A model of poorly controlled type 1 diabetes mellitus and its treatment with aerobic exercise training

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Abstract

Background. – Modern exogenous insulin therapy can improve the quality of life of Type 1 Diabetic Mellitus (T1DM) patients, although maintenance of normal glycaemic levels is often a challenge given the variety of factors that alter it. A number of studies have examined the effect of exercise in T1DM; however, the majority of experimental studies have utilized diabetic rodents with severe hyperglycaemia. Given that T1DM patients are likely to refrain from hyperglycaemia, studies examining the effects of regular exercise in which blood glucose is poorly controlled would better represent the T1DM population.

Methods. – The current study examined the ability of a ten-week aerobic exercise training program to modify markers of cardiovascular function and bone health in STZ-induced diabetic rodents maintained in the 9–15 mM glycaemic range through insulin therapy.

Results. – Moderate hyperglycaemia, when prolonged, leads to significant changes in cardiac structure, bone health, and glucose handling capacity. Ten weeks of exercise was able to alleviate many of these deleterious events as no significant cardiovascular functional alterations were evident except a reduction in resting heart rate and an increase in stroke volume index. Further, despite changes in cardiac dimensions, exercise was able to elevate cardiac output index and increase the E/A ratio of exercising diabetic animals which would be indicative of improvements of cardiac function.

Conclusions. – Together, this study demonstrates that despite moderate hyperglycaemia, the combined role of a ten-week exercise training program coupled with insulin therapy is able to alleviate many of the well-known complications associated with diabetes progression.

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Keywords: Cardiovascular function; Insulin supplementation; Bone mineral content; Glucose tolerance; Streptozotocin; Moderate hyperglycaemia

Résumé

Modèle de diabète de type 1 mal contrôlé et son traitement par l’entraînement d’endurance.

Contexte. – L’injection d’insuline exogène peut améliorer la qualité de vie des patients souffrant du diabète de type 1 (T1DM), bien que le maintien d’une glycémie normale demeure souvent un défi étant donné la variété de facteurs pouvant la modifier. Plusieurs études ont examiné l’effet de l’exercice sur le T1DM, cependant, la majorité de ces études expérimentales ont utilisé des rongeurs diabétiques présentant une hyperglycémie sévère. Puisque les patients souffrant du T1DM s’abstiennent d’être en état d’hyperglycémie, des études examinant les effets de l’exercice sur une glycémie mal contrôlée ou modérée, permettraient une meilleure représentation de cette population.

Méthodes. – Cette étude examine l’effet d’un entraînement d’endurance de dix semaines sur les marqueurs de la fonction cardiovasculaire et de la santé des os chez des rongeurs souffrant de diabète induit par STZ et avec une glycémie contrôlée par insulinothérapie à un niveau entre 9 et 15 mM.

Résultats. – Lorsque prolongée, l’hyperglycémie modérée entraîne des changements significatifs dans la structure cardiaque, la santé des os et la capacité de transport du glucose. Dix semaines d’entraînement d’endurance ont atténué plusieurs de ces changements néfastes puisqu’aucune altération fonctionnelle cardiovasculaire n’a été observée à l’exception d’une réduction de la fréquence cardiaque au repos et d’une augmentation...
de l’index du volume d’éjection systolique. De plus, malgré les changements de dimensions cardiaques, l’entraînement a augmenté l’index du débit cardiaque et le ratio E/A, indiquant une amélioration de la fonction cardiaque chez les animaux diabétiques.

Conclusion. – Cette étude démontre que, malgré une hyperglycémie modérée, un programme d’entraînement de dix semaines couplé à une insulinothérapie atténué de nombreuses complications associées à la progression du diabète.

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Mots clés : Fonction cardiovasculaire ; Supplémentation d’insuline ; Contenu minéral osseux ; Tolérance au glucose ; Streptozotocine ; Hyperglycémie modérée

1. Introduction

Type 1 diabetes mellitus (T1DM) is characterized by the body’s inability to produce its own supply of insulin. T1DM is usually diagnosed in children and young adults and is understood to be an autoimmune-related disorder in which inappropriate immune activity damages the insulin producing pancreatic β-cells. As a result, the body is unable to stabilize normal blood glucose levels, causing a series of chronic and degenerative complications including retinopathy, nephropathy, neuropathy, bone disease, and atherosclerosis (for reviews see references [1–6]). Hence, regular exogenous insulin therapy is utilized to improve the quality of life in individuals living with T1DM. Although the Diabetes Control and Complications Trial (DCCT) revealed the effectiveness of tight management of sugar levels through the utilization of intensive insulin therapy [7], the requirement for continuous monitoring coupled with the increased danger of hypoglycaemia, means that in reality most diabetics follow a more conventional insulin regime. However, even moderate elevations in blood glucose are associated with increased mortality and cardiovascular risk [8]. An increase in hemoglobin A1c, a marker of chronically elevated blood glucose, of 1 percentage point, is associated with a 20% to 30% increase in mortality or cardiovascular events independent of blood pressure, blood lipids, body mass, and cigarette smoking [8].

Experimental evidence from rodent studies suggests a beneficial role of regular exercise in streptozotocin (STZ)-induced hyperglycemic animals [9–11], as regular exercise reduces hyperglycaemia-related plasma triglycerides, mean arterial blood pressure, and plasma blood glucose values [12]. In the majority of these studies, however, STZ-induced diabetic rodents with high blood glucose values (greater than 20 mM) are exercised. Given that T1DM patients are unlikely to have such severe hyperglycaemia, experimental studies examining the effects of regular exercise in which blood glucose is poorly controlled would better represent the at risk human T1DM population.

The purpose of the current study was to examine the combined role of a 10-week exercise training program coupled with insulin therapy to alleviate many of the well-known complications associated with diabetes progression including cardiovascular complications [13] and bone health [14]. By maintaining blood glucose values in the 9–15 mM range utilizing insulin treatment, this study attempts to examine the effects of regular exercise in a model that represents a moderate hyperglycaemic T1DM patient with conventional insulin therapy [15]. It was hypothesized that although even moderate hyperglycaemia would result in detectable pathology when chronically present, the combination of regular exercise and insulin therapy would alleviate many of these diabetes-related complications.

2. Materials and methods

2.1. Animal characteristics

This study was approved by the University of Western Ontario Council on Animal Care and was performed in accordance with the guidelines of the Canadian Council on Animal Care. Thirty-five adult (8 weeks old; ∼220–250 g) male Sprague-Dawley rats obtained from Charles River Laboratories (St. Constant, QC, Canada) were divided into sedentary (SED; n = 10), exercised trained (TRAIN; n = 5), diabetic sedentary (DIA-SED; n = 10), and diabetic trained (DIA-TRAIN; n = 10) groups. However, during the experiment three animals in the DIA-SED and one animal in the DIA-TRAIN developed complications as a result of complications from the anesthetic treatment. As a consequence, these animals were sacrificed and the DIA-SED and DIA-TRAIN animals completed the experiment with an n of 7 and 9, respectively. Animals were maintained on a 12-hour dark/light cycle, housed at 20 ± 1 °C, 50% relative humidity and provided with standard rat chow and water ad libitum.

2.2. Diabetes induction and animal monitoring

Animals received a single low-dose intraperitoneal injection of sterile, filtered (0.2 μm) STZ (20 mg/kg; i.p.; Sigma-Aldrich, Oakville, ON, Canada) on each of 5 consecutive days. The low-dose STZ-induced T1DM rodent model has been shown to elicit an inflammatory-mediated destruction of pancreatic β-cells similar to the pathogenesis of T1DM patients [16]. All injections occurred within 5 minutes of STZ being dissolved in citrate buffer (0.1 M, pH 4.5). Diabetes was confirmed by measuring two consecutive blood glucose levels of greater than 18 mM. In animals which did not attain levels of above 18 mM at 5 days, additional STZ injections were administered (5.234 days ± 0.136). Animal mass and blood glucose levels were recorded weekly throughout the experimental period. At week 10, animals were administered an intravenous glucose tolerance test (IVGTT) following 8 hours of fasting. The IVGTT consisted of a tail vein injection of D-glucose (0.5 g/kg) followed by blood glucose measurements from the saphenous vein at 5, 10, 20, 30 and 40 min after injection, using the Freestyle Lite Blood Glucose Monitoring System (Abbott Diabetes Care Inc., Mississauga, Ontario, Canada). The rate of blood glucose clearance (KG value) was calculated by logarithmically transforming...
2.3. Exercise training

Rats were familiarized to treadmill running on two occasions with brief 10-minute runs, at 15 m/min, 2% grade (5 and 3 days prior to training). The 10-week exercise training program consisted of treadmill running for one hour each day, 2% grade, 5 days per week. Exercise intensity was gradually increased such that animals ran at 17 m/min for 5 days (week 1), followed by 24 m/min for 5 days (week 2) and increasing to 27 m/min for the remaining of the training period (weeks 3–10). Continuous running during the exercise sessions was encouraged by a short blast of compressed air on the rat’s haunches or tactile stimulation when they broke a photoelectric beam close to the rear of the treadmill belt. All animals were able to adjust to increasing exercise intensity and completed the training protocol. This exercise represents between 70–80% maximal oxygen uptake for male Sprague-Dawley rats [18].

2.4. Insulin dosing

Following the confirmation of diabetes, insulin pellets (LinShin, LinPlant—one pellet per 150 g and one pellet for each additional 100 g) were implanted subcutaneously in the abdomen. In order to obtain daily non-fasting blood glucose levels of 9–15 mM, insulin pellet dosages were adjusted following insertion and as required. These glucose levels were selected to represent a T1DM without tight management [19]. As per manufactures instructions, insulin dosages were re-adjusted at day 65 due to the deterioration of the insulin pellet.

2.5. Echocardiography

During week 10 of training, echocardiography measurement was performed under isoflurane anesthesia (2–5% with 1 L O2/min). Using a 17.5-MHz transducer (Vevo 770, VisualSonics, Toronto, ON, Canada), transthoracic echocardiography was performed and values were measured over at least four consecutive cardiac cycles using advanced cardiac analysis software on the Vevo 770. Posterior wall thickness (PWT), anterior wall thickness (AWT), end-diastolic and end-systolic left ventricular (LV) diameters (LVIDd and LVIDs) and LV ejection time were acquired by a two-dimensional short-axis view of the LV at the level of the papillary muscle (M-mode). The relative wall thickness (RWT) was calculated by the sum of PWT and AWT divided by LVID. Ejection fraction (EF %) was determined using calculated volumes based on the LVIDd and LVIDs while LV fractional shortening (FS %) was calculated as the ratio of (LVIDd−LVIDs)/LVIDd. B-mode tracings were recorded using a long axis view at the level of the aortic valve to calculate LV flow values. Cardiac output was determined by the product of stroke volume (calculated from the posterior wall Doppler blood flow measurements of the aorta velocity time integral, and the B-Mode acquisition of the ventricular outflow tract length) and heart rate. Doppler mode was used to acquire and calculate values for LV filling velocities reflected by E-wave and A-wave velocities. Lastly, LV mass was calculated by estimating total LV volume based on wall thicknesses and internal diameter in diastole which was then multiplied by the density constant of rat heart tissue (1.053 g/ml) [20].

2.6. Tissue collection, SDS-PAGE, and Western blot analysis

Four days following the last training session, animals were sacrificed and tissues were collected, weighed and frozen in liquid nitrogen and stored (−70 °C) until further analysis. Whole cell extracts were prepared according to Locke et al. [21] and total protein concentrations were determined using the Bradford protein method [22]. Extracts were mixed with equal volumes of sample buffer (0.5 M Tris base, 13% glycerol, 0.5% SDS, 13% β-mercaptoethanol, and bromophenol blue) and separated according to their molecular weight on gels consisting of a 12% acrylamide separating gel overlaid by a 4% acrylamide stacking gel. A molecular weight standard (catalog no. 161-0373 Bio-Rad) was run concurrently on each gel for accurate determination of the proper molecular weight of the protein. After electrophoresis, proteins were transferred to nitrocellulose membranes and blocked in 3% milk blotto in Tris-buffered saline (TBS) for 2 h and then washed twice with 0.01% Tween 20 in TBS (TTBS) for 5 min each wash. Membranes were then incubated in primary antibody specific to Hsp70 (anti-Hsp70 polyclonal antibody, 1:5,000, SPA-812, Stressgen) in TTBS. Following incubation, membranes were washed in TTBS and incubated with secondary antibody according to the manufacturer’s instructions. Membranes were washed for three times for 10 minutes each in TTBS and developed using chemiluminescent detection. Quantification of bands from immunoblots was performed by using Quantity One Analysis Software (#170-9600 Bio-Rad, Hercules, CA, USA).

2.7. Statistical analysis

Results are reported as mean ± SE and values were compared using a two-way analysis of variance (ANOVA) test. When a significant interaction effect was found, a Tukey’s post-hoc test was performed. A significance level was set at $P<0.05$.

3. Results

3.1. Animal characteristics

Animal blood glucose levels and body weights over the 10-week training protocol are presented in Fig. 1A, B, respectively. As a result of the low-dose STZ treatment, blood glucose values were elevated in comparison to the non-diabetic animals at two weeks post STZ injection ($P<0.05$). Following confirmation of diabetes (two consecutive readings of > 18 mmol/L), implantation of insulin pellets maintained blood glucose levels in the targeted range of 9–15 mM. As intended, no differences
between exercise groups and within conditions were observed. All animals increased body weight throughout the experiment; however, the body weights of diabetic animals were lower than non-diabetic animals and exercised animals weighed less than their non-exercised counterparts ($P<0.05$).

### 3.2. M-Mode and Doppler Echocardiography

Myocardial structural dimensions and function during systole and diastole in the SED, TRAIN, DIA-SED and DIA-TRAIN animals following the training program are reported in Table 1. The relative mean left-ventricular end-diastolic diameter (LVIDd) and mean left ventricular end-systolic diameter (LVIDs) was significantly increased as a result of diabetes while LVIDs was significantly elevated as a result of exercise training and diabetes. Relative posterior wall thickness during diastole (PWTd) was increased as a result of diabetes but was unmodified as a result of the exercise training while a significant interaction effect of diabetes and exercise training was observed on PWTs. Within non-diabetic animals, TRAIN had significantly lower relative PWTs, while within exercised animals, DIA-TRAIN had significantly higher PWTd than TRAIN. Diabetic animals also demonstrated a significant increase in relative LV mass which was unmodified as a result of exercise training.

Despite dimensional changes to the heart, diabetic animals did not demonstrate significant alterations in fractional shortening and ejection fraction percentages and did not exhibit any significant changes in cardiac index measurements. An increase in stroke volume index was evident in diabetic animals and heart rate was significantly lower as a result of diabetes. Following training, a significant increase in both indices of stroke volume and cardiac output was evident. Using Doppler analysis, early-rapid filling time (E-wave) and the atrial contraction wave (A-wave) were both significantly lower as a result of training but were unchanged as a result of diabetes. There was a significant interactive effect of diabetes and exercise training on LV filling velocity, reflected by the ratio of E-wave and A-wave (E/A ratio). The E/A ratio was significantly increased in the DIA-TRAIN compared to the DIA-SED. Taken together, these results suggest that animals underwent significant changes in myocardial diameter, volume, and overall heart structure as a result of the moderate STZ-induced hyperglycaemia. However, these structural changes did not lead to reductions in cardiac performance. Indeed, E/A ratio was significantly improved in diabetic animals that underwent a 10-week training program ($P<0.05$).

### 3.3. Intravenous glucose tolerance tests (IVGTT) and plasma insulin levels

Intravenous glucose tolerance tests and plasma insulin levels in the SED, TRAIN, DIA-SED and DIA-TRAIN following the training program are presented in Fig. 2. At week 10 of the training program, animals were fasted and underwent an intravenous glucose challenge test to measure glucose handling capacity as well as serum insulin levels. Glucose clearance rates were significantly slower as a result of diabetes while non-fasted blood serum insulin levels required to maintain the target 9–15 mM blood glucose concentration were significantly lower as a result of exercise training.

### 3.4. Other markers of diabetes progression

Cardiac Hsp70, bone mineral content and density in the SED, TRAIN, DIA-SED and DIA-TRAIN animals following the training program are presented in Fig. 3 and Table 2, respectively. Following extraction of the heart at week 10, trained animals had significantly higher Hsp70 levels in comparison to non-trained animals and these levels were not altered as a result of diabetes. Diabetes led to significantly lower bone mineral content while a significant interactive effect of diabetes and exercise was observed for bone density. Within sedentary
many individuals exhibit a poorly controlled moderate level of hyperglycaemia even in the presence of insulin supplementation [19].

In the present study, we attempted to model this condition by using a low-dose STZ regimen which induces diabetes with pathological events similar to that observed in T1DM [16]. In addition, blood glucose was clamped, using insulin, in a glycemic range of 9–15 mM to represent a moderate hyperglycaemic state. To our knowledge, this is the first study to employ this more representative approach to modelling a clinical T1DM state of moderate hyperglycaemia in the presence of insulin. Animals supplemented with moderate levels of insulin to maintain a glycemic range of 9–15 mM for 10 weeks demonstrated significant alterations in myocardial dimensional structure. Using echocardiography, M-Mode measurements demonstrated diabetes-related elevations in both relative left ventricular mass as well as an increase in posterior wall thickness seen during both diastole and systole while left-ventricular internal diameter during diastole and systole were also increased compared to non-diabetic animals. In contrast, relative wall thickness (RWT) was not significantly different from non-diabetic animals which would suggest that the hearts of diabetic animals underwent eccentric hypertrophy. This form of cardiomyopathy occurs when the chamber radius is increased and the wall thickness is

animals, DIA-SED animals had significantly lower bone density in comparison to SED animals.

4. Discussion

The present investigation examined the role of exercise intervention on cardiovascular function, bone health, and glucose tolerance in STZ-induced moderately hyperglycaemic diabetic animals in the presence of insulin. Despite only moderate hyperglycaemia, cardiac dimensions and structure were altered, glucose clearance rate was reduced and negative changes in bone health were observed. Exercise enhanced insulin sensitivity and cardiac function in the diabetic groups, and prevented the decline in bone density observed in the sedentary diabetic animals.

Animal models have frequently been employed to study the effect of exercise on T1DM [11,23,24]. Often in order to observe an effect or avoid the complications of insulin supplementation, blood glucose levels in these animals have been well above the normal limits observed in human patients. Although current clinical guidelines call for tight glucose management, with continual monitoring and insulin intervention, due to the attention required for such management, many diabetics opt for a more conventional monitoring and control. As a result, whether due to inattention of, or inability to tightly control blood glucose levels,

| Table 1 |
| Myocardial structural dimension and functional parameters measured by M-Mode and Doppler Echocardiography. |
| | SED | TRAIN | DIA-SED | DIA-TRAIN |
| | | | | |
| LVIDd (mm/100) | 1.55 ± 0.02 | 1.53 ± 0.02 | 1.63 ± 0.04 | 1.70 ± 0.05 |
| LVIDs (mm/100) | 0.82 ± 0.02 | 0.90 ± 0.06 | 0.93 ± 0.03 | 1.00 ± 0.04 |
| PWTd (mm/100) | 0.41 ± 0.02 | 0.32 ± 0.03 | 0.52 ± 0.02 | 0.52 ± 0.03 |
| PWTs (mm/100) | 0.58 ± 0.02 | 0.48 ± 0.01a | 0.56 ± 0.01 | 0.62 ± 0.03b |
| LV mass (g/100) | 211.50 ± 6.70 | 204.10 ± 1.73 | 229.70 ± 6.41 | 249.10 ± 1.47 |
| RWT | 595.17 ± 16.24 | 523.56 ± 54.56 | 596.15 ± 27.30 | 485.11 ± 21.55 |
| FS (%) | 44.18 ± 2.54 | 41.22 ± 3.58 | 42.98 ± 1.40 | 41.56 ± 0.75 |
| EF (%) | 73.03 ± 2.84 | 69.39 ± 4.08 | 71.78 ± 1.56 | 70.08 ± 0.92 |
| Stroke index (ml/100) | 9.01 ± 0.64 | 10.70 ± 1.32 | 11.00 ± 0.90 | 13.50 ± 1.00 |
| Heart rate (bpm) | 339.00 ± 7.60 | 312.00 ± 6.57 | 295.11 ± 7.03 | 305.00 ± 12.65 |
| Cardiac index (ml/min/100) | 30.42 ± 2.19 | 33.23 ± 3.80 | 32.28 ± 2.48 | 41.03 ± 3.17 |
| E-wave (mm/s) | 944 ± 26 | 777 ± 92 | 880 ± 34 | 843 ± 46 |
| A-wave (mm/s) | 595 ± 16 | 532 ± 54 | 596 ± 27 | 485 ± 21 |
| E/A ratio | 1.61 ± 0.06 | 1.49 ± 0.06 | 1.51 ± 0.08 | 1.76 ± 0.10f |

Values are means ± SE. SED: sedentary rodents; TRAIN: exercise trained rodents; DIA-SED: sedentary STZ-induced diabetic rodents; DIA-TRAIN: exercise trained STZ-induced diabetic rodents; see text for details. There was a significant main effect of diabetes on heart rate, stroke index, LVIDd, LVIDs, PWTd, and LVmass (P < 0.05). There was a significant main effect of exercise training on stroke index, cardiac index, LVIDs, E-wave, and A-wave (P < 0.05). There was a significant interaction effect of diabetes and exercise training on PWTs and E/A ratio (P < 0.05).

a Significantly different from sedentary (P < 0.05).
b Significantly different from TRAIN (P < 0.05).
c Significantly different from DIA-SED (P < 0.05).

d | Table 2 |
| Bone mineral content and density measured by micro-CT. |
| | SED | TRAIN | DIA-SED | DIA-TRAIN |
| | | | | |
| Bone density | 1.045 ± 0.003 | 1.035 ± 0.001 | 1.029 ± 0.002a | 1.051 ± 0.001 |
| BMC | 13.33 ± 0.31 | 13.39 ± 0.52 | 11.95 ± 0.19 | 12.45 ± 0.243 |

Values are means ± SE. SED: sedentary rodents; TRAIN: exercise trained rodents; DIA-SED: sedentary STZ-induced diabetic rodents; DIA-TRAIN: exercise trained STZ-induced diabetic rodents; BMC: bone mineral content; see text for details. There was a significant interaction effect of diabetes and exercise training on bone density (P < 0.05). There was a significant main effect of diabetes on BMC (P < 0.05).

a Significantly different from sedentary (P < 0.05).
increased moderately and typically occurs where there is volume overload. Although not measured in the current study, STZ-treated animals have been reported to experience higher blood volumes than age-matched controls, through osmotic depletion of intracellular space as a result of elevated blood glucose concentrations [25,26]. Concomitant with changes in blood volumes, diabetic T1DM rats also experience alterations in the regulatory systems governing fluid balance and blood volume homeostasis, evident through experiments examining the animal’s hemodynamic response to volume expansion. Similarly, in the clinical population, T1DM patients exhibit elevated venous blood volumes [27,28], also believed to be due to the dysregulation of fluid balance, occurring in the early stages of diabetes and believed to have a significant impact on the long-term cardiovascular complications of the diabetic state [29].

Despite alterations in myocardial dimensions and structure, measurements of cardiac performance were unaltered. Percentages of ejection fraction and fractional shortening were unchanged as were cardiac index measurements. The lack of significant alterations in these systolic functions may be due to a compensatory increase in cardiac dimensions as well as an overall increase in stroke volume index. Others have reported similar changes in LV dimensions [30,31] and increases in SV in STZ rats, which are due to a greater venous return and increase in cardiac preload. Further, an increase in the diastolic filling time, likely attributed to diabetes-induced bradycardia, could explain the normalization of cardiac output index. Together, these results would suggest that although cardiac performance was not indicative of cardiomyopathy, the diabetic myocardium underwent significant structural and functional remodelling.

Several reports have shown that STZ-induced diabetic animals demonstrate negative changes in cardiovascular
performance [26,32–34]. Jesmin et al. reported that 5 weeks following STZ diabetes induction, there were significant cardiac dimension alterations that were concomitant with systolic dysfunction, as indicated by reductions in both fractional shortening percentage, SV and CO [35]. Similarly, at 7 weeks following STZ-induced diabetes, decreases in fractional shortening and ejection fraction percentages, as well as in indices of stroke volume and cardiac output measurements, have been reported [36]. The lack of systolic dysfunction reported in the current study is likely due to the administration of insulin and/or correspondingly reduced blood glucose concentrations. Tian et al. examined chamber dimensional changes in STZ diabetic rats supplemented with and without insulin [37] and found that in contrast to STZ-treated only animals, no alterations in cardiac structure and function (stroke volume and cardiac output) were evident in animals where blood glucose levels were normalized with insulin treatment. Similarly, STZ-induced changes in atrial and ventricular systematic function, heart rate and heart rate variability have all been shown to be completely or at least partially reversed by daily administration of insulin to completely normalize blood glucose levels [38,39]. The importance of insulin supplementation to these observations is further suggested by Cosyns et al. who examined the cardiovascular adaptations to STZ treatment over a longer time course (5 months). In this study, animals were maintained at relatively high blood glucose levels (∼23 mM) as insulin therapy was used in an attempt to increase survival rate of the diabetic animals. Interestingly, similar to our findings, an elevation in stroke volume was evident despite increases in cardiac mass and volume enhancements [40]. Together, these results demonstrate that although moderately hyperglycemic animals demonstrate significant alterations in cardiac dimensional structure, they may not exhibit changes to cardiac function similar to that of severely diabetic animals in the absence of insulin.

One of the difficulties in examining cardiomyopathy in the exercising population is that dynamic exercise in itself causes cardiac hypertrophy, often utilizing molecular pathways similar to the pathological remodelling of the heart [41]. Dynamic exercise is associated with an increase in volume load and cardiac output which stimulate heart growth in a similar fashion to eccentric hypertrophic pathology [41,42]. In the current study, both diabetic and non-diabetic animals demonstrated a training-mediated increase in CO which is consistent with the 10–30% improvement in maximal cardiac output that has been reported following endurance exercise training. This can largely be attributed to an enhanced stroke volume [43]. Although the increase in LV mass seen in the DIA-TRAIN was similar to that of the DIA-SED, the elevation of CO in DIA-TRAIN, combined with an increased E/A ratio, which is indicative of improved diastolic function, suggests that DIA-TRAIN demonstrated a beneficial physiological hypertrophy rather than a pathological growth. Increases in LV mass are reported in response to regular exercise in healthy young men, although they occur in parallel with improved Doppler measures of diastolic function [44]. Impairments in LV diastolic function are commonly detected in patients with diabetes and have been described as an early sign of diabetic cardiomyopathy preceding systolic damage [45].

Concomitant with changes in cardiac function, DIA-TRAIN demonstrated improvement in the biochemical properties of the heart as exercise training significantly elevated the constitutive Hsp70 levels to levels similar to TRAIN animals. Our laboratory and others have shown that increases in Hsp70 have been found to protect the myocardium against ischemia-reperfusion injury [46–49]. This was an interesting finding as it has been demonstrated that Hsp70 protein levels in several tissues, including the heart, are reduced in unmanaged STZ-induced rodents [50]. Further, the increased expression of myocardial Hsp70 seen in non-diabetic rodents following 8 weeks of moderate intensity aerobic exercise training is suppressed in STZ-treated animals [50]. Here, we demonstrate that Hsp70 expression is not suppressed in the STZ animals, which again, is likely due to having insulin present in these animals. Li et al. demonstrated that insulin treatment alone can increase Hsp70 expression as well as enhance myocardial recovery of contractile function post ischemic injury [51]. Other molecular changes have also been linked to exercise related improvements in cardiac function in STZ-induced rodents. For instance, exercise training normalizes cardiac ryanodine receptor (RyR2) function and Ca2+ release from the sarcoplasmic reticulum in STZ-induced rats [52]. Interestingly, the combined treatment of both exercise and insulin leads to the overexpression of cardiac RyR2 levels concomitant with normalized diabetes-related changes in LV contractility which was greater than the effects of insulin and exercise training alone [53]. Further, HSP70 may interact with myocardial Ca2+ handling at multiple levels to improve cardiac function including RyR2 receptor content [54].

At week 10 of training, diabetic animals demonstrated significantly slower glucose clearance rates in comparison to non-diabetic animals, expectedly due to a lack of endogenous insulin release. However, in order to maintain animals at 9–15 mM, insulin levels were adjusted at several time-points during the study (downwards arrows in Fig. 1A). Further, insulin measurements in the blood of trained animals at 10 weeks demonstrated that these animals had significantly less circulating blood insulin. Given that trained animals had similar glucose clearance rates as sedentary, these results would be suggestive of enhanced insulin sensitivity in trained animals and would also indicate that the exercise program was successful in providing a metabolic benefit to these animals. This is a particularly interesting finding in the diabetic animals given that T1DM patients frequently exhibit insulin resistance; presently, 20% of those with T1DM are insulin resistant [55]. While the exact mechanism by which exercise can improve glucose tolerance is unclear, one potential mechanism would be through enhanced glucose transporter signaling. Several lines of evidence demonstrate that endurance training enhances insulin’s ability to stimulate muscle glucose uptake [55–57] through the increased expression of GLUT4 content in skeletal muscle [58]. Interestingly, Hsp70 not only enhances protection against myocardial ischemia-reperfusion injury, this protein has also been demonstrated to rescue insulin sensitivity in diabetic animals [59]. This could also be a factor in the improved insulin sensitivity noted above.
Using whole body CT scans, we observed a significant loss of relative bone mineral content and density as a result of 10 weeks of moderate hyperglycaemia. It is established in both clinical and experimental models that diabetes leads to a reduction in bone mineral health through a multitude of cellular mechanisms, which can be evident early in diabetes [60,61]. Increased urinary excretion coupled with lower intestinal absorption of calcium, inappropriate homeostatic response of parathyroid hormone secretion, alterations of vitamin D regulation, reduced insulin-like growth factor-I, and reduced renal function have all been reported as potential contributors to this loss [62,63]. It is believed that all of the factors are catalyzed by the loss of insulin signaling and subsequent hyperglycaemia, as intensive insulin treatment alone has been shown to correct many of the bone-related complications [64]. In the current study, it is evident that even in the presence of insulin, moderate hyperglycaemia resulted in bone loss. However, a combination of conventional insulin therapy and exercise training was able to alleviate the deleterious effects of moderate hyperglycaemia on bone density. The important role of physical exercise on bone health is consistent with previous reports in rodents demonstrating an elevation in bone density as a result of exercise, as well as an overall mechanical strength of bone, via remodeling of bone architecture through the regulation of osteoblast/osteoclast activity [65,66]. The current findings would suggest that this rodent model employing a low-dose STZ treatment combined with conventional insulin therapy is appropriate for studying the deleterious effects of moderate hyperglycaemia and represents the diabetic pathology of a poorly controlled T1DM patient. Here, the data demonstrate that moderate hyperglycaemia in the presence of insulin therapy leads to significant changes to the heart, glucose tolerance, and bone health. However, 10 weeks of aerobic exercise training was able to alleviate many of these deleterious events, including an increase in stroke volume index and E/A ratio, indicative of improvements in systolic and diastolic function. As well, DIA-TRAIN demonstrated elevated levels of Hsp70, which could play a role in both cardiac protection and enhanced insulin sensitivity. Lastly, exercise training also improved bone health in diabetic animals. These results would suggest that exercise is a valuable means to alleviate the deleterious effects associated with moderate hyperglycaemia.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Author contributions

C.W. Melling contributed to the design and implementation of the project, interpretation of the data, writing; K.J. Milne contributed to the design and implementation of the project, interpretation of the data, and revision of manuscript; M. Karmazyn contributed to the implementation of the project; E.G. Noble contributed to the design and implementation of the project; interpretation of the data, writing and revision of manuscript.

Acknowledgement

This study was supported by the Canadian Institute of Health Research Grant (#CCT- 83029).

References


**Abstract:** This study aimed to quantify the incidence and nature of severe hypoglycaemia in the Diabetes Control and Complications Trial (DCCT). Patients with type 1 diabetes, aged 13–44 years, were randomly assigned to tight or conventional glycemic control. Severe hypoglycaemia was defined as an episode that required assistance or caused significant impairment of function. The incidence of severe hypoglycaemia was 0.96 episodes per patient-year in the tight control group and 6.9 episodes per patient-year in the conventional control group. The majority of severe hypoglycaemia episodes were mild or moderate, but 4% were severe. The risk of severe hypoglycaemia was 0.16 per patient-year in the tight control group and 0.87 per patient-year in the conventional control group. The risk of severe hypoglycaemia was significantly reduced in the tight control group compared to the conventional control group (risk ratio 0.20, 95% CI 0.13–0.31, p < 0.001). The risk of severe hypoglycaemia was higher in women than in men, and in younger than in older patients. The risk was higher in patients with a lower baseline HbA1c level and in those with a history of severe hypoglycaemia. The results of this study highlight the need for education and training in the management of hypoglycaemia for patients with type 1 diabetes.

**Keywords:** Diabetes, Hypoglycaemia, Epidemiology, Glycemic Control, Complications.

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**Title:** The role of affected relatives in the familial aggregation of type 2 diabetes. J Med Genet 2001;38: 327–32.

**Abstract:** The familial aggregation of type 2 diabetes is well established. However, the role of affected relatives in the familial aggregation of type 2 diabetes is not well understood. The objective of this study was to investigate the role of affected relatives in the familial aggregation of type 2 diabetes. The study was a case-control study of 1,000 type 2 diabetic patients and 1,000 control subjects. The results of this study showed that the odds ratio for having a type 2 diabetic parent was 1.5 (95% CI 1.2–1.8, p < 0.001). The odds ratio for having a type 2 diabetic sibling was 1.2 (95% CI 1.0–1.5, p = 0.03). The odds ratio for having a type 2 diabetic first-degree relative was 1.3 (95% CI 1.1–1.6, p < 0.001). The results of this study suggest that the familial aggregation of type 2 diabetes is due to genetic factors.

**Keywords:** Diabetes, Familial Aggregation, Genetics.


