Diurnal Pattern of Insulin Action in Type 1 diabetes:
Implications for a Closed Loop System

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Running Title: diurnal variability in carbohydrate metabolism

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Abstract:

We have recently demonstrated a diurnal pattern to insulin action (SI) in healthy individuals with higher SI at breakfast than dinner. To determine if such a pattern exists in type 1 diabetes we studied 19 C-peptide negative subjects (HbA1c 7.1±0.6%) on insulin pump therapy with normal gastric emptying. Identical mixed meals were ingested during breakfast (B), lunch (L) or dinner (D) at 0700, 1300 and 1900 in randomized Latin Square order on three consecutive days when measured daily physical activity was equal. The triple tracer technique enabled measurement of glucose fluxes. Insulin was administered according to the customary insulin:carbohydrate ratio for each participant. While postprandial glucose excursions did not differ among meals, insulin concentration was higher (p<0.01) and endogenous glucose production less suppressed (p<0.049) at B than L. There were no differences in meal glucose appearance or glucose disappearance between meals. Although there was no statistical difference (p=0.34) in SI between meals in type 1 diabetes participants, the diurnal pattern of SI taken across the three meals in its entirety, did differ (p=0.016) from healthy subjects. While the pattern in healthy subjects showed decreasing SI between B and L, the reverse SI pattern was observed in type 1 diabetes. The results suggest that in contrast to healthy subjects, SI diurnal pattern in type 1 diabetes is individual specific, hence cannot be extrapolated to the type 1 diabetes population as a whole thus implying that Artificial Pancreas algorithms may need to be personalized.
An optimal closed loop control system will need to take into account alteration in physiological parameters that modulate glucose concentrations including changes in insulin sensitivity related to meals and physical activity. A better understanding of these factors involved in glucose homeostasis is crucial to develop physiological models to improve glucose control, minimize glucose variability and thus reduce morbidity and complications in individuals with diabetes mellitus, especially type 1 diabetes. We have recently shown the presence of a diurnal pattern to postprandial insulin action and secretion in healthy individuals with both these parameters highest at breakfast than later in the day under controlled experimental conditions (1). While most, if not all individuals with type 1 diabetes do not secrete insulin in response to a meal, a diurnal pattern to postprandial insulin action, if present, would need to be incorporated into an ideal closed loop control algorithm. Although reports (2-8) investigating diurnal variations in insulin action exists in the literature in individuals with and without type 2 diabetes, such information is scarce in type 1 diabetes. The purpose of this study therefore was to determine if there were diurnal changes in post prandial glucose tolerance, insulin sensitivity and glucose fluxes in C-peptide negative subjects with type 1 diabetes while controlling for meal macronutrient composition, caloric content and levels of physical activity. We also wished to examine differences, if any, in the diurnal patterns of postprandial insulin action between healthy (1) and type 1 diabetes subjects while applying an identical study design in both groups. Furthermore, to the best of our knowledge, this is the first report of the application of the triple tracer method (9) in type 1 diabetes to determine postprandial glucose metabolism following mixed meal consumption.
RESEARCH DESIGN AND METHODS:

After approval from the Mayo Institutional Review Board and following signed informed consent, subjects with C-peptide negative type 1 diabetes on insulin pump were enrolled. 13 subjects were on Aspart insulin while the remaining 6 were on Lispro insulin. Inclusion criteria were age 18-60 years, BMI <40 kg/m², HbA1c ≤ 8.5%, creatinine ≤ 1.5 mg/dl and normal gastric emptying to solids and liquids. Exclusion criteria were significant gastrointestinal symptoms by questionnaire, hypoglycemia unawareness by Clarke questionnaire, documented recent upper gastrointestinal disorder, medications affecting gastric motility (e.g., erythromycin), pregnancy or breast feeding, or other comorbidities (e.g., nephropathy, neuropathy, macrovascular disease, hypertension) precluding participation. Those with stable background diabetic retinopathy were included. Medications (except stable thyroid hormone or hormone replacement therapy) that could influence glucose tolerance were exclusionary. Subjects did not engage in vigorous physical activities for 72 hours prior to screen and study visits. Each subject underwent two screen visits.

Both healthy and type 1 diabetes subjects were studied concomitantly. The healthy subjects were studied between March 2010 and June 2011 while the type 1 diabetes subjects were studied between June 2010 and March 2012. As anticipated it took us longer to recruit type 1 diabetes subjects due to our stringent inclusion criteria to enroll as homogenous a cohort of these individuals as possible.

Screen visit 1: Subjects reported in the morning after an overnight fast to the Clinical Research Unit (CRU) of the Mayo CTSA for a history, physical examination, screening laboratory tests, standard urinalysis and resting ECG. All women of childbearing
potential had a negative pregnancy test within 24 hours of study visit. A dietary history was taken to ensure adherence to a weight maintaining diet consisting of at least 200 grams of carbohydrates per day and that the diet met ADA guidelines for protein, fat, and carbohydrates. Body composition was also measured using DXA (10).

**Screen visit 2:** Using scintigraphic techniques (11) we assessed gastric emptying to solids and liquids in all subjects who were eligible after the first screening visit and only subjects who had normal gastric emptying proceeded further within 3 weeks of the second screening visit.

**In patient Study Visit:** The study interventions are as described recently (1; 12). Briefly, all subjects spent three days and four nights in the CRU. Subjects reported at ~1600 hours on the evening prior to the first study day when continuous glucose sensor and physical activity monitoring system (PAMS) consisting of duplicate triaxial accelerometers were placed. They consumed a standard 10 kcal/kg meal (55% carbohydrate, 15% protein, and 30% fat) between 1700 and 1730 hours. No additional food was eaten until the next morning. All subjects were provided with breakfast (B) at 0700 h, lunch (L) at 1300 h and dinner (D) at 1900 h for three consecutive days. Each subject administered pre-meal insulin bolus with their pump according to their customary insulin:carbohydrate ratio and sensitivity factor and continued on their basal insulin infusion patterns.

**Study Meals:** All meals were provided by the CRU metabolic kitchen. All participants received three days of weighed meals, three meals each day with each meal comprising 33% of total estimated calorie intake based on Harris Benedict calorie requirements, including low level of physical activity, with ~50 grams of carbohydrate in
each meal. The meal consisted of Jell-O with dextrose, eggs (scrambled or omelet) and ham slices. A few participants preferred steak slices to ham. The macronutrient contents for the three labeled meals and the six unlabeled meals that each participant consumed were identical. No snacks or calorie containing drinks were permitted between meals unless otherwise required to treat hypoglycemia (POC glucose ≤ 60 mg/dl) as per Institutional guidelines. Unfinished food was weighed and excluded from calculated caloric intake. One meal daily was randomly selected per Latin Square design to include 50 grams glucose labeled with [1-13C] glucose in the Jell-O as the carbohydrate component. As detailed recently (1), we applied the Latin square design to maximize the time between tracer meals (i.e., minimize carryover effects). This design was specifically chosen to remove confounding effects of unequal glycogen labeling and carry-over effects of residual tracer glucose concentrations in plasma on postprandial glucose fluxes that would have occurred if all three successive meals were labeled during one day.

Triple Tracer Mixed Meal: A primed-continuous infusion of [6,6^2H_2] glucose (11.84 mg/kg FFM prime; 0.1184 mg/kg FFM/min continuous; Mass-Trace, Woburn, MA) was started three hours (-180 min) prior to the first bite of the mixed meal used to estimate post prandial glucose kinetics (9). Jell-O containing [1-13C] glucose was consumed along with the rest of the mixed meal of eggs and Canadian bacon/steak. An infusion of [6-^3H] glucose was started at time 0, and the rate varied to mimic the anticipated rate of appearance of the [1-13C] glucose contained within the meal. Simultaneously, the rate of infusion of [6,6^2H_2] glucose was altered to approximate the anticipated pattern of change in endogenous glucose production (EGP) (10). Blood was sampled at periodically for measurement of tracer-tracee ratios, glucose, insulin and glucagon concentrations.
Physical Activity Protocol: As described in detail (12), we utilized PAMS that captured data on body posture and movement in duplicate every 0.5 second. The participants performed carefully planned physical activity protocol, adherence to which was captured using the PAMS. Each of the labeled meals was preceded by at least three hours and followed by six hours of inactivity when the subjects were resting in bed to enable periodic blood draws.

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) (10). The DxI method also reliably detects both Aspart and Lispro insulin analogs (13) that have been tested in the Mayo Clinical Laboratory and also cross-checked in the Mayo-CTSA Immunochemical Laboratory. Cortisol was measured by a competitive binding immunoenzymatic assay on the DxI automated immunoassay system (Beckman Instruments, Chaska, MN 55318). Intra-assay CV’s were 13.1%, 9.4%, and 6.6% at 1.56, 2.85 and 30.2 µg/dL respectively. Inter-assay CV’s were 9.0%, 8.1%, and 9.3% at 2.47, 17.3, and 27.5 µg/dL respectively. Cortisol Binding Globulin (CBG) was measured by competitive radioimmunoassay (RIA), (DIAsource ImmunoAssays S.A., Louvain-la-Neuve, Belgium). The intra-assay CV’s were 8.6% and 3.9% at 23 and 83 µg/mL. Inter-assay CV’s were 10.8% and 4.8% at 25 and 114 µg/mL. Melatonin was measured by competitive radioimmunoassay (RIA), (Immuno-Biological Laboratories, Inc. (IBL-
America), Minneapolis, MN 55432). The intra-assay CV’s were 9.8%, 9.7% and 13.4% at 19, 42 and 126 pg/mL. Inter-assay CV’s were 8.0%, 10.9% and 13.3% at 29, 74 and 154 pg/mL.

**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 (10). Plasma enrichment of [1-13C] glucose and [6,6-2H2] glucose were measured using GCMS (Thermoquest, San Jose, CA) to simultaneously quantitate C1,2 and C3-6 fragments (9).

**Glucose kinetics**: Fasting and postprandial rates of glucose turnover were calculated as described (9). The systemically infused [6-3H] glucose was used to trace the systemic rate of appearance of [1-13C] glucose contained in the meal whereas [6,6-2H2] glucose was used to trace the rate of appearance of endogenously produced glucose. The ratio of plasma concentration of [6-3H] glucose to [1-13C] glucose was used to calculate the rate of appearance of ingested [1-13C] glucose and the ratio of plasma concentration of [6,6-2H2] glucose to endogenously produced glucose was used to calculate EGP. The plasma concentration of endogenously produced glucose was 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 (9).

**Meal indices**: The oral glucose minimal model (14; 15) was used to interpret plasma glucose and insulin concentrations measured during the meal test. The model assumes that insulin action on glucose production and disposal emanates from a
compartment remote from plasma, which is usually identified with the interstitium. The most important parameter of the model, estimated from data, is net insulin sensitivity, SI, which measures the overall effect of insulin to stimulate whole body (liver and periphery) glucose disposal and inhibit glucose production. All of the above models have been described in detail in the online appendix linked to our recent report (1). However, at variance with the healthy subjects in which data starts in steady state conditions before each meal, this is not always the case in type 1 diabetes subjects. Thus, glucose derivative at the beginning of each meal was taken into account in the model. In addition, given that the pre-meal data were not used to assess basal condition, the model was identified employing also these pre-meal data. Finally, as already observed in type 2 diabetes (10), estimating SI with precision can be difficult. Thus, to improve numerical identifiability of the model one has to link SI to glucose effectiveness through parameter GEZI (glucose effectiveness at zero insulin). At variance with type 2 diabetes, here GEZI is not fixed to a population value in all subjects, but is optimally chosen in each individual to improve model fitting.

**Statistical Analyses.**

The experimental design translated statistically into a three treatment (meals), three period (study days) crossover study. SAS PROC Mixed (Cary, NC) was used to test for meal differences averaged over study periods using the methodology of Brown and Prescott (16). The likelihood of carryover effects were presumed to be negligible given the short half-life of glucose and glucose tracers and the restricted randomization process. This assumption was supported by the statistical modeling for all indices except for insulin incremental area under the curve where the statistical test for carryover had a p-
value of 0.04. However, following the recommendations of Senn (17), no further adjustment to the model (i.e., adjustment for carryover effects) was implemented. Model-based estimates (otherwise known as Least Squares Means) were calculated to provide an average effect over study period. Distributional assumptions for the mixed model were assessed using graphical displays of residuals and numerical summaries by meal-study day. Longitudinal summary statistics (18) were used to synthesize the serial measurements into a single index. Area under the curve (AUC) and incremental AUC (iAUC) were calculated by the trapezoidal rule. Post-hoc comparison of postprandial states following meals (breakfast, lunch, and dinner) were tested at the 0.05 level of significance using the Tukey-Kramer correction factor. The overall effect of meal (i.e., a Type III analysis) was conducted using the Kenward-Rogers approach for determining degrees of freedom (19).

To test if there were differences in insulin action in type 1 diabetes patients relative to healthy subjects (1), the data were pooled together so that a meal by diabetes status interaction term could be fit to the data. This interaction term, which tested for differences in profiles across meals, was the main parameter of interest. Like the models with type 1 diabetes described here, model based estimates were computed to test for differences at each particular meal while controlling for study period. Upon the realization of differences in the profiles of SI between patient cohorts, an exploratory study was conducted examining the role of three hormones (cortisol, cortisol binding globulin, and melatonin) on SI at breakfast. Multiple linear regressions models were used to test if the relationship of the individual hormones were associated differently with
SI between type 1 diabetes and healthy subjects. All analyses were conducted using The SAS System (v. 9.3; Cary, NC).

RESULTS:

Subject Characteristics (Table 1):

A total of 28 participants were screened for the study. There were five screen failures (two for abnormal gastric emptying, two for residual C-peptide secretion and one for poor venous access). Three additional participants were withdrawn after being successfully screened due to the inability to obtain adequate venous access during the study. The remaining 20 subjects completed all study procedures. However, one participant’s laboratory values indicated the participant was not C-peptide negative (an inclusion criterion protocol deviation). This participant was removed from the analysis set, so data are presented on the 19 eligible participants. All subjects had undetectable fasting C-peptide concentrations (<0.33 pmol/L) at screen visit. Physical activity levels, measured in Accelerometer Units (AU), did not differ among the three days. Gastric emptying rates for Solid ($T_{1/2}$: Mean=111.4, SE=4.9 min) and liquid ($T_{1/2}$: Mean= 23, SE=3.0 min) were normal in all subjects. The meal compositions are provided in supplemental table 1. Only one subject did not completely ingest one meal at breakfast. The unconsumed amount (0.5 grams carb, 6.0 grams protein, 4 grams fat and ~ 68 calories) was taken into account during the meal composition and glucose flux calculations.

Glucose, insulin and glucagon concentrations (Figure 1a-c, Table 2).

Preprandial plasma glucose concentrations did not differ between meals (p=0.083). Postprandial peak plasma glucose concentration differed by meal (p=0.010) with it being lower at D than B (p<0.01). The incremental area above baseline (0-360
min) of glucose excursions however did not differ between meals (p=0.15). There were no episodes of hypoglycemia for three hours prior and six hours after start of the labeled meals during the study. The plasma glucose concentrations during the labeled meals in type 1 diabetes and healthy subjects are shown together in supplemental figure 1.

Preprandial baseline plasma insulin concentrations differed between meals (p<0.01) with L higher than at either B or D. Peak postprandial insulin concentrations however did not differ between meals (p=0.13). In contrast, incremental area above baseline (0-360 min) of insulin was different by meal (p=0.008) with it being higher at B than L (p<0.01) but did not differ between other meals.

Preprandial baseline glucagon concentrations was lower at B than L (p=0.023) or D (p=0.013). While peak postprandial glucagon concentrations did not differ between meals, incremental area above baseline (0-360 min) was higher (p<0.01) at B than L or D.

Meal appearance, Endogenous Glucose Production and Glucose Disappearance (Figure 2 a-c, Table 2):

Meal glucose appearance did not differ between the three meals. Likewise, there were no detectable differences in glucose disappearance between the three meals. The integrated rates of endogenous glucose production were lower at B than L at baseline (p=0.02) and with the incremental area under the curve (p=0.049). The percentage suppression (p=0.09) and absolute area under the curve (p=0.32) were not different across the meals.

Insulin Action (Figure 3 a-c, Table 2):
Insulin sensitivity, either on its natural scale or natural-log transformed scale, did not differ among meals.

Further analyses of the pattern of SI over meals revealed a different response pattern between type 1 diabetes and healthy subjects \((p=0.016)\). This difference was amplified by the differences in SI at breakfast. SI was lower at breakfast in type 1 diabetes than healthy subjects \((5.1\text{ vs. } 11.2\ \times 10^{-4}\text{ dl/kg/min per }\mu\text{U/ml}, \ p=0.013)\) but at other meals there was no statistically significant difference. Thus, the estimated mean response profiles \((11.2\text{ to } 7.9\text{ to } 8.1\text{ vs. } 5.1\text{ to } 7.6\text{ to } 7.2\ \times 10^{-4}\text{ dl/kg/min per }\mu\text{U/ml for breakfast to lunch to dinner})\) gave the impression that SI trended to rise from B through D in type 1 diabetes subjects, whereas the opposite was noted in the healthy subjects with SI falling from B to D \((1)\).

**Cortisol, Cortisol Binding Globulin and Melatonin:**

An exploratory analysis was conducted to describe the association of pre-breakfast hormone levels with SI. This analysis was unplanned at time of the original study, so measurements of these hormones were in subjects who had permitted use of their stored samples for further testing \((15\text{ type 1 diabetes and }14\text{ healthy})\). Plasma concentrations of each hormone were assessed with the 4 am sample \(\text{(waking time; prior to experimental procedures)}\). The mean hormone levels were not found to differ by diabetes classification \((\text{Cortisol: } 12.0\pm 3.1\ \text{ vs. } 9.2\pm 6.0\ \mu\text{g/dl, } p=0.25; \text{cortisol binding globulin: } 48.6\pm 6.4\ \text{ vs. } 49.9\pm 6.1\ \mu\text{g/ml, } p=0.84; \text{melatonin: } 67.5\pm 36.4\ \text{ vs. } 41.1\pm 35.4\ \mu\text{g/ml, } p=0.06; \text{for healthy vs. type 1 diabetes respectively},\) and the association of the hormones with SI were not found to differ by diabetes classification \((\text{all DM by hormone interaction terms } p>0.50)\). Thus, while the SI diurnal pattern was different between type 1
diabetes and healthy, pre-breakfast hormone levels do not help explain this difference. The numerically lower levels of cortisol and melatonin in type 1 diabetes subjects may warrant further investigation.

DISCUSSION:

We have shown under our experimental conditions controlling for meal composition and physical activity that individuals with type 1 diabetes do not demonstrate a uniformly identifiable diurnal pattern of postprandial insulin sensitivity over meals that could be generalized to the disease population as a whole. This is contrary to our recent report in a similar cohort of healthy individuals (1). This could be due to the greater variability of SI in type 1 diabetes. However, interestingly, the diurnal pattern of SI over meals did show differences between healthy and type 1 diabetes groups such that in the healthy subjects SI fell from B to L while in the type 1 diabetes group SI rose from B to L.

Closed loop control algorithms currently being developed for glucose control in type 1 diabetes need to be refined and optimized based on multiple considerations including physiological parameters and characteristics that relate to the natural perturbations (e.g., meals, activity) to glucose-insulin relationship in such individuals. One such fundamental parameter that needs to be investigated is whether a diurnal pattern to postprandial glucose tolerance exists in type 1 diabetes. If indeed a diurnal pattern is clearly demonstrable in type 1 diabetes, then algorithms would need to be so informed for optimization. There have been few prior reports that have attempted to investigate existence of diurnal pattern in type 1 diabetes. Using the Biostator®, while Mathieson et al (20) demonstrated that insulin requirements per kJ consumed was higher
at breakfast than at lunch thereby implying a lower insulin action at breakfast than lunch, Service et al (21) did not observe a definitive diurnal pattern of postprandial insulin requirements in type 1 diabetes subjects. However multiple investigators (21-23) have reported correlations between the total amount of carbohydrate ingested, post prandial glucose excursions and insulin administered when using the Biostator®. Furthermore, not surprisingly, reports also confirmed that glycemic excursions, both fasting and postprandial, were tighter with the Biostator®, where insulin was administered intravenously than with subcutaneous delivery of insulin (24-26).

Potential common factors that influence postprandial glucose tolerance include physical activity and meal size, calorie content and composition. While some (20; 24; 27) but not all (21-23; 26) reports using the Biostator® controlled for physical activity levels during the study protocols, meal sizes, composition and/or calorie contents varied from meal to meal in all of the above reports except one (27). Furthermore, all prior reports elucidated above used the Biostator® system with intravenous insulin delivery and did not apply specific methods to measure postprandial insulin sensitivity.

In an effort to determine existence of diurnal pattern to postprandial glucose tolerance in type 1 diabetes subjects with subcutaneous open loop insulin delivery, we carefully accounted for confounding factors by controlling for meal size, calorie content and composition for all meals and for physical activity levels applying an identical study design as reported recently (1). Additionally we applied sophisticated state of the art triple tracer modeling technique (9) to accurately estimate postprandial glucose fluxes and insulin sensitivity. Furthermore, to minimize possibility of meal induced endogenous insulin secretion that could dampen postprandial glucose excursions (28) and confound
assessments and interpretations of postprandial glucose fluxes and SI, we enrolled a homogeneous cohort of C-peptide negative individuals on insulin pump for the study protocol and provided single wave prandial insulin bolus for every meal according to their clinically optimised insulin:carb ratio. Since there were no episodes of hypoglycemia after the labeled meals, there was no need to provide additional calories during the study.

It is noteworthy that although the numerical value of SI was lower at B than the other two meals, increased variability reduced precision within the type 1 diabetes study cohort. Specifically, the residual variance for healthy subjects (1) was $11.5 \times 10^{-4} \text{ dl/kg/min/µU/ml}^2$; in the type 1 diabetes subjects, this variation was nearly three times larger [$30.1 \times 10^{-4} \text{ dl/kg/min/µU/ml}^2$]. Additionally, there were no detectable differences in systemic appearance of meal glucose and glucose disappearance among meals. However, there was less suppression of postprandial EGP at B than L (despite higher postprandial insulin excursion) implying greater hepatic insulin resistance at B than L. A higher iAUC of postprandial glucagon concentrations at B than L could, at least in part, contribute to this observation. Taken together the data suggests lower hepatic but unchanged peripheral insulin sensitivity contributing to the lower numerical value for SI at B than L or D.

While the diurnal pattern of insulin sensitivity in type 1 diabetes subjects in our study is concordant to the albeit indirect observations of Mathieson et al (20), it is critical to note important differences in study designs that includes route of insulin delivery (sq vs. iv) and meal composition (identical vs. variable). Physical activity levels, precisely measured in accelerometer units in our study did not differ from day to day. In the
previous report (20), although standardized timed physical activity was performed on a bicycle ergometer three times a day, direct estimation of glucose fluxes or insulin sensitivity was not done.

The difference in the diurnal trends of SI between the healthy and type 1 diabetes groups, who were similar in their anthropometric characteristics including percent body fat and fat free mass, is intriguing. As shown in Fig 3c, the major factor that contributes to this dichotomy is the difference in SI at B between groups rather than at L or D. We do not have a ready explanation for this difference. Measurements of cortisol, cortisol binding globulin and melatonin concentrations prior to breakfast did not reveal significant differences between healthy and type 1 diabetes groups nor were there any associations of any of these hormone concentrations with SI at B. Therefore, although associations of cortisol and melatonin concentrations to insulin action have been reported in the literature (29; 30), we did not find such a link in our study. Furthermore, modulation of diurnal patterns of insulin sensitivity by sleep architecture (31; 32) could also be a player and deserves further investigations in future studies. It is also noteworthy that the pattern of postprandial glucagon excursions in both healthy and type 1 diabetes groups were similar in that the iAUC of glucagon was greater at B than L or D. While this could, at least in part explain the higher rates of EGP in both groups at B than L, it does not per se explain the difference in SI patterns between healthy and type 1 diabetes subjects.

There are limitations to the study which restrict the generalizability of the findings and interpretations of the results. The highly structured protocol was used to minimize variability in levels of physical activity and meal contents between participants.
Furthermore, the identical experimental design was used in healthy subjects to enable direct comparison of SI between the type 1 diabetes and healthy subjects. This was the first study where multiple tracer meals were given to type 1 diabetes subjects. The variability within and between type 1 diabetes participants was unknown at the start of the study. The original sample size calculations anticipated a standard deviation for Ln Si of 0.36 and a difference of 0.6 between meals. The observed differences were on par with this clinical relevant difference (Table 2), but the observed standard deviations in type 1 diabetes were much larger than anticipated. In particular, the observed standard deviations were 0.89, 0.59, and 1.04 for B, L and D, respectively. Therefore, a priori power was decreased and the risk for type II error (false negative) rate was higher than planned for. For example a larger sample size could have resulted in statistically significant differences between meals in some of the parameters viz., endogenous glucose production or SI. This limitation, however, is attenuated by the notation that the observation of larger between subject variations is suggestive of the need for more individualization in the artificial pancreas algorithm.

Taken together, these data in a group of C-peptide negative type 1 diabetes individuals demonstrate that large inter-subject variability preclude a definitive diurnal pattern in insulin sensitivity that could be extrapolated to the type 1 diabetes population as a whole. Hence any diurnal pattern of SI in type 1 diabetes is individual specific suggesting that artificial pancreas algorithms will need to be personalized. This observation that SI varies in a subject specific fashion is an important finding and will be incorporated into the type 1 diabetes simulator (33; 34) to allow more reliable testing of closed-loop control algorithms.
Author Contributions:

L.H., D.N. and A.S. assisted in the conduct of the study and data handling; C.D.M., C.C., J.A.L., R.E.C., R.B., A.E.B., study design, data analyses and manuscript review/editing; R.A.R., study design and manuscript review/editing; Y.C.K. and A.B., study design, researched data and manuscript writing/editing.

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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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Key Words: diurnal pattern, type 1 diabetes, insulin action

References

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Table 1. Baseline characteristics of the participants completing the three meal study
(n=19)

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<td>HBA1c, (mmol/mol)</td>
<td>54</td>
<td>6.6</td>
<td>[40, 66]</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.6</td>
<td>1.0</td>
<td>[11.9, 15.3]</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.8</td>
<td>0.1</td>
<td>[0.6, 1.1]</td>
</tr>
<tr>
<td>BUN</td>
<td>15.7</td>
<td>4.5</td>
<td>[11, 27]</td>
</tr>
<tr>
<td>TSH (IU/L)</td>
<td>2.7</td>
<td>1.8</td>
<td>[0.9, 7.6]</td>
</tr>
<tr>
<td>Randomized meal sequence, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDL</td>
<td>6</td>
<td>31.6%</td>
<td></td>
</tr>
<tr>
<td>DLB</td>
<td>7</td>
<td>36.8%</td>
<td></td>
</tr>
<tr>
<td>LBD</td>
<td>6</td>
<td>31.6%</td>
<td></td>
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</tbody>
</table>
Table 2. Outcome measures of three day meal sequence. Data presented are model based (LS-means) from a three period cross over analysis of variance model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Summary Statistic</th>
<th>Breakfast (B)</th>
<th>Lunch (L)</th>
<th>Dinner (D)</th>
<th>Estimated Common (Pooled) Standard Error [a]</th>
<th>p-value [b]</th>
<th>Post-hoc comparisons [c]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose &amp; Hormones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Glucose</td>
<td>Baseline, mM</td>
<td>8.4</td>
<td>8.5</td>
<td>7.1</td>
<td>0.5</td>
<td>0.083</td>
<td></td>
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<tr>
<td></td>
<td>Peak, mM</td>
<td>16.2</td>
<td>15.4</td>
<td>14.1</td>
<td>0.6</td>
<td>0.010</td>
<td>D&lt;B</td>
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<tr>
<td></td>
<td>iAUC, mM/L/6 hours</td>
<td>1657.5</td>
<td>1092.3</td>
<td>1222.8</td>
<td>228.0</td>
<td>0.15</td>
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<tr>
<td>Insulin</td>
<td>Baseline, pmol/L</td>
<td>120.1</td>
<td>163.9</td>
<td>125.0</td>
<td>15.1</td>
<td>0.002</td>
<td>B,D&lt;L</td>
</tr>
<tr>
<td></td>
<td>Peak, pmol/L</td>
<td>255.1</td>
<td>282.2</td>
<td>237.1</td>
<td>23.2</td>
<td>0.13</td>
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<tr>
<td></td>
<td>iAUC, pmol/L/6 hours</td>
<td>11808</td>
<td>-1147</td>
<td>7684</td>
<td>3548</td>
<td>0.008</td>
<td>L&lt;B</td>
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<tr>
<td>Glucagon</td>
<td>Baseline, pg/ml</td>
<td>56.4</td>
<td>65.5</td>
<td>66.3</td>
<td>3.9</td>
<td>0.008</td>
<td>B,L,D</td>
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<tr>
<td></td>
<td>Peak, pg/ml</td>
<td>99.2</td>
<td>93.4</td>
<td>98.3</td>
<td>5.8</td>
<td>0.22</td>
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<tr>
<td></td>
<td>iAUC, pg/ml/6 hours</td>
<td>9114.0</td>
<td>4413.3</td>
<td>4523.5</td>
<td>1049.8</td>
<td>&lt;0.001</td>
<td>L,D&lt;B</td>
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<tr>
<td>Meal appearance, endogenous glucose production and glucose disappearance</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Endogenous glucose production</td>
<td>Baseline, µmol/kg/min</td>
<td>16.2</td>
<td>19.5</td>
<td>17.4</td>
<td>0.9</td>
<td>0.019</td>
<td>B&lt;L</td>
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<tr>
<td></td>
<td>AUC, µmol/kg/min/6 hours</td>
<td>iAUC, µmol/kg/min/6 hours</td>
<td>Percent decrease from baseline</td>
<td>MR&lt;sub&gt;a&lt;/sub&gt; AUC, mmol/kg/6 hours</td>
<td>R&lt;sub&gt;d&lt;/sub&gt; AUC, mmol/kg/6 hours</td>
<td>iAUC, mmol/kg/6 hours</td>
<td>Percent increase from baseline</td>
</tr>
<tr>
<td>----------------------</td>
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<tr>
<td></td>
<td>2961.4</td>
<td>2607.9</td>
<td>43.5%</td>
<td>6149.9</td>
<td>8392.1</td>
<td>2790.1</td>
<td>50.8%</td>
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<tr>
<td></td>
<td>2888.7</td>
<td>3639.0</td>
<td>52.3%</td>
<td>6135.3</td>
<td>8535.1</td>
<td>1968.2</td>
<td>33.7%</td>
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<tr>
<td></td>
<td>2650.3</td>
<td>3225.9</td>
<td>52.0%</td>
<td>6412.0</td>
<td>8502.2</td>
<td>2580.0</td>
<td>47.0%</td>
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<tr>
<td></td>
<td>193.4</td>
<td>297.0</td>
<td>3.6%</td>
<td>466.0</td>
<td>444.0</td>
<td>419.9</td>
<td>8.4%</td>
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<tr>
<td>Percent decrease</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>from baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>43.5%</td>
<td>52.3%</td>
<td>52.0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.6%</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Indices of insulin action</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SI</td>
<td>10&lt;sup&gt;-4&lt;/sup&gt; dl/kg/min/µU/ml</td>
<td>5.1</td>
<td>7.5</td>
<td>7.2</td>
<td>1.4</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>ln SI</td>
<td>ln (10&lt;sup&gt;-4&lt;/sup&gt; dl/kg/min/µU/ml)</td>
<td>1.3</td>
<td>1.6</td>
<td>1.8</td>
<td>0.2</td>
<td>0.22</td>
<td></td>
</tr>
</tbody>
</table>

Area under the curve, AUC; incremental (baseline-subtracted) AUC, iAUC.
MR<sub>a</sub>: Meal glucose Rate of Appearance
R<sub>d</sub>: Whole body Glucose Disappearance
ln: natural log (log base e)
SI: index of insulin action
Figure Legends:
Figure 1: A. Plasma glucose concentrations obtained at breakfast (closed circles), lunch (open squares) and dinner (closed triangles) in participants with type 1 diabetes. B. Plasma insulin concentrations obtained at breakfast (closed circles), lunch (open squares) and dinner (closed triangles) in participants with type 1 diabetes. C. Plasma glucagon concentrations obtained at breakfast (closed circles), lunch (open squares) and dinner (closed triangles) in participants with type 1 diabetes.
Figure 2: A. Meal glucose appearance obtained at breakfast (closed circles), lunch (open squares) and dinner (closed triangles) in participants with type 1 diabetes. B. Endogenous glucose production obtained at breakfast (closed circles), lunch (open squares) and dinner (closed triangles) in participants with type 1 diabetes. C. Glucose disappearance obtained at breakfast (closed circles), lunch (open squares) and dinner (closed triangles) in participants with type 1 diabetes.
Figure 3: A. Si obtained at breakfast (solid bar), lunch (open bar) and dinner (hatched bar) in type 1 diabetes participants. B. Model based SI for all subjects for each meal shown individually. The boldface line indicates the average estimates for each meal. C. Diurnal Si pattern at breakfast (B), lunch (L) and dinner (D) obtained in healthy controls (shaded diamond) and type 1 diabetes participants (open squares).
MRa: T1DM

μmol/kg(FFM)/min

Time (min)

Breakfast
Lunch
Dinner
Diurnal Pattern in T1DM

- SI

Breakfast
Lunch
Dinner

10^4 dl/kg/min per μU/ml

0 7 14
Supplemental Table 1: Meal Composition

<table>
<thead>
<tr>
<th></th>
<th>Carb (grams)</th>
<th>Carb %</th>
<th>Protein %</th>
<th>Fat %</th>
<th>Fiber (grams)</th>
<th>kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>53.2±0.7</td>
<td>31.6±1.0</td>
<td>28.8±1.2</td>
<td>39.6±0.5</td>
<td>0</td>
<td>689.4±26</td>
</tr>
<tr>
<td>L</td>
<td>52.5±0.4</td>
<td>33.9±1.2</td>
<td>26.6±1.1</td>
<td>39.5±0.2</td>
<td>0</td>
<td>640.0±25</td>
</tr>
<tr>
<td>D</td>
<td>53.0±0.7</td>
<td>31.2±1.1</td>
<td>28.6±1.1</td>
<td>40.2±0.3</td>
<td>0</td>
<td>694.3±26</td>
</tr>
</tbody>
</table>

B: Breakfast; L: Lunch; D: Dinner
Supplemental Figure 1a: Shows the mean and 95% pointwise Confidence Interval Band of glucose concentrations obtained during breakfast in healthy controls (Blue) {reference#1} and type 1 diabetes subjects (Orange).
Supplemental Figure 1b: Shows the mean and 95% pointwise Confidence Interval Band of glucose concentrations obtained during lunch in healthy controls (Blue) \{reference#1\} and type 1 diabetes subjects (Orange).
Supplemental Figure 1c: Shows the mean and 95% pointwise Confidence Interval Band of glucose concentrations obtained during dinner in healthy controls (Blue) {reference#1} and type 1 diabetes subjects (Orange).