Effects of voluntary exercise on heart function in streptozotocin (STZ) – induced diabetic rat

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Abstract

Exercise training improves cardiac performance and has been utilized as an effective adjunct to pharmacotherapy in the management of diabetes mellitus. The aims of this study were to characterize voluntary exercise habits in streptozotocin (STZ)-induced diabetic rats and investigate whether attained levels of voluntary exercise induce effects on diabetic heart function. Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg). Animals were divided into 4 groups: Control Sedentary (CS), Diabetic Sedentary (DS), Control Running (CR) and Diabetic Running (DR). DR and CR rats were provided with running wheels. Body weight and blood glucose were measured in running rats at the start and end of the 60-day exercise period. Ventricular action potentials were measured in spontaneously beating heart. Shortening and intracellular Ca^{2+} were measured in electrically stimulated ventricular myocytes. DR rats exercised less than CR rats. At 60 days the mean distance covered by DR rats was 1027 ± 289 m compared to 5975 ± 1117 m in CR rats. CR gained whilst DR rats lost weight at the end of the exercise period. Mean blood glucose in CR and DR rats at the start of the exercise period was 71 ± 4 and 426 ± 21 mg/dl, respectively. Glucose levels were not altered in CR rats but were reduced in DR rats at the end of the exercise period. The time to peak shortening and peak Ca^{2+} transient and time to half relaxation of the Ca^{2+} transient were prolonged in myocytes from DS compared to CS rats and were not additionally altered by voluntary exercise. In conclusion, diabetic rats were less inclined to partake in voluntary exercise compared to controls and the levels and duration of exercise were insufficient to significantly alter cardiac performance in either control or diabetic rats. (Int J Diabetes Metab 15: 32-37, 2007)

Key words: *Diabetes mellitus, streptozotocin, voluntary exercise, heart muscle, ventricular myocytes*

Introduction

It has been frequently demonstrated that chronic, dynamic exercise has beneficial effects on several structural and functional properties of both healthy and diabetic heart muscle. Hallmark adaptations include a training induced resting bradycardia, an increase in end-diastolic dimension and in maximal stroke volume and general improvement in ventricular performance and in contractile function. Exercise training has long since been utilized as an effective adjunct to pharmacotherapy in the management of diabetes mellitus and physical activity influences several aspects of diabetes mellitus including blood glucose metabolism, insulin action and cardiovascular risk factors.[1,2] Previous studies have demonstrated the beneficial effects of exercise on cardiac performance in diabetes mellitus. For example exercise training has been shown to reverse bradycardia and hypotension in diabetic rats.[3] Training also prevents the decreased end-diastolic volume and attenuates increased end-systolic volume that accompanies sedentary diabetes.[1] Reductions in left ventricular end-diastolic chamber and myocardial wall compliance, due to diabetes-induced changes in collagen, are also improved with exercise training.[4] Most of the studies carried out to date have utilised enforced exercise regimes. The aims of this study were to characterize voluntary exercise habits in streptozotocin (STZ)-induced diabetic rats and investigate whether attained levels of voluntary exercise induce effects on diabetic heart function.

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Methods & Materials

Animal model and exercise protocol

Diabetes was induced in male Wistar rats (210-230 g) with a single intraperitoneal injection of STZ (60 mg/kg body weight, Sigma, UK) dissolved in a citrate buffer. Weight-matched control animals received citrate buffer alone. Animals were divided into 4 groups: Control Sedentary (CS), Diabetic Sedentary (DS), Control Running (CR) and Diabetic Running (DR). Ten CR and 10 DR rats were provided with running wheels (Wobust Wodent Wheel, Transoniq, USA) equipped with digital wheel distance counters (Sigma, BC506) for a period of 60 days. Running distances were recorded at 2-day intervals from day 10 to day 60. Six CS and 6 DS rats were not provided with wheels. All animals were maintained on the same diet and

water *ad libitum*. Body weight and blood glucose (One Touch BasicPlus; Lifescan, Johnson & Johnson, USA) were measured at the start and end of the exercise period. Blood plasma electrolytes (sodium and potassium) and lipids (cholesterol, triacylglycerol and high-density lipoproteins) were measured with an autoanalyser (Beckman Synchron Clinical System, USA). Approval for this project was obtained from the Faculty of Medicine & Health Sciences Ethics Committee, United Arab Emirates University.

Measurement of heart rate and action potentials

After completion of the exercise period, running and sedentary rats were sacrificed humanely by use of a guillotine. Hearts were mounted on a Langendorff apparatus and perfused retrogradely at a constant flow of 8 ml.g heart^{-1} min^{-1} with a normal Tyrode solution containing 1.8 mM Ca^{2+} (see below) maintained at 35-36 ºC. Action potentials were recorded in spontaneously beating heart with a suction electrode positioned on the epicardial surface of the left ventricular myocardium.[5] Signals from the electrode were amplified (ML136 Bioamp) and conveyed via a Powerlab (PL410, ADInstruments, Australia) to a personal computer. Data were recorded and analysed with Chart software v 4.2 (Powerlab, ADInstruments). Action potential durations (APDs) were measured from threshold to 50 % (APD50) and 70 % (APD70) from the peak of the action potential.

Ventricular myocyte isolation

Ventricular myocytes were isolated according to previously described techniques with minor modifications [6]. After measuring action potentials, hearts were perfused for 4 min with a cell isolation solution (see below) containing 0.1 mM EGTA, and then for 6 min with solution containing 0.05 mM Ca^{2+}, 0.75 mg/ml collagenase (Type 2; Worthington, NJ, USA) and 0.075 mg/ml protease (Type X1V; Sigma). After this time, the ventricles were excised from the heart, minced and gently shaken in collagenase-containing isolation solution supplemented with 1 % bovine serum albumin. Cells were filtered from this solution at 4 min intervals and resuspended in 0.75 mM Ca^{2+}-containing isolation solution.

Measurement of ventricular myocyte shortening and intracellular Ca^{2+}

For electrophysiological studies myocytes were allowed to settle on the glass bottom of a Perspex chamber mounted on the stage of an inverted microscope (Axiovert 35, Zeiss, Germany). Myocytes were superfused (3-5 ml/min) with normal Tyrode containing 1.8 mM Ca^{2+}. Experiments were performed in electrically stimulated myocytes (1 Hz) at 35-36 °C). Unloaded myocyte shortening was followed using a video edge detection system (VED-114, Crystal Biotech, USA). The degree of shortening (expressed as a % of resting cell length), the time to peak shortening (TPK) and time from peak to half relaxation (THALF) were measured. Intracellular Ca^{2+} was measured in cells loaded with the fluorescent indicator fura-2 AM (F-1221, Molecular Probes, USA) as described previously [6]. Myocytes were alternately illuminated by 340 nm and 380 nm light using a monochromator (Cairn Research, England) which changed the excitation light every 2 ms. The resultant fluorescent emission at 510 nm was recorded by a photomultiplier tube and the ratio of the emitted fluorescence at the two excitation wavelengths (340/380 ratio) was calculated to provide an index of intracellular Ca^{2+} concentration. Data were recorded and analysed with Signal Averager software v 6.37 (Cambridge Electronic Design, UK). The amplitude, TPK and THALF of the Ca^{2+} transient were measured.

Solutions

The normal Tyrode solution contained (in mM): NaCl 140; KCl 5; $MgCl_2$ 1; glucose 10; HEPES 5; $CaCl_2$ 1.8 set to pH 7.4 with NaOH. The cell isolation solution contained (in mM) 130.0 NaCl, 5.4 KCl, 1.4 $MgCl_2$, 0.4 NaH_2PO_4, 5 HEPES, 10 glucose, 20 taurine and 10 creatine set to pH 7.3 with NaOH.

Statistics

Results were expressed as the mean ± SEM of 'n' observations; 'n' refers either to the number of animals, hearts or cells. Statistical comparisons were performed using either the Independent samples *t*-test or ANOVA followed by Bonferroni corrected t-tests for multiple comparisons, as appropriate. $p < 0.05$ was considered to indicate a significant difference.

Results

Exercise characteristics

The exercising animals were allowed free access to running wheels for 60 days. As expected in a voluntary exercise program the habits amongst animals were variable. Some control animals chose to run more than others and the same was true amongst diabetic animals (Figures 1a and 1b). On average, the DR rats ran significantly ($p < 0.01$) less than CR rats. At the end of the 60-day exercise period DR had covered a mean distance of 1027 ± 289 m (n = 10) compared to 5975 ± 1117 m (n = 10) in CR rats (Figure 1c).

Body weight and metabolic characteristics

At the end of the exercise period DR rats had lost 13 % (a decline from 214.4 ± 7.2 to 186.3 ± 7.9 g), while CR had gained 44 % (an increase from 229.6 ± 6.7 to 330.0 ± 13 g) of their initial weight (Figure 2a). The mean blood glucose level was significantly higher in DR compared to CR rats, both at the beginning (425.6 ± 21.2 mg/dl in DR and 71.3 ± 3.6 mg/dl in CR rats) and at the end of exercise period (319.3 ± 15.2 mg/dl in DR compared to 72.7 ± 3.5 mg/dl in CR rats). The blood glucose level was decreased substantially (~ - 25 %) in DR rats though not in CR rats at the end of the running period (Figure 2b).

Exercise had no significant effects on blood plasma electrolytes in either control or diabetic rats. Blood plasma sodium was 143.8 ± 2.0 mmol/L (n=4) in CS compared to 136.3 ± 1.9 mmol/L (n=6) in DS rats. Blood plasma potassium was 6.3 ± 0.4 mmol/L (n=4) in CS compared with 6.0 ± 0.1 mmol/L (n=6) in DS rats. In general blood plasma lipids including cholesterol, triacylglycerol and high-density lipoproteins were elevated in diabetic rats compared to controls. Cholesterol and triacylglycerol were further and significantly elevated by exercise in diabetic rats

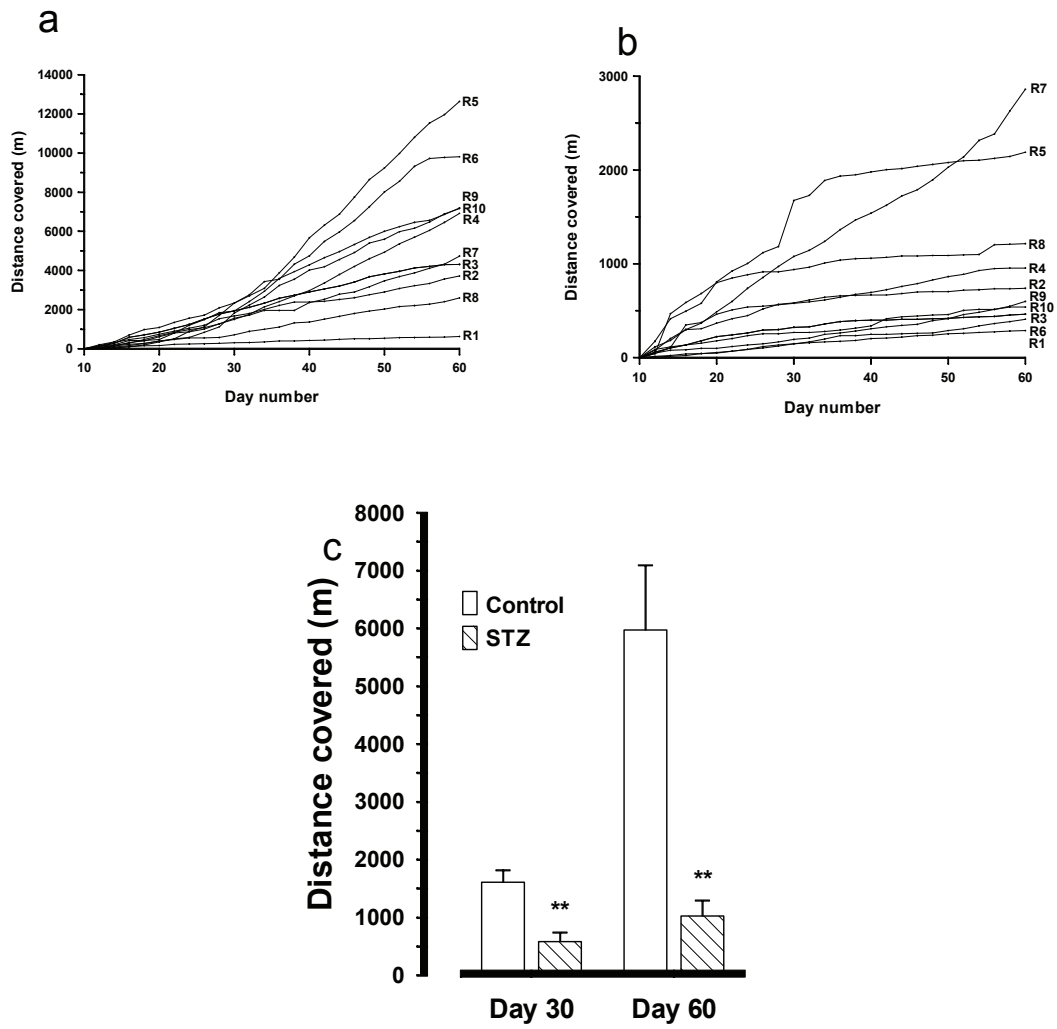

Figure 1: Voluntary exercise data for (a) control and (b) diabetic rats. Rats were provided with running wheels for a total of 60 days. Wheel rotations were recorded every 2 days from day 10 to day 60. (c) Mean distances ± SEM covered at day 30 and 60 of the running period (n=10). ** $p < 0.01$.

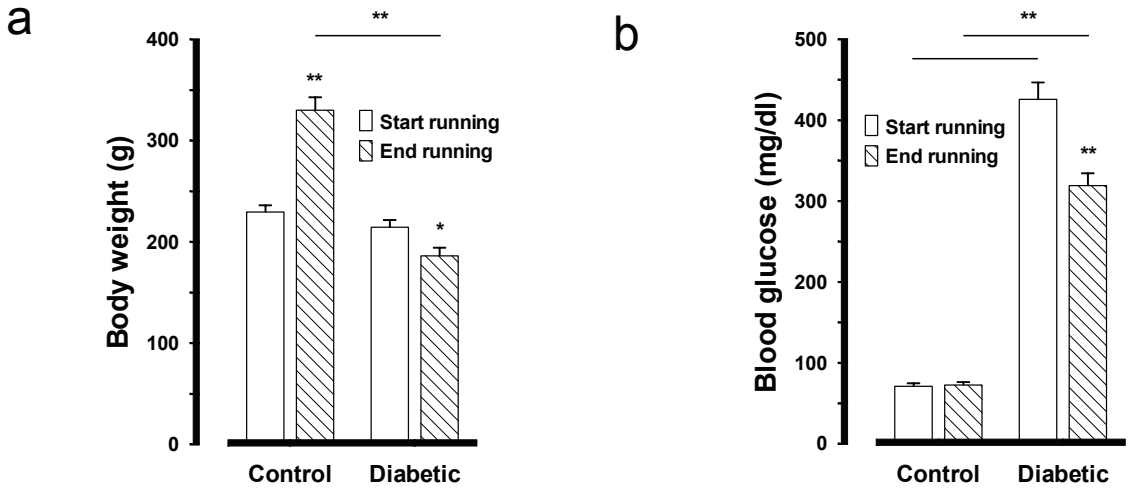

Figure 2: Effects of exercise on (a) body weight and (b) blood glucose in diabetic and control rats at the start and at the end of the running period. Data are mean ± SEM (n=10). * $p < 0.05$, ** $p < 0.01$

though not in controls. Blood plasma cholesterol was 44.8 ± 5.4 mg/dl (n=4) in CS compared to 63.0 ± 4.8 mg/dl (n=6) in DS rats. In the voluntary exercise groups, cholesterol decreased slightly to 38.8 ± 3.3 mg/dl (n=10) in CR but increased to 78.6 ± 5.5 mg/dl (n=10) in DR rats. Blood plasma triacyglycerol was 52.3 ±6.9 mg/dl (n=4) in CS compared to 171.7 ± 35.3 mg/dl (n=6) in DS rats. In the voluntary exercise groups triacylglycerol decreased slightly to 40.5 ± 7.3 mg/dl (n=10) in CR but increased to 340.0 ± 58.0 mg/dl (n=10) in DR rats. Blood plasma high-density lipoproteins were 36.4 ± 7.4 mg/dl (n=4) in CS compared to 49.4 ± 3.3 mg/dl (n=6) in DS rats.

Effects of exercise on heart rate and ventricular action potentials

Heart rate was not significantly ($p > 0.05$) altered in DS compared to CS rats and voluntary exercise had no additional effect on heart rate. Heart rate was 226 ± 33 beats per minute (BPM) (n=5) in CS compared to 184 ± 15 BPM (n=6) in DS rats. In the voluntary exercise groups heart rate was 244 ± 26 BPM (n=6) in CR and 146 ± 14 BPM (n=7) in DR rats (Figure 3a). The prolongations of APD50 and APD70 in DS and DR rats were consistent with observed reductions in heart rate in diabetic rats (Figures 3b & 3c).

Effects of exercise on myocyte shortening

Resting cell length of DS (117.3 ± 2.3 μm, n=70 cells from 5 hearts) was not significantly different from that of CS rat myocytes (120.5 ± 2.1 μm, n=51cells from 5 hearts). Exercise had no significant effects on resting cell length in either DR (116.0 ± 1.8 μm, n=72 cells from 6 hearts) or CR (126.6 ± 2.2 μm, n=59 cells from 6 hearts) myocytes. The time-course of shortening was significantly prolonged in ventricular myocytes from DS compared to CS rats and was not additionally altered by voluntary exercise (Figure 4a). The TPK shortening was 102.7 ± 2.1 ms (n=70 cells from 5 hearts) in DS compared to 84.1 ± 2.1 ms (n=51 cells from 5 hearts) in CS myocytes (Figure 4a). In the voluntary exercise groups the TPK shortening was 106.9 ± 2.5 ms (n=72 cells from 6 hearts) in diabetic compared to 92.4 ± 2.3 ms (n=59 cells from 6 hearts) in control myocytes. THALF relaxation was not significantly altered by either diabetes or by voluntary exercise (Figure 4b). Amplitude of myocyte shortening was not significantly altered by either diabetes or by voluntary exercise. Amplitude of myocyte shortening was 5.7 ± 0.3 % (n=70 cells from 5 hearts) in DS compared to 6.9 ± 0.4 % 9 (n=51 cells from 5 hearts) in CS rats (Figure 4c).

Effects of exercise on intracellular Ca^{2+}

Resting intracellular Ca^{2+} was not significantly altered by voluntary exercise. The time-course of the Ca^{2+} transient was significantly prolonged in DS compared to CS rats (Figure 5a & 5b). The TPK Ca^{2+} transient was 76.5 ± 2.9 ms (n=60 cells from 5 hearts) in DS compared to 61.0 ± 2.3 ms (n=36 cells from 5 hearts) in CS myocytes (Figure 5a). The THALF relaxation of the Ca^{2+} transient was 171.0 ± 5.5 ms (n=60 cells from 5 hearts) in DS compared to 122.6 ± 4.0 ms (n=36 cells from 5 hearts) in CS myocytes (Figure 5b). Neither the TPK nor the THALF relaxation of the Ca^{2+} transients were additionally altered by voluntary exercise in CR or DR rats. The amplitude of the Ca^{2+} transient was not significantly altered by either diabetes or by voluntary exercise (Figure 5c).

Discussion

The main findings of this study were: (1) Given free access to running wheels DR exercised less than CR rats, (2) CR rats gained weight whilst DR rats lost weight during the exercise period, (3) the hyperglycaemic effects of STZ-induced diabetes were partially reduced with exercise, (4) the prolonged time-course of myocyte shortening in DS compared to CS rats was not significantly altered by voluntary exercise and (5) amplitude of myocyte shortening was not significantly altered by either diabetes or voluntary exercise.

One of the aims of this study was to characterise the exercise habits of diabetic rats compared to healthy controls. In our study, given free access to running wheels, diabetic rats exercised significantly less than healthy controls. The distance covered in total and each day varied substantially between animals in both groups. What motivates exercise in general and why is this motivation reduced in STZ-induced diabetic rats is a difficult question to address and is likely to have a multifactorial explanation. STZ treatment causes damage to pancreatic β-cells which in turn reduces the ability of the pancreas to synthesise and release insulin.[7] The pathophysiology of STZ-induced diabetes includes hypoinsulinaemia, hyperglycaemia, change in eating habits, loss of weight, polyuria and glycosuria.[8] This collection of metabolic disturbances, which can be normalised with insulin treatment, may be partly responsible for a reduced enthusiasm for exercise. The loss of weight in STZ-treated animals may be partly attributed to loss of fluid (polyuria) but also due to a shift in metabolism from utilisation of carbohydrates to the use of fatty fuels to generate energy. The variability of exercise habits and general lack of enthusiasm for voluntary exercise amongst diabetic rats might explain why most previous studies have utilized enforced exercise regimes.[9]

Voluntary exercise reduced the hyperglycaemic effects of STZ treatment. Some previous studies, which have employed enforced exercise regimes, have also demonstrated improved fasting blood glucose and prevention of depression in myocardial glucose metabolism in diabetic rats which may in turn explain the benefits of exercise in preventing cardiac dysfunction in diabetes.[10]

The mild bradycardic effects of STZ-induced diabetes were not significantly altered by voluntary exercise. However, enforced exercise training regimes have been shown to reverse the bradycardia and generally improve myocardial function in diabetic rats.[3]

The prolonged time-course and small reduction in amplitude of shortening in ventricular myocytes from diabetic heart may be partly attributed to defects in Ca^{2+} transport including reduced L-type Ca^{2+} current, depressed

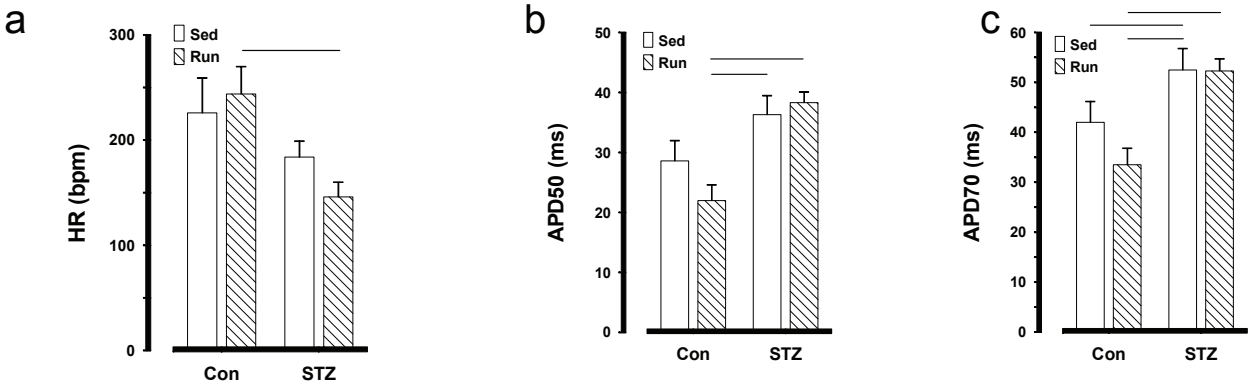

Figure 3: Effects of exercise on (a) heart rate and action potential duration at (b) 50 % and (c) 70 % from peak of action potential. Data are mean ± SEM (n=5-7). Lines above bars indicate $p < 0.05$

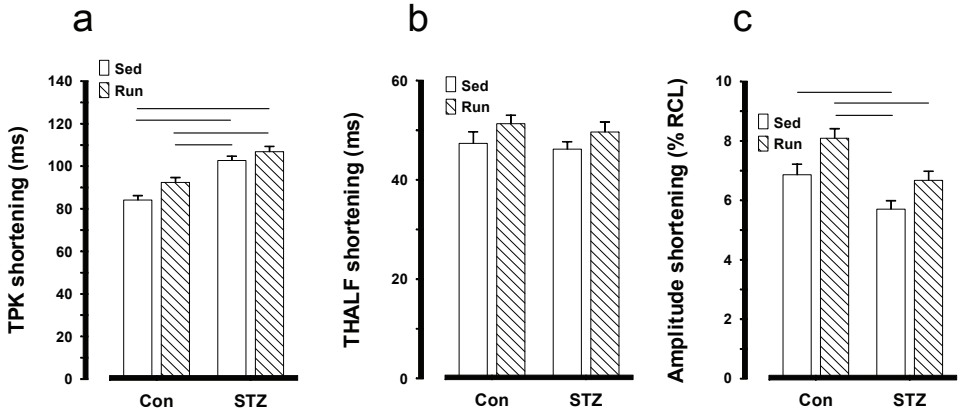

Figure 4: Effects of exercise on (a) time to peak (TPK) shortening, (b) time to half (THALF) relaxation of shortening and (c) amplitude of shortening expressed as a percentage of resting cell length (RCL). Data are mean ± SEM (n=51 - 72 cells from 5 – 6 hearts). Lines above bars indicate $p < 0.05$

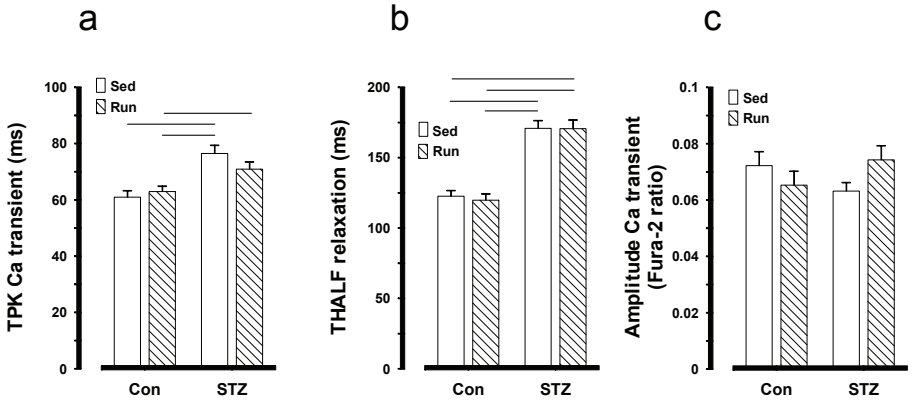

Figure 5: Effects of exercise on (a) time to peak (TPK) Ca^{2+} transient, (b) time to half (THALF) relaxation of Ca^{2+} transient (c) and amplitude of the Ca^{2+} transient. Data are mean ± SEM (n=36 - 60 cells from 5 – 6 hearts). Lines above bars indicate $p < 0.05$

sarcoplasmic reticulum Ca^{2+} release and uptake and Ca^{2+} transport on the Na/Ca exchange.[11, 12] Defects in myocyte contraction were not significantly improved with voluntary exercise.

In conclusion diabetic animals exercise less than controls and a 2-month voluntary exercise program was insufficient to significantly improve cardiac function in STZ-induced diabetic rat. However, even low levels of voluntary exercise, appeared to have desirable effects on glucose metabolism.

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