Title Page

Dietary Glycemic Index, Brain Function and Food Intake in Patients with Type 1 Diabetes Mellitus

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PART B
STUDY DESCRIPTION

TITLE OF PROTOCOL	Dietary Glycemic Index, Brain Function and Food Intake in Patients with Type 1 Diabetes Mellitus
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B1. PURPOSE OF PROTOCOL

Objective: To disentangle the postprandial effects of blood glucose and insulin on brain activation in relation to a high glycemic index (GI) meal.

Patients with type 1 diabetes mellitus (T1DM) have elevated rates of dysregulated eating and obesity (1,2). We hypothesize that these phenomena are exacerbated by effects of insulin and downstream metabolites on brain areas involved in food craving; specifically the Nucleus Accumbens (NAcc). If true, high GI meals might trigger craving and overeating, as this diet is associated with higher insulin requirements. We will dissect the role of insulin vs. glucose in mediating brain activation in response to a high GI meal by applying high vs. low GI meals with optimal (euglycemic clamp) vs. matched (primed-variable infusion) insulin coverage.

Aim 1: To examine the effects of insulin - in relation to a high GI meal - on brain activation and food intake in the early and late postprandial period.

Hypotheses:

1. (Primary) In the late postprandial period (4 hrs postprandial), blood flow to the NAcc will be greater after a high vs low GI meal when differences in glycemia are prevented by insulin.
2. In the late postprandial period, functional connectivity of the NAcc and prefrontal and limbic areas of intake control will be higher after a high vs low GI meal when preventing differences in glycemia.
3. Subjective food craving and ad libitum food intake will be associated with NAcc blood flow and prefrontal-NAcc functional connectivity in the late postprandial period.
4. In the early postprandial period (1 hr postprandial), blood flow will be decreased in the hypothalamus in response to a high vs. low GI meal.

Aim 2: To examine the effects of glucose - in relation to a high GI meal - on brain activation and food intake in the early and late postprandial period.

Hypotheses:

5. In the late postprandial period, NAcc activation will not differ between the high and low GI meal when insulin exposure is matched, in spite of the resulting hyperglycemia.
6. In the late postprandial period, functional connectivity of the NAcc and prefrontal and limbic areas of intake control will be similar after a high vs low GI meal.
7. In the early postprandial period, hypothalamus blood flow will be lower after the high vs. low GI meal.
8. In the early postprandial period, functional connectivity between the NAcc and prefrontal and limbic areas involved in intake control will be higher after a high vs low GI meal.
9. Subjective food craving and ad libitum food intake will be associated with NAcc blood flow and prefrontal-NAcc functional connectivity in the late postprandial period.

B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

1. OBESITY AND GLYCEMIC INDEX

Childhood obesity has reached epidemic proportions, with even higher levels in individuals with T1DM (1). Though multi-factorial in origin, highly processed carbohydrates in general and glycemic index (GI) in particular have been hypothesized to play an important role. The GI is a system for classifying carbohydrate-containing foods according to glycemic response (3,4). A high GI meal produces rapid elevations in blood glucose and insulin levels, followed by a period of low availability of metabolic fuels and even reactive hypoglycemia (5-7). This situation results in hunger as the body attempts to restore energy homeostasis (8-10). While there is continuing controversy regarding the effects of GI on body weight in the general population (11), individuals with high insulin secretion seem to be especially susceptible to modifications in GI (12), suggesting a role of insulin in mediating the effects of GI.

2. GLYCEMIC INDEX IN TYPE 1 DIABETES

With the rapid digestion of carbohydrate after a high GI meal, blood glucose peaks prior to achieving maximal insulin levels with standard therapy in individuals with TIDM. Insulin action may persist after nutrients from a high GI meal have been absorbed, resulting in an increased risk of hypoglycemia with subsequent need to eat carbohydrate to restore normal blood glucose. If this phenomenon recurs frequently, it could lead to excessive weight gain or possibly disordered/restrictive eating patterns in those who are attempting to avoid weight gain. Indeed, patients with T1DM have elevated rates of dysregulated eating and obesity (1,2). Several studies show benefits of GI in T1DM in terms of diet quality, postprandial hypoglycemia reduction and HbA1C (13,14). Because this diet lowers insulin requirement, there may be additional metabolic benefits including decreased anabolic drive at fat cells, and cerebral insulin signaling.

3. BRAIN CIRCUITS OF INTAKE CONTROL

Neural substrates of intake control comprise a network of hypothalamus, basal ganglia and cortical, limbic and paralimbic regions involved in reward and emotional connotation. An area of particular interest is the NAcc, which plays a role in addiction and craving, and in integrating hedonic and homeostatic intake control via insulin and dopamine signaling. In rodents, microinjections of opiate into the nucleus accumbens increase food intake and the reward value of food (17). In humans, NAcc activation is associated with poor weight-loss outcomes (18), and activation is greater n obese vs. lean individuals after viewing or consuming palatable, high calorie food (19-24).

The NAcc may play a special role in the late postprandial phase after the high GI meal: In rodent studies, extracellular concentrations of dopamine increased more after consumption of short cake (high GI) compared to standard rodent chow (low GI) (25). In clinical studies, the NAcc has been shown to be activated by hunger, hypoglycemia, and high insulin levels (15,16), as encountered in response to a high GI meal.

4. PRELIMINARY DATA

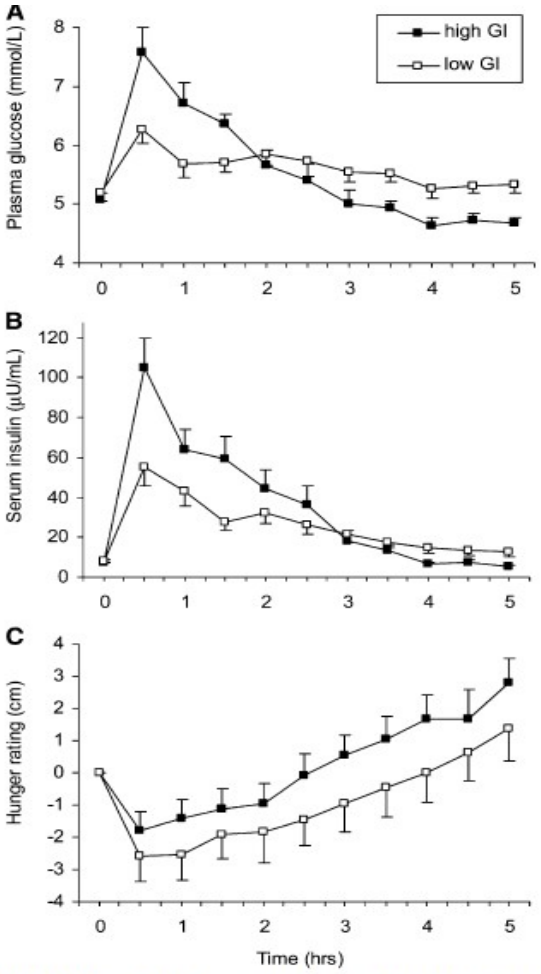


FIGURE 2. Mean ± SE changes in plasma glucose (A), serum insulin (B), and hunger (C) after test meals. Differences between high– and low–glycemic index meals were significant at 4 h (the time point of interest) for all 3 outcomes by using paired *t* tests. $n = 12$.

We conducted a randomized controlled trial and demonstrated that a high GI meal, independent of palatability, caloric content or macronutrient composition, increased NAcc blood flow (a proxy of brain activation) in the late postprandial period in healthy overweight and obese participants (15).

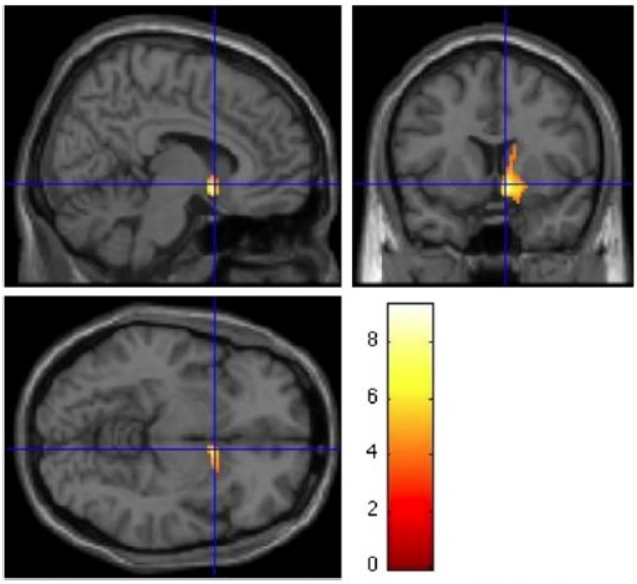


FIGURE 3. Regions with significantly different cerebral blood flow 4 h after test meals ($P \leq 0.002$). The color scale represents the value of the *t* statistic for the comparison between meals ($n = 11$) by using general linear model analyses as described in Subjects and Methods. For all areas depicted, the blood flow was greater after the high- than after the low-GI meal. GI, glycemic index.

Unresolved scientific questions involve (1) whether changes in blood glucose *per se* or the associated higher insulin levels mediate NAcc activation and the likely adverse downstream effects, and (2) how interactions with other areas of intake control are affected. While glucose and insulin both have direct effects on the brain, we hypothesize that insulin is the primary driver in this context (15,16). As both parameters are tightly correlated in healthy participants, this could not be addressed in our original study. T1DM is an ideal experimental setting to disentangle these two influences.

2.5. SIGNIFICANCE

We aim to disentangle the effects of variations in blood glucose and insulin on brain function in the postprandial state among youth with TIDM. The outcome of this work would have not only scientific but also clinical implications. If NAcc activation and its likely adverse effects are mediated by glucose excursion, optimal insulin coverage should be sufficient to prevent this problem. If it is mediated by insulin or its downstream effects, however, a high GI diet might trigger craving and overeating despite optimal insulin coverage. Thus, our findings will point the way to a heightened focus on improved insulin delivery to optimally control blood glucose *versus* measures to decrease insulin demand, including a low GI diet.

B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

Brief Overview of the Study

1. Overall Study Design and Research Question
This is an experimental study in humans using a randomized, double-blinded cross-over design to examine the effects of glucose and insulin on brain activation in relation to a high glycemic index (GI) meal in the early and late postprandial period.

2. Key Details of Study Implementation
Male participants with Type 1 diabetes will be enrolled to participate in 4 study visits involving standardized test meals and iv insulin infusions. Participants will be enrolled for 1-3 months. The primary endpoint is blood flow in the NAcc, a brain area that regulates food intake and craving.

3. Summary of Study Procedures
A total of 15 male participants (age 18-45) with T1DM will be recruited. Participants will be enrolled in the study for a total of 1-3 months, and participate in a pre-test visit and three test visits, each after a 10-12-hr overnight fast. Participants will be instructed to consume their regular, weight maintaining diet between visits.
At the ***pre-test visit***, the study director or PI will meet participants at the BIDMC GCRC, confirm eligibility and obtain informed consent. Participants will receive a low GI meal with optimal iv insulin coverage using a negative feedback algorhythm to maintain euglycemia (euglycemic clamp). Insulin requirement will be quantified. At some time during the visit, participants will present to the BIDMC research imaging facility (East Campus, Ansin 3) for a practice MRI session, during which they will undergo a brief imaging sequence to get accustomed to the scanning process and eliminate anxiety as a confounder of imaging data.
At each of ***3 test visits*** at the BIDMC GCRC and research imaging facility (East Campus, Ansin 3), one of the following experimental conditions will be applied in a randomized, blinded cross-over design: (a) high GI meal with euglycemic clamp, (b) low GI meal with euglycemic clamp, (c) high GI meal with primed-variable insulin infusion at the rate established during the pre-test visit. After steady state is established, baseline laboratory evaluation and MRI imaging will be obtained, followed by the test meal. Imaging will be repeated at 1 and 4 hours postprandial. Blood samples for pertinent metabolic and hormonal parameters will be obtained every 30 minutes. Each test-visit concludes with a standard weighed meal to quantify ad-libitum intake.

Detailed Study Procedures

A Pilot Phase
The pilot phase serves to establish feasibility of the insulin clamp and finalize clamp protocol in the setting of the test meals. 1-3 subjects will be recruited to undergo 3 test visits as outlined below, but no pre-test visit. Meal administration and insulin infusions with blood glucose checks will be as outlined. The order of visits will condition a → condition b → condition c and will not be randomized. The insulin dose administered during condition c will be determined during condition a.
Pilot phase subjects will undergo venous blood sampling for HgbA1C (once per visit) and C-peptide (once per study) only and will not undergo imaging. The same inclusion and exclusion criteria will apply for pilot phase subjects with the exception that the MRI compatibility exclusion criterion will be waved.

B Test Phase
1. Enrollment period
The enrollment period begins with the pre-test visit and ends with the 3rd test visit. The enrollment period will be limited to 3 months to decrease intra-subject variability. Throughout the study, energy balance and stable metabolic control are important because of their influence on insulin sensitivity,

hypoglycemia sensing, and hunger. Participants will therefore be asked to continue on their normal, weight-maintaining diet, exercise regimen and insulin plan. Participants will be instructed to wear a continuous glucose monitor or check blood glucose at a minimum of 6 times a day for 72 hours prior to each study visit to ascertain absence of hypoglycemia and near-normal BG control. Participants are advised to check blood or urine ketones according to their usually prescribed regimen. Customary reasons to check for ketones include hyperglycemia > 250 mg/dl, illness or preceding insulin omission or insulin pump failure. Subjects may check blood or urine ketones with their usual test strips. Participants will be instructed to call the study personnel and reschedule their visit if:

- they are ill
- they have symptomatic hypoglycemia below 50 mg/dl within 24 hours of the visit
- they have ketosis (blood ketones >0.6 or urine ketones >trace) in the setting of hyperglycemia or insulin omission within 24 hours of the visit
- they have hyperglycemia above 250 mg/dl the morning of the visit

2. Study Visits

At all visits, participants will present in the AM after a 10-12-h overnight fast to BIDMC CRC to standardize for circadian metabolic and hormonal fluctuations. Participants will be weighed and insulin pump and glucometer or continuous glucose monitoring data (if available) will be downloaded to evaluate energy balance and metabolic control at the beginning of each visit. In the CRC, an iv catheter will be inserted into an antecubital vein for the administration of insulin. Point of care glucose will be obtained and analyzed immediately. The visit will be rescheduled if blood glucose is below 50 or above 250 mg/dl. A second catheter will be inserted into a distal forearm or hand vein for the withdrawal of blood samples. A hotbox will be used to warm the hand to arterialize the venous blood. While in the MRI scanner, the hotbox will be replaced by a a heated gel pack. Insulin pump will be discontinued and iv insulin infusion will be given for 5 ½ hours at each of the four visits. At each visit, participants will consume a test-meal at baseline and an ad-libitum, standard weighed meal at the end of the visit.

2.1. Pre-test visit

The study director or PI will meet participants at the BIDMC GCRC, confirm eligibility and obtain informed consent. Participants will receive a low GI meal with optimal iv insulin coverage using a negative feedback algorhythm to maintain euglycemia (euglycemic clamp), as outlined in 3.3.1.. Insulin requirement will be quantified. At some time during the visit, participants will be transferred to the BIDMC imaging facility for a 5-minute practice MRI session, during which they will be positioned in the scanner and undergo a brief imaging sequence to get accustomed to the scanning process and eliminate anxiety as a confounder of imaging data. Insulin infusion may be briefly discontinued while in the MRI scanner.

2.2. Test visits

In a double-blinded cross-over design, three test visits will serve to evaluate brain function, metabolic, and hormonal parameters. Sessions will be at least 1 week apart to eliminate habituation to the scanning process and carry-over effects, but no more than 4 weeks apart to limit intra-subject variability. At each test visit at the BIDMC GCRC and imaging facility, one of the following experimental conditions will be applied in a randomized, blinded cross-over design: (a) high GI meal with euglycemic clamp, (b) low GI meal with euglycemic clamp, (c) high GI meal with primed-variable insulin infusion at the rate established during the pre-test visit. As the high GI meal is associated with higher insulin requirement, this condition will result in moderate hyperglycemia. Thus, the design will allow comparison between a high and low GI meal with matched blood glucose but different insulin levels (condition a vs. b) ***or*** matched insulin with different blood glucose levels (condition b vs. c) (figure 1). After steady state insulin infusion is achieved, baseline laboratory studies will be obtained and participants will be transferred to the BIDMC imaging facility for their baseline scan. Postprandial imaging times are chosen to study cerebral blood flow and functional connectivity between brain areas of interest at the peak of blood glucose and insulin requirement (+1 h) and at the nadir of blood glucose/ insulin (+4 h). Because compensatory mechanisms and potential overeating are expected in

the late postprandial period, this will be the main focus of the study. As most prior studies on intake regulation focus on the early postprandial phase, that time point will place the research in the context of the current literature. Test meals (3.1.) will be administered directly after the baseline imaging and participants will remain in the imaging facility through their 1-hour postprandial scan, and then transferred back to the GCRC. They will remain in the GCRC until the time of their 4-hr scan, for which they will be transferred to the imaging facility and then come back to the GCRC for the ad libitum weighed meal (3.2.) (see figure 2 for locations of respective procedures). Between imaging, participants will be allowed to engage in a quiet activity such as reading. Insulin infusion and glucose monitoring will be continued in the MRI scanner. For this purpose, the study physician and a CRC nurse will accompany the participant to the MRI suite and remain there for ~ 90 minutes for the first scanning period and 30 minutes for the second scanning period. In addition to frequent point of care blood glucose sampling, arterialized blood samples will be collected from an indwelling catheter at baseline and every 30 minutes for 4 hours. Samples will be processed and stored at -80 degrees Celsius for further analyses of pertinent metabolic and hormonal parameters including plasma glucose, insulin, C-peptide, FFA, ghrelin, GLP-1, PYY, CCK, glucagon, and leptin. To correlate brain activation with behavioral endpoints hunger will be assessed every 30 minutes on a 10-cm visual analog scale ranging from “not at all hungry” to “extremely hungry”. At the end of each visit, we will quantify ad libitum food intake of a standardized weighed meal.

3. Interventions

3.1. Test meals:

LOW GI	HIGH GI
Food	Food
Cornstarch	Corn syrup
Fructose	Fructose
Vanilla extract	Vanilla extract
Milk, 1% fat	Milk, 1% fat (+ lactase)
Egg white, dried	Egg white, dried
Olive oil	Olive oil

Table 2. Composition of test-meals

After baseline assessments, we will initiate timed consumption of a liquid test meal over 5 minutes, to facilitate conjunction with the MRI session. High and low GI test meals have been previously developed by our group, ingredients are listed in Table 2. The meals have similar macronutrient composition (60% carbohydrate, 15% protein, 25% fat), micronutrient profiles, physical properties, palatability and sweetness. Meals will provide 25% of individual daily energy requirements as estimated by the Harris Benedict equation. The high vs low GI meals have a predicted difference in GI of 90 vs 40. Consistent with this prediction, a pilot study in obese young adults found a 2.2-fold difference in glycemic response ($p<0.001$). This was confirmed in our previous study in healthy obese men (15).

3.2. Weighed ad libitum meals:

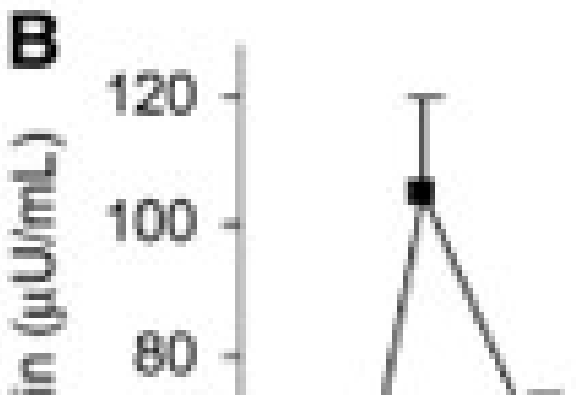
At the screening visit participants will select a meal according to their food preferences. The test meal will be prepared at CRC metabolic kitchen according to the following macronutrients breakdown: 60% carbohydrate, 15% protein, 25% fat. The meal will be large enough that it cannot be finished (~1000-1500 kcal), and served in multiple servings (e.g. quarters of a sandwich). Participants will be instructed to eat as much as they would like to satiate their hunger. Participants will self-administer insulin according to their usually prescribed regimen. The meal will be weighed before and after consumption and caloric intake and macronutrient composition will be calculated. The same meal will be used at each study visit.

3.3. Insulin administration

3.3.1. Euglycemic clamp - pre-test visit, experimental conditions a & b (figure 1)

During the entire clamp protocol, glucose levels will be measured every 5 minutes. A basal insulin infusion will be started at 80% of the patients insulin pump basal rate, and will be adjusted between 0.1 and 2.5 mU/kg·min, depending upon the patient’s plasma glucose level in relation to the target range target of 90-100 mg/dl according to a negative feedback mechanism. Once steady state is achieved, baseline laboratory studies and imaging will be obtained and test meal administered. Based on our preliminary study using the same test meals in 12 non-diabetic overweight and obese males (15), we anticipate a steep increment in insulin requirement within the first 30 minutes postprandial.

Figure 1: Anticipated serum insulin levels



Serum insulin peaked at 30 minutes postprandial and increment in plasma insulin was on average 110 μU/ml after the high GI meal and and 55 μU/ml after the low GI meal.

We will therefore implement a stepwise increase in insulin infusion rate within the first 30 minutes after the test meal. Increments are informed by our previous experience wherein each increment in the insulin infusion rate of 0.1 mU/kg·min raises the circulating insulin concentration by 6 μU/ml, as well as participants' individual insulin per carbohydrate ratios. We will aim to maintain postprandial blood glucose levels within a target range of 70-140 mg/dl.

3.3.1.1. High GI meal (condition a)
After consumption of the test meal, insulin infusion rate will be increased by 0.2-0.3 mU/kg/min every five minutes until blood glucose starts to fall, with further adjustments based on blood glucose trajectory. We then anticipate a stepwise decrease in insulin infusion rate, reaching basal rates at 2-3 hours postprandial. Blood glucose sampling and insulin infusion will be continued according to a negative-feedback mechanism for 4 1/2 hours postprandial. Then, insulin infusion will be discontinued and insulin pump therapy resumed according to the participants' home regimen using their own equipment.

3.3.1.2. Low GI meal (screening visit and condition b)
After consumption of the test meal, insulin infusion rate will be increased by 0.1-0.2 mU/kg/min every five minutes until blood glucose starts to fall, with further adjustments based on blood glucose trajectory. We then anticipate a stepwise decrease in insulin infusion rate, reaching basal rates at 3 hours postprandial. Blood glucose sampling and insulin infusion will be continued according to a negative-feedback mechanism. for 4 1/2 hours postprandial. Then insulin infusion will be discontinued and insulin pump therapy resumed according to the participants' home regimen using their own equipment.

Insulin administration chart
Planned insulin infusion rates to account for meals are depicted. Rates may vary based on individual insulin requirements and measured blood glucose as outlined above.

Time (minute)	**Insulin infusion rate (mU/kg/min)**	
	HGI	LGI
pre-meal	0.2	0.2
5	0.5	0.3
10	0.8	0.5
15	1.1	0.6
20	1.4	0.8
25	1.2	0.8
30	1.1	0.7
35	1.0	0.7
40	0.8	0.7
50	0.8	0.6
70	0.8	0.4
100	0.7	0.5
130	0.5	0.4
160	0.3	0.4
200	0.2	0.3
220-end	0.2	0.2

Example: Based on this chart, the total meal insulin administered for an 80 kg male would be 8 units for the high GI and 5 units for the low GI meal. These are customary amounts of mealtime insulin.

Due to individual differences in insulin requirement, actual administered rates of insulin will vary between 0.1 and 2.5 mU/kg·min. For patient safety, rates outside this specified range may be necessary at times.

3.3.2. Primed-variable insulin infusion - experimental condition c (figure 1)

In the run-in period, glucose levels will be measured every 5 minutes. A basal insulin infusion will be initially administered at 80% of the patients insulin pump basal rate, and will be adjusted between 0.1 and 2.5 mU/kg·min, depending upon the patient's plasma glucose level in relation to the target range target of 90-100 mg/dl. Once steady state is achieved, baseline laboratory studies and imaging will be obtained and test meal administered. Then, a primed-variable infusion of insulin will be administered at the rate established during the pre-test visit to achieve similar plasma insulin levels. For patient safety, glucose levels will be measured every 30 minutes. If glucose levels are > 400 mg/dl or < 60 mg/dl, insulin infusion will be adjusted to maintain glucose levels target of 60-400 mg/dl. Insulin infusion will continue for 4 1/2 hours postprandial. Then, Insulin infusion will be discontinued and insulin pump therapy resumed according to the participants' home regimen using their own equipment.

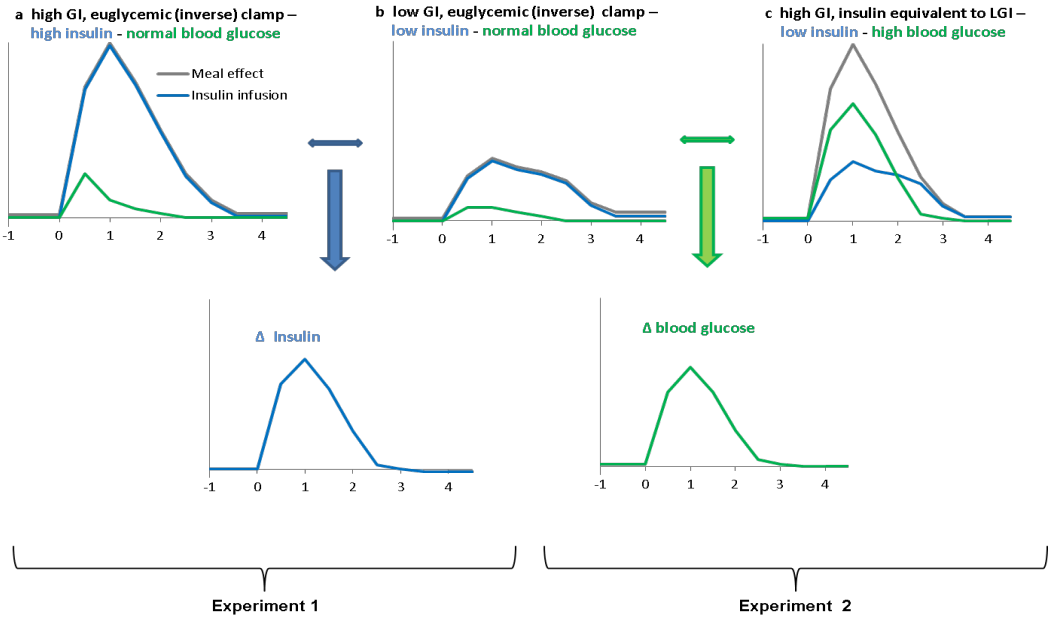


Figure 1:

Figure 1: Schematic depiction of anticipated glycemic changes and insulin levels during the different experimental conditions. Theoretical glycemic response to the meal is plotted in gray, anticipated insulin requirements in blue and resulting blood glucose in green. (a) High GI meal with euglycemic clamp, (b) low GI meal with euglycemic clamp, (c) high GI meal with primed-variable insulin infusion at the rate established during the pre-test visit. As the high GI meal is associated with higher insulin requirement, this condition will result in moderate hyperglycemia. Thus, the design will allow comparison between a high and low GI meal with matched blood glucose but different insulin levels (experiment 1 - condition a vs. b) ***or*** matched insulin with different blood glucose levels (experiment 2 - condition b vs. c)

4. Assessments

4.1. Data to confirm eligibility

4.1.1. Pre-enrollment medical record review (if patient recruited from participating institution)

- Age
- Sex
- Insulin pump use
- Dime of initial Diagnosis
- Total daily insulin dose
- Current Hemoglobin A1C (within 3 months)
- Weight (within 3 months and within 1 year), height
- Medical problems
- Medications
- Allergies / dietary restrictions
- Time of last episode of DKA
- Occurrence of hypoglycemia (BG <50 mg/dl)

4.1.2. At initial telephone screening

- ***Elicit if medical record unavailable:*** age, sex, date of initial diagnosis, weight (within 3 months and within 1 year), height, current Hemoglobin A1C (within 3 months). Participant will be asked to provide written confirmation of HbA1C (e.g. recent clinic note or laboratory printout).
- ***Confirm (or obtain if medical record unavailable):*** last episode of DKA, occurrence of hypoglycemia (BG <50 mg/dl), insulin pump use, total daily insulin dose, medical problems,

medications, allergies / dietary restrictions.
- ***Elicit always:*** Current complaints, smoking or illicit substance abuse, levels of physical activity, current weight loss diet, willing and able to: maintain weight and document for duration of the study, MRI exclusion criteria (according to BIDMC MRI checklist).

4.1.3. At the pre-test visit
- Height via double-measurement using a wall-mounted stadiometer.

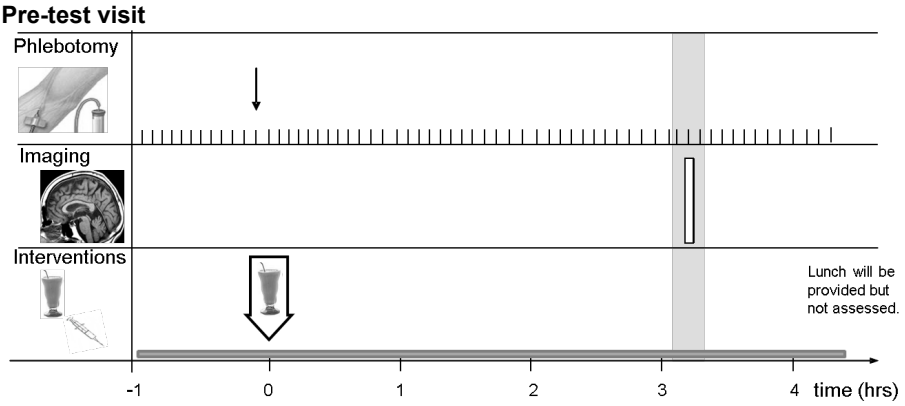
4.1.4. At each visit
- Hemoglobin A1C
- Weight wearing a hospital gown or minimal clothing and socks without shoes via double-measurement using an electronic scale
- Insulin pump and glucometer or continuous glucose monitoring date will be downloaded and reviewed.

4.2. Safety measures
- Vital signs and blood pressure will be determined at the beginning and end of each visit from the left arm using an automated system (Dinamap, Criticon Inc, Tampa, FL). The cuff will be sized appropriately for the diameter of the arm.
- Point of Care hemoglobin will be checked once at the beginning of the study to ascertain subjects are not anemic (Hemoglobin cut-off 12.5 mg/dl).

4.3. Process measures
- Fasting C-peptide via CLIA certified methods at the pre-test visit.
- Point of care blood glucose will be checked every 5 minutes during the pre-test visit and conditions a and b. Glucose checks will be spaced out to every 30 minutes after administration of the test meal during condition c.

4.4. Study outcomes

Figure 2:



Test visits - conditons (a) and (b)

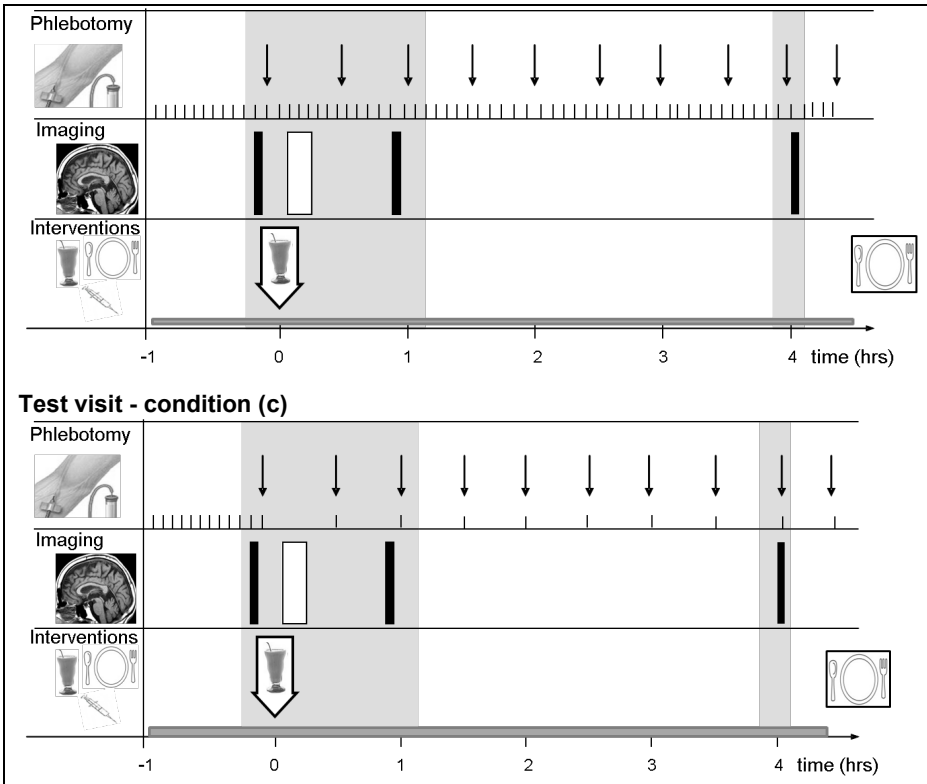


Figure 2: Schematic depiction of study visit flow. A – pre-test visit, B – study visit a and b. C – study visit c. Upper row: hash marks represent point of care glucose monitoring; arrows represent venous blood sampling. Blood glucose will be measured every 5 minutes during the clamp design and every 30 minutes during primed-variable insulin infusion. Middle-row: black boxes represent timed MRI scans, open boxes represent practice MRI session and MPRAGE sequence (time variable). Bottom row: Arrow represents test meal, box represents ad-libitum weighed meal, gray bar represents iv insulin infusion. Location of intervention is encoded: white background – GCRC; grey background- imaging facility.

4.4.1. Imaging

All imaging will be performed without contrast on the 3T scanner at the BIDMC research imaging facility (East Campus, Ansin 3). Participants will be asked to change into hospital gowns, to minimize the potential of unseen objects within pockets and metallic attachments to clothing. Participants will be instructed to lay still and rest with closed eyes during the image acquisition. They will then be positioned in a GE HD/X (GE Healthcare, Milwaukee, WI) in supine position. An imaging coil will be placed around their head. Participants will be given earplugs to minimize noise from the scanner and a squeeze ball that triggers an alarm to indicate a desire to exit. Participants will also be able to communicate by an intercom system to the operator. All scans will be performed by an experienced radiologic technologist.

Participants will undergo a 3 plane localizer, ASL and rs-fMRI scan at baseline, 1 hour and 4 hours postprandial. Between the baseline and 1-hour scan, an MPRAGE dataset will be obtained for anatomical correlation.

4.4.2. Laboratory Analyses

Arterialized venous blood samples will be collected at baseline and then every 30 minutes over a 4 1/2 hour period postprandially (figure 2). Blood volume will not exceed 140 ml per study visit or 420 ml over the study period. Samples will be processed and frozen at -80 degree Celsius for later batched analyses. We will use the following or comparable kits for sample analysis: plasma glucose, APEC Glucose Analyzer (APEC, Inc, Peabody, MA); serum insulin, Abbott IMx (Abbott Laboratories, Abbott Park, IL); serum fatty acids, Hitachi 917 Analyzer (Wako Chemicals USA, Inc, Richmond, VA. Plasma ghrelin, GLP-1, PYY, CCK, glucagon, and leptin will be analyzed as part of the metabolic

hormone panel (Luminex xMAP (Milliplex, Millipore, MA). To eliminate blood waste we will use a closed phlebotomy system (VAMP, Edwards Lifesciences Corp., Irving, CA).

4.4.3. Metabolic profiles

Samples will be obtained at baseline, 1 hour and 4 hours postprandial to be analyzed by the Metabolomics Core at Broad Institute. This Core has developed an LC-MS/MS methodology that uses several chromatographic stationary phases and analyzes > 400 sugars, sugar phosphates, nucleotides, amino acids, organic acids, and lipids (e.g. individual cholesterol esters, triglycerides, diacylglycerols, phospholipids, and lysophospholipids) in a targeted manner. The platform is designed on the principle that a metabolite can be identified by its parent ion mass (MS) and dominant product ion mass (MS/MS) on a sensitive mass spectrometer in combination with its retention time on an appropriate chromatography column. While none of these three parameters is individually sufficient to uniquely identify a metabolite in a biological fluid, the three in combination provide a "tag" that marks the metabolite to permit identification and quantification.

4.4.4. Psychophysics

Hunger will be assessed every 30 minutes throughout the session. We will ask participants, "How hungry are you right now?" To obtain a response to this question, we will use a 10-cm visual analog scale ranging from "not at all hungry" to "extremely hungry", consistent with methods used in our preliminary studies (15). Participants will be instructed to put a single vertical hash mark on the scale when responding.

5. Randomization

In this crossover trial, 3 conditions will be administered in 2 different sequences. As statistical comparison will only be made between condition (a) and (b), and (b) and (c), never between (a) and (c), the 2 orders will be (a)->(b)->(c), or (c)->(b)->(a). Condition b will always be adjacent to one of its comparators, limiting intra-subject variability, and the order of the compared conditions will be random. A randomization list of intervention sequences will be generated by the Clinical Research Program (CRP) at Boston Children's Hospital.

The study director will be supplied with a numbered log for assignment of sequential randomization numbers (RID) as participants are enrolled. This log will only contain the RID.

The randomization log will be given to the BIDMC GCRC nutrition core and not shown to any study staff. It will contain the RID, sequence of conditions and a letter-code for each condition, in which only condition b is identifiable (e.g. B, X and Y).

Participants are randomized the day before the first test-visit, after participation is confirmed by phone. As each participant is enrolled, the study director will enter the participant's unique study ID into the log, next to the next open RID. The study director will then communicate this information to the BIDMC GCRC nutrition core. The BIDMC GCRC nutrition core will reveal the experimental condition to the study physician to allow assignment to the appropriate insulin paradigm. Meals will be prepared accordingly and are labeled with the RID only and no information on the experimental condition.

For data pre-processing, the date of the MRI images will be obscured to maintain the blind. Only the letter code (e.g. B, X and Y) for each condition will be revealed to allow for single-subject and group analyses of brain response. After completion of all statistical analyses, the study director will be unblinded to the nature of the dietary intervention. If a participant withdraws before supplying usable data, the subject's RID will not be reused or assigned to another enrollee. If a participant withdraws after one test-visit, available data will be analyzed but not included in the group analysis. If a participant withdraws after two test-visits, available data will allow for full analysis of one experiment and will be included in the group analysis. The randomization list will be slightly longer than needed in order to allow for such extra entries.

B. Statistical Considerations

Sample Size Justification and Analysis Plan

Dr. Henry Feldman, a biostatistician based in the CHB Clinical Research Program and affiliated with the Endocrinology Division, collaborated with us on the power calculations and statistical analysis plan.

Multiple testing. Although Aims 1 and 2 cover several hypotheses and measures, one endpoint is primary: blood flow in the NAcc at 4 hr after feeding. Our power analysis thus primarily focuses on two comparisons, embodied in Hypothesis 1 (Aim 1) and Hypothesis 5 (Aim 2). The remaining hypotheses concern secondary outcomes. We would not consider changing the sample size for the sake of detecting effects in these outcomes and have therefore not pursued power analyses for them. We will of course conduct rigorous statistical analysis on the secondary outcome variables and deduce whatever we can from the available data, but our resource planning is aimed above all at the question of 4-hr postprandial blood flow in the NAcc.

Power analysis. We view the design, illustrated in Fig. 1, as a crossover study with two experimental conditions (A: high GI, high insulin, normal glucose; C: high GI, low insulin, high glucose) and a common comparison condition (B: low GI, low insulin, normal glucose).

Our original analytic plan (alternative #1) calls for two pre-specified comparisons (A vs B and C vs B), conducted as if they were independent experiments, using 5% type I error for each comparison without adjustment for multiple testing. The assumption of independence may seem questionable because of the correlation among each subject's three observed outcomes — $A+\delta+\varepsilon1$, $B+\delta+\varepsilon2$, and $C+\delta+\varepsilon3$ under a random-effects or compound symmetry assumption, where A, B, and C are fixed means for the three conditions and δ and εi are independent Gaussian deviates — but this assumption is justified by the fact that the crossover analysis will hinge on two within-subject contrasts that are statistically independent:

$\mathrm{Cov}((A + \delta + \varepsilon1) - (B+ \delta + \varepsilon2), (C + \delta + \varepsilon3) - (B+ \delta + \varepsilon2)) = 0.$

The detectable effect for each paired-sample comparison (A vs B or C vs B) is $(t\alpha/2+t\beta) \times SD(D)/n½$, where n is the sample size, SD(D) is the standard deviation of the intrasubject difference in mean flow between conditions, and t() are Student deviates for power $1–\beta$ and two-sided type I error α with $n–1$ df. Our prior study yielded SD(D)=20 units of blood flow. For these calculations we take $\alpha=0.05$ and a conservative value of SD(D)=30. The planned sample of n=15 gives us 80% power to detect an effect of $(2.1448 + 0.868) \times 30/15½ = 23.3$ units, or 4.3% of the mean flow (548 units in the prior study), and 90% power to detect an effect of 4.9%. These figures are comfortably below the 7.9% observed in our prior study. We therefore believe that the present protocol is adequately powered to detect clinically significant effects.

A still more conservative analytic plan (alternative #2) would be to apply the Bonferroni correction, halving the type I error rate for each of the two pre-planned comparisons. With this change, making $\alpha=0.025$, the detectable effects are 4.8% with 80% power and 5.4% with 90% power. These figures are negligibly different from the earlier results.

Finally (alternative #3), we have considered framing the analysis as a 3-group comparison (A vs B vs C) with the null hypothesis that all three conditions have equal mean. Pairwise comparisons would be pursued if and only if the hypothesis of three equal means was rejected. The type I error rate of 5% would be applied to the 3-group (2-df) hypothesis. The principle of closed testing enables us to use a $p<0.05$ criterion for the pairwise comparisons as well, should they be made, while preserving the family wise type I error rate at 5% for the overall procedure (Bender R, Lange S, J Clin Epidemiol 54:343, 2001; Marcus R et al., Biometrika 63:655, 1976). We conducted simulations of this plan, again assuming SD(D)=30 with n=15, and taking account of within-subject correlation across conditions, which we varied between $\rho=0$ and $\rho=0.8$. The detectable difference between condition A and the common comparison condition was similar to the figures cited above: 4.2-4.3% with 80% power, depending on ρ, and 4.8-5.0% with 90% power. Besides being relatively insensitive to the degree of within-subject correlation, these results held regardless of whether we assumed a null

effect for condition C or an effect equal in magnitude to that of condition A.
We conclude that our original analysis plan (alternative #1 above) is most satisfactory, having the advantage of simplicity as well as comparable inferential power to the more intricate strategies.

Data Analyses

Endpoints

Primary endpoint: Blood flow to the NAcc, as quantified by ASL.

Secondary endpoints: Blood flow to other brain areas relevant to intake control, connectivity of brain areas of intake control, metabolic fuels, hormones, hunger ratings, metabolomic profiles, ad libitum food intake.

Effect modifiers: insulin levels, glucose levels, metabolic fuels, hormones, hunger ratings, daily insulin requirement

Analysis of imaging data will be performed within the Statistical Parametric Mapping (SPM5, Welcome Department of Cognitive Neurology) statistical image analysis environment. The images will be converted to a standard naming convention using the OSIRIX DICOM viewer http://homepage.mac.com/rossetantoine/osirix/Index2.html) and then converted into Nifti format with appropriate spatial information in image headers. Once in the appropriate format, CBF images will be realigned to the first CBF image and then all CBF images will be transformed to a standard anatomical space using the registration parameters derived from the SPM "Normalization" algorithm applied to the mean image and using the gray matter a prior template as the target image. Normalization will be performed using the more standard preserve concentration option, which is acceptable since we do not expect significant atrophic changes during the study. Subsequently, images will be smoothed with an 8 mm FWHM kernel in preparation for statistical analysis. Statistical analysis will be performed on a voxel by voxel basis within SPM using the ANOVA algorithms of the general linear model and the SPM derived correction for multiple comparisons. A false discovery rate (FDR) threshold of 0.05 will be employed. Cluster size thresholding will not be initially employed to avoid loss of the small hypothalamus, but modest cluster thresholding may be applied to improve sensitivity of cortical activity change measurements. In addition, regional analysis of targeted regions will be performed by applying the regions defined with the AAL atlas on the spatially normalized images. MRI data will be correlated with data from venous sampling and hunger ratings using ANOVA algorithms.
To address the primary and secondary hypotheses, we will employ methods appropriate to crossover trials. Hypotheses 1,2,4-8 will be tested by paired T-Test, controlling for baseline and meal order. Hypothesis 3 and 9 will be addressed by the Pearson correlation between brain activation parameters and calculated caloric intake. Secondary analyses of associations between brain activation, metabolic and psychophysical parameters will be performed using an ANOVA design. All tests will be conducted with a 5% Type I error rate. SAS software (Cary, NC) will be used for all computations.

C. Subject Selection

Inclusion criteria

- Males age 18 to 45 years
- Type 1 diabetes for a minimum of 3 years
- BMI 20-35 kg/m^2
- Use of insulin pump
- Willing and able to: Maintain weight and document for duration of the study

Rationale for inclusion criteria. ***Age:*** We have chosen an age-range to minimize developmental and puberty effects on insulin sensitivity and brain activation, yet capture a relevant age-group for preventive measures where comorbidity isn't yet highly prevalent. ***Gender*** is a known confounder of neuroimaging data. Women with T1D are more prone to develop eating disorders and obesity (1,2), and gender differences in food-preferences and in neuro-humoral regulation of eating behavior have been observed (26). Furthermore, fluctuations in gonadal steroid hormones account for significant

changes in brain activation patterns depending on the menstrual cyclic phase in women (27). As such, women have to be studied on matching days in the late follicular phase. In a study that extends over 4 study visit, this would extend the enrollment period over a minimum of four months, with the associated intra-subject variability and seasonal changes in insulin requirement. We will therefore focus this pilot study on male participants. ***Time since diagnosis*** is set > 3 years to avoid participants that have persistent endogenous insulin production (honeymoon phase) that would confound the clamp design. The ***BMI range*** is chosen to exclude participants with underweight or extreme overweight who have alterations brain circuits of intake control (19-24). ***Insulin pump use*** will allow for tighter metabolic control and provide more detailed data on baseline insulin requirement to inform the insulin clamp design. It will also limit basal insulin on board that may confound plasma insulin levels.

Exclusion criteria

- Insulin resistance (current insulin requirement > 1.5 U/kg/d)
- Insulin requirement < 0.5 unit/kg/day (cut-off for preserved beta-cell function)
- HbA1C ≥ 8.0%
- DKA within 2 months
- Frequent hypoglycemia (BG <50 mg/dl), > 3 times per week
- Fluctuations in body weight >10% over preceding year
- Smoking or illicit substance abuse
- High levels of physical activity (≥60 minutes per day, ≥ 4 days per week)
- Current weight loss diet
- Medical problems, medications or dietary supplements that may affect metabolism, insulin action, body weight, appetite, energy expenditure, or gastrointestinal absorption (e.g. celiac disease)
- Allergies to compounds or intolerance of the liquid meals
- MRI exclusion criteria
- Other conditions according to self-report that would prohibit participation based and researcher assessment

Rationale for exclusion criteria. ***Insulin resistance*** is not a homogeneous phenomenon and can have different degrees in peripheral tissues and brain. Thus, insulin resistance with the concomitant higher insulin levels would have unforeseen effects on brain activation (e.g. higher insulin exposure, vs. concomitant brain insulin resistance with lower insulin exposure). Participants with overt insulin resistance will therefore be excluded. ***Very low insulin requirement and elevated C-peptide*** are markers of endogenous insulin production. As endogenous insulin production would confound the clamp design, we will limit the study to participants with minimal to no endogenous insulin production. ***Insulin deficiency (high HbA1C, DKA) and hypoglycemia*** have known effects on metabolism and intake regulation, and are exclusionary. ***Diet and recent weight change, use of medications, or dietary supplements, smoking, physical activity, or adherence to a special diet*** may affect metabolism and intake regulation, thereby confounding interpretation of study results.

B4. POSSIBLE BENEFITS

Benefits

Benefits to the participant include medical assessment that might identify a co-existing treatable illness. All clinical, laboratory and MRI studies will be reviewed by a qualified physician or radiologist respectively. We will notify patients and recommend for them to follow up with their physician if significant incidental abnormalities are detected.

The study may provide important information regarding the metabolic and cerebral effects of dietary GI, potentially informing dietary approaches to diabetes care. We believe that the benefits and knowledge to be gained considerably outweigh the minor risks and inconvenience to study participants.

Compensation

Participants will receive $ 150 upon completion of the pre-test visit, $ 200 upon completion of test-visit 1, $ 250 upon completion of test-visit 2, and $ 300 upon completion of test-visit 3, for a total of $ 900. Participants in the pilot study will receive $ 150 upon completion of test-visit 1, $ 200 upon completion of test-visit 2, and $ 200 upon completion of test-visit 3, for a total of $ 600. Compensation will be via check request through the BIDMC Accounts Payable department after each visit. In addition, participants will receive parking vouchers or reimbursement for public transportation.

B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

The protocol involves only moderate risks and discomforts to the participants. All participants will be screened prior to enrollment to rule-out the presence of any pre-existing or complicating medical condition, as described above. Slight pain and small bruises may be expected from insertion of the iv lines and blood drawing. The participant may feel lightheaded or nauseated during the iv sampling. There is a small chance of allergic reactions after the test meals. Minor discomfort might be caused by resting supine in the MRI scanner. High noise exposure will occur during MRI measurements and will be limited by providing earplugs.

Insulin will be administered iv, posing a potential risk of hypoglycemia. Insulin doses will be similar to participants' typical insulin regimen. We will monitor BG closely, and adjustments to insulin infusion rate will be made promptly. As such, we do not anticipate severe hypoglycemia. However, iv dextrose (D25%) will be available to correct severe hypoglycemia < 50 mg/dl that does not resolve with reduction or discontinuation of insulin infusion. The study physician and a study nurse will be present next to the participant during the entire clamp procedure of the study. During the primed-variable insulin infusion, patients will experience moderate hyperglycemia. Since the standard of care for diabetes treatment does not account for dietary glycemic index, similar hyperglycemia is expected to occur after day to day consumption of processed carbohydrates. However, we will monitor blood glucose and be prepared to intervene if blood glucose exceeds a threshold of 400 mg/dl. This is a safe value as no concurring ketosis or other complications would be expected in this setting.

B6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment

We will recruit participants from the community and diabetes programs at Boston Children's Hospital, and Beth Israel Deaconess Medical Center using a multi-step screening process to assess eligibility prior to enrollment. Advertisements will be posted online and in the community and be distributed to treating physicians.

1. Participant Identification

1.1. Participating Institutions:

- Eligible patients will be identified by chart research using data as outlined in 4.1.1.

- Their primary Diabetologist will be informed of study eligibility, and asked to present study information to the patient.
- A letter or email signed by the patient's treating physician will be sent to the patient.
- Interested subjects can contact the study team to arrange for a telephone interview.
- Other patients will be contacted by phone 2-3 weeks after the initial contact to inquire if they would like more information on the study. All calls will take place between 10am-9pm Monday thru Friday, or 10am – 5pm Saturday and Sunday. A telephone script will be used during the conversation or in case a voice message is left (attached).

1.2. Other locations:

- Advertisements will be posted online and in the community and be distributed to physicians.
- Physicians may give study information to potential participants, or
- Physicians may get patients permission to be contacted for the study and will then provide contact information to the study team.
- Participants may contact the study team directly by telephone or email to inquire about the study.

2. Enrollment

- A member of the study team will explain the study and discuss the required time commitment by phone or in person.
- This will be followed by a telephone screening (5 minutes) obtaining data as outlined in 4.1.2.
- At the pre-test visit, participants will lay in the scanner to assess whether they can be positioned correctly. Final eligibility data will be obtained as outlined in 4.1.3.

Consent

Information about the study design, venous sampling, test meals, MRI technique and scenario, and time commitment will be provided in word and writing to the participant prior to obtaining written consent. Written informed consent will be obtained from all participants by Dr. Belinda Lennerz MD PhD, at the screening visit at the BIDMC GCRC.

Subject Protection

This study has been designed to keep risks to the lowest level possible. A physician will be available on a 24-hr per day basis should any problems arise. The blood sampling and administration of test meals and insulin will be performed under strict supervision of the study physician and iv glucose will be available in the unlikely event of hypoglycemia. All adverse events will be evaluated by the safety officer and the PI within 72 hours (minor or moderate) or 24 hours (serious or fatal).

B7. STUDY LOCATION

Privacy / Physical Setting

For the preliminary telephone or in-person screening, participants will be asked if they are comfortable answering questions regarding their health and weight or if a callback at a different time is desired. If so, an appointment will be made. Participants will be given general information about the study and asked questions about their age, height, weight, fluctuations in body over preceding year, medical problems, medications, dietary supplements, smoking, illicit substance abuse, level of physical activity, and diet.

The study director will then meet with potential participants at the BIDMC GCRC in a private room to obtain consent. Anthropometric measures, vital signs and iv access will be obtained in a private room in the GCRC. Imaging will take place in the BIDMC imaging facility.

B8. DATA SECURITY

Subject confidentiality will be maintained using unique study identification codes to label blood samples, imaging data and hard copy case report forms. A study identification log will be the only link between a participant's identification codes and identifiable information, such as name and phone number. The log will be kept in a locked file accessible only to study staff for the purposes of enrolling participants. Tracking information will be stored in a separate database that is not linked to results.

Blood samples will be labeled with study identification code, visit number (no date), time point and type of specimen. No identifiable information will be included. Samples will be hand carried and transferred to freezer space at Boston Children's Hospital CRC by study staff.

Imaging data will be labeled with study identification code, visit number and time point. All fMRI reference and difference images will be exported from the DICOM database of the scanner onto a CD or DVD. CDs will be hand carried and transferred to a locked cabinet at 1 Autumn Street, Boston Children's Hospital by study staff.

Data collected during the study visit will be recorded on hard copy case report forms to ensure high-quality data and minimize missing information. Case report forms will be hand carried and transferred to a locked cabinet at 1 Autumn Street, Boston Children's Hospital by study staff. Data will be entered into an electronic database. A password protected study directory will be established on the server maintained by the Boston Children's Hospital CRP. All database files and SAS analysis files will be stored in this directory. Access to programs and archival datasets will be denied by password to non-study staff. All analytic and tracking database files will be backed up daily.

B9 Multi-Site Studies

Is the BIDMC the coordinating site? ☐ Yes ☒ No

Is the BIDMC PI the lead investigator of the multi-site study? ☐ Yes ☒ No

B10 Dissemination of Research Results

We aim to publish the results of this study in a peer-reviewed journal. Upon publication, participants will receive a thank-you note by email, along with a brief lay summary of the results and a link to the article. Participants will be able to request a hard copy of the publication.

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