Effects of Moderate Cycling Exercise on Blood Glucose Regulation Following Successful Clinical Islet Transplantation

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Funding: This project was funded by an Alberta Diabetes Institute Pilot Project grant.

Disclosure: JEY has received speaker's fees from Animas Canada, Dexcom Canada, and in-kind research support from Animas Canada, Medtronic Canada, Abbott Nutrition, and Ascensia Diabetes Care Canada. NB has received in-kind research support from Medtronic Canada and research funding from Almased. PAS has received consulting fees and/or speaker's fees from Abbott, Astra-Zeneca, Boehringer Ingelheim, Dexcom, Eli Lilly, Insulet, Janssen, Merck, Novo Nordisk and Sanofi; and grant support from Astra-Zeneca, Prometic, Novo Nordisk, Sanofi and Viacyte. The other authors report no conflicts of interest in this work

Short title: Glucose response to exercise post islet transplant

Abstract

Context: Islet transplantation is effective in preventing hypoglycemia in individuals with type 1 diabetes (T1D). However, it is currently unknown whether transplanted islets regulate plasma glucose concentrations appropriately during and after exercise in human islet transplant recipients (ITx).

***Objective*:** To determine the effect of exercise on plasma glucose, insulin, and glucagon concentrations in ITx compared to individuals without diabetes (CON).

Intervention: Participants completed two conditions in random order, 1) 45 minutes of aerobic exercise (60% VO_{2peak}), and 2) 45 minutes of seated rest. Blood samples were drawn at baseline, immediately post-exercise/rest, and every 15 minutes throughout a 60-minute recovery period. Post-exercise (24 hours) interstitial glucose was monitored using continuous glucose monitors.

Results*:* Twenty-four participants (12 ITx, 12 CON) completed the protocol. Plasma glucose decreased more over time with exercise in ITx compared to CON [main effects of treatment ($p=0.019$), time ($p=0.001$) and group ($p=0.012$)]. Plasma glucose was lower during exercise versus rest in ITx, but not CON [treatment by group interaction ($p=0.028$)]. Plasma glucose decreased more during exercise versus the rest session [treatment by time interaction ($p=0.001$)]. One ITx and one CON experienced plasma glucose concentrations <3.5 mmol/L at the end of exercise, both of whom returned above that threshold within 15 minutes. Nocturnal CGM glucose below 3.5 mmol/L was detected in two CON, but no ITx.

Conclusion: Despite a greater plasma glucose decline during exercise in ITx, hypoglycemia risk was similar during and following exercise in ITx compared to CON.

Keywords: Type 1 diabetes, islet cell transplant, aerobic exercise, glucoregulation

Précis

Blood glucose (BG) and insulin were measured after exercise and rest in ITx and CON. Insulin decreased less and BG decreased more in ITx than CON. ITx had no nocturnal hypoglycemia.

1. Introduction

Type 1 diabetes (T1D) is an autoimmune disorder that results in the destruction of insulin-secreting beta cells in the pancreas, leading to insulin deficiency and the necessity for exogenous insulin [1, 2]. Hypoglycemia subsequently becomes a major risk in this population, as insulin dosage must be carefully matched to carbohydrate intake while also considering physical activity levels [3, 4]. Islet transplantation (ITx) is an effective treatment for the prevention of severe hypoglycemia [5]. This procedure involves the intra-hepatic transplantation of pancreatic islets isolated from cadaveric organ donors into an individual with T1D, via the portal vein [6]. While not completely normalizing glucose regulation [7], this procedure can restore glucose-dependent insulin secretion [8, 9], and improve glucagon release in the face of declining blood glucose concentrations [10, 11].

In T1D, regular physical activity is inversely related to mortality risk, and may increase longevity while lowering the risk of diabetes-related complications [12, 13]. Nevertheless, aerobic exercise has also been shown to substantially increase the risk of hypoglycemia in individuals with T1D [3, 4]. In healthy individuals, glucose concentrations will remain relatively unchanged during an acute bout of moderate intensity aerobic exercise, despite the increased demands for glucose by working muscles [14]. This stability can be attributed to reductions in insulin secretion and increases in hepatic glucose production to match the increased glucose uptake [15]. In individuals with T1D, the risk of exercise-induced hypoglycemia is of concern [16, 17] due to exogenous hyperinsulinemia combined with impaired, or even absent glucagon response from the alpha cells [18].

Animal studies suggest that islet cell transplantation may not provide adequate glucoregulation during moderate intensity aerobic exercise. A recent review by Funk et al.

(2017) identified five studies of exercise following islet cell transplantation in animal models of T1D [19]. Despite four of the five studies observing euglycemia [20-23], three noted impaired insulin suppression or a slower return to baseline of insulin concentrations following exercise in the islet transplant recipient (ITx) animals, as compared to controls without diabetes [21, 23, 24]. Similarly, a recent study involving human participants having undergone a total pancreatectomy and islet autotransplantation, found significant decreases in insulin levels during exercise in control participants, while no change in insulin levels was found in the transplant recipients [25]. This finding hints at the importance of the sympathetic nervous system's role in suppressing insulin release from native beta cells in the pancreas during exercise [26-28], which may be delayed, incomplete, or even absent when these cells are transplanted to the liver. Moreover, two animal studies that measured glucagon found its response to be highly variable [23, 24], and the one study involving human autotranplant recipients found no changes in glucagon with moderate exercise [25].

In humans, insulin clamp studies demonstrate impaired glucagon and epinephrine responses to hypoglycemia in ITx [10, 29, 30], with greater recovery of epinephrine responses upon longer-term follow-up [11]. To date there are no studies examining the effects of exercise on glucose regulation in clinical ITx. The present study addresses this gap by examining the plasma glucose response, as well as the related changes in insulin, c-peptide and glucagon concentrations, to moderate intensity aerobic exercise following successful clinical ITx. It is hypothesized that ITx recipients will show less of a decline in circulating insulin during exercise, along with a greater decrease in plasma glucose, compared to individuals without diabetes (CON).

2. Material & Methods

Insulin independent islet transplant recipients were identified through the University of Alberta Clinical Islet Transplant Program. Matched healthy controls were recruited using snowball sampling and convenience sampling (i.e. family members, colleagues, friends, etc.). Participants were matched for sex with the goal of also matching closely for height (±10 cm), weight (±2 kg), age (±5 years), and physical activity level (Godin Leisure-Time Questionnaire). Islet recipients were taking tacrolimus (target trough levels 8-10 ng/ml or as clinically indicated) and mycophenolate (up to 1g bid) for immunosuppression, and had not received corticosteroids. Exclusion criteria included taking exogenous insulin, or having a glycated hemoglobin (A1C) >7.5% (58.5 mmol/mol), blood pressure >150/95 mmHg, angina, and/or any other condition or injury that would contraindicate exercise (e.g., lower limb injury). All volunteers provided written informed consent. The study was approved by the University of Alberta Health Research Ethics Board (Biomedical), in accordance with the Declaration of Helsinki.

Experimental design

Participants performed resting and exercise conditions on two separate days, at least 48 hours apart, and in random order as determined by coin flip. Testing took place at the same time of day for both treatments (late afternoon or early evening, ~1700h). Participants were provided with food logs to assist them in replicating the composition and timing of food consumption on both testing days, and for 24 hours after each testing session. At least 24 hours before the first testing session, participants visited the laboratory for baseline assessment and to have a blinded continuous glucose monitoring (CGM) sensor (Enlite™ with iPro®2 CGM, Medtronic, Northridge, CA, USA) inserted subcutaneously in the abdominal region. Participants were

instructed to test and record their capillary glucose values on the provided food logs four times per day with a OneTouch® Ultra®2 glucose meter and test strips (LifeScan Milpitas, CA, USA). Participants were given a pedometer (Yamax DigiWalker 200, Yamax Corporation, Tokyo, Japan) to monitor their daily step count, and record it in the provided log.

Baseline Testing & Measurement of VO_{2peak}

The baseline visit (non-fasting) was included to establish consent and eligibility. Height, weight and seated blood pressure measurements were taken, and a peak oxygen consumption (VO_{2peak}) test was completed on a Monark Ergomedic 894E Peak Bike (Monark, Varberg, Sweden) with a weight basket. During the test, resistance was increased each minute until volitional fatigue was reached. Males began at 1.0 kiloponds (kp) and 0.3 kp was added each minute while females began at 0.5 kp and 0.2 kp was added each minute. The rate of perceived exertion (RPE) was assessed using a numerical scale. Heart rate (HR) was monitored using a Polar heart rate monitor belt and watch. A Parvo Medics TrueOne® 2400 Metabolic Measurement System (Sandy, Utah, USA) was used to measure oxygen consumption and carbon dioxide production. Capillary glucose was also measured pre- and post-exercise.

Exercise and Resting Interventions

The exercise session consisted of a 45-minute bout of moderate intensity (60% of the participant's predetermined VO_{2peak}) aerobic exercise on a cycle ergometer. One hour of seated recovery followed the exercise session. The resting control session was of equal duration, during which the participant sat quietly in a chair. Indirect calorimetry was completed from minutes 5-10 and 35-40 during the exercise and seated rest conditions to confirm participant effort levels. The rate of perceived exertion was assessed every 5 minutes during exercise to assess the perceived difficulty of the exercise session.

Upon arrival at the laboratory an IV catheter was inserted to facilitate the drawing of blood samples throughout exercise and recovery. Venous blood samples were taken at the beginning of exercise or seated rest (time 0), end of exercise or seated rest (minute 45), and every 15 minutes during recovery (minutes 60, 75, 90, and 105). Each blood sample was collected into a 10-mL EDTA vacutainer tube. Subsequently, 2.0 mL were transferred into a tube with 6.7 µL aprotinin (Millipore, MA, USA). A further 0.25 mL whole blood was transferred into 1.0 mL ice-cold 8% perchloric acid. Aprotinin and perchloric acid were added to inhibit proteases known to interfere with the determination of glucagon and to deproteinize the samples, respectively. The EDTA tubes were centrifuged at 1500 x g for 10 minutes at 4°C. The tubes containing aprotinin and perchloric acid were centrifuged at 2000 x g for 15 minutes at 4°C. Following centrifugation, the samples were immediately moved to a -80°C freezer until assays were completed.

Glucose was measured using the hexokinase timed end point method on a Siemens ADVIA 1800 chemistry system with Siemens ADVIA chemistry glucose hexokinase_3 concentrated (GLUH-c) reagent. Glucagon, c-peptide and insulin were measured using a Multi-Spot® Assay System with a Sector® Imager 2400 (Meso Scale Discovery®, MD, USA). All assays were run in duplicate and the average was reported. With the glucagon assays some values were close to the lower detection limit. Where one value was available these were included in the analysis. Where both values fell below the detection level, that participant was excluded from the analysis.

Statistical Analysis

The analyses differed according to the comparison of interest. For the descriptive characteristics and most CGM outcomes, ITx and CON were compared by one-way ANOVA.

Background physical activity (steps per day) was compared using a 2×2 ANOVA. Data included in the CGM analysis (i.e., mean 24-hour glucose, MAGE, SD, time spent above 9.9 mmol/L or below 3.5 mmol/L) commenced at 'minute 0' following the exercise and seated rest conditions and persisted for a 24-hour period. Mean 6-hour, 12-hour and 24-hour glucose also commenced at 'minute 0' following exercise. CGM standard deviation and mean amplitude of glycemic excursions (MAGE) were calculated using EasyGV© software (www.easygv.co.uk). The percent of time spent above 9.9 mmol/L, below 3.5 mmol/L and in range (i.e. 3.5 to 9.9 mmol/L) as measured by CGM are presented as median±IQR. These outcomes were compared between conditions by Wilcoxon Signed Rank Test and compared between groups by Mann-Whitney U Test.

For the blood samples collected immediately before and after exercise, 2×2×2 factorial ANOVAs were used to examine the main and interaction effects among treatments (i.e., exercise vs. rest; repeated measures), group (i.e., ITx vs. CON), and time (i.e., pre vs. post exercise; repeated measures) for plasma glucose, insulin, C-peptide, and glucagon. When examining these outcomes during the 1-hour recovery period, a 2×2×4 was used to examine the main and interaction effects among treatments, groups and time (i.e., 4 blood samples taken 15 minutes apart). Insulin and C-peptide concentration as well as the insulin to C-peptide ratio (ICR) showed skewed distribution and were log transformed before the analyses. Other data are presented as mean ± SD. The α- was set at 0.05 and two-tailed tests were chosen. Data were analyzed using SPSS 25.0 software (IBM, Amonk, New York, USA).

3. Results

Twelve insulin independent ITx and 12 CON of the same sex (i.e., 5 males and 7 females per group) completed the protocol at the University of Alberta between September 21, 2015 and February 3, 2017. There were no significant differences between the groups with respect to height, weight, and age (Table 1). Glycated hemoglobin was lower in CON ($p<0.0001$). Despite efforts to match for physical activity levels (no difference in Godin Leisure Time Questionnaire scores), aerobic capacity (VO_{2peak}) was lower ($p=0.002$) in ITx. Hematocrit ($p=0.0004$) was also lower in ITx, while resting heart rate was higher ($p=0.01$) compared to CON.

Plasma glucose

Plasma glucose levels were not significantly different between groups at the beginning of the exercise session (ITx = 6.1 ± 1.0 mmol/L vs. CON = 5.5 ± 1.0 mmol/l; $p=0.16$). Glucose levels were, however, higher in the ITx group compared to the CON group at the beginning of the rest session (ITx = 6.9 ± 2.0 vs. CON = 5.1 ± 1.0; $p=0.02$). Within the CON and ITx groups, there were no significant differences between the baseline exercise and rest session plasma glucose concentrations ($p=0.12$ and 0.15 respectively).

The effects of exercise and rest on plasma glucose are presented in Figure 1A. The 2×2×2 ANOVA demonstrated that plasma glucose levels were higher in ITx compared with CON ($p=0.012$, ITx=11, CON=10); lower on exercise compared with rest days ($p=0.019$); with a very clear decline in plasma glucose over time ($p=0.001$). The decrease in glucose was more pronounced during exercise compared to rest (time by condition interaction; $p=0.001$). Plasma glucose was lower during exercise versus rest in ITx but not CON (treatment by group interaction; $p=0.028$). During the 60-minute recovery period, plasma glucose was higher in ITx than CON (main effect of group; $p=0.008$). Compared with CON, plasma glucose levels

increased during recovery in ITx after exercise, while plasma glucose levels were unchanged following rest (interaction of time by treatment by group; p=0.005).

Plasma glucose concentrations below 3.5 mmol/L was only observed in one ITx at the completion of exercise (3.2 mmol/L). Blood glucose levels increased to over 3.8 mmol/L within 15 minutes of exercise completion without intervention. One CON experienced a blood glucose decline to 3.5 mmol/L, and also recovered within 15 minutes.

Glucagon

There were no baseline differences in glucagon between ITx and CON in the exercise condition (p=0.57) or the rest condition (p=0.30). Glucagon concentrations (ITx=11, CON=9) were similar in ITx compared with CON (effect of group: p=0.46), but were higher on exercise days compared with rest days (effect of treatment: p=0.013) (Figure 1B). There was no clear change in glucagon levels during the 45 minutes of exercise or rest (effect of time: p=0.082). There was an overall trend towards greater increases in glucagon during exercise compared to rest [time and treatment interaction approaching statistical significance (p=0.066)].

However, during the 60-minute recovery period glucagon levels were higher after exercise (effect of treatment, p=0.001) and decreased throughout recovery [main effect of time (p=0.01, ITx=9, CON=7)]. Glucagon levels decreased after exercise but remained stable after rest [interaction of time and treatment approached statistical significance (p=0.066)]. It should be noted that several of the samples for CON fell either close to or below the detection limit of our assay. As such duplicate results were not always available and outcomes should be interpreted with caution.

Insulin, C-peptide and Insulin:C-peptide Ratio

There were no baseline differences in insulin or c-peptide between ITx and CON in the exercise condition ($p=0.35$, $p=0.28$, respectively), while in the rest condition there was no difference for c-peptide ($p=0.18$) but insulin was higher in ITx vs CON ($p=0.048$). Plasma insulin levels (Figure 1C) and c-peptide levels (Figure 1D) were both higher in ITx compared to CON (effect of group: $p=0.011$, $p=0.047$, respectively). Exercise was associated with lower insulin and c-peptide levels compared with rest (effect of treatment: $p=0.005$, $p=0.016$, respectively) with a significant decrease in both insulin and c-peptide levels during exercise in both groups (effect of time; $p<0.001$ for both). Insulin and c-peptide levels decreased more during exercise compared to the resting control session, but the decrease in insulin levels was less in ITx compared to CON over time [interactions of time by treatment ($p<0.001$), a time by group interaction ($p=0.021$), and a time by treatment by group interaction ($p=0.017$)]. C-peptide was lower during exercise in CON than in ITx (treatment by group interaction, $p=0.015$). During recovery (ITx=9, CON=8) exercise was associated with lower c-peptide compared to the resting session (effect of treatment: $p=0.01$).During the 60-minute recovery period there were no significant differences in insulin levels between ITx and CON or between exercise and rest days although c-peptide levels were lower after exercise compared to the resting session (effect of treatment: $p=0.01$).

Overall, while c-peptide showed a similar pattern to insulin, differences were examined using insulin:c-peptide ratio (ICR; ITx=11, CON=11, figure 1E). ICR was higher in ITx compared to CON (effect of group: $p=0.02$) and changed over time during each session (effect of time: $p<0.01$). There was also an effect of treatment, with ICR being lower during the exercise session compared to the resting control session ($p=0.03$). In addition, the ICR increased during resting control in CON while it decreased in ITx [significant time by treatment ($p=0.001$), and

time by group (p=0.035) interactions], and decreased to a greater extent over time during exercise in CON compared to ITx [time by treatment by group (p=0.008)]. During recovery (ITx=9, CON=8) there was a significant effect of time (p=0.01) and a time by group interaction (p=0.05) as ICR decreased over time in CON while remaining unchanged in ITx.

Continuous glucose monitoring

A summary of CGM data can be found in Table 2. Overall, ITx had significantly higher mean interstitial glucose concentrations (Figure 2) in the 24-hour period following testing sessions compared to CON (p<0.001). They also had greater glucose variability compared to CON, as measured by SD (p=<0.001) and MAGE (p=0.001).

With respect to time spent in range for interstitial glucose, CON spent more time in range than ITx in the 6 hours following testing sessions. This difference was mostly due to ITx spending more time above 10.0 mmol/L (Table 2). Contrary to our hypothesis, however, interstitial glucose values below 3.5 mmol/L were not more common in ITx than in CON, and no statistically significant differences between groups were seen. Overnight, two CON experienced CGM glucose < 3.5 mmol/L compared to no ITx after the resting control session, and one CON versus no ITx after the exercise session.

Background Physical Activity

In assessing self-reported steps per day (measured by study pedometers) there was no effect of treatment (p=0.262). Step counts were lower (p=0.048) in ITx (n=9; exercise = 6567 ± 2858 steps; rest = 5184 ± 3493 steps) compared to CON (n=11; exercise = 8894 ± 3869 steps, rest = 8307 ± 3341 steps), however there was no condition by group interaction.

4. Discussion

This is one of the first studies examining the effects of moderate intensity aerobic exercise on blood glucose regulation in clinical ITx. In line with our hypothesis, plasma glucose concentrations decreased to a greater extent in ITx compared to CON during 45-minutes of exercise, albeit from a higher baseline level. Although insulin independent, ITx had higher plasma glucose levels compared to CON, consistent with sub-normal beta cell mass. Despite this, ITx also had higher circulating insulin concentrations and ICR. Consistent with one other study in humans [25], there were no differences in glucagon concentrations between ITx compared to CON during 45-minutes of exercise and 60 minutes of recovery.

Perhaps one of the most striking findings from this study was that overall, despite higher circulating insulin concentration and greater declines in plasma glucose during exercise compared to CON, only one ITx experienced blood glucose concentrations below 3.5 mmol/L during exercise. In addition, the one participant that did drop below this threshold recovered quickly once exercise stopped, without requiring glucose intake. Reassuringly, ITx did not experience nocturnal hypoglycemia post-exercise, thereby alleviating the major fear associated with exercise and physical activity for individuals with T1D.

The smaller change in insulin levels despite a greater decrease in glucose in ITx vs CON during exercise suggests that inhibition of insulin secretion from intrahepatic islets may not be completely normal. Whether this is because of lack of innervation or altered microenvironment including paracrine factors is not known. In native beta cells, the sympathetic nervous system plays an important role in insulin suppression during exercise. As a result, insulin levels will decrease throughout moderate intensity exercise [28, 31], and will not increase in proportion to rising blood glucose levels during high intensity efforts [26, 27]. The possibility that hepatic clearance of insulin may be reduced in ITx, where insulin is secreted within the liver from intra-

hepatic islets, compared with portal delivery of insulin to the liver from the native pancreas of CON should be considered [32]. This inference is supported by the differences in ICR between ITx and CON observed both during a period of rest, as well as during exercise. In addition, higher plasma glucose levels despite higher insulin levels in ITx may suggest some degree of insulin resistance relative to CON. This might be as a result of the diabetogenic effects of immunosuppressant drugs (particularly tacrolimus) or differences in body composition.

In the present study, insulin concentrations decreased in ITx during exercise, but not to the same extent as they did in CON. While insulin suppression during exercise has been found in all animal models to date regardless of exercise duration, exercise modality, or transplantation site [19], three studies have found that insulin suppression is either less pronounced in ITx compared to controls [23, 24] or that ITx take longer to return to baseline post-exercise [21]. In addition, a recent human study found that individuals with autotransplanted islets did not demonstrate a decrease in insulin levels during exercise, where significant declines in insulin were seen in control participants [25]. It has been proposed that since epinephrine and norepinephrine infusion have had little impact on insulin suppression [21], that sympathetic neural influences may be playing a role [21, 22]. While animal studies would suggest that sympathetic re-innervation of transplanted islets may occur within weeks of the procedure [21, 22], it is possible that it is insufficient for appropriate suppression of insulin secretion during exercise. Although the higher circulating insulin concentrations in the present study may simply reflect higher blood glucose levels, there may also be a contribution from incomplete innervation of transplanted islets.

Sympathetic stimulation of alpha cells in the native islets might also explain why glucagon increased in response to exercise, and the subsequent 60-minute recovery, in a similar

way in both ITx and CON. Although Bellin et al, [33] found defective glucagon responses to stepped hypoglycemia in autoislet transplant recipients these studies were conducted in a resting state using exogenous insulin to induce hypoglycemia in a population with significant risk factors for hypoglycemia (total pancreatectomy and gastroenteric reanastamosis). Most studies involving exercise are in rodent models, where significant increases in glucagon in response to 30 minutes of treadmill running were found in STZ-induced diabetic rats after islet allo-transplantation [24]. The glucagon response to exercise was consistent whether islets were transplanted into the liver, the kidney, or the peritoneum despite the fact that insulin responses to exercise were different across each of these transplant sites, raising questions about the relative roles of native alpha cells versus transplanted alpha cells.

An unexpected finding of our study was that ITx experienced hyperglycemia to a greater extent after exercise than after the resting control session. This rebound in blood glucose concentrations has also been noted after aerobic exercise in T1D individuals who have not undergone islet transplantation [34]. The higher blood glucose concentrations in ITx compared to CON after the resting control session is most likely caused by the evening meal. This post-prandial hyperglycemia may reflect the relatively limited functional beta cell mass in ITx [9]. One explanation as to why this was exacerbated after the exercise session is that native alpha cells, in the absence of the suppressing effect of insulin from neighboring beta cells, may have continued to produce glucagon for an extended period post-exercise [35]. While there are currently no studies that can support or refute this speculation, a further examination of this phenomenon is warranted.

While not statistically significant, the finding that CON would experience more CGM glucose measurements below 3.5 mmol/L than ITx overnight following exercise was reassuring

for ITx. Previous CGM studies of individuals with normal glucose tolerance have shown that it is not unusual for people without diabetes to experience interstitial glucose concentrations <3.9 mmol/L [36-39]. One study by Wang et al. (2012) found that 49% of individuals assessed as having normal glucose tolerance experienced interstitial glucose measurements below 3.9 mmol/L during a 3-day period of sensor wear [36]. Another study of 74 participants wearing CGM for 3 to 7 days, found that 1.1% of daytime CGM readings in nondiabetic individuals were below 3.9 mmol/L with twice the number of readings below the same threshold at night [37]. These values can increase to over 9% during endurance exercise [38]. While it is possible that some of the nocturnal values occur when participants fall asleep in a position that deprives blood flow to the area of the sensor [40], daytime values below 3.9 mmol/L still occur in individuals without diabetes. Our CON CGM data are thus in line with existing literature. The fact that ITx experienced no CGM glucose values below 3.5 mmol/L following exercise can be interpreted as an indication that the transplant procedure is successfully preventing low blood glucose from occurring.

Participants were matched as closely as possible for physical activity level, but the aerobic capacity of ITx was significantly lower than CON, likely due to the low hematocrit. Lower VO_{2peak} values are consistent with another study comparing pancreatectomized and islet autotransplanted individuals to controls without diabetes [25]. In the present study, the low hematocrit is likely multi-factorial with contributions from immunosuppression (particularly mycophenolate mofetil), autonomic neuropathy (where erythropoietin release is blunted [41], and suggested by the high resting and low peak heart rate) and anemia of chronic disease. In theory, however, this means that CON would have expended more energy during exercise than ITx, which could have decreased the difference between the two groups with respect to changes

in blood glucose during exercise. In addition, a higher level of aerobic fitness has been associated with less suppression of insulin during exercise, and a less pronounced glucagon response [31, 42]. Taken together, these studies would indicate that a greater difference could be expected between our ITx and CON groups in insulin concentrations (and potentially plasma glucose as a result) had their fitness levels been more closely matched.

While the sample size for this particular study was adequate for a repeated measures design, a larger sample would be required for subgroup comparisons. For example, it would be beneficial to determine whether or not sex, age, physical fitness, or the time elapsed since the transplant procedure have an influence on blood glucose regulation during exercise in ITx. Future studies examining these aspects individually or as part of a study with a larger sample size are warranted.

The small number of blood samples taken during the exercise sessions also proved to be a limitation in the interpretation of the data. More frequent samples throughout may have provided more clarity in terms of the rates of change for the hormones involved, as well as for the blood glucose concentrations. These details could have provided more room for extrapolation and further interpretation. In spite of these limitations, this is the first study to examine how transplanted islets respond to the stress of exercise in human ITx and highlights the need for future studies of different exercise intensity, modality, and duration.

5. Conclusion

Our data suggest that individuals having undergone islet transplantation are capable of performing exercise to lower blood glucose levels with no increase in the risk of hypoglycemia. Notwithstanding greater changes in blood glucose during and following exercise, ITx experienced few blood glucose concentrations lower than 3.5 mmol/L during exercise and no

interstitial glucose concentrations below this threshold in the 24-hour period following exercise. As fear of hypoglycemia is the main barrier to physical activity in T1D individuals pre-transplant, the islet transplant procedure could make exercise and physical activity (along with its many benefits) more accessible in this population. This may be especially important for ITx, as it has been suggested that increased physical activity may be a factor involved in improved insulin sensitivity, which could improve blood glucose control and increase graft survival by decreasing metabolic demand on the transplanted islets [43]. Further studies are encouraged to examine different exercise modalities and durations in order to determine what type of exercise regimen is most beneficial in this population.

Acknowledgments

This study was supported by a Pilot Study Grant from the Alberta Diabetes Institute. JLR was supported by a Canada Graduate Scholarship. DRF was supported by a Mazankowski Summer Studentship from the Augustana Faculty of the University of Alberta. PAS is supported by AMHSP. The clinical islet transplant program has research funding from AIHS, JDRF and the Stem Cell Network. We would also like to thank Ms. Becca Dyck and Ms. Chufan Zhang for their help in data collection.

The original research question was formulated by PAS and JEY. NGB, PAS and JEY designed the study. PAS, JEY, JLR and DRF were responsible for participant recruitment. DRF, JLR, SRT and JEY collected the data. NGB analyzed the data. All authors contributed to the drafting and revising of the manuscript.

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Legends for Tables and Figures

Table 1. Participant characteristics

*Data presented are mean ± SD. SD=standard deviation, ITx=islet cell transplant recipients, CON=control, M=males, F=females, BMI=body mass index, A1C=glycated hemoglobin, VO_{2peak}=peak volume of oxygen uptake, T1D=type 1 diabetes. *n=10 for CON*

Table 2. Continuous glucose monitoring data the 6-hour, 12-hour and 24-hour period following the exercise or resting control sessions

*Data are presented as median [IQR], except for * where data are mean±SD. ITx=Islet cell transplant recipients, CON=control participants, SD=standard deviation, MAGE=mean amplitude of glycemic excursion, % high = % of time spent >9.9 mmol/L; %low = % of time spent <3.5 mmol/L; % in range = % of time spent between 3.5 and 9.9 mmol/L*

Figure 1 – Data are presented as mean ± SD. Changes in blood glucose, glucagon, insulin and c-peptide before and after rest and exercise treatments (gray box) and recovery in ITx (black squares) and CON (white squares).

Figure 2. CGM glucose from 30 minutes pre-exercise until 12 hours post-exercise

Table 1. Participant characteristics

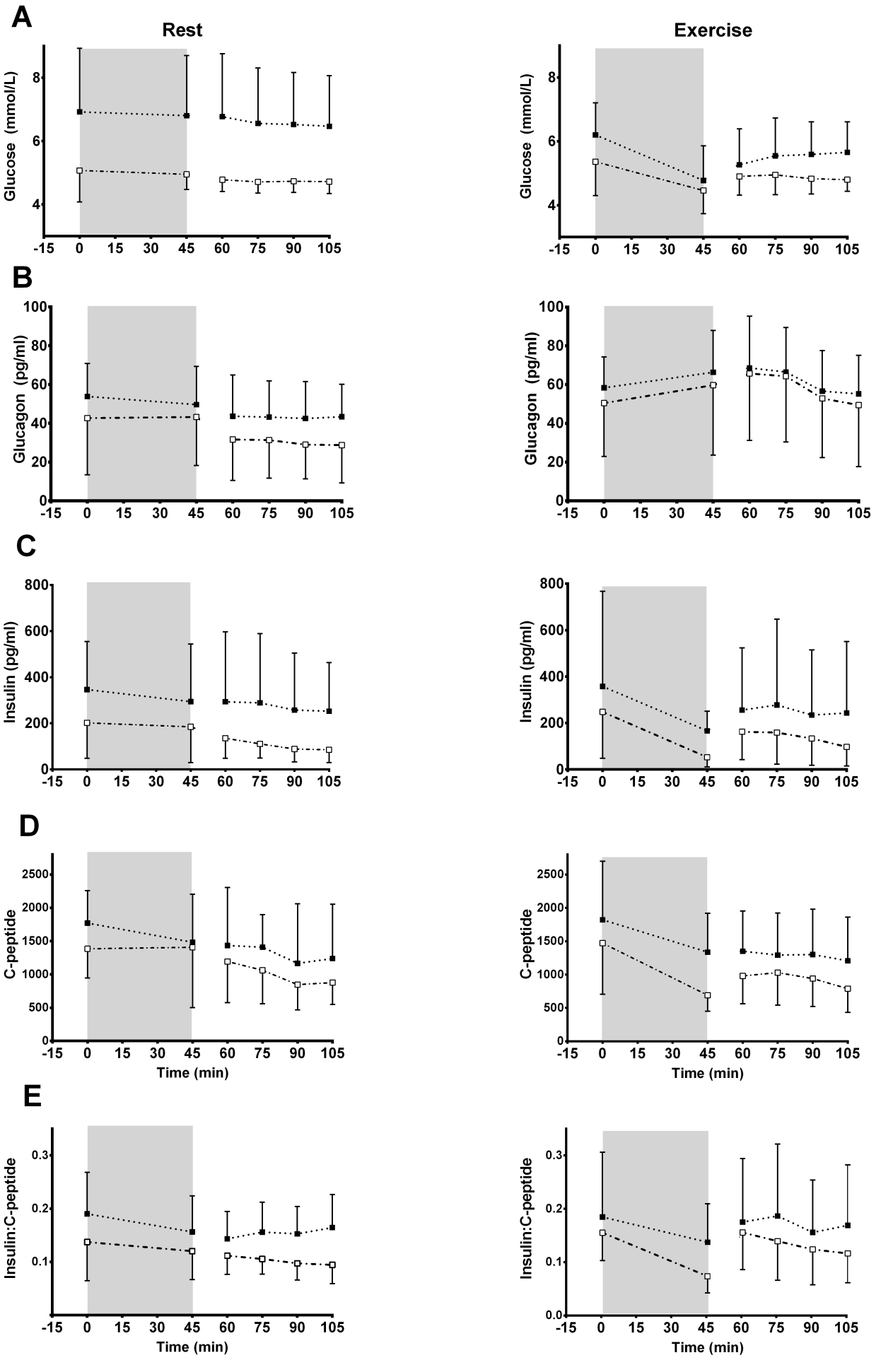
	ITx	**CON**	**p-values**
M/F, (n)	5/7	5/7	1.00
Age (yrs)	52.7 ± 7.9	55.3 ± 11.3	0.51
Height (cm)	165.9 ± 7.1	167.7 ± 8.8	0.60
Weight (kg)	64.0 ± 9.0	64.6 ± 10.7	0.88
BMI ($kg \cdot m^{-2}$)	23.2± 2.7	22.9 ± 3.2	0.82
A1C (%)	6.3 ± 0.5	5.4 ± 0.2*	<0.0001
Hematocrit	34.2 ± 3.8	40.5 ± 3.6	0.0004
Resting heart rate (bpm)	78 ± 10	65 ± 11	0.01
$VO_{2peak,}$ $mL \cdot kg^{-1} \cdot min^{-1}$	24.0 ± 6.0	35.7 ± 9.7	0.002
Heart rate at VO_{2peak} (bpm)	144 ± 19	174 ± 12	0.0005
RPE at VO_{2peak}	17 ± 1	18 ± 1	0.22
RER at VO_{2peak}	1.14 ± 0.07	1.21 ± 0.09	0.14
Godin Leisure Time Questionnaire	48.2 ± 41.5	47.7 ± 28.0	0.96
T1D diagnosis duration (yrs)	36.2 ± 12.7	-	-
Time since last transplant (months)	10.4 ± 17.8	-	-
Number of transplant procedures	3.0 ± 1.7	-	-
Total number of islets (IEQ)	1297219 ± 331889	-	-
Islets per kilogram of body weight (IEQ/kg)	19553 ± 5516	-	-

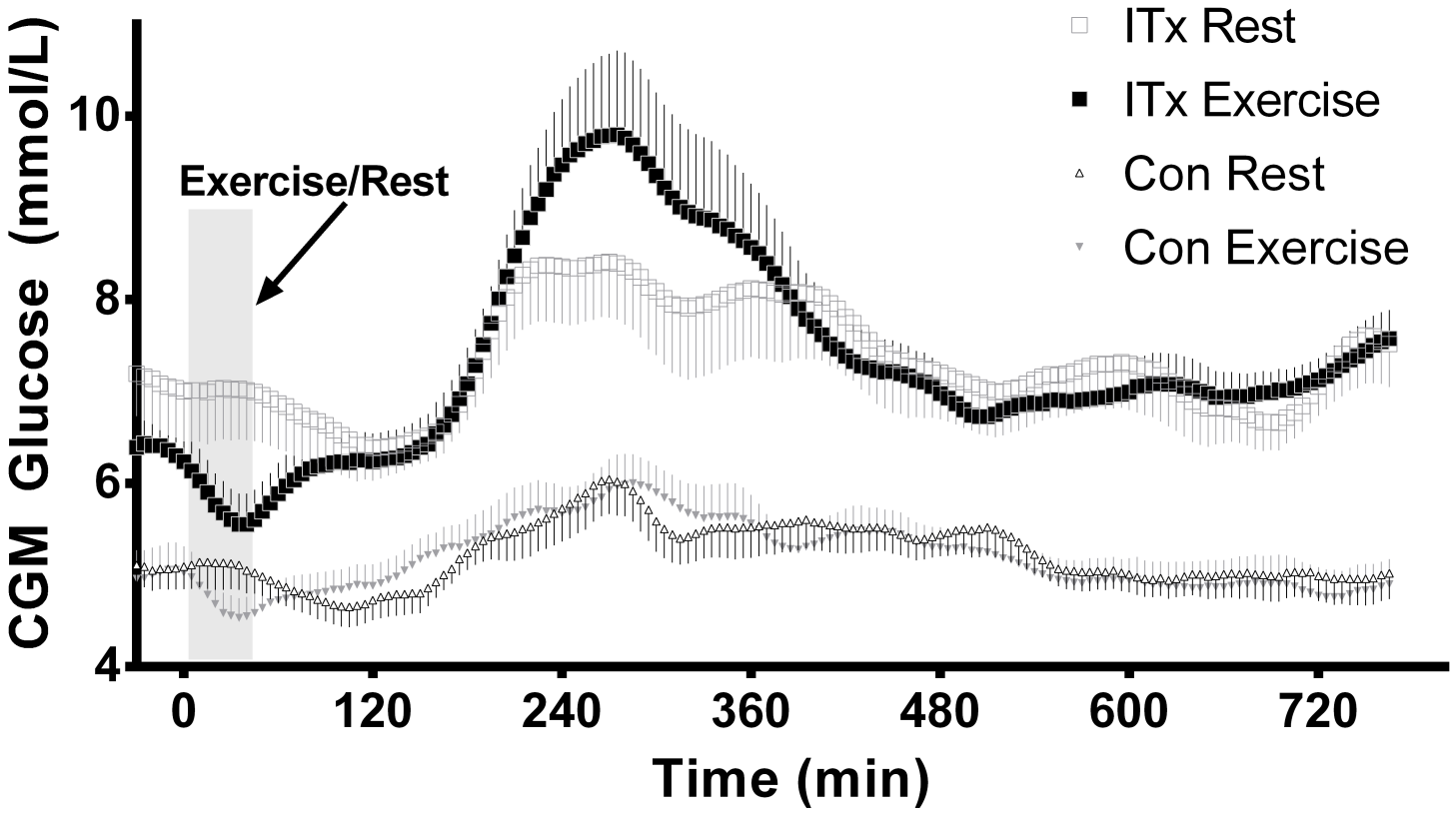
*Data presented are mean ± SD. SD=standard deviation, ITx=islet cell transplant recipients, CON=control, M=males, F=females, BMI=body mass index, A1C=glycated hemoglobin, VO_{2peak}=peak volume of oxygen uptake, T1D=type 1 diabetes, BPM=beats per minute. *n=10 for CON*

Table 2. Continuous glucose monitoring data the 6-hour, 12-hour and 24-hour period following the exercise or resting control sessions

	ITx (n=12)		CON (n=12)		p-values		
	Exercise	**Rest**	**Exercise**	**Rest**	**Group**	**Treatment**	**Interaction**
***6-hr glucose (mmol/L)**	7.8±1.6	7.5±1.5	5.4±0.7	5.3±0.7	<0.001	0.34	0.52
***12-hr glucose (mmol/L)**	7.4±1.1	7.4±1.4	5.2±0.8	5.2±0.7	<0.001	0.92	0.73
***24-hr glucose (mmol/L)**	7.8±1.0	7.5±1.2	5.3±0.8	5.3±0.7	<0.001	0.28	0.22
***SD (mmol/L)**	1.5±0.7	1.5±0.6	0.7±0.3	0.7±0.3	<0.001	0.96	0.67
***MAGE (mmol/L)**	4.1±2.2	4.0±1.7	2.0±0.9	1.9±0.4	0.001	0.75	0.86
% high (6 hr)	6.9[0.0-45.5]	5.6[0.0-25.0]	0.0[0.0-0.0]	0.0[0.0-0.0]	0.014	0.18	0.32
% low (6 hr)	0.0[0.0-0.0]	0.0[0.0-0.0]	0.0[0.0-0.0]	0.0[0.0-0.0]	0.51	0.66	1.00
% in range (6 hr)	93.1[54.9-100]	94.4[75.0-100.0]	100.0[100.0-100.0]	100.0[100.0-100.0]	0.05	0.21	0.35
% high (12 hr)	4.5[0.0-22.57]	5.2[0.0-12.5]	0.0[0.0-0.0]	0.0[0.0-0.0]	0.014	0.60	0.51
% low (12 hr)	0.0[0.0-0.0]	0.0[0.0-0.0]	0.0[0.0-0.0]	0.0[0.0-0.0]	0.51	0.66	1.00
% in range (12 hr)	95.5[77.4-100]	94.8[87.5-100.0]	100.0[100.0-100.0]	100.0[100.0-100.0]	0.09	0.68	0.60
% high (24 hr)	12.7[0.4-24.1]	8.0[0.0-21.4]	0.0[0.0-0.0]	0.0[0.0-0.0]	<0.001	0.59	0.16
% low (24 hr)	0.0[0.0-0.0]	0.0[0.0-0.0]	0.0[0.0-0.0]	0.0[0.0-0.8]	0.18	0.72	1.00
% in range (24 hr)	87.3[76.0-99.6]	92.0[78.6-100.0]	100.0[100.0-100.0]	100.0[99.1-100.0]	0.14	0.43	0.35

*Data are presented as median [IQR], except for * where data are mean±SD. ITx=Islet cell transplant recipients, CON=control participants, SD=standard deviation, MAGE=mean amplitude of glycemic excursion, % high = % of time spent >9.9 mmol/L; %low = % of time spent <3.5 mmol/L; % in range = % of time spent between 3.5 and 9.9 mmol/L*


A
Rest
Exercise
Glucose (mmol/L)
B
Glucagon (pg/ml)
C
Insulin (pg/ml)
D
C-peptide
Time (min)
E
Insulin:C-peptide
Time (min)


ITx Rest
ITx Exercise
Con Rest
Con Exercise
Exercise/Rest
CGM Glucose (mmol/L)
10
8
6
4
0
120
240
360
480
600
720
Time (min)