PROTOCOL

Title: Effect of Dietary Glycemic Index on Beta-cell Function

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INTRODUCTION AND RATIONALE

Beta-cell secretory dysfunction is a critical feature of type 2 diabetes mellitus (T2DM) [1] and is already present in subjects with pre-diabetes (impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG)) [2-10]. Both IGT and IFG increase the risk of future development of type 2 diabetes [11]. Hyperglycemia itself can exacerbate beta-cell dysfunction [12, 13] with oxidative stress proposed as a major mediator of this "glucotoxic" effect [13]. Further, it has been suggested that glucose fluctuations, frequently referred to as "glycemic variability", may be more harmful than chronic sustained hyperglycemia in inducing oxidative stress [14, 15] and therefore could be important with regard to deteriorating beta-cell function. However, there are no data specifically examining whether glycemic variability contributes to deterioration of beta-cell function.

One way to modulate glycemic variability is to vary dietary glycemic load using foods with either a high or low glycemic index (HGI or LGI). Low glycemic load (LGL) diets result in lower post-prandial glucose levels (see preliminary data and [16-19]) and improved glycemic control in patients with T2DM [20, 21]. Post-prandial glucose levels are the main factor influencing glycemic variability in T2DM patients [22]. Short term studies suggest that LGL diets decrease glycemic variability in subjects with IGT or T2DM [23-25] and decrease oxidative stress in healthy subjects [18, 19]. Dietary advice to follow a LGL diet has also been shown to improve beta-cell function in subjects with IGT [26] and T2DM [27], but whether or not this is due to a decrease in glycemic variability is not known. Furthermore, whether high glycemic load (HGL) diets worsen beta-cell function has not been tested.

Hypothesis: Increased glycemic variability results in increased oxidative stress and thereby exacerbates beta-cell dysfunction in individuals with pre-diabetes.

To examine this hypothesis we plan to experimentally manipulate post-prandial glucose excursions using HGL and LGL diets in a 4-week controlled dietary intervention during which we will measure changes in oxidative stress and beta-cell function. Further, to determine whether oxidative stress is an important mechanism whereby a HGL diet and increased glycemic variability contribute to beta-cell dysfunction, we will treat a subset of subjects with the anti-oxidant N-acetylcysteine (NAC) to determine whether NAC can prevent beta-cell dysfunction. We propose to study subjects with pre-diabetes as they already manifest defects in beta-cell function and therefore are more likely to demonstrate further deterioration on a HGL diet or improvement on a LGL diet. Furthermore, they are a population that is highly relevant as they are at high risk of developing T2DM.

Specific Aim 1: To determine if a HGL diet worsens and a LGL diet improves beta-cell function compared to a baseline control diet in subjects with pre-diabetes.

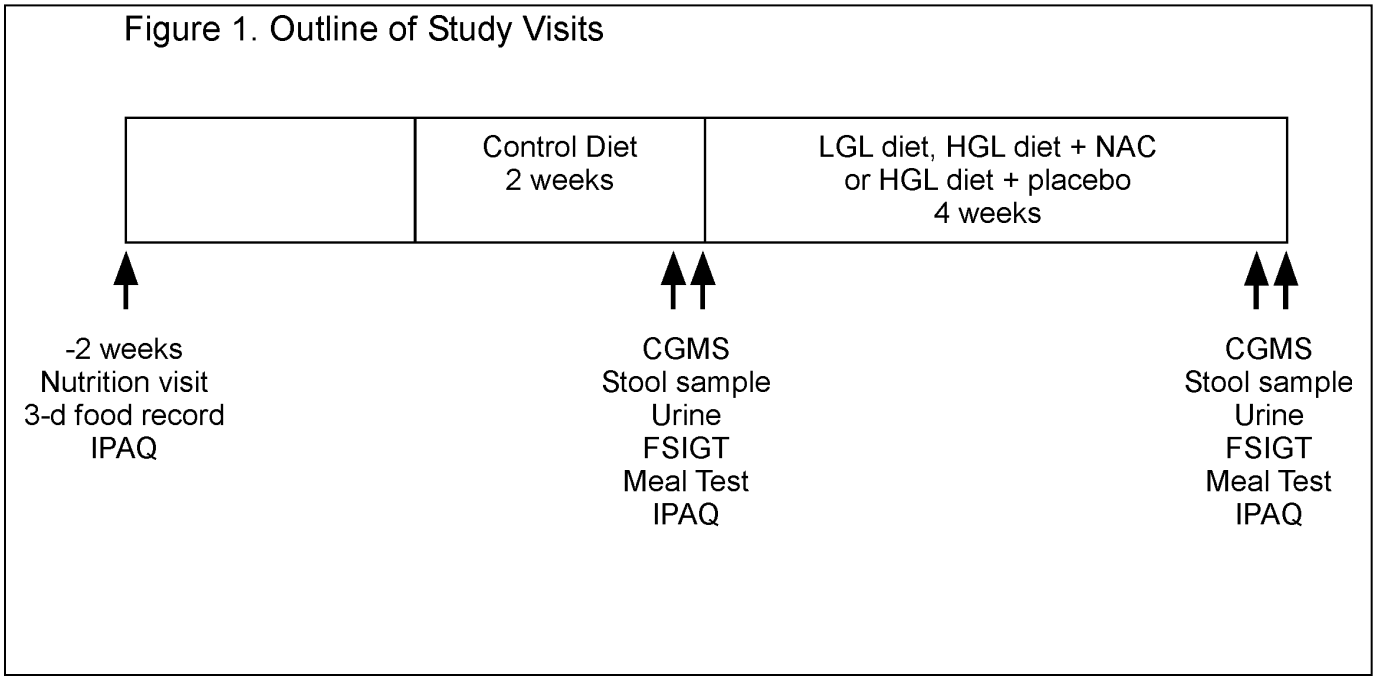
Specific Aim 2: To determine if increased glycemic variability produced by a HGL diet is associated with decreased beta-cell function, and conversely if decreased glycemic variability on a LGL diet is associated with improved beta-cell function in subjects with pre-diabetes.

Specific Aim 3: To determine if oxidative stress induced by a HGL diet mediates reductions in beta-cell function by examining if 1) systemic markers of oxidative stress are associated with beta-cell function; 2) the relationship between glycemic variability and beta-cell function is at least partially explained by oxidative stress; and 3) the anti-oxidant NAC prevents the reduction in beta-cell function on a HGL diet.

RESEARCH DESIGN AND METHODS

Overview: The study will be a randomized, parallel-design feeding study in men and women with pre-diabetes. Subjects will be randomly assigned to one of 3 separate arms (n=20 subjects/arm): 1) 4 weeks on a LGL diet (glycemic index [GI] <35); 2) 4 weeks on a HGL diet (GI >70) + placebo twice daily; or 3) 4 weeks on a HGL diet (GI >70) + NAC 1200 mg twice daily. The placebo vs. NAC arms will be double-blinded. Subjects will be studied after a 2 week baseline control diet with a moderate glycemic load (GI 55-58) for comparison and all diets will be weight stable with the same

macronutrient composition (55% carbohydrate/30% fat/15% protein). Beta-cell function will be assessed by both a frequently sampled intravenous glucose tolerance test (IVGTT) and a meal test. Glycemic variability and mean glucose levels will be assessed by a 3 day Continuous Glucose Monitoring System (CGMS) and glycemic control by fructosamine. Markers of oxidative stress, vascular endothelial function and inflammation and incretin hormone levels will be measured. A stool sample will be collected to assess changes in the gut microbiome on the diets. Acetaminophen absorption will be assessed during the meal test to assess changes in gastric emptying.



Study design: The study will be a randomized, parallel design, controlled feeding study of subjects with pre-diabetes. Randomization will be stratified by sex. The randomization code will be computer generated and kept by the study coordinator. Because the LGL and HGL diets will vary dramatically in food content, it will be impossible to blind subjects to diet assignment, but both subjects and investigators will be blinded to whether they receive NAC or placebo while on the HGL diet. Subjects will undergo study procedures after 2 weeks on a control diet (baseline) and after 4 weeks on the intervention diets (Figure 1). Menstruating women will be studied during the follicular phase of their menstrual cycle.

Subjects:

Inclusion criteria: We will enroll 20 subjects in each study arm (3 arms) with a goal to complete studies on 15 subjects/study arm. We conservatively estimate a drop-out rate of 25%. We will enroll and study subjects with documented IGT (2 hour glucose 140-199 mg/dl after a standard 75 gram OGTT) and/or IFG (fasting plasma glucose 100-115 mg/dl and 2 hour glucose >100 mg/dl). Both men and women, age 18-65 years will be eligible.

Exclusion criteria: Diabetes, alanine aminotransferase (ALT) >1.5 times the upper limit of normal, hematocrit <33%, creatinine >1.5 men or >1.3 women, multiple food allergies or intolerances, other serious medical or inflammatory conditions, pregnancy or lactation and tobacco use. Subjects will be excluded if they take medications that affect insulin sensitivity and secretion (niacin, diabetes medications or glucocorticoids) or inflammation (anti-inflammatories). People with significant gastroesophageal reflux, swallowing problems or stomach ulcers, including those taking medication

for these indications, will be excluded as this is a contra-indication for use of NAC. Subjects taking other investigational drugs currently or within the past 30 days will not be enrolled. Subjects with fasting glucose >115 mg/dl will be excluded as they have typically lost the first phase insulin response [28] and that would preclude the ability to detect a decrease during the study.

Recruitment: The VA, Group Health Cooperative and the UW outpatient clinics in the greater Seattle area have large numbers of patients who are at risk for glucose intolerance. Using the electronic record we will be able to identify and contact patients who have had an HbA1c of 5.8-6.8% within the past three years and who meet all other inclusion/exclusion criteria. We will also recruit subjects with known pre-diabetes who have undergone OGTT screening for other research studies being conducted by the PI. We will recruit from subjects who are part of the Diabetes Registry as well as the Diabetes Endocrinology Research Center (DERC) Registry. We will also use direct advertising targeting subjects who may be at risk for pre-diabetes (such as people who are obese, have a history of gestational diabetes or a family history of T2DM) utilizing ads and fliers posted at the VA medical center in Seattle as well as in local newspapers and on bulletin boards in the greater Seattle area and on-line. We will also send mailings with a letter and fliers to local physicians for referral of interested patients with pre-diabetes.

Study procedures:

Screening: Subjects will undergo an initial brief telephone screening to determine if they may be eligible for the study. Subjects who come in for the screening visit will provide written informed consent and undergo a history and physical exam, blood draw (hematocrit, creatinine, ALT, glucose, and TSH if the subject is on thyroid replacement medication or has a history of thyroid problems or if there is clinical concern for possible abnormal thyroid function during the screening exam.) and a 75 gram OGTT. They will sample study foods and review the menu to ensure that they will be able to tolerate the diets. Eligible subjects will undergo the following interventions and procedures (as illustrated in Figure 1).

Diets: All study diets will contain the same nutrient composition (55% energy from carbohydrate/30% fat/15% protein) and similar percentages of saturated fat, mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA), fructose, vitamin E and vitamin C. Fiber content will vary as this is inextricably bound with the glycemic index of foods. The average daily GI for each diet will be 55-58 for the 2 week baseline control diet, >70 for the HGL diet and <35 for the LGL diet. The diets have already been developed by the Fred Hutchinson Cancer Research Center (FHCRC) Human Nutrition Lab using the 2002 international table of glycemic index and glycemic load values [29]. These diets have been used in a previous diet study and were well tolerated. Subjects will come to the Human Nutrition Lab twice weekly to be weighed and to pick up their food. Individual energy requirements will be calculated using the Mifflin-St. Jeor formula adjusted for activity level. Subjects will be given individualized dietary instructions to keep their weight stable and told to maintain their usual level of physical activity. Subjects will be weighed twice a week when they come to pick up their food and the diets will be adjusted in 200 calorie increments if needed to maintain a stable weight. They will be told not to take any vitamins (including multivitamins) throughout the duration of the study as these may impact our measures of oxidative stress.

Drug: Subjects on the HGL diet will take either NAC 1200 mg (two 600 mg capsules) orally twice daily or matching placebo capsules orally twice daily. NAC and matching placebo capsules will be obtained from Twinlabs (Utah). If study subjects are not able to tolerate the 1200 mg twice daily dose, the dose will be decreased to 600 mg twice daily. If this were to occur one of the study staff would follow-up with the study subject by phone within 5 days of the change in dose to assess for side-effects. NAC is FDA approved as a mucolytic agent and for treatment of acetaminophen overdose and is also available over-the-counter as a dietary supplement. It is rapidly absorbed with peak plasma levels occurring after approximately 1 hour. We will monitor for drug activity by measuring erythrocyte GSH and GSSG levels.

Compliance: Dietary compliance will be monitored by having subjects check each food they eat on a daily menu. They will be asked to note, in as much detail as possible, all deviations from the study regimen on these daily food records. They will also be asked to return all food they might not be able or willing to eat. Any returned food will be weighed by the Human Nutrition Lab for accurate assessment of quantity and quality of foods that were actually consumed. Drug compliance will be monitored by having subjects on the HGL diet return any unused capsules at the end of the 4 week intervention period. Subjects who take <80% of the study medication will be considered as drop-outs and will be replaced.

Nutrition visit: Subjects will report to the Human Nutrition Lab two weeks before starting the control diet period to review and sign Nutrition visit consent form, review their 3-day food record, measure body weight and calculate caloric needs.

Study procedures: All study procedures will be performed while on the control diet and again while on the intervention diet (see Figure 1). The IPAQ will be performed at the nutrition visit as well as on the CGMS days. Subjects will be instructed to avoid taking vitamins or anti-inflammatory medications during the study.

CGMS: The CGMS (iPro by Medtronic, Northridge CA) will be inserted by trained study staff and subjects will receive instruction on its use and care. The CGMS will be removed 3 days later at the time of the IVGTT. The iPro provides blinded interstitial glucose profiles. Subjects will also be instructed to keep a daily food and physical activity diary (such as time and type of exercise performed or food consumed) for the 3 days that they are wearing the CGMS. For proper calibration of the CGMS, subjects will be provided with a glucometer (ACCU-CHEK Aviva) and instructed to check their fingerstick blood glucose four times daily for the 3 days while they are wearing the CGMS. The CGMS will be removed by study staff before the IVGTT is performed and the data downloaded and analyzed using iPro software. The mean glucose levels as well as the SD are calculated by the iPro software. The MAGE will be calculated as described by Service et al [30].

IPAQ: The international physical activity questionnaire (IPAQ) [31] will be administered to monitor and adjust for changes in physical activity on the control diet and at the end of the intervention diet. Subjects will be told to try to maintain a stable activity level throughout.

Stool sample: Because changes in the stool microbiota may be associated with inflammation and could be altered by changes in diet composition, subjects will be asked to collect a stool sample during the last few days on the control and intervention diets. Subjects will be provided with stool sample collection kits and detailed instructions.

Fasting urine sample: One aliquot will be sent to the clinical lab for measurement of urine creatinine and a second aliquot will be frozen at -80°C for subsequent measurement of F_2-isoprostanes by gas chromatography/mass spectrometry (GC/MS). A urine pregnancy test will be performed in women with child-bearing potential.

IVGTT: Subjects will arrive after at least a 12 hour overnight fast. An insulin-modified IVGTT will be performed as we have done previously [32]. For subjects on the HGL diet, they will take study medication (either NAC or placebo) 30 minutes prior to glucose injection. The sampling arm will be wrapped in a heating pad to “arterialize” the blood. After obtaining 3 baseline samples, 50% dextrose (11.4 mg/body surface area) will be administered intravenously over 60 seconds. From 20-25 minutes post glucose injection, insulin (0.03 units/kg) will be infused. Blood samples will be drawn for glucose and insulin at 35 time points over 240 minutes.

Meal test: The meal test will immediately follow the IVGTT. Subjects will consume a standardized lunch meal containing 75 grams carbohydrate and the same glycemic index and macronutrient composition the subject has been eating. Acetaminophen 1.5 grams (given as 3 x 500 mg tablets with water) will be given at the start of the test meal. The test meal will be consumed within 15 minutes.

Blood will be drawn for glucose, insulin, C-peptide, total GLP-1, total GIP and free fatty acid measurements at -10, -5, -1, 10, 20, 30, 60, 90, 120, 180 and 240 minutes after the start of the meal. Plasma nitrotyrosine, endothelial and inflammatory markers will be measured at -1, 60, 120, 180 and 240 minutes. Acetaminophen levels will be measured at 0, 30, 60, 90, 120, 180 and 240 minutes to estimate gastric emptying. Blood will be collected at -30, -1, 60, 120, 180 and 240 minutes for the erythrocyte GSH and GSSG assays. Computer modeling of the meal test data will be performed using SAAM II software under the guidance of David Foster.

Safety Assessment: A side-effect questionnaire will be utilized to assess for side-effects from the diets or NAC. This will be performed twice, once before starting the study diet +/- NAC and again after four weeks on the study diet +/- NAC. An independent safety officer will be appointed to review safety data every 6 months. The safety officer will be notified in the event of pregnancy, an unanticipated adverse event or a serious adverse event, in addition to the IRB. Women with child-bearing potential will undergo a urine pregnancy test.

Study withdrawal criteria: Subjects will be withdrawn from the study if they develop serious or intolerable side-effects from study medications or study diets, development of any serious medical condition during the study or pregnancy. Subjects who are not compliant with the protocol will also be withdrawn.

Study stopping rules: The appointed safety officer will make the decision to stop the study prematurely if he/she determines that there are serious side-effects associated with the study.

Primary endpoints:

- The disposition index (insulin sensitivity index [S_I] x AIRg) from the IVGTT

Secondary endpoints:

- The disposition indices (static, dynamic and total beta-cell responsivity [Φ] x $S_{I\ MT}$) derived from minimal modeling of the meal test [33]
- Glycemic variability measured by CGMS: SD of glucose and MAGE
- Mean glucose from the CGMS
- Post prandial glucose AUC (0-240 minutes) during the meal test
- Plasma fructosamine levels
- Markers of oxidative stress including urine isoprostanes, plasma nitrotyrosine and plasma protein carbonyls
- Anti-oxidant status: Total plasma anti-oxidant capacity and erythrocyte GSH and GSSG levels and the GSH/GSSG ratio
- Insulin sensitivity: S_I will be determined from data from the IVGTT using Bergman's minimal model [34] and $S_{I\ MT}$ will be determined from the meal test data using the model developed by Cobelli [33]
- AIRg will be calculated as the incremental insulin response from 0-10 minutes after glucose injection during the IVGTT
- Markers of inflammation including TNFα, IL-6, MCP-1, PAI-1, IL-1β
- Markers of endothelial function including VCAM, ICAM-1 and endothelin-1
- Free fatty acids levels during the meal test
- Plasma acetaminophen levels (maximal concentration and area under the curve) as a measure of gastric emptying
- Stool microbiota profiles
- Incretin hormone levels during the meal test (GIP and GLP-1)

Sample size calculations: Sample sizes were calculated using Stata (Version 9.1). For computation of samples sizes for the IVGTT we used data from repeat IVGTTs that yielded a mean disposition index of 2517 and a standard deviation for the change in the disposition index of 829 [35]. Sample size for Specific aim 1 was calculated for a one-sample comparison (paired test) with two-sided

α=0.05 and power=0.80 for the change in disposition index from baseline. Using a sample size of 15 subjects/arm, we will have 94% power to detect a 30% change in the IVGTT disposition index from baseline. For Specific Aim 3, a sample size of 15/group was calculated using a two-sample comparison (independent t-test) with one-sided α=0.05 and power=0.80 for comparison of the change in the disposition index (intervention – baseline) on the HGL + placebo vs. HGL + NAC. A one-sided α was used for Specific Aim 3 as we anticipate that the NAC will lead to an improvement in β-cell function. Using a conservative estimate of a drop-out rate of 25% we will enroll 20 subjects in each arm to ensure that we complete studies on 15 in each arm.

Statistical analysis: The data will be tabulated and changes calculated (intervention diet – baseline) for each variable. For Specific Aim 1 paired t-test analysis will be used to assess for significant changes in each variable between baseline and each diet intervention. Specifically we will determine if the LGL diet increases the disposition index compared to the control diet and if the HGL diet + placebo decreases the disposition index compared to the control diet. Using general linear model with repeated measures we will test for diet group effect adjusting for possible covariates such as changes in weight. We will use similar statistical approaches to assess for significant diet effects on other measures of beta-cell function, insulin sensitivity, markers of inflammation and oxidative stress and glycemic variability. For Specific Aim 2 we will assess the relationship between changes in the disposition indices and measures of glycemic variability, post-prandial glucose levels, mean glucose and fructosamine levels on the HGL + placebo and LGL diet using simple and multiple linear regression analysis. For Specific Aim 3 we will compare changes in the disposition index from baseline between the HGL + placebo vs. HGL + NAC arms by independent t-test analysis. The relationship between markers of oxidative stress and measures of glycemic variability, glucose levels, insulin sensitivity and beta-cell function will be determined using simple and multiple linear regression models and Sobel's test of the mediating role of oxidative stress in the relationship between glycemic variability and beta-cell function will be conducted. We will also determine if baseline beta-cell function is associated with changes in post-prandial glucose AUC and measures of glycemic variability using regression analysis. If baseline beta-cell function predicts the response to the diets, we will adjust for this in our final analyses. Exploratory analyses will investigate the effects of the high and low GL diets compared to the control diet on gastric emptying, markers of endothelial function, the stool microbiome and incretin hormone responses. These changes will be correlated with changes in beta-cell function, insulin sensitivity and insulin secretion measures. If any of these variables are found to have a significant association with beta-cell function, they will be included in the multiple regression models listed above. Regression analyses will also adjust for potential confounders such as age, BMI and gender. Data that are not normally distributed will be transformed to approximate a normal distribution. A p value <0.05 will be considered significant.

References

1. Kahn, S.E., et al., *The beta cell lesion in type 2 diabetes: there has to be a primary functional abnormality.* Diabetologia, 2009. **52**(6): p. 1003-12.
2. Festa, A., et al., *The natural course of β-cell function in nondiabetic and diabetic individuals: the Insulin Resistance Atherosclerosis Study.* Diabetes, 2006. **55**(4): p. 1114-20.
3. Byrne, M.M., et al., *Elevated plasma glucose 2 h postchallenge predicts defects in ß-cell function.* Am J Physiol, 1996. **270**(4 Pt 1): p. E572-9.
4. Ehrmann, D.A., et al., *Insulin secretory responses to rising and falling glucose concentrations are delayed in subjects with impaired glucose tolerance.* Diabetologia, 2002. **45**(4): p. 509-17.
5. Breda, E., et al., *Insulin release in impaired glucose tolerance: oral minimal model predicts normal sensitivity to glucose but defective response times.* Diabetes, 2002. **51 Suppl 1**: p. S227-33.

6. Ferrannini, E., et al., *Beta-cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis.* J Clin Endocrinol Metab, 2005. **90**(1): p. 493-500.
7. Gastaldelli, A., et al., *Beta-cell dysfunction and glucose intolerance: results from the San Antonio metabolism (SAM) study.* Diabetologia, 2004. **47**(1): p. 31-9.
8. Utzschneider, K.M., et al., *Impact of differences in fasting glucose and glucose tolerance on the hyperbolic relationship between insulin sensitivity and insulin responses.* Diabetes Care, 2006. **29**(2): p. 356-62.
9. Jensen, C.C., et al., *ß-cell function is a major contributor to oral glucose tolerance in high-risk relatives of four ethnic groups in the U.S.* Diabetes, 2002. **51**(7): p. 2170-8.
10. Kanat, M., et al., *Impaired early- but not late-phase insulin secretion in subjects with impaired fasting glucose.* Acta Diabetol, 2011. **48**(3): p. 209-17.
11. de Vegt, F., et al., *Relation of impaired fasting and postload glucose with incident type 2 diabetes in a Dutch population: The Hoorn Study.* JAMA, 2001. **285**(16): p. 2109-13.
12. Boden, G., et al., *Effects of prolonged glucose infusion on insulin secretion, clearance, and action in normal subjects.* Am J Physiol, 1996. **270**(2 Pt 1): p. E251-8.
13. Poitout, V. and R.P. Robertson, *Glucolipotoxicity: fuel excess and beta-cell dysfunction.* Endocr Rev, 2008. **29**(3): p. 351-66.
14. Ceriello, A., et al., *Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients.* Diabetes, 2008. **57**(5): p. 1349-54.
15. Monnier, L., et al., *Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes.* Jama, 2006. **295**(14): p. 1681-7.
16. Bouche, C., et al., *Five-week, low-glycemic index diet decreases total fat mass and improves plasma lipid profile in moderately overweight nondiabetic men.* Diabetes Care, 2002. **25**(5): p. 822-8.
17. Wolever, T.M. and C. Mehling, *Long-term effect of varying the source or amount of dietary carbohydrate on postprandial plasma glucose, insulin, triacylglycerol, and free fatty acid concentrations in subjects with impaired glucose tolerance.* Am J Clin Nutr, 2003. **77**(3): p. 612-21.
18. Dickinson, S., et al., *High-glycemic index carbohydrate increases nuclear factor-kappaB activation in mononuclear cells of young, lean healthy subjects.* Am J Clin Nutr, 2008. **87**(5): p. 1188-93.
19. Botero, D., et al., *Acute effects of dietary glycemic index on antioxidant capacity in a nutrient-controlled feeding study.* Obesity (Silver Spring), 2009. **17**(9): p. 1664-70.
20. Thomas, D.E. and E.J. Elliott, *The use of low-glycaemic index diets in diabetes control.* Br J Nutr: p. 1-6.
21. Livesey, G., et al., *Glycemic response and health--a systematic review and meta-analysis: relations between dietary glycemic properties and health outcomes.* Am J Clin Nutr, 2008. **87**(1): p. 258S-268S.
22. Zhou, J., et al., *Glycemic variability and its responses to intensive insulin treatment in newly diagnosed type 2 diabetes.* Med Sci Monit, 2008. **14**(11): p. CR552-8.
23. Nansel, T.R., L. Gellar, and A. McGill, *Effect of varying glycemic index meals on blood glucose control assessed with continuous glucose monitoring in youth with type 1 diabetes on basal-bolus insulin regimens.* Diabetes Care, 2008. **31**(4): p. 695-7.
24. Gellar, L. and T.R. Nansel, *High and low glycemic index mixed meals and blood glucose in youth with type 2 diabetes or impaired glucose tolerance.* J Pediatr, 2009. **154**(3): p. 455-8.
25. Brynes, A.E., et al., *A low glycemic diet significantly improves the 24-h blood glucose profile in people with type 2 diabetes, as assessed using the continuous glucose MiniMed monitor.* Diabetes Care, 2003. **26**(2): p. 548-9.
26. Wolever, T.M. and C. Mehling, *High-carbohydrate-low-glycaemic index dietary advice improves glucose disposition index in subjects with impaired glucose tolerance.* Br J Nutr, 2002. **87**(5): p. 477-87.
27. Wolever, T.M., et al., *Low glycaemic index diet and disposition index in type 2 diabetes (the Canadian trial of carbohydrates in diabetes): a randomised controlled trial.* Diabetologia, 2008. **51**(9): p. 1607-15.
28. Brunzell, J.D., et al., *Relationships between fasting plasma glucose levels and insulin secretion during intravenous glucose tolerance tests.* J Clin Endocrinol Metab, 1976. **42**(2): p. 222-9.

29. Foster-Powell, K., S.H. Holt, and J.C. Brand-Miller, *International table of glycemic index and glycemic load values: 2002.* Am J Clin Nutr, 2002. **76**(1): p. 5-56.
30. Service, F.J., et al., *Mean amplitude of glycemic excursions, a measure of diabetic instability.* Diabetes, 1970. **19**(9): p. 644-55.
31. Craig, C.L., et al., *International physical activity questionnaire: 12-country reliability and validity.* Med Sci Sports Exerc, 2003. **35**(8): p. 1381-95.
32. Utzschneider, K.M., et al., *The Dipeptidyl Peptidase-4 Inhibitor Vildagliptin Improves β-cell Function and Insulin Sensitivity in Subjects with Impaired Fasting Glucose.* Diabetes Care, 2007.
33. Dalla Man, C., et al., *Two-hour seven-sample oral glucose tolerance test and meal protocol: minimal model assessment of beta-cell responsivity and insulin sensitivity in nondiabetic individuals.* Diabetes, 2005. **54**(11): p. 3265-73.
34. Bergman, R.N., L.S. Phillips, and C. Cobelli, *Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and ß-cell glucose sensitivity from the response to intravenous glucose.* J Clin Invest, 1981. **68**(6): p. 1456-67.
35. Abbate, S.L., et al., *Effect of heparin on insulin-glucose interactions measured by the minimal model technique: implications for reproducibility using this method.* Metabolism, 1993. **42**(3): p. 353-7.