Exercise Effects on Postprandial Glucose Metabolism in Type 1 Diabetes:

*A Triple Tracer Approach*

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Abstract:

To determine the effects of exercise on postprandial glucose metabolism and insulin action in type 1 diabetes (T1D) we applied the triple tracer technique to study 16 T1D subjects on insulin pump therapy before, during and after 75 min of moderate intensity exercise (50% VO₂ max) that started 120 min after a mixed meal containing 75 g labeled glucose. Prandial insulin bolus was administered as per each subject’s customary insulin: carbohydrate ratio adjusted for meal-time meter glucose and the level of physical activity. Basal insulin infusion rates were not altered. There were no episodes of hypoglycemia during the study. Plasma dopamine and norepinephrine concentrations rose during exercise. During exercise, rates of endogenous glucose production rose rapidly to baseline levels despite high circulating insulin and glucose concentrations. Interestingly, plasma insulin concentrations increased during exercise despite no changes in insulin pump infusion rates implying increased mobilization of insulin from subcutaneous depots. Glucagon concentrations rose before and during exercise. Therapeutic approaches for T1D management during exercise will need to account for its effects on glucose turnover, insulin mobilization, glucagon and sympathetic response and possibly other blood-borne feedback and afferent reflex mechanisms to improve both hypoglycemia and hyperglycemia.
INTRODUCTION:

Exercise increases peripheral glucose uptake (Rd) via insulin dependent and independent mechanisms. Simultaneously, rates of endogenous glucose production (EGP) increase to minimize risks of hypoglycemia (5, 9, 31, 33). These changes in glucose fluxes are facilitated by falling insulin and rising glucagon and catecholamine (34) concentrations in plasma together with emerging roles for potential blood-borne feedback and afferent reflex mechanisms in stimulating glucose rate of appearance (Ra) (6, 18, 19) and pancreatic islet hormone secretion (24).

However, the increment in endogenous glucose production may not sufficiently compensate for the increase in glucose disposal thus predisposing to exercise-induced hypoglycemia in type 1 diabetes (T1D) (27). This could, at least in part, be due to impaired glucagon and/or catecholamine secretion and responsiveness because of concomitant dysfunction of alpha cell or autonomic systems respectively that often afflicts patients with T1D (8, 17). Furthermore, the increase in insulin sensitivity can persist for several hours after cessation of exercise in a rat study (14) hence further predisposing individuals with T1D to delayed hypoglycemia (20, 22). While there have been numerous reports (11, 12, 25) evaluating glucose kinetics during and after exercise in individuals without diabetes, a comprehensive assessment of glucose turnover during and immediately after exercise applying state of the art isotope dilution techniques has, to the best of our knowledge, not been conducted in individuals with T1D. In this context, in a study assessing individuals with and without T1D during exercise, Petersen et al (21), applying magnetic resonance technology, suggested that compared to healthy individuals, those with T1D had higher rates of EGP due to increased rates of gluconeogenesis.
However, detailed assessment of carbohydrate turnover when exercise is conducted in the postprandial state has been a challenge to exercise physiologists. This is because accurate estimations of essential components of postprandial glucose turnover, i.e., systemic appearance of meal glucose (MRa), rates of EGP and Rd require complex isotope dilution technique to minimize non-steady state errors in the estimation of these parameters. While we have recently reported use of the triple-tracer technique (25) to measure the components of postprandial glucose turnover during moderate intensity exercise (50% VO2 max) in healthy adults, we describe here, applying the same technique and similar protocol, measurement of postprandial glucose kinetics during and after moderate intensity exercise in a cohort of C-peptide negative individuals with T1D on insulin pump therapy. Subjects exercised on a treadmill at 50% VO2 max for 75 minutes, two hours after consuming a mixed meal breakfast. No subjects developed hypoglycemia during or after exercise. We report estimations of components of postprandial glucose turnover (MRa, EGP and Rd) in a continuous fashion together with insulin and glucagon excursions before, during and after exercise constituting the six hours duration of the study. Finally, we also compare the responses during this period with those in healthy subjects (25) recently reported.

**MATERIALS AND METHODS:**

After approval from the Institutional Review Board and following signed informed consent, subjects with T1D on continuous subcutaneous insulin infusion pumps (CSII) were screened for eligibility. Inclusion criteria were age 18-65 years, BMI 19-40 kg/m2, HbA1c ≤ 10%, creatinine ≤ 1.5 mg/dL and normal gastric emptying. Exclusion criteria were significant gastrointestinal symptoms by questionnaire, documented recent gastrointestinal disorders, medications affecting gastric motility, pregnancy, unaccustomed physical activity, or on an
active weight loss program or any other comorbidities precluding participation. Medications (except stable thyroid hormone or hormone replacement therapy) that could influence glucose tolerance were exclusionary. Those with stable diabetic retinopathy were included. Subjects did not engage in vigorous physical activities for 72 h before screen and study visits. Each subject underwent two screen visits. A total of 18 people were recruited. One failed enrollment criteria due to C-peptide level and another withdrew from the study. Two subjects’ data on MRa and Rd were excluded from the analyses of glucose turnover because of malfunction of the pump infusing [6-3H] glucose.

**Experimental Protocol:**

*Screen Visit 1*

Subjects reported in the morning after an overnight fast to the Clinical Research Unit (CRU) of the Mayo Clinic Center for Clinical and Translational Research (CCaTS) for a history, physical examination, screening laboratory tests, standard urinalysis and resting ECG to ensure good health. All women with reproductive capacity had a negative urine pregnancy test within 24 hours of the study visit. Dietary histories were obtained to ensure adherence to a weight maintaining diet consisting of at least 200 grams of carbohydrates per day and meeting the American Diabetes Association guidelines for protein, fat and carbohydrates. Body composition was also measured using DXA (23). Subjects performed a graded exercise test on a treadmill to determine VO2 max according to guidelines (American College of Sports Medicine Guidelines for Exercise Testing and Prescription, 7th Edition) and to ensure stable cardiac status. Expired gases were collected and analyzed using indirect calorimetry. VO2 max was determined when at least two of the following three criteria were met: 1) subjects were too tired to continue exercise
based on Borg’s perceived exertion scale, 2) Respiratory exchange ratio > 1.1 or 3) a plateau was reached in oxygen consumption with increasing workload. The purpose of this test was to use individualized VO₂ max data to determine workload during the moderate intensity (~ 50% VO₂ max) protocol during study day.

Screen Visit 2

Subjects eligible to enroll based on screening results during visit 1 were scheduled for screen visit 2. Using scintigraphic techniques (3), gastric emptying to solids and liquids were assessed; results were summarized as the time required for 50% of solids and liquids separately to empty (T₁/₂). Thereafter, subjects who had normal gastric emptying for solids and liquids proceeded to the inpatient study visit within 3 weeks of this visit.

Study Visit

All subjects spent ~40 hours in the Clinical Research Unit (CRU).

Day 1. Subjects were admitted to the CRU at ~ 4 PM. A point of care urine pregnancy test was performed where appropriate to ensure that the test was negative before proceeding any further. They were then provided a standard 10 kcal/kg meal (55% carbohydrate, 15% protein, and 30% fat) consumed between 5 and 5-30 PM. Subjects administered pre-meal insulin bolus guided by their customary insulin: carbohydrate ratio. No additional food was given until the next morning unless required in the context of hypoglycemia. However, there was no hypoglycemia during the study period.

Day 2. At 6 AM, an intravenous cannula was inserted in a retrograde fashion into a dorsal hand vein and the hand placed in a heated (55° C) Plexiglas box to enable drawing of arterialized
venous blood for glucose, glucose tracer and hormone analyses. At 7 AM, a triple tracer mixed meal study was performed (2). Briefly, a mixed meal containing 75 grams of glucose enriched with [1-$^{13}$C] glucose was ingested at time 0. The meal provided ~33% of daily estimated calorie intake. Subjects administered pre-meal insulin bolus with their CSII taking into account their customary insulin: carbohydrate ratio adjusted for the ambient glucose concentrations and the planned exercise. To be pragmatic and to reflect as much of a real-world scenario as possible and to facilitate incorporation of these data into closed control simulation as we have done for previous studies in T1D (16, 30), each individual subject was permitted to decide on their prandial insulin dose as they would have normally done during free-living situation. Basal insulin pump rates were not changed during the study duration. Simultaneously, an intravenous infusion of [6-$^{3}$H] glucose was started and continued for the next 6 h at variable rates to mimic the anticipated rate of appearance of the ingested [1-$^{13}$C] glucose. Concurrently, the [6, 6-$^{2}$H$_{2}$] glucose infusion rate, started at 4 AM at a primed-constant rate until 7 AM, was varied thereafter to mimic the anticipated rate of EGP. At 120 min following the first bite, subjects started a treadmill exercise at 50% VO$_{2}$ max: i.e., four bouts of walking at 3-4 miles/h for 15 min with rest periods of 5 min between each walking bout: total duration 75 min, i.e., 120-195 minutes (25). The workload during physical activity was continuously monitored by measurements of oxygen consumption (VO$_{2}$) to maintain a target of 50% of VO$_{2}$ max. The [6, 6-$^{2}$H$_{2}$] glucose and [6-$^{3}$H] glucose infusion rates were modified from the start of physical activity at 120 min for the next 4 h to mimic the anticipated changes in EGP and MRa during and after physical activity. To determine the optimal tracer infusion rates necessary to minimize changes in tracer/trace concentration for determination of postprandial EGP and MRa, we analyzed data from the first two subjects and modified the tracer infusion rates accordingly. Before modification of the
glucose tracer infusion rates, infusion rates (ml/min) for [6,6-2H2] glucose were: T0 to T5: 0.1, T5 to T10: 0.09, T10 to T20: 0.08, T20 to T30: 0.06, T30 to T210: 0.03, T210 to T 250: 0.04, T250 to T290: 0.066, T290 to T360: 0.075; infusion rates (ml/min) for [6-3H] glucose were T0 to T10: 0.2, T 10 to T20: 0.52, T20 to T30: 0.35, T30 to T 60: 0.2, T60 to T120: 0.1, T 120 to T180: 0.05, T180 to T360: 0.02. These above rates were identical to those infused in the healthy controls. After modification, infusion rates (ml/min) for [6,6-2H2] glucose were:T0 to T5:0.1, T 5 to T10:0.08, T 10 to T20:0.07, T20 to T30: 0.05, T30 to T120: 0.015, T120 to T150: 0.04, T 150 to T170: 0.07, T170 to T195: 0.085, T 195 to T250: 0.07, T 250 to T360: 0.09; infusion rates (ml/min) for [6-3H] glucose were: T0 to T10: 0.2, T10 to T60: 0.48, T60 to T120: 0.38, T120 to T180: 0.25, T180 to T230: 0.1, T230 to T360: 0.02. Following the last blood draw, all tracer infusions were stopped, the hand vein cannula removed, lunch provided and the study completed.

Analytical Techniques

Hormone analyses: C-Peptide was measured on the Cobas e411 (Roche Diagnostics, Indianapolis, IN) using a 2-site electrochemiluminescence immunometric assay. Insulin was measured by a two-site immunoenzymatic assay performed on the DxI automated system (Beckman Instruments, Chaska, MN) and Glucagon by a direct, double antibody radioimmunoassay (Linco Research, St. Charles, MO) (23). Catecholamines were measured by reversed phase HPLC with electrochemical detection after extraction with activated alumina.

Glucose and Glucose tracers: Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at –80°C until assay. Plasma glucose concentration was measured using a glucose oxidase method (YSI, Inc., Yellow Springs, OH). Plasma [6-3H] glucose specific activity was measured by liquid scintillation counting as described (1). Plasma enrichment of [1-13C] glucose
and [6,6-2H₂] glucose were measured using GCMS (Thermoquest, San Jose, CA) to
simultaneously quantitate C-1 to C-2 and C-3 to C-6 fragments (23).

**Calculations:** Glucose turnover using the triple tracer approach was calculated as described (2, 25). Briefly, [6-3H] glucose was used to estimate the systemic rate of appearance of the ingested [1-13C] glucose in the mixed meal. Thereafter, these rates were divided by the [1-13C] glucose enrichment in the meal to obtain systemic appearance of meal glucose. [6,6-2H₂] glucose was used to trace the rate of EGP after estimation of endogenous glucose concentration (calculated by subtracting the concentration of exogenously derived (ingested) glucose (i.e. plasma [1-13C] glucose concentration multiplied by meal [1-13C] glucose enrichment) from total plasma glucose concentration). Rates of glucose disappearance were calculated by Steele’s non steady state equation (28).

**Statistical Analyses**
The primary scientific goal of the study was to validate the triple tracer mixed meal experimental protocol during exercise in patients with TID. To complement that goal, exploratory analyses were conducted to examine the estimated flux values in terms of clinical relevance and expected directions as a part of the validation of the protocol. To do this, longitudinal models consisting of a random subject effect and a fixed effect for time as a factor were used to quantify changes in hormones and other measurements over time. Post hoc comparisons of mean values at each time point were estimated from this model. These general regression models were also supported by more focused comparisons that related to specific hypotheses of interest. These analyses included the iAUC, which was determined by the difference between the area under the curve
relative to zero, as determined by the trapezoidal rule, and baseline area (baseline value * length of time).

In addition, subject characteristics for T1D were compared to previously published data on healthy individuals using Wilcoxon rank sum and chi-square tests, and two-sample t-tests were used to compare the iAUC values between T1D and healthy individuals. Key differences were summarized with 95% confidence intervals in the results. P-values less than 0.05 were considered to be supportive of an initial finding; no correction for multiple testing has been applied to reported p-values. All statistical analyses were conducted using the SAS System version 9.4 (Cary, NC).

RESULTS:

Subject Characteristics (Table 1)

Table 1 depicts the anthropometric characteristics of the T1D study subjects. Briefly, 50% of the participants were female, and the mean age was 45 years. The mean (SD) duration of T1D was 26.9 (12.3) years. Subjects had a mean (SD) fasting plasma glucose and HbA1c values of 9.9 (3.4) mM and 7.6 (0.7) % at screen visit, respectively.

Gastric Emptying Rates:

For liquid emptying (50% empty T1/2) mean (sd) was 19.8 (6.7) min (normal range: 33-75 min) while for solids, (50% empty T1/2), mean (sd) was 98.9 (32.4) min (normal range: 71-198 min)

Glucose, Insulin, and Glucagon Concentrations (Figures 1A)

Mean (sd) fasting plasma glucose concentrations were elevated at the start of the meal 9.9 (3.1) mM. Peak mean (range) glucose for all subjects was 21.1 (14.7 to 27.8) mM. The glucose
concentrations gradually fell during exercise to reach baseline levels at the end of exercise. Thereafter, glucose concentrations remained flat until the end of the study. The plasma glucose concentrations that were elevated after the meal, prior to exercise (time=20 to 120) and during exercise (120 to 195 minutes) were no longer statistically different from baseline (0 minutes) after exercise (p>0.07, 240 to 360 minutes).

As expected, plasma insulin concentrations rose after the prandial bolus with mean (range) of peak measures of all subjects being 241.2 (115.8 to 374.4) pM. The peak concentrations were reached 90 (20 to 195) minutes after the bolus given at the start of the meal. Thereafter, insulin concentrations gradually fell until the start of exercise. During exercise (120 to 195 minutes), however, plasma insulin concentrations unexpectedly rose (p=0.005, 150 minutes vs. 120 minutes) compared to the concentrations prior to the initiation of exercise despite no changes in basal insulin infusion rates. After exercise completion, the concentrations gradually fell and returned to near baseline levels by the end of the study (p=0.11).

Glucagon levels, on the other hand, rose 20 minutes after the meal to a mean level of 84.6 pg/ml, which was an increase from baseline of 18.4 pg/ml (95% CI, 10.7 to 26.2 pg/ml; p<0.001). During exercise there was also a gentle rise in glucagon concentrations before gradually declining to baseline levels (p=0.18, t=0 vs 360 minutes) at the end of the study (360 minutes, 71.6 pg/ml).

**Dopamine, Epinephrine and Nor-epinephrine concentrations (Figure 1B)**

Plasma dopamine and norepinephrine concentrations increased (p<0.01) but epinephrine concentrations did not change (p=0.1) pre vs. post exercise.

**Tracer-Tracee Ratios (Figure 2A and 2B)**
The [\textsuperscript{6-\textsuperscript{3}H}] glucose/ [\textsuperscript{1-\textsuperscript{13}C}] glucose and [\textsuperscript{6,6-\textsuperscript{2}H\textsubscript{2}}] glucose/ endogenous glucose tracer-tracee ratios rose slightly but not significantly (p=0.1) during the study, thereby minimizing non-steady state errors hence enabling accurate estimations of MRa, EGP and Rd throughout the study period. Figure 2B represents the individual tracer-tracee ratios during the study.

**Endogenous Glucose Production (Figures 3)**

Integrated rates of EGP rapidly fell after the start of the meal (p<0.003 for all comparisons, t=30 to 110 minutes) reaching a mean (range) time to nadir at 76 (45 to 130) minutes. Thereafter, EGP rose from the nadir time to 200 minutes after the meal, with values measured between 180 to 210 minutes being not statistically different from baseline. After 210 minutes, EGP decreased to be less than baseline rates (p<0.05 for all comparisons).

**Meal Glucose Appearance, Glucose Uptake and Glucose Clearance (Figures 3)**

As explained earlier, due to malfunction of the [\textsuperscript{6-\textsuperscript{3}H}] glucose infusion pump, data for MRa, Rd and hence glucose clearance was unavailable for two subjects. Therefore, on the subset of subjects (n=14) with complete tracer infusions, MRa reached peak rates with mean (range) being 87.7 (70.7 to 130.6) µM/kgFFM/min and time at 57 (30 to 120) minutes after start of the meal. During exercise (120 to 195 minutes) MRa maintained a plateau before rapidly returning close to baseline levels shortly after cessation of exercise.

Rates of glucose disappearance (Rd) increased with mean (range) for all subjects being 78.3 (50.1 to 107.3) µM/kgFFM/min after the meal reaching a peak rate at 94 (60 to 150) minutes. During exercise, Rd rose gently for the most (125 to 180 minutes) of the exercise period before rapidly falling to near baseline levels shortly after cessation of exercise (240 minutes). When considered in the light of the high plasma glucose concentrations, rates of glucose clearance rose...
gently during exercise, peaking at ~ 170 minutes before returning to baseline levels shortly after completion of exercise. The glucose turnover data from the first 2 subjects did not differ from the rest (MRa: p=0.53, EGP: p=0.14, Rd: p=0.52).

Comparisons between T1D and Healthy Participants (ref #18) (Tables 2A, 2B and figures 4,5):

Table 2A presents the differences between the healthy individuals and T1D participants. T1D subjects had a higher body mass index, fasting plasma glucose, HbA1c but a lower level of fitness (as measured by VO2 max) than healthy participants. However, the age, fat free mass, and percent body fat did not differ statistically between groups. There were striking differences in the integrated excursions and patterns of plasma glucose, insulin, glucagon and rates of glucose disappearance and glucose clearance between T1D and healthy subjects as detailed in Table 2B.

DISCUSSION:

We have demonstrated the successful application of the triple tracer technique (2) to estimate postprandial glucose turnover during moderate intensity exercise in both healthy (25) and now in T1D subjects. This was possible with appropriate adjustments to glucose tracer infusion rates of [6,6-2H2] glucose to match anticipated changes in endogenous glucose and of [6-3H] glucose to match anticipated changes in MRa during and after exercise based on results obtained from initial few experiments. The resultant tracer-tracee ratios obtained were constant and unchanging, thereby permitting accurate estimates of glucose turnover while minimizing non-steady state errors in calculations. This iterative process has for the first time, to the best of our knowledge, enabled us to obtain a unique and virtually continuous temporal profile of the components of postprandial glucose metabolism before, during and immediately after exercise in T1D subjects. Furthermore, careful and frequent sampling of arterialized venous blood before,
during and after exercise has permitted measurements of glucose, insulin and glucagon concentrations thus providing valuable data to further our understanding of glucose-insulin-glucagon interactions under these circumstances. Further analyses of the data obtained in this study in T1D subjects and comparison with those obtained in healthy nondiabetic subjects undergoing a similar exercise protocol reported recently (25) has provided several striking differences discussed below.

Fasting glucose concentrations were significantly higher despite higher fasting insulin concentrations in T1D than healthy subjects implying insulin resistance in T1D subjects in the baseline rested state as has been demonstrated before (4, 15). Detailed examination of the concentration profiles of the underlying hormones (insulin and glucagon) provides interesting perspectives and differences. Despite the fact that T1D subjects administered their customary insulin dose at the start of the meal based on the carbohydrate content adjusted for the degree of planned exercise and their pre-meal glucose levels, it was clearly inadequate to prevent hyperglycemia in the early postprandial (0-120 min) period. This is corroborated by the observation that the insulin excursion during this period was lower but glucagon excursion higher, in T1D than healthy subjects implying that co-existing α cell dysfunction that has been described in T1D (16, 26) likely also contributed to postprandial hyperglycemia in these individuals.

There were also striking differences in glucose, insulin and glucagon profiles during (120-195 min) and after exercise (195-360 min). While glucose concentrations fell rapidly in T1D subjects throughout exercise, it also fell in healthy subjects before rising to baseline levels and maintained a plateau thereafter. In T1D subjects plasma glucose concentrations also reached a plateau after end of exercise before slowly dropping to baseline concentrations. It was
noteworthy that in these untrained subjects, plasma glucose concentrations did not continue to fall immediately after completion of exercise. None of the T1D subjects developed hypoglycemia during or after exercise.

Plasma insulin concentration profiles during exercise reveal intriguing differences between groups. As anticipated, in healthy subjects, plasma insulin concentrations rapidly fell to fasting levels (in appropriate response to ambient glucose concentrations), by the end of exercise and remained at this level for the rest of the study (25). In contrast, in T1D subjects, there was a distinct non-trivial rise in plasma insulin concentrations during exercise despite the fact that insulin pump infusion rates remained at baseline levels in all subjects. Since as per inclusion criteria, all enrolled subjects were C-peptide negative, it is safe to assume that the only source of insulin in these subjects was exogenous via their insulin pumps. Furthermore, since the insulin pump basal infusion rates did not alter either before or during exercise, it is reasonable to speculate that there was an increased mobilization of subcutaneously delivered insulin from the insulin pump infusion sites in the abdominal subcutaneous fat depots (since all subjects had their pumps inserted in their abdomen) presumably due to increased subcutaneous adipose tissue blood flow during exercise (10). To the best of our knowledge, this increase in plasma insulin concentrations during exercise in T1D subjects has not been described previously and could contribute to enhanced insulin action over and above the independent effects of exercise per se (11, 12, 32), both during and after exercise. This important observation however needs to be considered by both patients, care providers and scientists responsible for developing next generations of open loop and closed loop therapeutic strategies and algorithms. It is also noteworthy that plasma insulin concentrations at the end of the study remained 50% higher than at the start of the study period. This could be due to either continued increased mobilization of
insulin from subcutaneous fat depots, reduced insulin clearance, reduced volume of distribution or a combination thereof despite prior observations that insulin clearance increases during exercise in T1D subjects (29). This observation assumes even more importance because in sharp contrast to our recent report in T1D subjects (16) where plasma insulin concentrations returned to baseline pre-prandial levels, in the absence of exercise, within three hours after a mixed meal, in our current study plasma insulin concentrations did not return to baseline even after completion of exercise and remained substantially higher than pre-meal concentrations.

Plasma glucagon excursions also show intriguing differences between healthy and T1D subjects that support the notion of coexisting \(\alpha\) cell dysfunction in T1D (26). Glucagon concentrations were higher in the postprandial, pre-exercise period (0-120 min) in T1D than healthy subjects despite the higher plasma glucose concentrations during this time. During exercise, while glucagon concentrations rose briskly (~2 fold) in healthy subjects, in T1D subjects the rise was blunted (~1.3 fold) and slower implying persistent \(\alpha\) cell dysfunction during exercise in these individuals. While this observation, coupled with higher plasma insulin concentrations and lower hepatic glycogen reserves (17) could expose T1D subjects to greater risk of hypoglycemia during exercise, we did not observe clinical or biochemical hypoglycemia in this study likely because of postprandial hyperglycemia. However, it is possible that if the subjects had administered a higher meal insulin bolus to lower postprandial glucose concentrations and/or exercised for a longer duration or intensity, their risk of hypoglycemia would have increased substantially.

In the postprandial period in vivo it has been estimated using arterio-venous difference across the leg, that greater than 80% of glucose disposal during hyper-insulinemia is accounted for by the skeletal muscle with other tissues (i.e., adipose or liver) accounting for a much lower
percentage (7). However the arterio-venous difference measurements (7) were made in relatively
slim type 2 diabetes subjects that were close to their ideal weight and matched with a control
group. The current study reports measurements made in type 1 diabetes patients with 35% body
fat and matched with a control group with 29% body fat with a high likelihood of higher levels
of perivascular adipocytes. Therefore we cannot exclude that adipose tissue could account for a
larger percentage of glucose disposal in this study.

There are several intriguing inferences that arise from these observations in this cohort of
untrained individuals with and without T1D. First, T1D subjects appear to be insulin resistant in
the post prandial state and particularly during exercise as has been observed in prior studies (4,
15). This is especially relevant during exercise when both plasma insulin and glucose
concentrations were higher in T1D than healthy subjects. Second, both Rd and glucose clearance
rapidly return to baseline pre-prandial levels within 30-45 minutes after completion of exercise
in both groups thus implying that exercise induced changes in tissue glucose uptake (both insulin
and non-insulin mediated) do not persist for a prolonged period after cessation of exercise.
However, this observation will need to be carefully considered in the light of differing insulin
concentrations between the two groups, i.e., glucose clearance was numerically same in both
groups after exercise in the presence of significantly lower peripheral insulin concentrations in
the healthy subjects. Taken together, this implies, but does not prove that if one could match the
glucose and insulin concentrations in both groups, Rd and clearance would have been higher in
healthy than T1D subjects. Third, there was a brisk rise in rates of EGP during exercise in both
groups. In healthy subjects, this was naturally facilitated by falling insulin and glucose levels and
rapidly rising glucagon concentrations. Interestingly, the equally rapid increase in EGP in T1D
subjects during exercise despite higher glucose levels and lower glucagon concentrations, all of
which would normally suppress EGP, implies robust exercise induced hepatic responsivity despite adverse hormonal and substrate milieu, which necessitates further investigations. Factors that could contribute to this adaptive process in T1D subjects could be related to increased hepatic glucagon sensitivity stimulating glycogenolysis, increased hepatic gluconeogenesis due to enhanced substrate availability (e.g., lactate, free fatty acids), or potential blood-borne feedback and afferent mechanisms that have more recently shown to modulate glucose Ra (6, 18, 19).

We did not measure lactate turnover nor rates of gluconeogenesis during the study, hence cannot comment on the relative contributions of these metabolic processes to rates of EGP. An additional factor that needs to be considered is that while in T1D subjects, peripheral and portal insulin concentrations maybe similar, it is different in healthy subjects. Hence the wide difference in insulin concentrations observed between groups in the peripheral circulation during exercise would be lesser in the portal circulation. In contrast, it is possible that the difference between groups in glucagon concentrations in the peripheral circulation during exercise would be similar to that in the portal circulation since it is likely (but not proven) that hepatic glucagon clearance does not differ between healthy and T1D subjects. Therefore insulin : glucagon ratio in the portal circulation, that directly modulates EGP, would be different than that observed in the peripheral circulation between healthy and T1D subjects. Future studies are necessary to elucidate these aspects further in individuals with T1D.

The primary goal of our study was to refine the triple tracer technique to accurately estimate glucose turnover continuously during and after exercise in the postprandial period under physiologically relevant conditions (i.e., without performing the conventional but non-physiological insulin clamp technique) in individuals with T1D on insulin pump therapy. Based
on the relatively unchanging tracer-tracee ratios that enable accurate estimation of glucose turnover, we were successful in achieving those goals. That said, a limitation of this study is the absence of a no-exercise control visit in the same study cohort. With the current design, it is also not possible to conclude whether both the T1D and controls experience the benefit of exercise with skeletal muscle glycogen depletion leading to increases in insulin stimulated glucose uptake for up to 48 h after the exercise bout via described mechanisms (13). This implies that the question whether the T1D subjects benefit from a single bout of exercise as much as healthy individuals cannot be answered without further research. Additionally, study of the glucose, insulin and glucagon plasma profiles under the experimental conditions also permitted insights into novel observations that include suggestion of mobilization of insulin from subcutaneous injection depots and suboptimal glucagon rise during exercise and highlight the possibility of yet to be identified blood-borne feedback or afferent mechanisms that might stimulate Ra during exercise. Furthermore the maintenance of the ‘normal’ rise in EGP during exercise despite unfavorable glucose and hormonal milieu in these subjects provides material for further investigations in this area.

Although there were no statistical differences between the two groups in age, weight and percent body fat, these values tended to be higher in T1D subjects. BMI was higher in T1D subjects although fat free mass did not differ between groups. These anthropometric differences could have, at least in part, contributed to the changes in patterns of postprandial glucose turnover between groups.

Taken together, information on exercise effects on glucose kinetics could be useful to inform and modify the gain function of an insulin controller of an artificial pancreas system in the future. Finally, comparing the hormonal and glucose turnover data between healthy (25) and
T1D subjects undergoing identical study design offers valuable new hypothesis generating ideas that deserve to be tested in future studies.

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Dr. Ananda Basu is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of data and the accuracy of data analysis. There are no conflicts of interest to declare for any of the authors.

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Figure Legends:

**Figure 1 A:** Glucose (upper panel), Insulin (middle panel) and glucagon (lower panel) concentrations obtained from time 0-360 minutes in T1D subjects. Shaded box between 120-195 minutes represents exercise period at 50% VO$_2$ max. The inset in middle panel shows the mean insulin pump infusion rates during the study.

**Figure 1B:** Plasma Dopamine (upper panel), epinephrine (middle panel) and nor-epinephrine (lower panel) concentrations obtained from time 0-360 minutes in T1D subjects. Shaded box between 120-195 minutes represents exercise period at 50% VO$_2$ max.

**Figure 2A:** [6-$^3$H] glucose / [1-$^{13}$C] glucose ratio (upper panel) and [6,6-$^2$H$_2$] glucose / endogenous glucose ratio (lower panel) obtained from time 0-360 minutes in T1D subjects. Shaded box between 120-195 minutes represents exercise period at 50% VO$_2$ max.

**Figure 2B:** Shows individual tracer-tracee ratios during the study.

**Figure 3:** Rates of Meal Appearance (MRa) (upper left panel), Endogenous Glucose Production (EGP) (upper right panel), Glucose Disappearance (Rd) (lower left panel) obtained from time 0-360 minutes in T1D subjects. Shaded box between 120-195 minutes represents exercise period at 50% VO$_2$ max.

**Figure 4:** Glucose (upper panel), Insulin (middle panel) and glucagon (lower panel) concentrations obtained from time 0-360 minutes in T1D subjects (solid line-circles) and healthy controls (dotted line-squares). Shaded box between 120-195 minutes represents exercise period at 50% VO$_2$ max. The inset in middle panel shows the mean insulin pump infusion rates during the study in T1D subjects.
Figure 5: Rates of Meal Appearance (MRa) (upper left panel), Endogenous Glucose Production (EGP) (upper right panel), Glucose Disappearance (Rd) (lower left panel) obtained from time 0-360 minutes in T1D subjects (solid line-circles) and healthy controls (dotted line-squares). Shaded box between 120-195 minutes represents exercise period at 50% VO$_2$ max.
Figure 1A

**Plasma Glucose**

- Graph showing plasma glucose levels over time (0-360 minutes).
- The data points are marked with black dots, and error bars are present.
- The shaded area indicates a specific time period of interest.

**Plasma Insulin**

- Graph showing plasma insulin levels over time (0-360 minutes).
- The data points are marked with black dots, and error bars are present.
- The shaded area indicates a specific time period of interest.

**Plasma Glucagon**

- Graph showing plasma glucagon levels over time (0-360 minutes).
- The data points are marked with black dots, and error bars are present.
- The shaded area indicates a specific time period of interest.
Figure 2A

Ratio of [6-$^3$H] Glucose / [1-$^{13}$C] Glucose

Ratio of [6,6-$^2$H$_2$]-Glucose / Endogenous Glucose

Time (min)
<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>44.9 (12.5)</td>
</tr>
<tr>
<td>Sex (Male, N (%))</td>
<td>8 (50.0%)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7 (0.1)</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>84.8 (21.0)</td>
</tr>
<tr>
<td>Fat Free Mass (Kg)</td>
<td>54.9 (13.0)</td>
</tr>
<tr>
<td>% Body Fat</td>
<td>34.9 (8.6)</td>
</tr>
<tr>
<td>VO₂max (ml/min/Kg)</td>
<td>25.5 (5.2)</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>28.6 (5.5)</td>
</tr>
<tr>
<td>Fasting Blood Glucose (mg/dl)</td>
<td>177.4 (61.1)</td>
</tr>
<tr>
<td>Fasting Blood Glucose (mM)</td>
<td>9.9 (3.4)</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>7.6 (0.7)</td>
</tr>
<tr>
<td>HbA1C (mmol/mol)</td>
<td>60.0 (7.5)</td>
</tr>
<tr>
<td>Duration of Type1 Diabetes (years)</td>
<td>26.9 (12.3)</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.9 (1.3)</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>15.1 (4.5)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.8 (0.1)</td>
</tr>
<tr>
<td>Thyroid Stimulating Hormone (mIU/L)</td>
<td>2.2 (1.1)</td>
</tr>
</tbody>
</table>
Table 2A: Subject Characteristics for Healthy (n=12) and Type 1 Diabetes (n=16) Participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy Participants¹ (Mean (SD))</th>
<th>Type1 Diabetes (Mean (SD))</th>
<th>p-value²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>37.1 (13.0)</td>
<td>44.9 (12.5)</td>
<td>0.1313</td>
</tr>
<tr>
<td>Sex (Male, N (%))</td>
<td>5 (41.7%)</td>
<td>8 (50.0%)</td>
<td>0.6617</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7 (0.1)</td>
<td>1.7 (0.1)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>70.9 (16.6)</td>
<td>84.8 (21.0)</td>
<td>0.1090</td>
</tr>
<tr>
<td>Fat Free Mass (Kg)</td>
<td>50.9 (13.5)</td>
<td>54.9 (13.0)</td>
<td>0.4718</td>
</tr>
<tr>
<td>VO2max (ml/(min*Kg))</td>
<td>32.2 (7.2)</td>
<td>25.5 (5.2)</td>
<td>0.0114</td>
</tr>
<tr>
<td>% Body Fat</td>
<td>29.1 (7.0)</td>
<td>34.9 (8.6)</td>
<td>0.1041</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>24.1 (3.7)</td>
<td>28.6 (5.5)</td>
<td>0.0485</td>
</tr>
<tr>
<td>Fasting Blood Glucose (mg/dl)</td>
<td>84.3 (9.9)</td>
<td>177.4 (61.1)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fasting Blood Glucose (mM)</td>
<td>4.7 (0.5)</td>
<td>9.9 (3.4)</td>
<td>0.0001</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>5.1 (0.2)</td>
<td>7.6 (0.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbA1C (mmol/mol)</td>
<td>31.9 (2.6)</td>
<td>60.0 (7.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.9 (1.5)</td>
<td>13.9 (1.3)</td>
<td>0.8526</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>14.4 (4.8)</td>
<td>15.1 (4.5)</td>
<td>0.5758</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.8 (0.1)</td>
<td>0.8 (0.1)</td>
<td>0.9038</td>
</tr>
<tr>
<td>Thyroid Stimulating Hormone (mIU/L)</td>
<td>2.4 (1.2)</td>
<td>2.2 (1.1)</td>
<td>0.6093</td>
</tr>
</tbody>
</table>

¹Data on the healthy participants have been published previously in Ref # 18.
²p-values are from Wilcoxon rank sum/chi-square tests
Table 2B: iAUC for Hormones and Measures of Glucose Turnover in T1D and Healthy Participants

<table>
<thead>
<tr>
<th>Measure</th>
<th>Type-1 Diabetes</th>
<th>Healthy Participants</th>
<th>T1D vs. Healthy Individuals</th>
<th>p-value $^\dagger$</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>95% CL of Mean</td>
</tr>
<tr>
<td>Glucose (mM/minutes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 120</td>
<td>16</td>
<td>823.1</td>
<td>233.0</td>
<td>(698.89, 947.20)</td>
</tr>
<tr>
<td>0 to 360</td>
<td>16</td>
<td>139.1</td>
<td>1182.6</td>
<td>(754.02, 2014.39)</td>
</tr>
<tr>
<td>120 to 195</td>
<td>16</td>
<td>381.2</td>
<td>299.9</td>
<td>(221.36, 540.99)</td>
</tr>
<tr>
<td>195 to 360</td>
<td>16</td>
<td>100.0</td>
<td>733.1</td>
<td>(-210.66, 570.63)</td>
</tr>
<tr>
<td>Insulin (pM/minutes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 120</td>
<td>16</td>
<td>11835.5</td>
<td>5015.3</td>
<td>(9163.01, 14507.89)</td>
</tr>
<tr>
<td>0 to 360</td>
<td>16</td>
<td>26322.9</td>
<td>10253.5</td>
<td>(20859.18, 31786.54)</td>
</tr>
<tr>
<td>120 to 195</td>
<td>16</td>
<td>6891.5</td>
<td>3739.1</td>
<td>(4899.10, 8883.91)</td>
</tr>
<tr>
<td>195 to 360</td>
<td>16</td>
<td>7595.9</td>
<td>4731.5</td>
<td>(5074.66, 10117.14)</td>
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<tr>
<td>Glucagon (pg/(ml *minutes))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 120</td>
<td>16</td>
<td>1039.8</td>
<td>1132.0</td>
<td>(436.66, 1643.02)</td>
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<tr>
<td>0 to 360</td>
<td>16</td>
<td>5291.1</td>
<td>4560.1</td>
<td>(2861.20, 7720.99)</td>
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<tr>
<td>120 to 195</td>
<td>16</td>
<td>1145.5</td>
<td>1083.5</td>
<td>(568.12, 1722.82)</td>
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<tr>
<td>195 to 360</td>
<td>16</td>
<td>3105.8</td>
<td>2809.1</td>
<td>(1608.90, 4602.66)</td>
</tr>
<tr>
<td>Meal Rate of Appearance (µM/(kgFFM *minutes))</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>0 to 120</td>
<td>14</td>
<td>5712.8</td>
<td>1702.3</td>
<td>(4729.98, 6695.69)</td>
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<tr>
<td>0 to 360</td>
<td>14</td>
<td>7898.6</td>
<td>2471.7</td>
<td>(6471.50, 9325.75)</td>
</tr>
<tr>
<td>120 to 195</td>
<td>14</td>
<td>1433.5</td>
<td>746.8</td>
<td>(1002.29, 1864.71)</td>
</tr>
<tr>
<td>195 to 360</td>
<td>14</td>
<td>752.3</td>
<td>452.2</td>
<td>(491.18, 1013.39)</td>
</tr>
<tr>
<td>Endogenous Glucose Production (µM/(kgFFM *minutes))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 120</td>
<td>16</td>
<td>-1377.2</td>
<td>582.3</td>
<td>(-1687.50, -1066.93)</td>
</tr>
<tr>
<td>0 to 360</td>
<td>16</td>
<td>-2856.2</td>
<td>1771.9</td>
<td>(-3800.31, -1911.98)</td>
</tr>
<tr>
<td>120 to 195</td>
<td>16</td>
<td>-458.3</td>
<td>488.1</td>
<td>(-718.36, -198.16)</td>
</tr>
<tr>
<td>Measure</td>
<td>Type1- Diabetes</td>
<td>Healthy Participants</td>
<td>T1D vs. Healthy Individuals</td>
<td>p-value $^\S$</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------------</td>
<td>----------------------</td>
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</tr>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>95% CL of Mean</td>
</tr>
<tr>
<td>195 to 360</td>
<td>16</td>
<td>-1020.7</td>
<td>945.1</td>
<td>(-1524.29, -517.05)</td>
</tr>
<tr>
<td>Rate of Disappearance (µM/(kgFFM *minutes))</td>
<td>0 to 120</td>
<td>14</td>
<td>3769.1</td>
<td>1346.2</td>
</tr>
<tr>
<td></td>
<td>0 to 360</td>
<td>14</td>
<td>6424.7</td>
<td>1769.1</td>
</tr>
<tr>
<td></td>
<td>120 to 195</td>
<td>14</td>
<td>2360.1</td>
<td>742.6</td>
</tr>
<tr>
<td></td>
<td>195 to 360</td>
<td>14</td>
<td>295.5</td>
<td>532.6</td>
</tr>
<tr>
<td>Glucose Clearance (µM/(kgFFM *minutes))</td>
<td>0 to 120</td>
<td>14</td>
<td>123.1</td>
<td>77.2</td>
</tr>
<tr>
<td></td>
<td>0 to 360</td>
<td>14</td>
<td>254.6</td>
<td>256.7</td>
</tr>
<tr>
<td></td>
<td>120 to 195</td>
<td>14</td>
<td>132.4</td>
<td>113.0</td>
</tr>
<tr>
<td></td>
<td>195 to 360</td>
<td>14</td>
<td>-0.8</td>
<td>126.5</td>
</tr>
</tbody>
</table>

$^\#$Healthy Participants

$^\S$p-values are from two-sample t-tests