Exercise Training Improves Cardiac Performance in Diabetic Rats (43593)

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Abstract. **Diabetes mellitus is often associated with a cardiomyopathy characterized by alterations in cardiac metabolism and declines in cardiac performance. We sought to determine whether exercise training would attenuate the depressed cardiac performance seen in diabetic animals. Female rats were divided into four groups: sedentary control, trained control, sedentary diabetics, and trained diabetics. After 1 week of training, we induced diabetes by intravenous injection of streptozotocin (65 mg/kg). We trained animals on a treadmill using a progressive protocol that plateaued at 27 m/min for 1 hr/day, 5 days/week for a total of 8 weeks. We measured cardiac output at a variety of left atrial filling pressures with an isolated working heart apparatus; glucose was the sole metabolic substrate for the heart. Training increased succinate dehydrogenase activity in the soleus muscle of exercised rats, but did not change heart and body weights or plasma glucose and thyroid hormone levels. The diabetic groups exhibited depressed cardiac outputs at high workloads compared to nondiabetics. Training increased the cardiac output of both sedentary and diabetic animals at high, but not low, preloads. We suggest that exercise can attenuate the severity of diabetic cardiomyopathy.** [P.S.E.B.M. 1993, Vol 203]

Diabetes is a chronic metabolic disorder associated with secondary complications involving end-organ damage, including congestive heart failure (1). Cardiomyopathy, independent of coronary atherosclerosis, is a well known consequence of diabetes (2–7). Physical training often improves glucose homeostasis in human diabetics (8) and animal models with mild (9, 10), but not severe (11), diabetes. Furthermore, aerobic training of sufficient intensity and duration increases cardiac performance in normal animals (12–14). In the present study, we used the isolated heart preparation to determine whether endurance training of rats could ameliorate the cardiac dysfunction associated with diabetes when glucose was available as the sole substrate. We found that exercise training attenuated the reduction in cardiac output associated with diabetes without affecting heart and body weights or plasma glucose and thyroid hormone levels.

Materials and Methods

Training. Female Sprague-Dawley rats (200–250 g) were obtained from Charles Rivers (Wilmington, DE). We provided Purina rat chow and water *ad libitum* and maintained animals on a 12-hr light, 12-hr dark cycle in a constant temperature environment. Rats were randomly selected for a training regimen consisting of treadmill running (8.5% grade) for 60 min/day, 5 days/week, starting at 18 m/min and progressively increasing to 27 m/min, for 8 weeks to obtain a training effect. We have demonstrated that a similar training regimen increases muscle succinate dehydrogenase activity (15). We occasionally used mild electrical shock to encourage the animals to run. Animals still refusing to run were eliminated from the study. After 1 week of running, we further separated animals into diabetics and nondiabetics to constitute four groups: (i) trained controls (TC), (ii) sedentary controls (SC), (iii) trained diabetics (TD), and (iv) sedentary diabetics (SD).

Induction of Diabetes. Nonfasted rats were lightly

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anesthetized with ether. Animals in the diabetic group were injected with streptozotocin (STZ) at a dose of 65 mg/kg into the tail vein. We prepared the STZ (kindly provided by Upjohn, Kalamazoo, MI) in 0.5-ml aliquots (50 m*M* citrate buffer, pH 4.5) immediately before injection to avoid oxidation. The control animals received an equal volume of vehicle. We confirmed the diabetic state 24–48 hr after injection using the Diastix test for glucosuria. All animals with plasma glucose concentrations exceeding 300 mg/dl at the time of sacrifice were considered diabetic.

Heart Perfusion. After 8 weeks of training, we anesthetized animals with sodium pentobarbital (60 mg/kg, ip). After opening the abdominal cavity, we drew an abdominal aortic blood sample (~~3 ml) into a heparinized syringe for measurement of plasma glucose, glycosylated hemoglobin (GHb), triiodothyronine (T_3), thyroxine (T_4), and insulin levels. We immediately removed the heart to an isolated perfused working heart apparatus similar to that described by Fintel and Burns (16), and Barbee *et al.* (2). Briefly, the heart was excised, rinsed in cold saline (5°C), and mounted on a Langendorff perfusion apparatus using a stainless steel cannula in the aorta. Retrograde perfusion of the coronary arteries began immediately. After placing an additional cannula in the left atrium, we perfused the heart in the antegrade direction via the left atrial cannula with a modified Krebs-Henseleit bicarbonate buffer containing the following concentration of salts (in millimolar concentrations): NaCl, 118.7; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; EDTA, 0.05; and $CaCl_2$, 1.25. Glucose (5.5 m*M*) was the sole substrate. We increased cardiac output by elevating left atrial filling pressure from 10 to 30 cm of H_2O in 5-cm increments. Perfusate entering the left atrium was pumped out of the aorta and either perfused the coronary arteries or was collected from the aortic outflow, which consisted of a length of tubing with a 23-gauge needle and a 1.5-in barrel. The outflow tract represented a constant resistance against which the left ventricle pumped the stroke volume. We chose this resistance to achieve physiological pressure development by the left ventricle. We determined coronary flow, aortic flow, heart rate, and peak systolic and diastolic pressures at various left atrial filling pressures in spontaneously beating hearts. After perfusion at the highest pressure, we returned all hearts to a left atrial filling pressure of 15 cm of H_2O; any heart that did not generate systolic pressures similar to that measured earlier at 15 cm were discarded and not included in the study.

Assays. We removed soleus muscles from each animal after heart removal and later measured succinate dehydrogenase activity to document a training effect (15). After blood samples were centrifuged at 1000*g* for 10 min at 4°C, plasma was removed and stored at −70°C. We then measured plasma glucose using a Beckman glucose analyzer (Palo Alto, CA) (17), and plasma insulin, T_3, and T_4 concentrations with commercially available RIA kits (Micromedic Systems [Horsham, PA] and Diagnostic Products Corp. [Los Angeles, CA], respectively). We washed the packed cells and stored them at 4°C. GHb was measured spectrophotometrically within 1 week after separation from non-GHb on boronate-agarose affinity columns (Isolab, Akron, OH).

Statistics. Data are reported as the mean ± standard error of the mean. We compared single parameters in the diabetic model (such as glucose, T_3, etc.) using a one-way analysis of variance followed by Tukey's test when significant F ratios were found. We compared all indices of cardiac work at various preloads using a split-plot analysis of variance that allowed analysis of the effect of both diabetes and exercise, and the interaction of these treatments across various levels of left atrial filling pressure. We made comparisons of both main effects (control versus diabetes, exercise versus sedentary group, and preload) and interactions (such as exercise versus diabetes and exercise or diabetes versus preload). Duncan's multiple range test was used to make group comparisons when a main effect or interaction was significant. This analysis was performed using the most recent release (5.08) of the statistical analysis system at the Louisiana State University Medical Center IBM mainframe computer. We set the level of significance at $P < 0.05$. All experiments were approved in advance by the institutional animal care and use committee and conducted according to the guiding principles in the *Care and Use of Animals*, approved by the American Physiological Society.

Results

Effects of STZ and Training. The major characteristics of the trained and sedentary control and diabetic groups at the time of sacrifice are listed in Table I. Mean body weights were not different between the sedentary control animals and the trained control animals. Yet, we noted a failure to increase body weight in both diabetic groups, with the sedentary diabetic animals demonstrating the lowest body weights of the four groups. Mean heart weight/body weight ratios (Table I) were slightly, but not significantly, higher in the sedentary diabetic group compared with those in the sedentary controls. T_3, but not T_4, levels were significantly lower in both diabetic groups compared to control values. Our measurements of plasma glucose levels indicated a greater than 2-fold elevation in diabetics compared to those in controls. Because a single blood glucose determination is often an insensitive indicator of metabolic control, we also measured GHb concentrations. GHb levels were elevated more than 3-fold in the diabetic animals compared to those in controls. The GHb levels we measured in the nondi-

Table 1. Effects of Diabetes and Training on Heart Weights, Body Weights, and Various Metabolic Parameters

Group	SC	SD	TC	TD
Body wt (g)	301 ± 7	229 ± 8[a]	303 ± 10	243 ± 16[a]
Dry heart wt (g)	0.18 ± 0.02	0.15 ± 0.01	0.18 ± 0.01	0.16 ± 0.01
Heart wt/body wt (mg/g)	0.61 ± 0.05	0.67 ± 0.03	0.61 ± 0.01	0.66 ± 0.03
Glucose (mg/100 ml)	213 ± 8	468 ± 11[a]	209 ± 5	480 ± 17[a]
GHb (%)	5.19 ± 0.17	17.72 ± 0.48[a]	5.02 ± 0.16	17.16 ± 0.52[a]
Insulin (μU/ml)	41.9 ± 5.8	5.2 ± 1.3[a]	59.9 ± 8.0	3.2 ± 0.8[a]
T_4 (μg/dl)	60.0 ± 1.0	55.6 ± 2.8	60.3 ± 4.9	54.2 ± 4.5
T_3 (ng/dl)	53.7 ± 2.9	41.6 ± 2.7[a]	56.6 ± 2.9	41.9 ± 3.0[a]
SDH (μmol/g)	8.24 ± 0.35	7.04 ± 0.4	12.18 ± 0.71[a,b]	10.14 ± 0.8[a,b]

All values are the mean ± SE (n = 7–11 animals/group).
[a] $P < 0.05$ vs SC.
[b] $P < 0.05$ vs SD.

abetic animals indicated excellent blood glucose control. However, the plasma glucose levels measured at sacrifice were somewhat elevated in controls. This is probably due to the acute stress associated with intraperitoneal injection of pentobarbital sodium for anesthesia (18). Based upon these data along with the marked differences in insulin levels between groups, we concluded that STZ injection induced a state of severe diabetes. Succinate dehydrogenase activity was significantly elevated in both the trained control and trained diabetic animals compared to levels in the respective sedentary groups. Therefore, we conclude that treadmill running imposed a significant training effect.

Heart Perfusion. We compared cardiac output (aortic plus coronary flow) in unpaced hearts among the four groups using 5.5 mM glucose as the sole substrate (Fig. 1). Increasing the left atrial preload from 10 to 30 cm. H_2O caused significant increases in cardiac output in all groups ($P < 0.0001$). Cardiac output was depressed in hearts from diabetic animals compared to that in controls ($P < 0.04$); this variable was increased in hearts from trained animals compared to that in sedentary rats ($P < 0.05$). There was no interaction between the groups with regard to these variables. That is, the diabetic state did not have a different effect on the cardiac output of trained and untrained animals, and training increased the cardiac output of diabetic and nondiabetic animals by amounts that were not significantly different from each other. However, there was an interaction of control and diabetic animals versus load ($P < 0.006$) and sedentary versus trained animals versus load ($P = 0.0001$). These differences occurred at high (25 and 30 cm; $P < 0.05$), but not low (10, 15, and 20 cm), preloads. Despite the differences in cardiac output, other measures of cardiac work (heart rate × peak systolic pressure, cardiac output × peak systolic pressure, etc.) were slightly, but not significantly, different between groups. When cardiac work was expressed as flow/gram dry weight or stroke volume/gram dry weight, there were, again, no statistically significant differences between groups. The overall

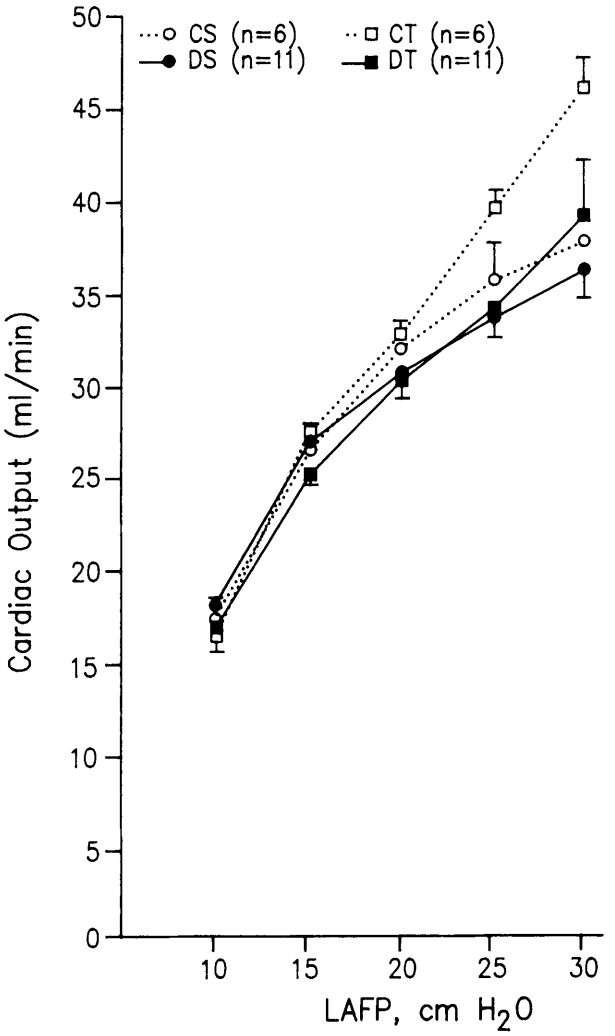

Figure 1. Cardiac output in hearts perfused with glucose from four experimental groups. LAFP, Left atrial filling pressure. ○, CS; ●, DS; □, CT; ■, DT. Values represent the mean ± SE of 6–11 rats/group. There was a significant overall difference in cardiac output between trained and sedentary rats, and a significant overall difference in cardiac output between diabetic and nondiabetic rats.

spontaneous heart rate was not significantly altered by diabetes or training; heart rates for the four groups (beats/min) were as follows: TC, 256 ± 14, SC, 256 ± 9, TD, 238 ± 12, and SD, 222 ± 8.

Discussion

Myocardial abnormalities are common in diabetic patients (19) and experimental diabetes (2–7). The major clinical findings in diabetic humans include reductions in both systolic (19) and diastolic (20) function. The hallmark of experimentally induced diabetes is a decreased cardiac reserve (2, 4, 6). Papillary muscles from diabetic rats exhibit reduced isotonic shortening velocity in addition to decreased rates of isometric contraction and relaxation (3). We found a significant depression of cardiac output, but not other indices of cardiac work, in hearts from chronically diabetic animals. This depression was significant only at high left atrial filling pressures, indicating the presence of a decreased cardiac reserve. We were somewhat surprised to find that diabetes caused only a mild decrease in cardiac output in these animals compared to the large reductions in both cardiac output and other indices of cardiac work seen previously (2). The reason for these differences is unclear, but could be related to the rat strain (Wistar versus Sprague=Dawley) or sex (female versus male). We have also confirmed the salutary effect of endurance exercise training on myocardial function (12). These effects were evident at high, but not low, preloads. Original to this study is the finding that the diabetes-associated decrement in cardiac function tends to normalize with endurance exercise training when hearts are perfused with glucose as the sole substrate.

The effects of exercise on myocardial performance are influenced by a number of factors, including sex, exercise intensity and duration, and type of exercise. For instance, other investigators found that females trained with the same running regimen as male rats showed less myocardial improvement (21). This may explain the small increase in volume output that exercise produced in this study. The effect of exercise on heart weight could be due to the type and intensity of exercise. Apparently, swim training in female rats leads to greater increases in cardiac mass than does running (22). Furthermore, Baldwin *et al.* found that changes in myosin ATPase activity (a biochemical correlate of contractility changes) occurred in rats undergoing intense, but not modest, training (23).

Tahiliani and McNeill reviewed the salient features of diabetes-induced myocardial dysfunction (24) and concluded that these abnormalities are due in part to a combination of depressed myosin ATPase and decreased calcium uptake by the sarcoplasmic reticulum (SR). The reduced myosin ATPase activity is associated with diabetes-induced hypothyroidism and is corrected by T_3 treatment (25). The depressed SR calcium ATPase is partly caused by elevated levels of long chain acyl carnitines due to elevation of free fatty acids (26, 27).

Regarding these two mechanisms, hypothyroidism is probably not the principal cause of reduced cardiac output in hearts from these diabetic animals. T_4 levels were not affected by STZ treatment, and T_3 levels manifested only small (although statistically significant) decreases in diabetic rats, which were not affected by exercise. The decline in T_3 levels between sedentary control and diabetic animals is somewhat less (23%) than we observed previously (37%). Furthermore, we have previously shown that T_3 treatment does not correct the diabetes-induced decrease in cardiac function when glucose is used as the sole substrate (2).

Paulson *et al.* (5) previously reported that exercise training improved cardiac function in diabetic animals. Hearts from these animals were perfused with a mixture of both glucose and free fatty acids. The authors suggested that exercise training had decreased the severity of the diabetic state. This may not be the mechanism of exercise-induced alterations in cardiac output in the present study. Injection of STZ at a dose of 65 mg/kg induced a state of severe diabetes in both trained and sedentary rats, characterized by decreases in body weight and T_3 and insulin levels and striking increases in glucose and GHb levels. This corroborates the findings reported by other investigators that exercise training does not improve glucose homeostasis in severe diabetics (11). However, exercise training could have lowered plasma lipid levels in diabetic animals, as reported by Paulson *et al.* (5), leading to improved myocardial SR function.

Exercise training also could have improved myocardial insulin sensitivity or otherwise affected glucose utilization of the heart. Previous studies have shown that exercise training increases whole body insulin sensitivity and glucose oxidation by skeletal (28) and cardiac (29) muscle.

Finally, exercise training may have changed the mechanical or geometric characteristics of the left ventricle. Bakth *et al.* (30) reported that exercise training normalized myocardial collagen levels in diabetic dogs. However, these findings may not be relevant to diabetic rats, since Litwin *et al.* (31) indicated that total chamber stiffness was decreased in diabetic rats, as opposed to the increase stiffness in human diabetics (19, 20). Whereas our data suggest that exercise may improve cardiovascular function, exercise could have deleterious myocardial effects in diabetes. Some types of endurance training can exacerbate the diabetes-induced decrease in myocardial Ca^{2+}-activated ATPase and β-adrenergic receptor number (32, 33).

Although our *in vitro* assessment of cardiac output could suggest a decrease in cardiac performance in diabetic animals and an increase in trained animals,

the physiological significance of these changes is unclear. Exercise increased cardiac output 8–12% only at high preloads, while diabetes attenuated cardiac output 11–12% at the same preloads. We should emphasize that the left atrial filling pressures imposed using our heart apparatus do not necessarily reflect identical *in vitro*, much less *in vivo*, left ventricular end-diastolic pressures. We designed the perfusion apparatus to study cardiac reserve over a range of left atrial pressures that probably overlap *in vivo* ventricular end-diastolic pressures. These cardiac output changes might suggest that basal cardiac performance is unaffected by diabetes. However, Litwin *et al.* (31) reported that chronic diabetes increases left ventricular end-diastolic pressure in the rat. This shifts the diabetic myocardium to a higher point on the Starling curve, where functional decrements, as measured by *in vitro* techniques, are more likely. At this point, endurance training may have a protective effect. Further assessment is needed *in vivo* under a variety of loading and metabolic conditions to determine the relationship between *in vitro* dysfunction and *in vivo* cardiac performance.

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1. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: The Framingham study. Am J Cardiol **334**:29–34, 1974.
2. Barbee RW, Shepherd RE, Burns AH. T_3 treatment does not prevent myocardial dysfunction in chronically diabetic rats. Am J Physiol **254**:H265–H273, 1988.
3. Fein FS, Kornstein LB, Strobeck JE, Capasso JM, Sonnenblick EH. Altered myocardial mechanics in diabetic rats. Circ Res **47**:922–933, 1980.
4. Miller TB. Cardiac performance of isolated perfused hearts from alloxan diabetic rats. Am J Physiol **236**:H808–H812, 1979.
5. Paulson DJ, Kopp SJ, Peace DG, Tow JP. Myocardial adaptation to endurance exercise training in diabetic rats. Am J Physiol **252**:R1073–R1081, 1987.
6. Penpargkul S, Schaible T, Yipintsoi T, Scheuer J. The effect of diabetes on performance and metabolism of rat hearts. Circ Res **47**:911–921, 1980.
7. Vadlamudi RVSV, Rogers RL, McNeill JH. The effects of chronic alloxan and streptozotocin-induced diabetes on isolated rat heart performance. Can J Physiol Pharmacol **60**:902–911, 1982.
8. Horton ES. The role and management of exercise in diabetes mellitus. Diabetes Care **11**:201–211, 1988.
9. Tan MH, Bonen A, Garner JB, Belcastro AN. Physical training in diabetic rats: Effects on glucose tolerance and serum lipids. J Appl Physiol **52**:1514–1518, 1982.
10. Tancrede G, Rousseau-Migneron S, Nadeau A. Beneficial effects of physical training in rats with a mild streptozotocin-induced diabetes mellitus. Diabetes **31**:406–409, 1982.
11. Goodyear LJ, Hirshman MF, Knutson SM, Horton ED, Horton ES. Effect of exercise training on glucose homeostasis in normal and insulin-deficient diabetic rats. J Appl Physiol **65**:844–851, 1988.
12. Bersohn MM, Scheuer J. Effects of physical training on end-diastolic volume and myocardial performance of isolated rat hearts. Circ Res **40**:510–516, 1977.
13. Guisti R, Bersohn M, Malhotra A, Scheuer J. Cardiac function and actomyosin ATPase activity in hearts of conditioned and deconditioned rats. J Appl Physiol **44**:171–174, 1978.
14. Schaible TF, Scheuer J. Effects of physical training by running or swimming on ventricular performance of rat hearts. J Appl Physiol **46**:854–860, 1979.
15. Shepherd RE, Green HE, Sembrowich WH, Gollnick PD. Effects of physical training on control mechanisms of lipolysis in rat fat cell ghosts. J Appl Physiol **42**:884–888, 1977.
16. Fintel MC, Burns AH. A simplified working heart apparatus specialized for use with radioisotopes and oxygen electrodes. Alabama J Med Sci **19**:129–135, 1982.
17. Kadish AH, Little RL Sternberg JC. A new and rapid method for determination of glucose by measurement of rate of oxygen consumption. Clin Chem **14**:116–121, 1968.
18. Winder WW, Fuller EO, Conlee RK. Adrenal hormones and liver cAMP in exercising rats–different modes of anesthesia. J Appl Physiol Respirat Environ Exercise Physiol **55**:1634–1636, 1983.
19. Regan TJ, Lyons MM, Ahmed SS. Evidence for cardiomyopathy in familial diabetes mellitus. J Clin Invest **60**:885–899, 1977.
20. Zarich SW, Arbuckle BE, Cohen LR, Roberts M, Nesto RW. Diastolic abnormalities in young asymptomatic diabetic patients assessed by pulsed Doppler echocardiography. J Am Coll Cardiol **12**:114–120, 1988.
21. Schaible TF, Penpargkul S, Scheuer J. Differences in male and female rats in cardiac conditioning. J Appl Physiol **50**:112–117, 1981.
22. Schaible TF, Scheuer J. Cardiac adaptations to chronic exercise. Prog Cardiovasc Dis **27**:297–324, 1985.
23. Baldwin KM, Cooke DA, Cheadle WG. Time course adaptation in cardiac and skeletal muscle in different running programs. J Appl Physiol **42**:267–272, 1977.
24. Tahiliani AG, McNeill JH. Diabetes-induced abnormalities in the myocardium. Life Sci **38**:959–974, 1986.
25. Garber DW, Everett AW, Neely JR. Cardiac function and myosin ATPase in diabetic rats treated with insulin, T3, and T4. Am J Physiol **244**:H592–H598, 1983.
26. Adams RJ, Cohen DW, Gupte J, Johnson D, Wallick ET, Wang T, Schwartz A. *In vitro* effects of palmitylcarnitine on cardiac plasma membrane Na,K-ATPase, and sarcoplasmic reticulum Ca^{2+}-ATPase and Ca^{2+} transport. J Biol Chem **25**:12404–12410, 1979.
27. Lopaschuk GD, Katz S, McNeill JH. The effect of alloxan- and streptozotocin-induced diabetes on calcium transport in rat cardiac sarcoplasmic reticulum. The possible involvement of long chain acyl carnitines. Can J Physiol Pharmacol **61**:439–448, 1983.
28. James DE, Kraegen EW, Chisholm DJ. Effects of exercise training on *in vivo* insulin action in individual tissues in the rat. J Clin Invest **76**:657–666, 1985.
29. Shepherd RE, Burns AH. Metabolic responsiveness in hearts from exercise trained rats. Med Sci Sports **15**:125, 1983.
30. Bakth S, Arena J, Lee W, Torres R, Haider B, Patel BC, Lyons MM, Regan TJ. Arrhythmia susceptibility and myocardial composition in diabetes. J Clin Invest **77**:382–395, 1986.
31. Litwin SE, Raya TE, Anderson PG, Daugherty S, Goldman S. Abnormal cardiac function in the streptozotocin-diabetic rat. J Clin Invest **86**:481–488, 1990.
32. Belcastro AN, Maybank P, Rossiter M, Secord D. Effect of endurance swimming on rat cardiac myofibrillar ATPase with experimental diabetes. Can J Physiol Pharmacol **63**:1202–1205, 1985.
33. Sylvestre-Gervais L, Nadeau G, Tancrede M Nuyen, Rousseau-Migneron S. Decrease in ventricular beta-adrenergic receptors in trained diabetic rats. Basic Res Cardiol **79**:432–439, 1984.