or heart rate in lean rabbits. Resting heart rate was lower ($P \leq 0.05$) and blood pressure tended to be lower ($P = 0.10$) in obese animals trained under the high-volume program, compared to obese controls. Citrate synthase activity did not differ between lean and obese controls but was increased by exercise training (overall means for controls vs. exercise-trained rabbits were 12.4 ± 0.8 vs. 16.3 ± 0.9 μmol/min/g, respectively).

Table 1. Group Characteristics[a]

	LC ($n = 17$)	OC ($n = 21$)	L-LO[b] ($n = 9$)	L-HI ($n = 7$)	O-LO ($n = 8$)	O-HI ($n = 7$)
BW (kg)	3.71 ± 0.03	5.13 ± 0.09*	3.73 ± 0.03	3.62 ± 0.1	4.99 ± 0.13	4.82 ± 0.10
BP (mm Hg)	82.5 ± 1.2	94.0 ± 1.4*	82.1 ± 2.2	83.4 ± 1.6	91.5 ± 1.8	88.6 ± 2.2**
HR (bpm)	190 ± 6	224 ± 5*	173 ± 9	189 ± 7	213 ± 7	195 ± 11***
Dry RV (g)	0.46 ± 0.04	0.72 ± 0.05*	0.50 ± 0.06	0.40 ± 0.04	0.72 ± 0.06	0.62 ± 0.04
Dry LV (g)	0.97 ± 0.04	1.32 ± 0.06*	1.07 ± 0.07	1.00 ± 0.03	1.41 ± 0.06	1.23 ± 0.09

[a] Values are mean ± SE. LC, lean controls (combined group); OC, obese controls (combined group); L-LO, lean, exercise-trained, lower training volume; L-HI, lean, exercise-trained, higher training volume; O-LO, obese, exercise-trained, lower training volume; O-HI, obese, exercise-trained, higher training volume; BW, body weight; BP, blood pressure; HR, heart rate; RV, right ventricle; LV, left ventricle.
[b] Used by permission (Carroll JF. Isolated heart responsiveness to β-stimulation after exercise training in obesity. Med Sci Sports Exerc 35:548–554, 2003).
* $P \leq 0.05$, different from LC; ** $P \leq 0.10$, different from OC; *** $P \leq 0.05$, different from OC.

Table 2. Logistic Regression Parameters[a]

	LC (n = 17)	OC (n = 21)	L-LO[b] (n = 9)	L-HI (n = 7)	O-LO (n = 8)	O-HI (n = 7)
Developed pressure						
Min (mm Hg)	67.2 ± 3.8	63.3 ± 2.6	76.2 ± 5.0	46.3 ± 7.0***	66.4 ± 5.5	53.1 ± 3.0
Max (mm Hg)	110.1 ± 7.1	109.8 ± 4.4	107.7 ± 9.4	96.6 ± 10.9	97.8 ± 8.1	109.0 ± 9.6
EC_{50} (Log M)	−8.3 ± 0.1	−8.2 ± 0.1	−8.6 ± 0.1	−7.5 ± 0.02***	8.1 ± 0.1	−7.8 ± 0.1
Slope	1.43 ± 0.26	1.26 ± 0.21	2.26 ± 0.53	2.07 ± 0.59	2.54 ± 0.52	1.04 ± 0.14
$+dP/dt_{max}$						
Min (mm Hg/sec)	1302 ± 73	1319 ± 57	1422 ± 90	974 ± 114	1238 ± 94	1095 ± 28**
Max (mm Hg/sec)	2992 ± 146	3207 ± 232	2888 ± 218	2675 ± 299	2632 ± 270	3406 ± 392
EC_{50} (Log M)	−7.9 ± 0.1	−7.7 ± 0.1*	−8.1 ± 0.1	−7.4 ± 0.2***	−7.6 ± 0.1	−7.4±0.1****
Slope	1.33 ± 0.09	1.74 ± 0.20	1.85 ± 0.41	2.64 ± 0.65*	1.77 ± 0.29	1.35 ± 0.16
$-dP/dt_{max}$						
Min (mm Hg/sec)	983 ± 54	915 ± 39	1138 ± 80	642 ± 87***	931 ± 106	830 ± 50
Max (mm Hg/sec)	2194 ± 99	2532 ± 181	2135 ± 136	1932 ± 201	2084 ± 215	2640 ± 258
EC_{50} (Log M)	−8.1 ± 0.1	−7.9 ± 0.1	−8.3 ± 0.1	−7.4 ± 0.2***	−7.8 ± 0.1	−7.5± 0.1****
Slope	1.22 ± 0.10	1.41 ± 0.24	1.30 ± 0.18	1.94 ± 0.47	2.00 ± 0.45	0.96 ± 0.12
+dP/dt/P						
Min (mm Hg/sec/mm Hg)	18.8 ± 0.2	19.5 ± 0.2	18.5 ± 0.4	21.0 ± 1.2***	20.6 ± 1.1	20.0 ± 0.7
Max (mm Hg/sec/mm Hg)	27.5 ± 0.6	29.0 ± 0.8	27.9 ± 1.1	28.0 ± 1.4	28.8 ± 2.3	30.6 ± 1.2
EC_{50} (Log M)	−7.7 ± 0.1	−7.5 ± 0.04*	−7.8 ± 0.1	−7.4 ± 0.2***	−7.4 ± 0.1	−7.3 ± 0.1**
Slope	2.10 ± 0.31	2.50 ± 0.27	1.58 ± 0.13	4.28 ± 0.48***	3.05 ± 0.50	2.32 ± 0.49

[a] Values are mean ± SE. Group designations as listed in Table 1. Logistic regression parameters determined by the formula: $y = ([min\ max]/[1 + (x/EC_{50})^{slope}]) + max$, where min = minimum value of the sigmoidal relationship, max = maximal value of the sigmoidal relationship, and x = isoproterenol concentration.
[b] Used by permission (Carroll JF. Isolated heart responsiveness to β-stimulation after exercise training in obesity. Med Sci Sports Exerc 35:548–554, 2003).
* $P \leq 0.05$, different from LC; ** $P \leq 0.05$, different from OC; *** $P \leq 0.05$, different from LC and L-LO; **** $P \leq 0.05$, different from OC and O-LO.

Isolated Heart Data. Comparisons of lean and obese controls indicated that obese controls had higher EC_{50} values for $+dP/dt_{max}$ and +dP/dt/P (Table 2), confirming our earlier observation that there is reduced isolated heart sensitivity to isoproterenol in obesity (11). Comparisons of lean controls with lean animals trained under the low– and high–training volume programs indicated that lean animals trained under the high–training volume program had a higher EC_{50} for developed pressure, $+dP/dt_{max}$, $-dP/dt_{max}$, and +dP/dt/P compared with the other two lean groups

Table 3. Plasma Hormone Concentrations in Sedentary and Exercise-Trained Lean and Obese Rabbits[a]

Group	Lean control	Obese control	Lean (HI) exercise-trained	Obese (HI) exercise-trained
Plasma renin activity (ng Angl/ml/hr)	1.92 ± 0.51 (6)	7.91 ± 1.61* (8)	2.35 ± 0.90 (5)	3.93 ± 0.61 (3)
Aldosterone (pmol/l)	245 ± 42 (7)	584 ± 71* (11)	315 ± 55 (7)	385 ± 105 (7)
Cortisol (μg/dl)	1.00 ± 0.25 (8)	2.72 ± 0.55* (12)	0.60 ± 0.12 (7)	2.26 ± 0.62 (7)
Atrial natriuretic peptide (p*M*)	8.99 ± 2.41 (8)	2.85 ± 0.60* (12)	2.94 ± 0.79* (7)	3.53 ± 1.16 (7)
Norepinephrine (pmol/ml)	5.53 ± 1.33 (6)	3.29 ± 0.57 (9)	2.28 ± 0.77 (5)	2.63 ± 1.38 (3)
Epinephrine (pmol/ml)	0.39 ± 0.13 (6)	0.25 ± 0.08 (9)	0.58 ± 0.20 (5)	0.35 ± 0.18 (3)

[a] Values are mean ± SE; numbers in parentheses indicate sample sizes. Angl, angiotensin I; HI, animals trained under higher workload program.
* $P \leq 0.05$, different from lean control.

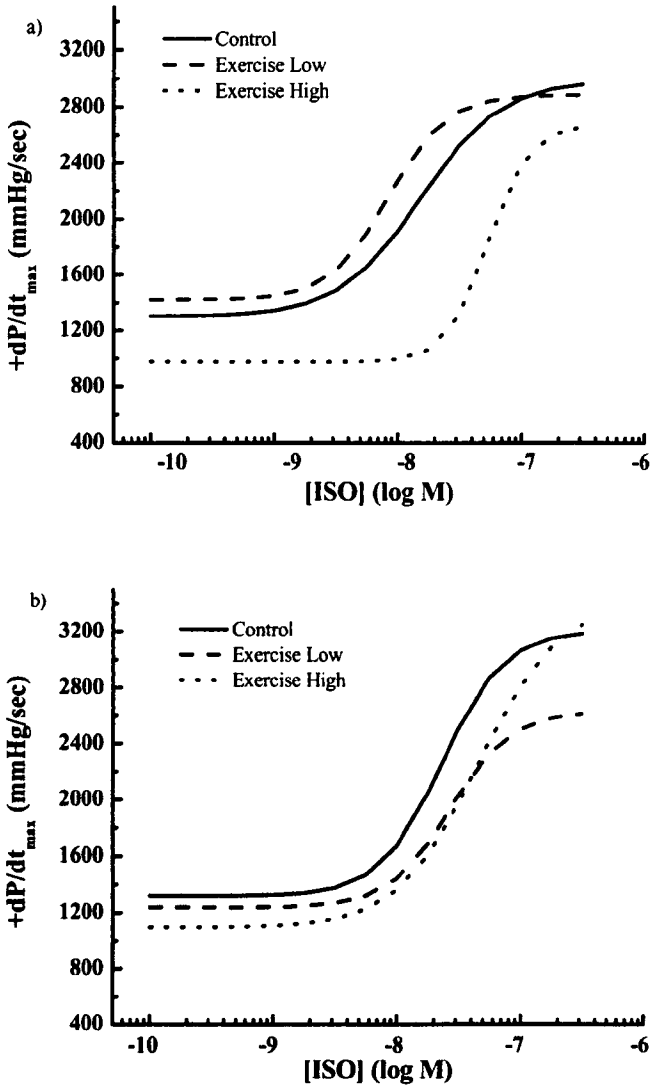

Figure 2. Dose-response relationship between isoproterenol (ISO) and +dP/dt$_{max}$ in isolated hearts of (a) lean and (b) obese rabbits. In each graph, hearts from controls are compared with hearts from rabbits trained with lower and higher training volumes. Curves were constructed using group averages for minimum, maximum, EC_{50}, and slope parameters. Solid line depicts combined control group, dashed line depicts lower training volume group, and dotted line depicts higher training volume group.

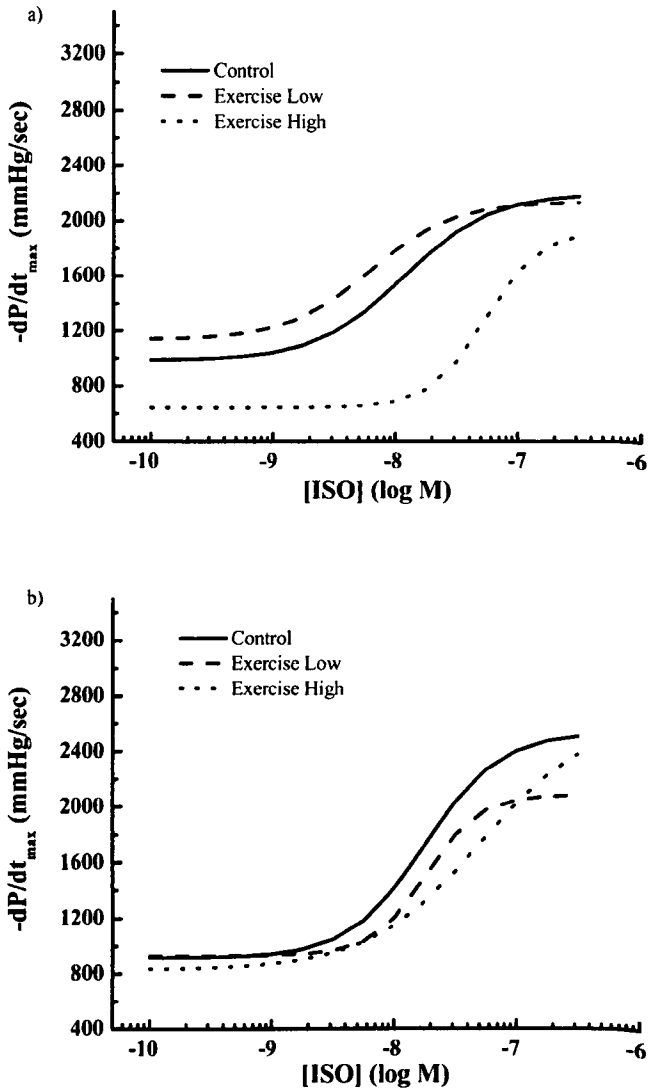

Figure 3. Dose-response relationship between isoproterenol (ISO) and –dP/dt$_{max}$ in isolated hearts of (a) lean and (b) obese rabbits. In each graph, hearts from controls are compared with hearts from rabbits trained with lower and higher training volumes. Curves were constructed using group averages for minimum, maximum, EC_{50}, and slope parameters. Solid line depicts combined control group, dashed line depicts lower training volume group, and dotted line depicts higher training volume group.

(Table 2 and Figs. 2 and 3). Lean animals trained under the high–training volume program also had higher minimum values for dP/dt/P and lower minimum values for developed pressure and –dP/dt$_{max}$ compared with the other two lean groups. Slope values for +dP/dt$_{max}$ and +dP/dt/P were higher in lean animals trained under the high–training volume program compared with lean controls (Table 2 and Figs. 2 and 3).

Comparisons of obese controls with obese animals trained under the low– and high–training volume programs indicated that obese animals trained under the high–training volume program had higher EC_{50} values for +dP/dt$_{max}$ and –dP/dt$_{max}$ compared with the other two obese groups and had a higher EC_{50} value for +dP/dt/P compared with obese controls. Obese animals trained under the high–training volume program also had a lower minimum value for +dP/dt$_{max}$ compared with obese controls (Table 2 and Figs. 2 and 3).

Hormone Analyses. Because in this study we used different assay procedures than we did in our previous study (10), hormone concentrations are presented for animals from the present study only. Obese controls had higher PRA, ALDO, and cortisol and lower ANP compared with lean controls ($P \leq 0.05$; Table 3). There was a *diet* × *exercise status* interaction on ANP. Training resulted in lower ANP in lean animals, but while ANP was 24% higher in obese exercisers compared with obese controls, this difference was not significant. In main effects models, there were higher

Table 4. Body Composition Components in Sedentary and Exercise-Trained Lean and Obese Rabbits[a]

	Lean control (n = 14)	Obese control (n = 18)	Lean (HI) exercise-trained (n = 10)	Obese (HI) exercise-trained (n = 8)
Water (%)	62.8 ± 0.8	48.1 ± 1.1*	64.7 ± 1.0***	51.9 ± 1.3*,***
Fat (%)	12.9 ± 1.1	30.6 ± 1.5**	9.2 ± 1.5****	26.4 ± 2.0**,****
Solids (%)	19.3 ± 0.4	16.1 ± 0.5*	19.8 ± 0.4	16.0 ± 0.3*
Ash (%)	3.9 ± 0.2	2.4 ± 0.1*	4.1 ± 0.2	2.7 ± 0.1*
Water (kg)	2.32 ± 0.04	2.45 ± 0.04**	2.38 ± 0.07	2.52 ± 0.06**
Fat (kg)	0.47 ± 0.045	1.58 ± 0.10**	0.34± 0.05****	1.28 ± 0.10**,****
Solids (kg)	0.71 ± 0.02	0.82 ± 0.03**	0.73 ± 0.02	0.78 ± 0.02**
Ash (kg)	0.15 ± 0.007	0.12 ± 0.006*	0.15 ± 0.009	0.13 ± 0.004*

[a] Values are means ± SE. HI, animals trained under higher workload program.
* $P \leq 0.05$, combined obese < combined lean; ** $P \leq 0.05$, combined obese > combined lean; *** $P \leq 0.05$, combined exercise groups > combined controls; **** $P \leq 0.05$, combined exercise groups < combined controls.

PRA, ALDO, and cortisol concentrations in obese compared with lean animals ($P \leq 0.05$), but there was no effect related to exercise training. There was a trend for lower norepinephrine in exercise-trained animals compared with controls ($P = 0.06$).

Body Composition. Group means for body composition components are presented in Table 4 for animals in the present study only. 2 × 2 ANOVAs indicated that exercise-trained animals had lower percent body fat and fat weight and higher percent body water compared with controls ($P \leq 0.05$). As expected, obese animals had higher percent body fat, fat weight, water weight, and solids weight, while lean animals had higher percent water, percent solids, percent ash, and weight of ash ($P \leq 0.05$).

Receptor Binding. Group means for B_{max} or K_d are presented in Table 5. 2 × 2 ANOVA results indicated that there was a body weight effect on K_d, with lower values in obese animals compared with lean animals ($P \leq 0.05$). There was no exercise training effect on K_d. There were no body weight or exercise training effects on B_{max}.

Discussion

Our previous studies demonstrated that obesity was associated with reduced isolated heart responsiveness to β-adrenergic stimulation and that a lower training stimulus did not alter this relationship (11). The present hypothesis that a higher training stimulus was required to attenuate obesity-related decreases in intrinsic cardiac responsiveness to isoproterenol was not supported. In fact, isolated heart responses to isoproterenol were reduced, as evidenced by increased EC_{50} values for $+dP/dt_{max}$, $-dP/dt_{max}$, and +dP/dt/P in both lean and obese animals trained under the high-training volume program compared with same-weight controls and animals trained under the low-training volume program.

The present study equalized weekly training volume between lean and obese groups. Use of equal training volumes among groups is a common training paradigm, with weekly kJ/kg or kcal/kg used to measure training workload (12, 19, 20). Since increased body weight increases the metabolic cost of weight-bearing exercise, total work/week in the present study was calculated in kpm, and daily duration was modified based on running speed and body weight. The present training protocol involved a total increase in training volume of 27% compared with an earlier study (11); this increase was achieved primarily by increasing daily duration up to Week 10. Training duration has been demonstrated to have significant effects on a variety of physiological variables, including cardiac hypertrophy and insulin sensitivity (12, 19). While this program resulted in a substantially increased overall workload, we do not preclude the possibility that different results might be obtained if the increased training workload were achieved by varying intensity or frequency.

Data regarding cardiac responses to β-adrenergic stimulation after exercise training in obesity, particularly

Table 5. β-Adrenoceptor ^{125}I-CYP Binding in Cardiac Membranes from Lean and Obese Control and Exercise-Trained Rabbits[A]

	Lean control (n = 5)	Obese control (n = 6)	Lean (HI) exercise-trained (n = 4)	Obese (HI) exercise-trained (n = 6)
K_d (pM)	55.2 ± 9.9	35.7 ± 6.6*	50.7 ± 13.4	28.8 ± 5.6*
B_{max} (fmol/mg)	61.2 ± 17.9	57.7 ± 8.3	64.3 ± 23.3	31.9 ± 6.2
B_{max} (pmol/ventricle)	16.8 ± 5.8	24.1 ± 5.0	14.7 ± 4.5	13.9 ± 3.0

[A] Values are mean ± SE. HI, animals trained under higher workload program; B_{max}, receptor density; K_d, receptor sensitivity to agonist.
* $P \leq 0.05$, combined obese < combined lean.

in the absence of weight loss, are scarce and equivocal. One study demonstrated that heart rate and blood pressure responses to a submaximal dose of isoproterenol in obese subjects were increased after training (6). In contrast, neither low- nor high-intensity training altered heart rate responses to β-adrenergic stimulation in obese subjects (21). However, these studies either failed to include a control group (6) or failed to demonstrate initial decrements due to obesity (21). In the present study, reduced responsiveness to isoproterenol was seen both in lean and obese animals trained under the high–training volume program, indicating a generalized, rather than an obesity-specific, response to the greater training stimulus. These results are in agreement with those of studies demonstrating reduced responsiveness to adrenergic stimuli after training (7, 8). In rats, papillary muscles from young trained animals exhibited depressed peak developed tension and $+dT/dt_{max}$ under control conditions and in response to calcium and norepinephrine (8). Trained rats also had reduced *in situ* left ventricular pressure and $+dP/dt_{max}$ responses to tyramine infusion and aortic occlusion, along with reduced calcium-regulated myofibrillar ATPase (7).

A reduced heart rate response to isoproterenol is a common manifestation of decreased training-induced responsiveness to β-adrenergic stimulation. This has been demonstrated in both younger and older men (5, 22). In trained pigs, maximal isoproterenol-stimulated heart rate decreased 18%, and the slope of the isoproterenol dose–heart rate response relationship was reduced (23). Despite reduced heart rate responsiveness, other indices of left ventricular systolic performance were often improved in response to isoproterenol after training (5).

In contrast, there are studies indicating that exercise training either increases (4, 5) or does not change (24–26) β-adrenergic responsiveness. In older men, 6 months of training did not alter age-related decreases in heart rate, blood pressure, ejection fraction, and cardiac output responses to β-adrenergic stimulation (25). Similarly, aged trained rats did not differ from their sedentary counterparts in terms of isoproterenol-stimulated papillary muscle function (26). Conversely, increased responsiveness to β-adrenergic stimuli has been found in young (4) and older persons. In older men, training enhanced isoproterenol-induced fractional shortening at a given level of wall stress (5).

The reasons for the discrepancies between our study and these latter studies are unclear. Factors that may cause different responses are numerous and include intensity; frequency and/or duration of the training program; the testing protocol (e.g., *in vivo* vs. *in vitro* studies, drug dosages [maximal or submaximal], single or multiple doses, use of atropine to block training-induced increases in parasympathetic tone); subject characteristics such as age, weight, and gender; species/gait; or presence of underlying pathology. The choice of outcome variable also may affect conclusions, since some variables may demonstrate training-related changes while others do not (24). *In vivo* studies may make it difficult to differentiate contributions of primary hemodynamic effects from secondary reflex responses. Lack of pretreatment with atropine (22) may mask training-induced alterations in isoproterenol responses. Use of a single or maximal drug dosage may also mask training-induced adaptations, since maximum responses may be unaltered, whereas the sensitivity of a system, as estimated by the EC_{50}, may be changed by exercise training. In our study, use of the Langendorff isolated heart eliminates the influence of preload, afterload, rate, and neural and hormonal influences and therefore should reflect the intrinsic responsiveness of the heart. Few studies have taken this approach to evaluating training-related responsiveness.

Mechanisms responsible for reduced sensitivity to isoproterenol in animals trained under a higher workload program (HI) are unclear. Receptor binding did not appear to play a role, since there were no exercise training-related effects on either B_{max} or K_d. One possibility is that endurance training may depress myocardial catecholamine stores (27). Another possibility is that training- or obesity-induced alterations in muscarinic receptor density/sensitivity may affect responses to isoproterenol because of its interaction with the β-adrenergic signal transduction pathway *via* G_i. However, training-induced changes in muscarinic responsiveness are inconsistent. Exercise training has been demonstrated to reverse the decrease in parasympathetic drive that occurs with aging (28) and to attenuate the increase in muscarinic receptor density that occurs with altitude acclimatization (29, 30). However, normoxic exercise training did not affect left ventricular cholinergic receptor density or affinity for ligand (29). Exercise training also had no effect on atropine-induced cardiac acceleration or muscarinic receptor binding characteristics in control or insulin-deficient rats (31). These studies evaluated muscarinic receptor influence by testing heart rate responses to maximal exercise or atropine stimulation (29–31). The potential influence of muscarinic receptors in the present *in vitro* protocol, where the heart rate was fixed and where there was no endogenous ligand, is questionable. Therefore, alterations in muscarinic receptors likely played a minor role in the present study. However, determination of the influence of muscarinic receptors in an *in vivo* obese, exercise-trained model requires further study.

The relationship between intrinsic β-adrenergic responsiveness and *in vivo* cardiac performance remains unresolved. Reduced β-adrenergic responsiveness may not be a liability under present training conditions, in which HI increased workload over 12 weeks principally by increasing duration. It is likely that heart rate during training was reduced, which would allow for an increased diastolic filling period and increased stroke volume. If peripheral oxygen extraction also increased, increased intrinsic contractility would not be essential. These data indicate that increased intrinsic cardiac responsiveness to β-adrenergic stimulation

is not a crucial adaptation to support increased cardiovascular performance.

Exercise training in the present study was associated with decreased body fat and increased body water, even as control and respective HI groups did not differ significantly in body weight. In obese humans, weight loss is the focal point of exercise training, and changes in body composition frequently occur. Similarly, exercise-trained obese Zucker rats had reduced body fat, but also lighter body weight, compared with obese controls (32). In these instances, it is difficult to determine the contribution of exercise training to body composition changes. Few exercise training studies have the *a priori* purpose of examining body composition changes in the absence of weight loss (33). However, this is a relevant issue because of the difficulty of achieving and maintaining weight loss in obesity.

The mechanism responsible for decreased body fat in animals trained under the high–training volume program is uncertain. Total feed consumption over 12 weeks did not differ significantly between the present obese controls and obese animals trained under the high–training volume program (13.5 ± 0.4 vs. 13.5 ± 0.6 kg, respectively); lean controls and lean animals trained under the high–training volume program were fed a maintenance diet. Body weights in animals trained under the high–training volume program were slightly, but not significantly, lower than those observed in respective controls, indicating that reduced body fat may have been due to a negative calorie balance. Increased sympathetic activation, which might affect lipolysis, did not appear to be the mechanism by which body fat was reduced by training, since there tended to be lower plasma norepinephrine and no difference in plasma epinephrine in exercisers. However, a single measure of plasma catecholamines may not adequately reflect the chronic state of sympathetic nervous system activation in individual tissues/organs. Whether there was an upregulation of the β-adrenergic signaling pathway in adipose tissue, as seen in exercise-trained rodents (34), requires further study.

Similar to previous findings (10), obese controls had higher PRA, ALDO, and cortisol compared with lean controls. ANP was lower in obese controls than lean controls, a finding which is in accord with data in obese humans (35). Plasma ANP was lower in exercise-trained lean rabbits compared with lean sedentary controls, but this same adaptation did not occur in exercise-trained obese rabbits. ANP responses to exercise training are variable, with studies showing reduced (36) or increased (37) plasma ANP. Training in obese animals trained under the high–training volume program resulted in decreases of 50% in PRA, 34% in ALDO, and 17% in cortisol and increases of 24% in ANP. Although these differences were not individually significant, the combined *in vivo* effect may represent an attenuation of the abnormal hormonal activation of obesity. Of particular interest are the directionally opposite changes in PRA and ANP, because of the known inhibition of the renin-angiotensin system by ANP. Although these results are intriguing, caution must be taken in their interpretation because of the sample size limitations for some of the hormone analyses.

In summary, these data reinforced our previous findings that obesity is associated with decreased isolated heart responsiveness to β-adrenergic stimulation (11), and they indicated that an increased volume of training, compared with that of a prior program (10, 11), reduced isolated heart sensitivity to isoproterenol in both lean and obese rabbits. Other training-induced adaptations included reduced body fat, a trend for reduced plasma norepinephrine, reduced ANP in lean rabbits, and reduced heart rate and blood pressure in obese rabbits. These results indicate that the training threshold for adaptations in β-adrenergic sensitivity is different than that for hemodynamic adaptations such as reduced blood pressure (10). However, the *in vivo* significance of reduced cardiac responsiveness to β-adrenergic stimulation remains to be determined.

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