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Effects of physical training on the myocardium of streptozotocin-induced diabetic rats

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Summary: Effects of endurance swimming training on myocardial contractility and left ventricular myosin isoenzymes were examined in diabetic rats. A diabetic condition was induced in 15-week-old male Wistar rats, by intravenous injection of streptozotocin (50 mg/kg). Swimming training was carried out for five to six weeks (90 min/day, 6 days/week). In order to estimate myocardial contractility, the isometric developed tension of the isolated left ventricular papillary muscle was measured. Myosin isoenzymes were obtained by pyrophosphate gel electrophoresis. Fasting blood glucose of the trained group was significantly lower than that of the sedentary group (sedentary vs. trained = 409.6 ± 25.9 vs. 266.3 ± 20.5 mg/dl, $p < 0.001$). There was no significant difference in isometric developed tension (T) between the two groups, and the dT/dt_{max} of the trained group showed a tendency to increase (sedentary vs. trained, T: 2.8 ± 0.8 vs. 2.9 ± 0.8 g/mm^2, dT/dt_{max}: 23.1 ± 3.6 vs. 26.2 ± 3.5 g/mm^2 · s, $p < 0.1$). Myocardial mechanical responses to isoproterenol and dibutyryl cAMP were increased in the trained group. Left ventricular myosin isoenzyme pattern was shifted towards VM-1 by endurance swimming (sedentary vs. trained, VM-1: 5.6 ± 4.5 vs. 19.6 ± 8.8%, $p < 0.001$, VM-3: 75.1 ± 10.0 vs. 54.9 ± 14.7%, $p < 0.001$). These results indicate that endurance swimming can improve disordered glucose metabolism and also influence myocardial contractility, myocardial catecholamine responsiveness, and energetics in myocardial contraction.

Key words: swimming training, streptozotocin, diabetic rat, myocardial contractility, myosin isoenzyme

Introduction

Physical training can improve disordered glucose metabolism in diabetes (1, 11, 12, 13, 20), but only a few studies have been made concerning the effects of endurance training on t-ihe myocardium in diabetes (14, 17). The present study examined the influence of swimming training on myocardial contractility, and myocardial catecholamine responsiveness and ventricular myosin isoenzymes in relation to myocardial contractile energetics, using rats with streptozotocin-induced diabetes. In order to estimate myocardial contractility, isometric developed tension of the isolated left ventricular papillary muscle was measured. Ventricular myosin isoenzymes were determined by pyrophosphate gel electrophoresis. In addition, myocardial mechanical responses to dibutyryl cAMP (DBcAMP), which passes the myocardial surface membrane and exerts its positive inotropic effect without direct stimulation of the beta-receptors (9), were also examined in order to find out the extent to which post-receptor processes play a role in myocardial catecholamine responsiveness in diabetic rats.

Materials and Methods

15-week-old male Wistar rats were made diabetic by an intravenous injection of streptozotocin (50 mg/kg). The diabetic rats were divided into two groups, i.e., sedentary controls and swim-trained rats. The swimming training was carried out with water temperature of 33–35 °C, according to our previous program (18, 19). The training time was 10 min the first day, and was increased by 10 min daily to 90 min per day. The program was continued for five to six weeks (6 days/week), and at the end of the fasting program blood glucose was measured by the glucose oxidase method.

Left ventricular papillary muscles were removed under the microscope and were mounted vertically between a motionless lever and a force transducer using silk ligatures and small steel hooks. Papillary muscles were stimulated at a frequency of 0.2 Hz and with a voltage 30 % above threshold, while being perfused with Tyrode solution (composition in mM: glucose 25.0, NaCl 130.0, $NaHCO_3$ 20.0, NaH_2PO_4 1.2, KCl 4.1, $CaCl_2$ 1.1, $MgCl_2$ 1.5, pH 7.4, 32 °C) bubbled with 95 % O_2 and 5 % CO_2. After the steady state was obtained at L_{max}, isometric developed tension (T), dT/dt_{max}, time to peak tension (TPT), and total contraction time (TCT) were measured. Mechanical responses to isoproterenol (10^{-7} M) were estimated at L_{max}, and following the interposition of Tyrode solution for 30–35 min, the responses to DBcAMP (10^{-5} M) administration were also measured. The measure of each parameter was obtained by comparing two paired values, one being that of the steady state prior to isoproterenol or DBcAMP administration, and the other, the maximum value after isoproterenol or DBcAMP administration.

Pyrophosphate gel electrophoresis was carried out as described elsewhere (3, 7, 15). The gel contained 3.8 % acrylamide and 0.12 % N,N'-methylene-bis-acrylamide. The electrophoresis buffer was 20 mM $Na_4P_2O_7$ (pH 8.8) in the presence of 10 % glycerol. Native myosin from the left ventricle was extracted with a solution consisting of 100 mM $Na_4P_2O_7$ (pH 8.8), 5 mM 1,4-dithiothreitol, 5 mM EGTA, 5 µg/ml leupeptin. Electrophoresis was carried out for 30 h at 2 °C and a voltage gradient of 13.3 V/cm.

Statistical comparisons were carried out using Student's *t*-test.

Results

Ten swim-trained and 10 sedentary rats were examined. Body weight of the trained diabetic group was significantly heavier than that of the sedentary diabetic group. Ventricu-

Table 1. Body weight, ventricular weight and papillary muscle size.

	BW (g)	VW (mg)	VW (mg) / BW (g)	Papillary muscle size	
				L (mm)	CSA (mm^2)
sedentary	341.5 ± 25.9	946.0 ± 78.2	2.78 ± 0.24	5.48 ± 0.56	0.87 ± 0.15
trained	380.5 ± 28.5	997.1 ± 103.5	2.56 ± 0.25	5.43 ± 0.30	0.84 ± 0.18
	$P < 0.005$	ns	ns ($P < 0.1$)	ns	ns

BW: body weight, VW: ventricular weight, L: length, CSA: cross sectional area. Values are means ± SD, ns: not significant.

Table 2. Fasting blood glucose.

	Bood glucose (mg/dl)
sedentary	409.6 ± 25.9
trained	266.3 ± 20.5
	$P < 0.001$

Values are means ± SD.

Table 3. Myocardial mechanics.

	DT (g/mm^2)	RT (g/mm^2)	dT/d_{tmax} ($g/mm^2 \cdot s$)	TPT (msec)	TCT (msec)
sedentary	2.8 ± 0.8	1.0 ± 0.3	23.1 ± 3.6	167.5 ± 13.4	588.5 ± 45.8
trained	2.9 ± 0.8	0.9 ± 0.3	26.2 ± 3.5	152.0 ± 14.4	545.0 ± 49.5
	ns	ns	ns ($P < 0.1$)	$P < 0.05$	ns ($P < 0.1$)

DT: developed tension, RT: resting tension, TPT: time to peak tension, TCT: total contraction time. Values are means ± SD, ns: not significant.

lar weight of the trained group showed a tendency to increase, but was not significantly heavier than that of the sedentary group (Table 1). There was no difference in papillary muscle size between the two groups (Table 1). Fasting blood glucose of the trained group was significantly lower than that of the sedentary group (Table 2). Measures of myocardial mechanics obtained from isolated left ventricular papillary muscles are shown in Table 3. There was no significant difference in isometric developed tension (T) between the trained and sedentary groups, while the dT/dt_{max} of the trained group showed a tendency to increase. The time to peak tension (TPT) and total contraction time (TCT) of the trained group were shorter than those of the sedentary group. Myocardial mechanical responses to isoproterenol and DBcAMP are shown in Figs. 1 and 2. The response of developed tension to isoproterenol of the trained group had a tendency to increase as compared with the sedentary group (sedentary vs. trained = 7.4 ± 4.1 vs. 10.2 ± 3.0 %, $p < 0.1$). The change in dT/dt_{max} in response to isoproterenol administration was significantly greater in the trained than in the sedentary group (sedentary vs. trained = 9.8 ± 3.4 vs. 12.9 ± 3.2 %, $p < 0.05$). The change in developed tension in response to DBcAMP administration did not differ significantly between the two groups (sedentary vs. trained = 6.6 ± 3.6 vs. 9.0 ± 3.2 %), but

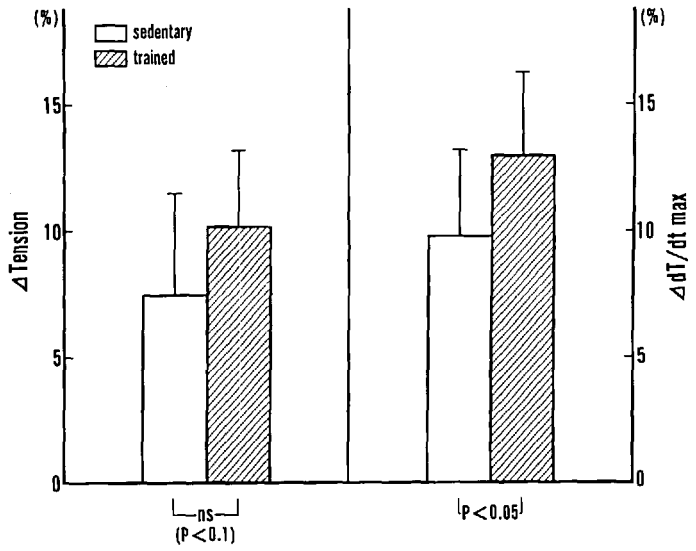

Fig. 1. Comparisons in Δtension, $\Delta dT/dt_{max}$ due to isoproterenol (10^{-7} M) between the swim-trained and sedentary rats. Vertical lines indicate SD; ns: not significant.

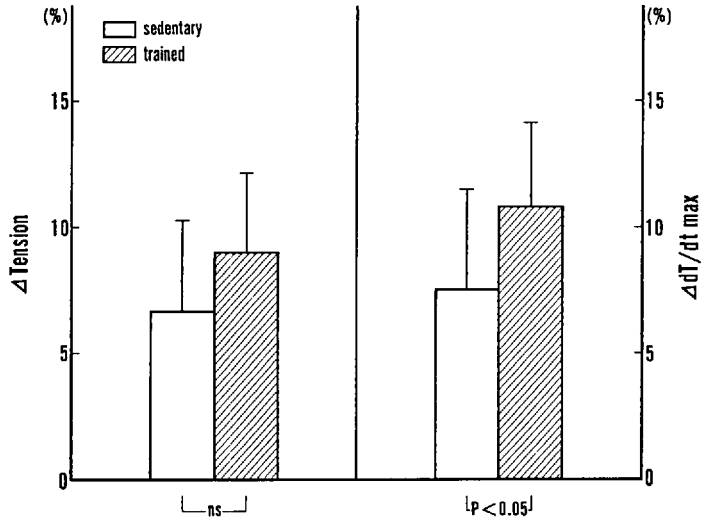

Fig. 2. Comparisons in Δtension, $\Delta dT/dt_{max}$ due to dibutyryl c AMP (10^{-5} M) between swim-trained and sedentary rats. Vertical lines indicate SD; ns: not significant.

the change in dT/dt_{max} following DBcAMP administration was significantly greater in the trained group than in the sedentary group (sedentary vs. trained = 7.5 ± 3.9 vs. 10.8 ± 3.2 %, $p < 0.05$). Left ventricular myosin isoenzyme pattern obtained by pyrophosphate gel electrophoresis showed that VM-3 was predominant in the diabetic group, and the pattern was significantly shifted towards VM-1 by endurance swimming training. (Fig. 3).

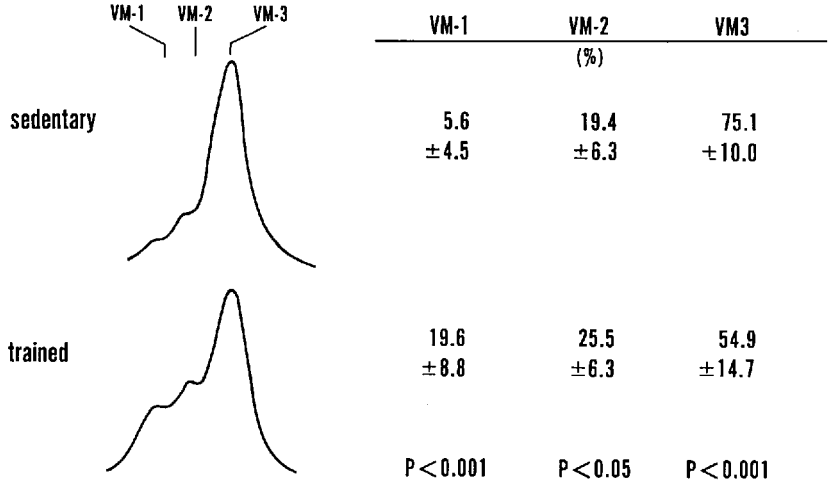

Fig. 3. Left ventricular myosin isoenzymes. Left panel shows representative absorbancy profiles of pyrophosphate gel electrophoresis. Values are means ± SD.

Discussion

From results of the present study, swimming appears to improve (i.e., reduce) metabolic abnormalities in dieabetic rats in that body weight was increased and fasting blood glucose was decreased by the training. These changes might be due to an enhanced insulin sensitivity in tisssues brought about by exercise-training (1, 12, 13). According to previous literature, swimming enhances myocardial contractility (2, 5, 6, 10, 12, 16). In the present study, however, the isometric developed tension of isolated left ventricular papillary muscles was not altered by swimming in diabetic rats, although dT/dt_{max} was increased. This may partly be because the daily training time was relatively short as compared with that of previous reports. Increased dT/dt_{max} in the trained group can be explained by the idea that the velocity parameters are influenced more by myocardial transformation than by developed tension and working capacity (4,8). The left ventricular myosin isoenzyme pattern of diabetic rats with predominant VM-3, which has the lowest electrophoretical mobility and ATPase activity, shifted towards VM-1 with the fastest mobility and the highest enzymic activity, following swimming training. Isometric developed tension showed a tendency to increase in the trained group following isoproterenol administration and the response of dT/dt_{max} was significantly greater in the trained group than in the sedentary one. These results are similar to previous reports which showed increased myocardial mechanical catecholamine sensitivity in swim-trained normal or spontaneously hypertensive rats (18, 19). Myocardial mechanical responses to DBcAMP increased in the trained group. As DBcAMP is thought to pass the myocardial surface membrane and to exert its positive inotropic effect without directly stimulating the beta- receptors (9), it may be said that postreceptor processes also play a role in enhanced myocardial sensitivity to catecholamines in swim-trained diabetic rats. Alteration in ventricular myosin isoenzyme pattern in swim-trained diabetic rats can be given as an example, because there is a report that the activated cAMP-regulated system can be influenced by changes in myosin isoenzyme distribution (21).

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