Myocardial adaptation to endurance exercise training in diabetic rats

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Paulson, Dennis J., Stephen J. Kopp, Donna G. Peace, and June P. Tow. Myocardial adaptation to endurance exercise training in diabetic rats. Am. J. Physiol. 252 (Regulatory Integrative Comp. Physiol. 21): R1073–R1081, 1987.—The purpose of this study was to determine whether exercise training would prevent the progressive functional decline in pump function of hearts from diabetic rats. Four groups were studied: sedentary control, trained control, sedentary diabetic, and trained diabetic. Trained rats were adapted to the treadmill prior to induction of diabetes in half of the group streptozotocin injected (50 mg/kg). Thereafter the duration, speed, and grade were then progressively increased until the trained rats could run for 60 min at 27 m/min, 5% grade (wk 8). Cardiac output and work were measured in isolated working hearts perfused at various left atrial filling pressures and with buffer containing the concentrations of glucose and fatty acids found in vivo. Sedentary diabetic rats had lowered body weight, elevated plasma glucose, triacylglycerol, and cholesterol. Exercise training of diabetic rats lowered plasma triacylglycerol levels. Training increased plantaris muscle cytochrome oxidase activity significantly in both the trained control and trained diabetic groups. Cardiac pump function was impaired in hearts from the sedentary diabetic rats perfused with either normal or diabetic substrate conditions, but the impairment was larger under diabetic conditions. Training of diabetic rats prevented this depression. Myocardial carnitine content was decreased in hearts from sedentary diabetic rats. Exercise training increased carnitine content in both control and diabetic rats. This training protocol did not affect cardiac pump function of the trained control group. These results suggest that exercise training may limit the myocardial contractile dysfunction associated with diabetes mellitus.

Cardiac output; myocardial performance; perfused heart; carnitine

Persons with diabetes mellitus have a greater risk of developing cardiovascular disease, including cardiomyopathy (10). Studies in experimental animals (8, 21, 28, 33) and humans (10, 11, 13) have shown that diabetics exhibit depressed myocardial contractile function under a variety of stress conditions. This depression was not believed to be due to any overt preexisting coronary artery disease (10) but rather a direct effect of diabetes on the myocardium, thus suggesting a diabetic cardiomyopathy. Exercise has long been recommended as an important form of treatment for diabetics, although evidence that support its effectiveness has been limited. Several studies have shown that exercise training of diabetics will diminish basal glucose levels, improve glucose tolerance, and increase sensitivity to insulin (5, 6, 26, 34, 35). Many reports have indicated that exercise training of normal individuals will lower the prevalence of heart disease and increase myocardial contractile function (see Ref. 31). These studies suggest that exercise training may prevent the myocardial contractile dysfunction associated with diabetes mellitus. Therefore the purpose of this study was to determine whether exercise training of diabetic rats would prevent or limit the progressive myocardial decline in pump function associated with this disease.

Materials and Methods

Induction of diabetes mellitus. Diabetes was induced in male rats of the Sprague-Dawley strain (300–350 g) and was dissolved in a 0.01 M citrate buffer (pH 4.5) and was injected within 5 min. Control rats were untreated. Seventy-two hours later the induction of diabetes was confirmed by measuring urine glucose with Ames-Keto-Diastixs.

Exercise training protocol. For 2 wk prior to the induction of diabetes, all rats assigned to the exercise regimen were run on a Stanhope rodent treadmill for 15 min/day, 25 m/min, 5 days/wk. One-half of the exercise-conditioned rats were assigned, at random, to a trained diabetic group and were injected with streptozotocin as described above. The remaining exercise-conditioned rats served as a control trained group. Sedentary rats were similarly divided into sedentary control and sedentary diabetic groups. The trained groups continued to run on the treadmill 6 days/wk for 8 wk. Body weight was recorded on a weekly basis. The duration, speed, and grade were progressively increased as indicated in Table 1. Sedentary rats were untreated. All animals had free access to rodent laboratory chow and water.

Perfusion of hearts. Each rat was anesthetized with ketamine (80 mg/kg) and xylazine (8 mg/kg). The chest was opened, the heart excised, placed in cold bicarbonate buffer, and mounted on the perfusion apparatus within 60 s. The basic design of the rat working heart perfusion apparatus has been described previously (14), but certain modifications were made in order that cardiac output measurements could be made by timed collections. Each heart was perfused initially in a nonrecirculating retro-
were assayed for the total level of carnitine using the method described by Parvin and Pande (18). The plantaris muscle was also excised and frozen. This muscle was homogenized in an isolation medium containing 0.1 M KCl, 50 mM 2-(N-morpholino)propane sulfonic acid, 10 mM MgSO₄ and 1 mM ethylenediaminetetraacetic acid. Cytochrome oxidase activity was measured spectrophotometrically at 25°C as described by Wharton and Tzagoloff (37).

Statistical analysis. Data obtained for each group were compared to determine statistically significant differences using a one-way analysis of variance test (ANOVA) and a least-significant difference (LSD) test for post hoc comparisons among treatment groups. A repeated measures two-way factorial analysis of variance was used to separately determine whether exercise training had a significant main effect on cardiac output and work curves at various left atrial filling pressures in both control and diabetic hearts. A probability value of P<0.05 was accepted as significant.

RESULTS

Table 1 shows the amount of exercise performed by the trained control and diabetic rats during the 8-wk period of training. The duration, speed, and grade were progressively increased until all rats could run for 60 min at 27 m/min and 5% grade, wk 8. This final exercise intensity has been shown to represent ~65% of an untrained rat's maximum O₂ consumption (3). The effects of this training protocol on body weights of control and diabetic rats is also shown in Table 1. Sedentary control rats showed a progressive increase in mean body weight during the 8-wk period. Exercise training significantly attenuated this increase in body weight. Sedentary diabetic rats either lost weight or maintained a relatively constant weight. Exercise training of diabetic rats did not significantly affect body weight.

Sedentary diabetic rats exhibited a marked elevation in plasma levels of glucose, triacylglycerol, and cholesterol (Table 2). Exercise training of diabetic rats had no significant effect on plasma glucose but significantly lowered plasma triacylglycerol levels. Cholesterol levels tended to be lowered by exercise training, but the decrease was not statistically significant. Exercise training of control rats had no significant effect on any of the plasma components.

Cytochrome oxidase activity of the plantaris muscle was assayed to determine whether the exercise protocol produced a significant training effect (Table 2). Exercise training significantly increased skeletal muscle activity of this enzyme in both control and diabetic rats. Sedentary diabetic rats had a slightly lower activity compared with sedentary control rats, but the decrease was not statistically significant.
MYOCARDIAL ADAPTATION TO TRAINING

TABLE 2. Effects of diabetes and exercise training on plasma glucose, triacylglycerol, and cholesterol and plantaris muscle cytochrome oxidase activity

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Glucose (mg/dl)</th>
<th>Triacylglycerol (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Muscle Cytochrome Oxidase, pmol·min⁻¹·g wet wt⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary control</td>
<td>12</td>
<td>254±19</td>
<td>75±8</td>
<td>56±3</td>
<td>24±2</td>
</tr>
<tr>
<td>Trained control</td>
<td>11</td>
<td>230±12</td>
<td>46±8</td>
<td>55±4</td>
<td>33±2*</td>
</tr>
<tr>
<td>Sedentary diabetic</td>
<td>12</td>
<td>621±39*</td>
<td>288±52*</td>
<td>83±3*</td>
<td>19±1†</td>
</tr>
<tr>
<td>Trained diabetic</td>
<td>12</td>
<td>590±30</td>
<td>68±11†</td>
<td>66±9</td>
<td>30±2§</td>
</tr>
</tbody>
</table>

Values are means ± SE; n is no. of rats; P < 0.05. * Significantly different from sedentary control group. † Significantly different from trained control group.

Hearts from sedentary diabetic rats had significantly lower dry weights and elevated heart weight-to-body ratios (Table 3). Trained diabetic rats also had significantly decreased heart weights relative to controls, but the heart weight-to-body weight ratio was not significantly affected. Exercise training of control rats had no significant effect on heart weight or heart weight-to-body weight.

The effects of diabetes and exercise training on cardiac pump function of isolated hearts perfused under diabetic substrate conditions are shown in Figs. 1 and 2. The perfusion medium contained 1.2 mM palmitate and 22 mM glucose. Hearts from sedentary diabetic rats had significantly lowered coronary flow during perfusion than the sedentary control group. Hearts from trained diabetic rats also had lowered coronary flow at low left atrial filling pressures, but at higher filling pressures coronary flow increased to control levels. Increasing left atrial filling pressure produced a progressive increase in aortic flow, stroke volume, and cardiac output of all hearts, but the increase in these parameters was significantly attenuated in hearts from the sedentary diabetic group, particularly at the higher left atrial filling pressure (using ANOVA and LSD tests). The cardiac outputs at 15-, 20-, and 25-cM H₂O left atrial filling were significantly lower in hearts from sedentary diabetic rats compared with sedentary control rats. Exercise training of diabetic rats effectively limited this depression in cardiac pump function. The mean cardiac outputs of hearts from this group at each atrial filling pressure were inbetween the sedentary control and sedentary diabetic groups. No significant differences (using one-way ANOVA and LSD tests) were detected at any left atrial filling pressure between the trained diabetic group and either the sedentary control or sedentary diabetic groups. A two-way factorial analysis of variance test for repeated measures was used to separately determine whether there was a significant main effect of training on the cardiac output curve in both control and diabetic rats (Table 4). A statistically significant main effect of training was found on the cardiac output curve of diabetic rats. As expected a significant effect of left atrial filling pressure was also found. Exercise training had no effect on the cardiac output curve of control rats.

When the cardiac output data were expressed on a heart weight and work basis, a similar pattern was found (Fig. 2). Increasing left atrial filling pressure produced a progressive increase in cardiac output per gram dry weight of heart and cardiac work in all groups, but the depression of hearts from sedentary diabetic rats was somewhat diminished. Because these parameters were expressed on a heart dry weight basis and the hearts from sedentary diabetic rats were lower in weight than controls, the magnitude of the cardiac output and work depression in hearts from sedentary diabetic rats was decreased. Only at the 20-cM H₂O left atrial filling pressure was a significant difference in cardiac work found between hearts from sedentary control and diabetic rats. No significant differences were found for cardiac output per gram dry weight. Exercise training of diabetic rats significantly increased cardiac output per gram dry weight and cardiac work at the 10-, 20-, and 25-cM H₂O left atrial filling pressures compared with sedentary diabetic rats (using one-way ANOVA and LSD tests). Training of control rats had no effect on either parameter. The two-way factorial analysis test for repeated measures showed that exercise training had a significant main effect on the cardiac work curve in diabetic rats but not control rats (Table 4).

These perfused hearts were analyzed for total carnitine content (Fig. 3). Hearts from sedentary diabetic rats had significantly decreased levels of carnitine. Exercise training of diabetic rats prevented this deficiency. Total myocardial carnitine content was also significantly elevated in trained control rats.

In another experiment hearts from sedentary control and diabetic rats were perfused under identical conditions except that the perfusion medium contained normal concentrations of fatty acids (0.4 mM) and glucose (5.5 mM) (Fig. 4). The results showed that aortic flow, stroke volume, and cardiac output were still depressed in diabetic hearts perfused under normal substrate conditions, although the depression in cardiac pump function did not appear to be as large (compare Figs. 1 and 3). When cardiac work, which is expressed on a per gram heart
FIG. 1. Effects of diabetes and exercise training on coronary flow, aortic flow, stroke volume, and cardiac output of isolated hearts perfused at various left atrial filling pressures. Perfusion medium contained diabetic plasma concentrations of free fatty acids (1.2 mM) and glucose (22 mM). All values are means ± SE; n = 11-12. *P < 0.05 significantly different from sedentary control. †P < 0.05 significantly different from trained control. $P < 0.05 significantly different from sedentary diabetic.

weight was calculated, no significant differences were found between the sedentary control and sedentary diabetic hearts at any left atrial filling pressure (data not shown). Neely et al. (14) reported that in the isolated perfused working rat heart, pump performances per gram heart is greater if the heart is smaller. Since the cardiac work was not greater in the smaller diabetic hearts, this finding suggests that the diabetic hearts perfused under normal substrate conditions have depressed pump performance compared with normal hearts.

DISCUSSION

Several studies have shown depressed myocardial contractile function in hearts from alloxan- and streptozotocin-induced diabetic animals (8, 21, 28, 33). Perfused hearts from diabetic animals showed a decrease in their ability to respond to increasing filling pressures and afterloads (21, 33). Papillary muscles isolated from diabetic animals have depressed velocity of shortening and delayed onset of relaxation (8). These studies primarily used pressure or tension indices to assess cardiac contractile activity, and the hearts or muscle strips were perfused with glucose as the only exogenous substrate. Although the present study used volume output as an index of cardiac function, the results are generally consistent with those of the previous studies. Cardiac output was depressed in hearts from sedentary diabetic rats compared with sedentary controls, particularly at the higher left atrial filling pressures. The new findings of
the present study were that this depression in myocardial pump function could be limited by exercise training of diabetic rats and that the magnitude of this depression was dependent on the perfusion medium substrate composition. Hearts from sedentary diabetic rats, perfused under diabetic substrate conditions, had Starling curves for cardiac output and work that were depressed relative to sedentary control rats. Hearts from trained diabetic rats perfused under these conditions had Starling curves for cardiac output and work that were significantly greater than hearts from sedentary diabetic rats and within the control range. Perfusion hearts from sedentary diabetic rats with medium containing normal levels of glucose and free fatty acids decreased the magnitude of the cardiac output depression. The potential mechanisms that could account for the impairment of pump function of hearts from sedentary diabetic rats as well as the enhancement of pump function of hearts from trained diabetic rats cannot be determined from the results of the present study. Since ventricular diastolic volume measurements were not made, it is impossible to determine whether the effects of exercise training were due to changes in contractility or compliance.

Many reports have indicated that normal individuals with high levels of physical activity tend to have a lower prevalence of heart disease (See Ref. 31). A preliminary study has also shown that diabetic patients who engaged in physical activity have a lower risk of developing cardiovascular complications compared with diabetic patients who did not participate in physical activity (11). Other studies have shown that exercise training of diabetic patients will increase maximum O2 consumption and lower submaximal heart rate (5). Recently Bakth et al. (1) have shown that exercise training of diabetic dogs will normalize the fibrillation threshold and reduce the susceptibility to arrhythmias. These findings and the results of the present study provide evidence that exercise training of the diabetic rat is beneficial at least in terms of reducing the cardiac complications associated with this disease.

There are several mechanisms that could account for the beneficial effect of exercise training on myocardial performance of diabetic rats. These mechanisms include but are not limited to exercise-induced alterations in the myocardial contractile protein subunit composition, reduced severity of the diabetic state, improved myocardial metabolism, and altered myocardial ultrastructure. The present study cannot differentiate which mechanism or combination of mechanisms is most likely responsible for this improvement.

Several studies in both human and experimental animals have shown that exercise training of diabetic rats
will diminish basal glucose levels, improve glucose tolerance, and increase the sensitivity to insulin (5, 6, 26, 34, 35). Plasma lipid levels have been shown to be decreased by exercise training in both normal and diabetics (20, 26, 34). In the present study no effect of exercise training was found on plasma glucose. However, this result is not necessarily inconsistent with exercise training reducing the severity of the diabetic state for two reasons. 1) Plasma glucose was measured at only one time point, which may not be a true reflection of the diabetic conditions and 2) it is possible for exercise training to increase peripheral insulin sensitivity but have no effect on blood glucose concentration (36). Diabetic plasma triacylglycerol levels were significantly decreased by exercise training in the present study. Cholesterol levels tended to be lower but the decrease was not significant. These results suggest that exercise training decreased the severity of the diabetic state; however, this conclusion cannot be made with certainty, since the plasma data may also reflect the effects of anesthesia and thoracotomy. A more comprehensive assessment of the diabetic state, such as a glucose tolerance test or the levels of glycosylated hemoglobin, is needed. An improvement of the diabetic condition would tend to minimize the detrimental effects of this disease on the heart, thus preserving myocardial contractile function.

Impaired myocardial performance in diabetic animals has been associated with depressed levels of cardiac actomyosin and myosin adenosinetriphosphatase (ATPase) activities (7, 8, 25). Since swimming exercise training has been shown to increase myocardial contractility and increase contractile protein ATPase activity (31), it is conceivable that the exercise training enhancement of cardiac performance of diabetic rats may be mediated through effects on contractile protein ATPase activity. However, not all forms of exercise training produce the same alteration in contractile protein ATPase activity; e.g., the running-induced enhancement of cardiac contractility has not been correlated to changes in these enzymes (31). In addition, treatment of diabetic rats with thyroxine has been shown to correct the depression in contractile protein ATPase but did not improve myocardial contractility (33). A study by Belcastro et al. (2) found that exercise swim training of diabetic rats produced a further decrease in calcium-activated myocardial ATPase activity. These findings suggest that it is unlikely that the improvement in myocardial pump function produced by exercise training is mediated primarily by effects on contractile protein ATPase activity.

The finding that exercise training increased myocardial carnitine content in both control and diabetic rats suggests that the beneficial mechanism may be mediated through effects on cardiac metabolism. Carnitine is involved in a number of aspects of cardiac metabolism, most notably it is required for the transport of long-chain fatty acids across the inner mitochondrial membrane (28). Several clinical studies have shown a close association between carnitine deficiency and cardiomyopathy (28). Because the diabetic heart predominantly utilizes free fatty acid for its energy supply (33), the deficiency of carnitine, even though small, may be of particular importance. Previously Paulson et al. (18) have shown that treating diabetic rats for 2 wk with L-carnitine (ip) prevented the myocardial carnitine deficiency and improved the ability of diabetic hearts to recover from a period of ischemia and reperfusion. Pieper et al. (22) have shown that the addition of L-carnitine to the perfusion medium of fatty acid-perfused diabetic hearts prevented the decline in ATP. In contrast Lopaschuk et al. (12) found that the addition of DL-carnitine to the diet of diabetic rats had no effect on heart function. Thus, at this point in time, the role of carnitine deficiency in the dysfunction of the diabetic heart remains unresolved.

In diabetic dogs the contractile dysfunction was associated with collagen accumulation causing increased stiffness of the heart (27). Recently Bakth et al. (1) have shown that exercise training of diabetic dogs normalized myocardial collagen levels, thus suggesting that exercise training may alter myocardial structure. Exercise training has also been shown to alter myocardial mass, histology, and coronary vessel density in normal animals.
Figure 4. Coronary flow, aortic flow, stroke volume, and cardiac output of hearts isolated from sedentary control and diabetic rats perfused with normal plasma concentrations of free fatty acid (0.4 mM) and glucose (5.5 mM). All values are means ± SE; n = 7-8. *P < 0.05 significantly different from sedentary control.

Thus it is also conceivable that the beneficial effects of exercise training diabetic rats may be mediated through changes in myocardial ultrastructure.

The exercise-training protocol used in the present study had no effect on cardiac pump function of control rats. This result is not surprising, since other studies employing treadmill running as a mode of exercise have not found an effect on cardiac performance (15). The cardiac adaptation to exercise training appears to be intensity related and may be more prominent when imposed on a lower base-line functional status. In this regard it has been recently shown that exercise training will also ameliorate the cardiac dysfunction associated with hypertension in rats (30, 32).

The second important finding of the present study was the observation that the magnitude of cardiac output depression of sedentary diabetic rats may have been dependent on perfusion medium concentrations of glucose and free fatty acids. When the results of the two experiments shown in Figs. 2 and 4 were compared, it appeared that the cardiac output curves were depressed in hearts from sedentary diabetic rats perfused with either normal or diabetic concentrations of free fatty acids and glucose, but the depression was larger in diabetic hearts perfused under diabetic substrate conditions. However, because these experiments were not carried out...
concurrently, this conclusion cannot be made with certainty. Similar results have been obtained by Fields et al. (9). They showed that myocardial performance was impaired in hearts from diabetic rabbits perfused with 0.4 mM palmitate, 13 mM glucose, and 1 μU/ml insulin. If the concentration of exogenous fatty acids was lowered to 0.06 mM, myocardial function of the diabetic heart was improved and comparable to control levels. These results are consistent with the findings of Pieper et al. (23) who showed that perfusing control and diabetic hearts with only glucose produced constant and normal levels of creatine phosphate and ATP. Adding progressively higher concentrations of palmitic acid to the perfusion medium had no effect on control hearts but produced a progressive decrease in ATP in diabetic hearts despite normal levels of creatine phosphate. This reduction in myocardial ATP correlated with the accumulation of long-chain acyl-CoA (24), which has been shown to inhibit in isolated mitochondria the adenine nucleotide translocator (19). Other studies have also shown that the perfusion medium substrate concentration will affect tricaprylglycerol metabolism of the diabetic heart (17). Other studies have shown that treatment of diabetics with drugs such as hydroxaline (an antihypertensive agent), which lower plasma lipid levels but do not affect blood glucose concentrations, will prevent the diabetes-induced cardiac changes (29). These findings suggest that the deleterious effects elevated by plasma lipids and/or glucose may be at least partially responsible for the impairment in cardiac performance associated with diabetes mellitus.

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