Post prandial glycemia following high protein and high fat meals among subjects with T1DM

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Chapter 1: Specific Aims

Type 1 diabetes (T1D) education focuses on managing blood glucose by estimating carbohydrate (CHO) meal content and dosing mealtime insulin based on an insulin to carbohydrate ratio. In general, one unit of rapid-acting insulin is administered per an individualized gram amount of carbohydrates in a meal. Although the primary macronutrient affecting postprandial glycemia is considered carbohydrates, other dietary factors including proteins and fats also have an impact. Several feeding studies have demonstrated high protein and fat meals delay the time to peak glucose and increase postprandial glucose levels. However, there is limited information about how high protein and fat meals influence postprandial glucose among adults with T1D in a free-living setting. This project fills the gap to identify how, in a free-living situation, high fat and high protein meals affect postprandial glycemia in people living with type 1 diabetics.

A delay in the time to postprandial glucose (PPG) peak and an increase of postprandial glucose area under the curve (AUC) has been reported in feeding studies focused on high protein and high fat meals. Increasing fat content resulted in a delayed peak and longer duration of PPG. Likewise, increasing protein in a protein drink resulted in a late peak in PPG. For meals that contained both high protein and high fat, there was a twofold increase in the glucose incremental AUC. To date, feeding studies in a controlled environment have examined variable protein and fat content impacts on PPG but how these different macronutrient distributions effect PPG in a free-living situation is currently unknown.

However, measuring both dietary intake and postprandial glycemia in a free-living situation is prone to high amounts of measurement error. Current dietary recall methods such

as a 24-hour dietary recall or recorded food diary, require participants to have sufficient memory skills and or be diligent in recording intake. Incomplete documentation and inaccurate food measurements can impact or skew dietary intake results. These traditional dietary reporting methods result in underreporting ranging from 11 to 41 percent.[1] Overreporting is not common. Similarly, measuring PPG is problematic and frequent measures have in the past required multiple blood draws or finger sticks. The advent of continuous glucose monitoring has dramatically changed the landscape for measuring PPG.[2] The T1Dexi pilot study was designed to address these issues by using innovative technologies to measure dietary intake and PPG.

The overall goal of the Type 1 Diabetes Exercise Initiative (T1Dexi) pilot study was to establish methods to measure the impact of different types of exercise on glycemic control among individuals with T1D. The pilot study enrolled 48 participants and used a novel phone app for collecting exercise and dietary intake data. Glycemia was measured with continuous glucose monitoring. Thus, the T1Dexi pilot data is a unique data set that captured both nutrient intake and continuous glucose data to measure post-prandial glycemic response in subjects with T1D. We examined the effects of high protein and high fat meals within subjects on postprandial glycemia using this data set. We hypothesized that increased protein and fat will delay time to peak PPG and increase the total PPG response similar to studies in several controlled feeding studies. To test this hypothesis, we looked at the following specific aims:

Aim 1: To determine the effects of protein and fat in mixed meals on time to peak glucose in participants with type 1 diabetes.

Hypothesis: We hypothesize that meals higher in protein and fat will delay time to peak postprandial glucose.

Aim 2: To determine the effects of protein and fat in mixed meals on post-prandial glucose AUC in participants with type 1 diabetes.

Hypothesis: We hypothesize that meals higher in protein and fat will increase postprandial glucose AUC.

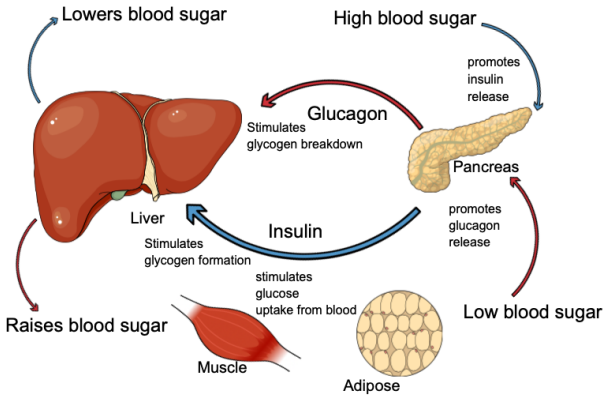
Chapter 2: Background and Review of the Literature

Type 1 diabetes mellitus, T1DM, is a condition which results in the inability secrete adequate insulin to maintain normal blood glucose. During 2016, it was estimated that T1DM affected 0.55 percent or 1.3 million adults in the United States of America.[3,4] Individuals with T1DM experience the destruction of pancreatic beta-cells by the immune system which results in insulin insufficiency.[3] Without insulin, the body is unable to properly utilize the glucose that accumulates in the bloodstream. Nutrition management for T1DM focuses on managing blood glucose by estimating carbohydrate meal content and dosing mealtime insulin based on an insulin-to-carbohydrate ratio.[5,6] To prevent ketoacidosis and mortality, individuals with T1DM are dependent on exogenous insulin. In general, one unit of rapid-acting insulin dose is administered per an individualized gram amount of carbohydrates in a meal. Although carbohydrates are closely monitored for insulin administration estimations, individuals with T1DM continue to experience difficulty in controlling blood glucose when consuming mixed meals containing proteins and fats.[2,7,8] Some studies have suggested that the efficacy of mealtime insulin administration should be determined with the consideration of macronutrient distribution consumed at each meal.[9-12] However, guidelines have not been established to account for those additional meal components.

2.1 Insulin

Insulin is a peptide hormone that is secreted by pancreatic beta-cells in response to the rise in blood glucose to initiate glucose disposal and maintain euglycemia (Figure 1). The glucose-mediated insulin secretion process begins with glucose entering the cell through the glucose transporter GLUT-2.[13] Glucokinase, found in the cell, will respond to cytosolic glucose by phosphorylating it into glucose-6-phosphate (G6P). Rapid rise in intracellular ATP from glycolysis of G6P initiates depolarization of the beta-cell. The depolarization of the cell will activate the voltage gated Ca^{2+} channel to open and increase the Ca^{2+} concentration. The influx of Ca^{2+} will signal for the release of insulin into the blood.[14] From the blood, the insulin will bind to the insulin receptor on the adipose or muscle tissue cells and intracellular signaling will result in GLUT-4 translocation to the plasma membrane to allow glucose uptake by the cell. Insulin signaling in the hepatocytes suppresses endogenous glucose release into the blood. Thus, insulin secretion will cause glucose disposal into muscle and adipose with a decrease in endogenous glucose release from the liver resulting in lower blood glucose concentrations. Patients with T1DM have a loss of pancreatic beta-cells leading to the absence of insulin secretion in response to hyperglycemia and reliance on exogenous insulin to manage blood glucose concentrations.

Figure 1: Insulin action to lower blood sugar



Insulin resistance occurs when higher than normal insulin levels are required to maintain normal blood glucose.[13] Although commonly seen in individuals with T2DM, insulin resistance can occur in those with T1DM. The effects of insulin resistance results in the inability of target tissues such as muscle and adipose to effectively respond to insulin and promote glucose uptake. Insulin levels are low while in basal state and high during the fed sates in the normal condition; insulin levels are higher during fed states with insulin resistance.[13] Increased insulin resistance makes it difficult for individuals to maintain euglycemia.

In response to glucose, insulin secretion in a healthy individual is biphasic. During the first phase, insulin will be released within 1 minute, peak between 3-5 minutes, and last a duration of approximately 10 minutes.[13] This is a rapid response that represents the insulin stored in secretory granules. During the second phase, insulin will be released after a glucose bolus, approximately 10 minutes later and last the duration of the hyperglycemic event. The second phase represents the insulin that is being newly synthesized.[13] The ultimate goal of T1DM management is to mimic the action of the pancreas with exogenous insulin delivery and maintain normal blood glucose levels. Several different methods are currently used by patients to deliver exogenous insulin.

2.2 Insulin: MDI vs. Pump

Individuals with diabetes can receive supplemental exogenous insulin through injection or by pump. Multiple daily injections, MDI, therapy involves administering exogenous insulin throughout the day by way of syringe, pen, or prefilled pen. Injections are administered subcutaneously at the abdominal, arms, and thigh. However, subcutaneous injections at the abdomen is preferred due to the quicker absorption into the bloodstream.[15] MDI for the treatment of T1DM is a combination of bolus and basal insulin and vary by its onset, peak, and duration.[16] Bolus insulin is given during meals and is considered rapid-acting. Basal insulin is given at set times throughout the day and can be intermediate- or long-acting. Basal insulin analogues are a sub-group of human insulin medications that are generated in the lab and are often preferred over Neutral Protamine Hagedorn or NPH, due to the efficacy, safety and patient satisfaction.[16] NPH insulin is produced through the precipitation of recombinant synthesized human insulin with zinc and protamine.[15] Either NPH or a long-acting analog are typically used along with meal-time short-acting bolus insulin to manage glycemia via MDI.

An insulin pump is another method used to deliver insulin. Individuals who use a pump will receive continuous supply of insulin. The pump is often used by those who have difficulty meeting treatment targets or experience frequent or severe hypoglycemia.[16] Insulin pumps provide insulin through normal, square-wave, and dual-wave boluses.[17] The continuous nature of the pump may mimic physiologic insulin delivery more closely when compared to the MDI method. Temporary changes to the infusion rate of the pump can be conducted to prevent imminent hypoglycemia or glucose swings.[16] Pump technology is advancing and modern pumps can have sensor-augmented systems to prevent insulin delivery to prevent hypoglycemia during

low blood glucose levels.[16] Automated insulin delivery systems are now available, including 670G and Control IQ. Automatic bolus calculators can be used by those who have insulin pumps to determine bolus insulin dose by entering estimated carbohydrate intake for the meal, information about recent glucose concentration, and considering estimates of insulin sensitivity.[12] This type of insulin dosing is beneficial for aiding with PPG concentration control. Regardless of the method of delivery, either MDI or pump, exogenous insulin therapy does not completely replicate the endogenous glucose control and further study to improve these systems such as improved bolus calculators and artificial pancreas systems are ongoing.

2.3 Nutrition Intervention

Insulin therapy and glucose monitoring is needed to achieve optimal glycemic control. Prescribed insulin dose regimens range from 0.4-1.0 units/kg/day.[16] If an individual has an elevated pre-prandial blood glucose reading, an insulin "correction dose" may be administered to ensure blood glucose does not remain elevated.[16] An insulin correction dose can be determined by the individuals' insulin correction factor or insulin sensitivity factor. Bolus insulin dosing is based on carbohydrate counting and insulin-to-carbohydrate regimens to aid with maintaining appropriate blood sugar levels after eating.[18] Other considerations for bolus insulin administration includes planned exercise and illness.[16] The method of carbohydrate counting involves estimating the number of carbohydrate grams in a meal or snack and matching it to the insulin dose. In general, 1 unit of rapid-acting insulin is administered per 12-15g of carbohyrdates.[16] Carbohydrate counting with adjusted insulin boluses improves glycemic control when insulin is adjusted accordingly.[6] The insulin-to-carbohydrate ratio is dependent upon the individual's insulin sensitivity. Insulin dosage per gram of carbohydrate is typically

prescribed by the patient's physician and is individualized based on the patient's glycemic control. This method allows for more freedom when choosing foods to eat, but does leave room for inconsistent carbohydrate intake throughout the day.[6] Patients may experience episodes of hyper- and hypoglycemia when under- and overestimating carbohydrates in a meal.[19,20] Although carbohydrates are identified as the primary macronutrient effecting PPG, it has been observed that other meal components, such as protein and fat, can alter the postprandial glycemic curve and may lead to delayed glucose peaks. It has been suggested that adjusting insulin dosage to include protein and fat may further improve glycemic control.[9,10]

2.4 Postprandial Glycemia

Postprandial glycemia, PPG, is the concentration of plasma glucose after eating.[21] In non-diabetic individuals, fasting glucose concentrations are typically less than 100 mg/dL. After the start of a meal, approximately 10 minutes, the absorption of dietary carbohydrates begins and the concentration of blood glucose rises.[21] The composition, quality, and timing of meals eaten impact the extent and time of blood glucose concentrations.[21] In non-diabetic individuals, the peak of blood glucose may reach up to 140mg/dL approximately 1 hour after the start of the meal and descends to pre-prandial levels after 2-3 hours.[21] For individuals with T1DM, blood glucose peak and duration are determined by the amount of exogenous insulin administered. Uncontrollable blood glucose, such as hyper- or hypoglycemia, can lead to adverse health effects.[22]

2.5 Hyper- vs Hypoglycemia

Either hyper- or hypoglycemia can have adverse effects and the goal of insulin therapy is to avoid glycemic extremes. Hyperglycemia is described as elevated blood glucose and can be a

result of insulin deficiency.[23] It is described as having blood glucose reading of >130mg/dL after fasting or >180mg/dL two-hours after a meal. Uncontrolled hyperglycemia is a risk factor for microvascular complications that can manifest into nephropathy, neuropathy, and retinopathy.[12,23] Symptoms of hypoglycemia or low blood glucose include palpitations, cognitive impairment, seizures and unconsciousness.[23] Mild hypoglycemia is described by a blood glucose reading of <70mg/dL, moderate hypoglycemia is a reading of 55-70 mg/dL, and severe hypoglycemia is <55mg/dL.

2.6 PPG for T1DM and T2DM

Suboptimal glycemic control contributes to elevated PPG. When compared to nondiabetic individuals, both T1DM and T2DM experience elevated and more prolonged PPG excursions. This change in PPG excursions is related to the abnormal secretion of insulin and glucagon, hepatic glucose uptake, reduction in hepatic glucose production, and peripheral glucose uptake (figure 1).[21] There are slight differences between the PPG excursions of individuals with T1DM and T2DM. For T1DM individuals, the time and height of peak of insulin concentration and PPG is dependent upon the type, amount, and route of the administered exogenous insulin.[21] Those with non-insulin dependent T2DM experience a delay of peak insulin levels and insufficient control of PPG excursions due to inadequate endogenous insulin.[21] Several additional factors can influence PPG including meal composition and gastric emptying that will be discussed in more detail below.

2.7 Gastric Emptying

Gastric emptying impacts the time it takes to reach glycemia peaks. Blood glucose and gastric emptying have a bidirectional relationship; the rate of the gastric emptying influences

the level of PPG and the amount of blood glucose present in the bloodstream will impact the rate of gastric emptying.[8] Patients with T1DM often experience delayed gastric emptying that can lead to prolonged hyperglycemia.[8] Gastric emptying is influenced by the composition of the meal whether solid or liquid, the amount of energy present, and mixture of macronutrient components.[8] Delayed gastric emptying times are seen for solid foods due to the duration needed for mechanical digestion whereas liquids increase gastric emptying.[8] Consuming meals that have a high energy content will delay gastric emptying. Dietary fiber, fat, and protein have also been shown to delay gastric emptying due to the slower breakdown of those foods. Dietary fats and proteins have been shown to increase PPG excursions, potentially related to the delayed gastric emptying.[5,24] Although dietary fat and proteins impact glycemia independently, meals are often eaten with combination of macronutrients and both have an additive effect on the PPG.[24,25]

2.8 Protein and Glycemia

Dietary protein it is broken down into amino acids to be used in processes such as synthesis of bodily proteins for the building of cell structures, production of hormones and enzymes, and glucose production. Amino acid digestion, absorption and uptake into b-cells can independently stimulate a postprandial insulin response.[26] The consumption of protein in a mixed meal or alone had been shown to delay, sustain, and increase PPG concentrations and excursions.[24] Protein tends to impact PPG towards the end of the glycemic excursion, several hours after a meal.[2,12] There are two methods in which protein is thought to influence PPG: alteration of hormones that are used to maintain glucose homeostasis and the conversion of amino acids to glucose through gluconeogenesis.[24]

The hormones involved with PPG include glucagon and cortisol. Eating a high protein meal increases plasma glucagon levels in the blood.[24,27] For those with T1DM, insufficient insulin and an increase in glucagon will cause hyperglycemia. Although not well understood, it is speculated that the hormone cortisol may also effect PPG.[24] It is speculated that high-protein meals increase cortisol concentrations which can increase insulin requirements. High cortisol is thought to play a role in insulin resistance.[27]

High protein meals may increase PPG by stimulating gluconeogenesis in the liver. Gluconeogenesis is the production of glucose through non-carbohydrate sources including the keto acids of a variety of different amino acids. Amino acids are utilized as an energy source through the conversion into glucose or production of ketone bodies.[24] Insulin inhibits gluconeogenesis, however inadequate insulin will promote the increase in gluconeogenesis which will result in an increase in hepatic glucose output and potentially an increase in blood glucose.[24]

High-protein meals appear to increase the time to peak glucose after a meal. It was observed in one study that the addition of 35 grams of protein to 30 grams of carbohydrates in a meal lead to an increase in PPG levels by 48mg/dl at 5 hours post-prandially.[25] In another study using 30g carbohydrate drinks with varying amounts of protein (12.5, 25, 50, and 75g), it was observed that the addition of protein led to glycemic excursions between 150 and 300 minutes post-prandially. [28] These studies suggest that the addition of protein increases the duration of blood glucose elevation (5 hours rather than 2-3) and delays time to peak glucose. The combination of carbohydrates with protein has an additive effect that is different than when protein is eaten alone.[2,29] Subjects consuming small amounts of protein alone did not

have a change in PPG; when subjects consumed more than 75 grams of protein alone there was a significant elevated PPG excursion.[29] In contrast, as little as 12.5 grams of protein with carbohydrate delayed peak PPG. Meals containing only protein may need a different insulin dosing regimen when compared to a mixed meal of carbohydrates and protein.[2] However, it should be noted that most meals are mixed containing carbohydrates, proteins and fats. There have been different suggestions to how insulin dosages can be adjusted to account for protein in meals. One study has suggested that for a meal with 50 grams of protein with carbohydrates and no fat, adding 30 percent more insulin to the dose will help with glycemic control.[30] However, it has also been suggested that 100 kcals of protein and fat is equivalent to the bolus insulin dosage for 10 grams of carbohydrates.[31]

2.9 Lipids and Glycemia

Dietary fat is broken down into fatty acids from triglycerides and absorbed across the GI, circulate as part of lipoprotein molecules or free fatty acids throughout the body and stored in the body for future energy use.[24] There are four methods in which glycemic response can be influenced by dietary triglycerides: delayed gastric emptying, increased free fatty acids, changes in hormones, and increased gluconeogenesis from the glycerol. When dietary fat is consumed, gastric emptying is delayed which causes a delayed glycemic peak.[2,12,24] Elevated free fatty acids that are in circulation stimulate a greater insulin response among those without T1DM. Among T1DM individuals the absence of a normal beta cell response when free fatty acids are elevated suggests that bolus insulin doses based solely on carbohydrates will be insufficient to prevent postprandial hyperglycemia. In regards to hormones, dietary fats play a role in the release of glucagon which increases gluconeogenesis.[24,32] Lipids can be utilized in

gluconeogenesis though the use glycerol. Glycerol comes from triglycerides and is broken down into pyruvate which can be used in gluconeogenesis for energy production.[24]

How insulin dosage could potentially be adjusted to account for the changes in blood glucose caused by a diet high in lipids is currently debated. In a randomized within-subject trial, the relationship between dietary lipid amount and glycemia was investigated to determine the optimal insulin adjustment for dietary lipid.[7] It was determined that there was not a significant difference in glycemia based on the source of dietary lipid such as saturated, monosaturated or polysaturated fats. However, increasing the amount of fat did change the glucose curve; the early postprandial glucose response is lower and late postprandial response is higher with a high fat meal.[7] The findings of the this study suggest that adjusting insulin dosage will aid in minimizing the risk of hyperglycemia and that an additional 20 percent of insulin be given when consuming a meal with 60 grams or more of fat.[7] In another study, participants required 42 percent more insulin when consuming a high-fat meal compared with consuming a similar low-fat meal with a similar amounts of carbohydates.[11]

2.10 Controlled feeding-study vs. Free-living situations

Feeding studies refers to a specific study design in which food of known composition is provided to the participants. In a controlled feeding-study, food and beverages are precisely prepared by the study staff for the individual to consume.[33,34] Individuals will be either be admitted to a clinical research center or come to the center to eat a meal and pick up additional meals of a specified nutrient content. For a controlled setting, there is less variability when estimating nutrient and energy but participants have limited food choices. Challenges include adhering to the diet and refraining from temptations from foods not a part of the study.[33]

However, all the current studies available to assess the effects of varied macronutrient distributions on PPG in subjects with T1DM have used the controlled-feeding method. Although the controlled-feeding method is ideal for studying specific dietary effects on biological processes, the generalizability of these studies is limited.

In a free-living situation, the study staff monitor the participant's dietary intake. Estimating energy intake for individuals partaking in a free-living situation is plagued with difficulties due to the requirement of self-reporting.[35] Variability of the diet intake and inaccuracies of food recalls make it difficult to accurately measure both the types of foods and how much the individual actually consumed. However, free-living dietary intake studies are able to provide insight into typical dietary patterns of participants.

2.11 Methods to measure dietary intake

Traditional dietary assessment tools such as 24-hour recalls, diet records, and food frequency questionnaires are reliant upon the participants' ability to recall all foods eaten and provide estimations of the amount. For a 24-hour recall, an interview is conducted and responses are recorded by the interviewer.[36] This method of diet assessment requires participants to recall and report all foods and beverages consumed in the last 24 hours. Limitations to this method includes the inaccurate relay of diet consumption related to knowledge, memory, or interview probing. A single 24-hour recall may not be representative of the participant's typical diet and multiple days of recalls may be warranted. Diet records require participants to record food and beverage amounts, ideally at the time of consumption, over one or more days.[36] Limitations include the alteration of food choice and amount consumed related to reactivity bias and burden of the participant to diligently record intake.[36]

Food frequency questionnaires require participants to recall usual intake of a specific list of foods and beverages during a specific period of time. Limitations include having a food list that is appropriate for the population being observed, such as the inclusion of culturally specific foods, and the results of the assessment may provide crude estimates of intake and may not be reflective of the portion sizes the individual would typically choose.[36] Traditional methods all have limitations and novel methods to accurately measure dietary intake are being explored including digital food photography.

Digital food photography is an innovative method for collecting and estimating dietary intake. This method utilizes mobile technology such as a smartphone or other similar device to self-report dietary intake and provide documentation for food portion sizes using visual estimations.[37] A digital diet record is a valid and feasible method for dietary assessment and improves estimations of macronutrient content in meals. [38,39] Studies validating digital food photography demonstrated energy intake using this method was better than traditional methods.[40]

The T1 Dexi pilot project uses the Remote Food Photography Method © (RFPM©) which is a validated method for assessing diet intake[41] in free-living and laboratory conditions.[40,42] The use of the RFPM provides detailed information about food selection, consumption and waste.[40] RFPM© uses ecological momentary assessment (EMA) methods to improve data quality and reduce missing data through automated reminders for participants to capture images of their foods.[43,44] This method of food recall does not required the participant to estimate portion sizes. Trained evaluators use the before and after meal photos to estimate portion sizes which increases accuracy and improves estimation of nutrient intake.[41,43]

The use of remote food photography has the advantage of reducing patient burden and incorporating computer automation for improving accuracy.[44] Energy intake calculated using the RFPM was compared to measured total energy expenditure by doubly labeled water; RFPM captured total energy intake within 3.7% of the TEE representing a significant improvement over traditional methods of dietary intake assessment.[43] Specific foods and portion sizes are entered into The Food Photography Application© software that calculates the energy and nutrient content of those foods based on values from the Food and Nutrient Database for Dietary Studies.[44] The process for estimating intake involves the use of trained registered dietitians (RDs) who compare the images of food selections and waste to images of standard portions.[44]

2.12 T1-Dexi Publications

Two manuscripts that have been published based on the T1-Dexi pilot data set. The primary findings of these publications are summarized below.

The ability of patients with T1DM to accurately estimate meal content, particularly the protein and fat content of meals is relatively unknown. In this analysis, participant estimates the meal were compared with expert nutrition analyses performed via the Remote Food Photography Method© (RFPM©). Participants were asked to take photos of meals/snacks on the day of and day after scheduled exercise, enter carbohydrate estimates, and categorize meals as low, typical, or high protein and fat. Glycemia was measured via continuous glucose monitoring. Participants (n=48) were 15-68 years (34± 14 years); 40% were female. The majority (70%) of both low protein and low-fat meals were accurately classified as such by participants. However, only 22% of high protein meals and 17% of high fat meals were

accurately classified. Forty-nine percent of meals with <30 g of carbohydrates was overestimated by an average of 25.7±17.2 g. The majority (64%) of large carbohydrate meals (≥60 g) were underestimated by an average of 53.6±33.8 g.[45] Glycemic response to large carbohydrate meals was similar between participants who underestimated or overestimated carbohydrate content, suggesting that factors beyond carbohydrate counting may impact postprandial glycemic response.[45] This is an interesting finding in the context of the current proposal. It is possible that other factors such as pre-prandial glucose, exercise or other factors will impact PPG regardless of the protein and fat content of the meal. Controlling for these factors will be an important aspect of this analysis.

A subsequent study examined the 24-h effects of exercise on glycemic control as measured by continuous glucose monitoring (CGM). Participants in the Ti-Dexi pilot study were randomly assigned to complete twice-weekly aerobic, high-intensity. interval, or resistance-based exercise sessions in addition to their personal exercise sessions for a period of 4 weeks. Exercise was tracked with wearables and glucose concentrations assessed using CGM. An exercise day was defined as a 24-h period after the end of exercise, while a sedentary day was defined as any 24-h period with no recorded exercise +/-10 minutes long. Sedentary days start at least 24 h after the end of exercise. Mean glucose was lower (150 – 45 vs. 166 – 49 mg/dL, P = 0.01), % time in range [70–180 mg/dL] higher (62% – 23% vs. 56% – 25%, P = 0.03), % time >180 mg/dL lower (28% – 23% vs. 37% – 26%, P = 0.01), and % time <70 mg/dL higher (9.3% – 11.0% vs. 7.1% – 9.1%, P = 0.04) on exercise days compared with sedentary days.[46] Glucose variability and % time <54 mg/dL did not differ significantly between exercise and sedentary

days. No significant differences in glucose control by exercise type were observed.[46] This study illustrates the substantial impact of exercise on PPG independent of macronutrient intake.

2.13 Conclusion

In conclusion, we investigated how protein and fat content of meals impacts PPG of subjects with T1DM enrolled in the T1Dexi Pilot study. This analysis addressed the gap in the current literature on the impact of high protein and high fat meals on PPG among subjects with T1DM. There is some evidence that high protein and high fat meals delay time to peak PPG and increase total AUC of PPG in patients with T1 DM. To date, studies have tested the effects of protein and fat on PPG using controlled feeding study designs. The analysis in this project will use data collected from free-living subjects with T1DM collected using the T1-Dexi app and CGM.

Chapter 3: Methods

This was a secondary analysis of a previously collected dataset. The study was a prospective cohort of adolescents and adults with T1DM. Participants were recruited and randomized into groups of: aerobic, resistance, or High Intensity Interval Training *HIIT), and followed prospectively. Inclusion criteria included: age between 15-70 years, use of either multiple daily insulin injections (MDI) or an insulin pump, and a diabetes duration for at least 2 years. For this analysis, the key outcome variables included macronutrient content of the meal, the time to peak glucose, and the area under the curve of post-prandial glucose. A brief description of how the data was collected is provided below.

3.1 Primary Study Description

In the primary study, participants were trained to use the T-1 Dexi app, to record exercises and collect images/photos of food selection before meals and plate waste after meals for up to 16 days during the 4-week protocol. The 48 participants ranked meals as low, typical or high protein; low, typical, or high fat; small, medium or large meal size; and entered the estimated grams of carbohydrates for that meal into the app. Meal insulin bolus data was collected through Tidepool/Medtronic if the subject was using an insulin pump and by Clipsulin or written logs if the subject was an MDI user. The 2,731 meal photos were analyzed by the Ingestive Behavior Laboratory at the Pennington Biomedical Research Center using the Remote Food Photography Method© (RFPM©) to estimate nutrient content.

3.2 Tidepool and Carelink

Bolus and basal insulin data were collected through the server software Tidepool (Palo Alto, CA) or Carelink by Medtronic MiniMed (Devonshire, CA). This data was collected through the participants use of a Medtronic insulin pump and wireless Bluetooth-enabled smart insulin pen (Clipsulin). For participants using the MDI method, data was collected through written logs.

3.3 Continuous Glucose Monitoring

Participants' blood glucose was estimated using continuous glucose monitoring (CGM). The participants used their personal CGM (50% Dexcom; 10% Medtronic, 2% Abbott) and those who were not current CGM users had a blinded GCM (38% Dexcom G4 with 505 or G5). Meal entries were compared to the glucose levels to observe PPG excursion were related to the carbohydrate estimation and nutrient intake data captured by RFPM©.

3.4 Remote Food Photography Method

Participants utilized the T1-Dexi App to document and measure nutrient intake. A smartphone was used by the participant to capture images of their food before consumption and food waste after. When using the app, food images were captured at an arm's distance away and at a 45-degree angle with a reference card or fiduciary marker. A reference card or fiduciary marker was provided to the participant before the data collection and was used as a reference point of measure. Two images were captured per eating occurrence and transmitted to the server for analysis. The food images were analyzed by a trained human rater that used a computer program that linked the foods in the images to the Food and Nutrient Database for Dietary Studies and other sources, such as manufacturer's information.

The human rater assessed the foods in the image and linked them to a nutrient reference in the Food and Nutrient Database for Dietary Studies.[47] Reference images were from a food image archive and had known quantities of foods. The rater compared the reference image to the participant image and estimated food selection, portion sizes, and plate waste. This method of estimation relied on existing and validated visual comparison methodology.[37]

3.5 Statistical Methods

The statistical software, STATA IC 16, was used for the analyses. Summary descriptive statistics included mean, standard deviation, and 95% confidence intervals. Primary dependent variables included time to peak postprandial glucose and PPG area under the curve. Independent variables included pre-meal glucose concentration, meal energy, carbohydrate, protein and fat content, insulin bolus, sex, age, body weight, previous exercise and time of meal consumption. Variable distributions were tested for normality and co-linearity before analysis.

Several decisions regarding data inclusion/exclusion were determined after data alignment and cleaning. These included PPG data from end of the meal until the beginning of the next meal; meals with less than 180 minutes of PPG were excluded. Meals with rescue carbohydrate intake within 60 minutes, and meals with missing insulin data.

Mixed effect multiple linear regression models were implemented for each specific hypothesis. Time to peak glucose was the response variable for the first model. Fixed effects included meal energy, carbohydrate, protein and fat with participant as a random effect.

PPG area under the curve (AUC) was calculated using the trapezoidal method. PPG AUC was the response variable for the second model. Similar to the first model, fixed effects included meal energy, carbohydrate, protein and fat content with participant as a random effect. The model residuals were assessed for skewness; no transformation was employed due to normal distribution.

Missing CGM data for the glucose reading at minute 0 and 180 was interpolated using linear interpolation. For the linear interpolation method, the nearest 3 CGM measurements were used to interpolate the missing value. Missing pre-meal and time 180 glucose values were interpolated before total area under the curve (AUC) was calculated. Incremental AUC was calculated by subtracting the area below the pre-meal glucose concentration as a measure of glucose excursion after a meal.

Chapter 4: Results

4.1: Subject characteristics

Characteristics of the study participants are given in Table 1. The study enrolled 48 participants. Thirteen participants were excluded due to the absence of quality meal photos

from which to analyze nutrient intake. The remaining participants (n=35) had from 2-40 meals, with an average of 18.7 meals per participant with corresponding nutrient analysis. Participants ranged in age from 16 to 68 years and 68.6% were male. They had a mean T1DM duration of 20.4 ± 13.6 years. All the participants identified as white non-Hispanic. They had a mean HbA1c of 7.2%.

Table 1. Subject Characteristics (N = 35)

Characteristics	Summary Statistics
Age (yrs.)	
Mean (SD)	35.9 (15)
Range	16 to 68
15-25	10 (28.6%)
26-44	16 (45.7%)
45-70	9 (25.7%)
Gender	
Male n (%)	24 (68.6)
Female n (%)	11 (31.4)
TID Duration (yrs.)	
Mean (SD)	20.4 (13.6)
Range	3 to 57
Age at Diagnosis (yrs.)	
Mean (SD)	15.4 (10.2)
Range	2 to 53
Body-Mass Index (kg/m^2)	
Male	N = 24
Mean (SD)	26.6 (4.3)
Range	18.5 to 39.9
Female	N = 11
Mean (SD)	26.5 (2.9)
Range	23.9 to 34.2
Height (cm)	
Male	N=24
Mean (SD)	178.5 (7.5)
Range	167 to 198
Female	N=11
Mean (SD)	165 (6.4)
Range	152 to 173
Weight (kg)	
Male	N=24
Mean (SD)	84.9 (14.5)
Range	65 to 119.3
Female	N=11
Mean (SD)	72.4 (13.0)
Range	58 to 102.1
Race/Ethnicity *n (%)*	
White Non-Hispanic or Latino	35 (100)
Daily Insulin Units *mean (SD)*	
Basal	21.7 (9.3)
Bolus	7.2 (4.5)
Total	42.7 (17.4)

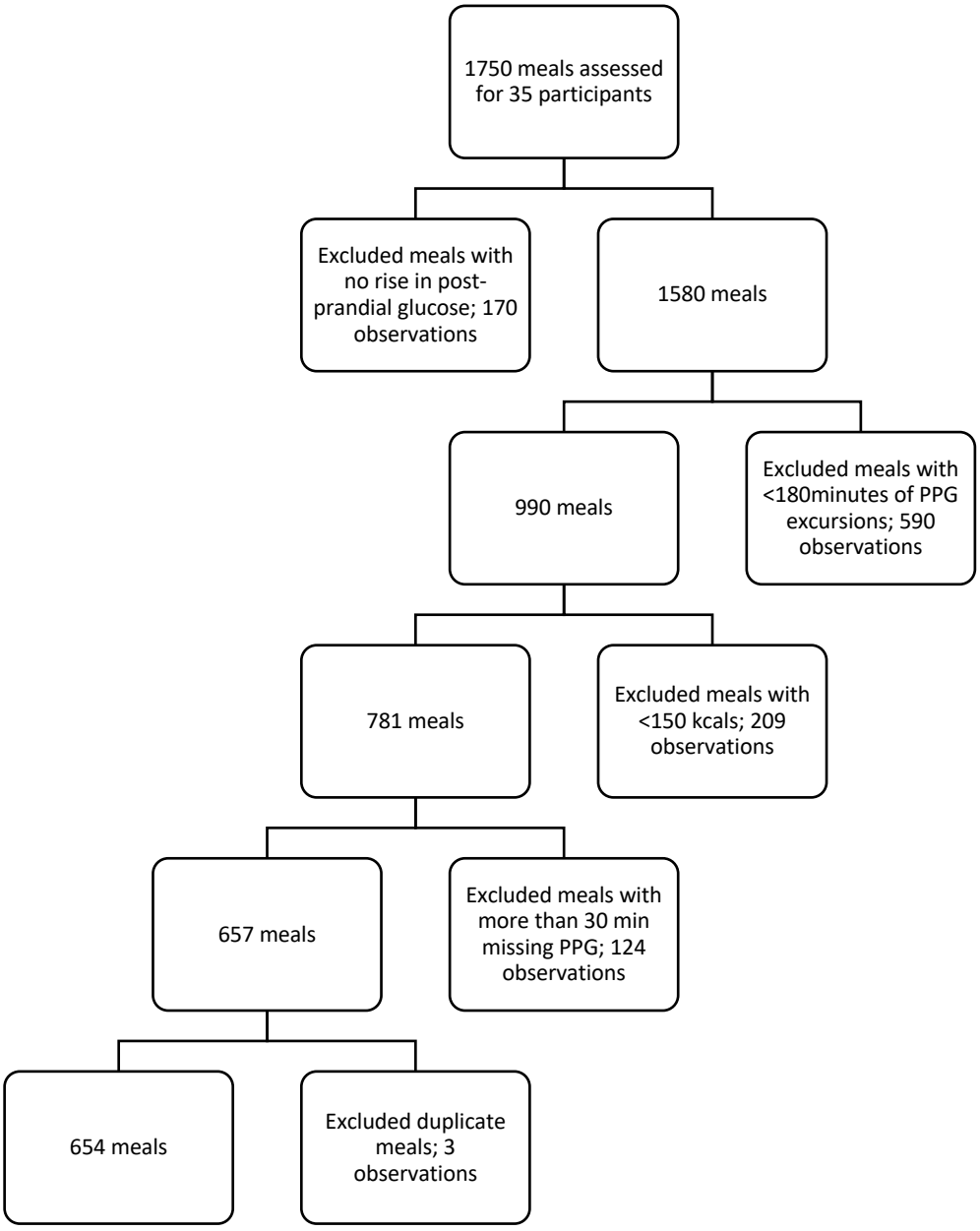
Insulin Modality at Enrollment *n (%)*	
Injections	29 (54.3)
Pump	7 (20.0)
Both	1 (2.9)
Not Reported	8 (22.9)
HbA1c Test	
Mean (SD)	7.2 (1.1)
Range	5.8 to 10.4

A comparison of characteristics between subjects with Type I Diabetes Mellitus (N=35) enrolled in the T1Dexi Pilot study.
Results are means ± standard deviation of the mean (SD). Range of values expressed as the *low – high* observations.

4.2: Inclusion and exclusion criteria for meals

To address our specific research questions, meals were reviewed and exclusion criteria applied to arrive at the final meal data for the analysis (Figure 2). Based on previous research, high protein and high fat meals delay time to peak glucose and increase post-prandial glucose excursions several hours after the meal. We initially excluded all meals with a time to peak glucose prior to the minute 0 and those with less than 180 minutes of post-prandial glucose CGM data before the next meal. We then excluded "meals" with less than 150 kcals with the reasoning that fat and protein are unlikely to impact post-prandial glycemia of very small meals or snacks and we excluded meals that had substantial missing post-prandial CGM readings (>30 minutes) due to CGM errors or data acquisition and download gaps. Finally, we deleted duplicate meals. The final group of meals that met our inclusion criteria was 654 meals.

Figure 2. Flow diagram of the approach used to identify meals for assessment.



4.3: Meal Description

The characteristics of the meals assessed are given in Table 2. The (n=654) meals ranged from 150 to 2444 calories. Of the total calories, an average meal contained 47.3% carbohydrates, 15.9% protein, and 36.2% fat.

Table 2. Meal Composition	
Characteristics	**Summary Statistics** (N=654)
Calories *(kcals)*	
Mean (SD)	498.6 (338.9)
Range	150 to 2444
Carbohydrates *(% of total kcals)*	
Mean (SD)	47.9 (20.5)
Range	3.7 to 100
Protein *(% of total kcals)*	
Mean (SD)	15.9 (9.9)
Range	0 to 57.5
Fat *(% of total kcals)*	
Mean (SD)	36.2 (16.9)
Range	0 to 86.9
Total AUC *(PPG excursion 0-180 minutes, mg/dL*minute)*	
Mean (SD)	28495.8 (11175.2)
Range	7515 to 70099.0
iAUC *(incremental AUC, mg/dL*minute)*	
Mean (SD)	5460.45 (9951.0)
Range	-30063.5 to 40732.9
Time to Peak *(PPG excursion 0-180 minutes)*	
Mean (SD)	105.6 (57.8)
Range	0 to 180
Peak Post Prandial Glucose	
Mean (SD)	205.9 (73.2)
Range	62 to 401
Pre-meal Glucose	
Mean (SD)	128.5 (65.7)
Range	39 to 401

Dietary intake of the subjects was based on food photography captured with the T1-Dexi App. Nutrient content was measured by a human rater that analyzed foods in the images and linked them to a nutrient reference in the Food and Nutrient Database for Dietary Studies.
Results are means ± standard deviation of the mean (SD). Range of values expressed as the *low – high* observations.
* = iAUC was calculated by subtracting the area below the pre-meal glucose concentrations from the total AUC.
Pre-meal glucose describes the glucose reading at the start of the meal.

4.4: Effects of protein and fat on time to peak PPG

We hypothesized that meals higher in protein and fat would delay time to peak postprandial glucose. The distribution for time to peak glucose was skewed. A variety of transformation were assessed but none normalized the results. We ran a mixed effects model with the following fixed effects: total calories consumed, carbohydrates (% total calories), protein (% total calories), fat (% total calories), and pre-meal glucose. Carbohydrates (% total calories) was calculated using the following equation: *100-(% meal protein + % meal fat)*. The

random effect was participant. The residuals of the mixed effects model were also skewed. Thus, parametric analysis with a mixed effects model was not valid for the dependent variable with time to peak glucose.

Next, we analyzed the data with a nonparametric approach. Meals were classified as *low*, *medium*, or *high* protein, based on a previous study.[45] The time to peak glucose was compared by categories of low: <13% protein (n=262), medium: 14-18% protein (n=183), and high: >19% protein (n=209) by a Kruskal Wallis test. There was not a significant difference (p=0.23) in time to peak glucose between the *low*, *medium,* and *high* protein groups, described in Figure 3A.

Next, meals were classified as *low*, *medium* or *high* fat. The time to peak glucose was compared by categories of low: <25% fat (n=166), medium: 26-32% fat (n=89), and high: >33% fat (n=399) by a Kruskal Wallis test. There was no significant difference (p=0.79) in time to peak glucose between the *low*, *medium,* and *high* fat groups, based on a previous study,[45] described in Figure 3B.

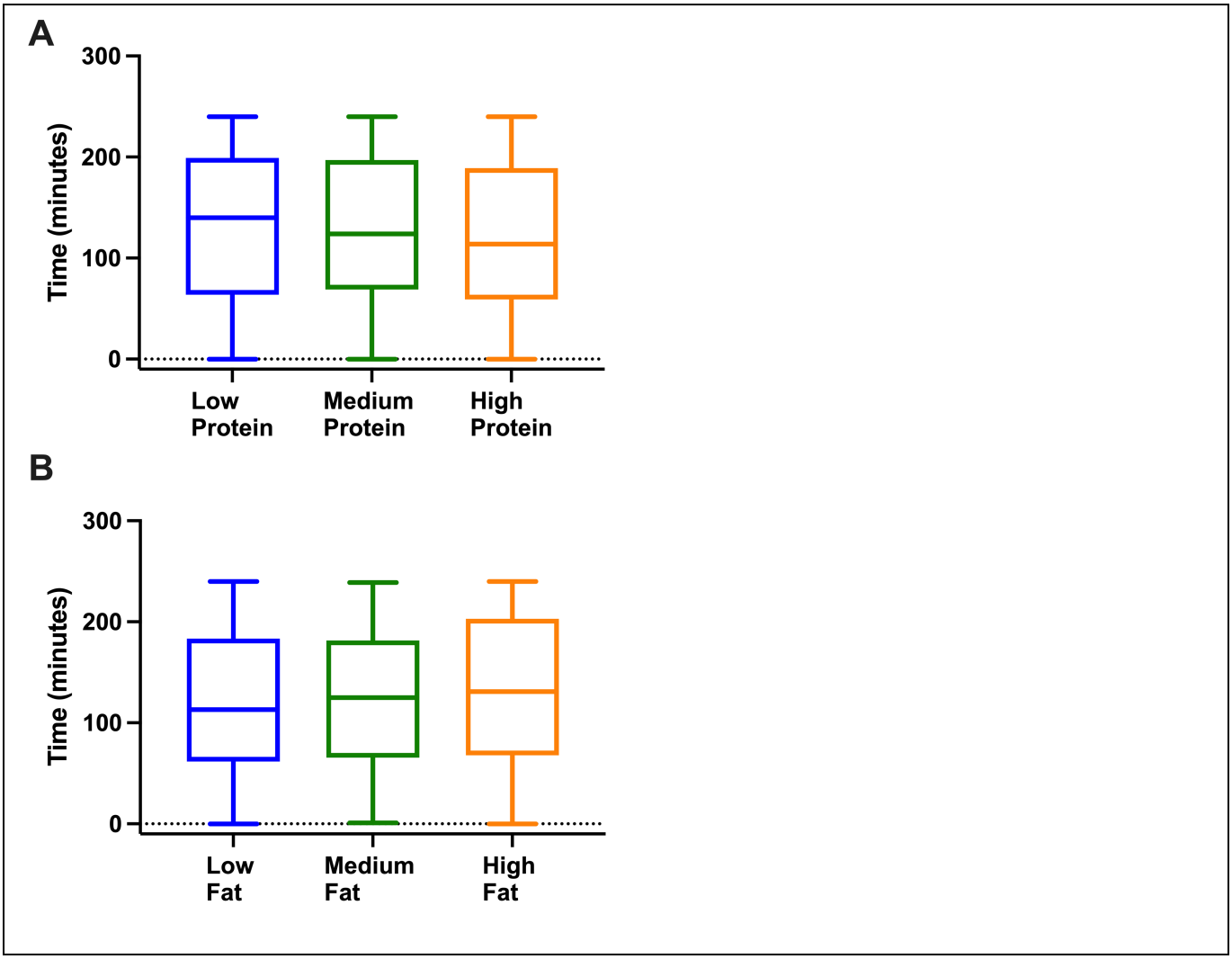


Figure 3. Boxplot distribution of time to peak glucose A) grouped by *low, medium,* and *high* protein. Blue represents low protein (<13%, n=262). Green represents medium protein (14-18%, n=183). Orange represents high protein (>19%, n=209). B) grouped by *low, medium* and *high* fat. Blue represents low fat (<25%, n=166). Green represents medium fat (26-32%, n=89). Orange represents high fat (>33%, n=399). Bars represent the lowest and highest time to peak value of each category. There is no significant difference seen for time to peak glucose by protein or fat.

4.5: Does protein or fat content of the meal increase post-prandial glucose excursion when expressed as a percent of total calories?

We hypothesized that meals higher in protein and fat would increase postprandial glucose excursion as measured by area under the post-prandial glucose curve (AUC). Total AUC

was calculated by the trapezoid method in STATA. For meals with missing pre-meal glucose a linear interpolation was used to estimate the value. To account for pre-meal glucose concentration, an incremental AUC (iAUC) was calculated by subtracting the area below the pre-meal glucose concentration. iAUC represents the total area above or below the pre-meal glucose concentration or glucose excursion after the meal. The iAUC was normally distributed and used as the dependent variable in the following models. Based on a scatterplot matrix, no outliers were identified. The variables carbohydrate (% total calories) and fat (% total calories) of the meal where co-correlated (R^2=0.77) and only 1 variable was entered into the model.

A mixed effects model was run with the following fixed effects: total calories consumed, protein (% total calories), carbohydrate (% total calories), and pre-meal glucose. The random effect was the participant (Table 4 model 1). A reduced model that removed protein (% total calories), a non-significant effect, was run (model 2). A simple multiple-linear regression model was used for model 3. The R^2 of the simple multiple-linear model was 0.25, which indicated that 25% of the change in PPG was explained by the percent of total calories, carbohydrates of the meal, and pre-meal glucose concentrations. The protein of the meals did not have a significant effect on PPG in the mixed effect models. Figure 4 illustrates the linear relationships between iAUC and A) total calories consumed, B) carbohydrates (% total calories), C) protein (% total calories), and D) pre-meal glucose (glucose reading at the start of the meal).

Table 4: Incremental AUC mixed effects models using percent

	Mixed Effects Model						Simple Linear Regression		
	Model 1			Model 2			Model 3		
	Coef.	95% CI	p>|t|	Coef.	95% CI	p>|t|	Coef.	95% CI	p>|t|
Fixed effects									
Calories Consumed	3.719669	1.779735 5.659602	0.000	3.735493	1.799625 5.67136	0.000	3.628491	1.637123 5.619859	0.000
Carbohydrate*	50.79333	12.44948 89.13718	0.009	47.78314	15.13358 80.43271	0.004	65.66605	32.59586 98.73624	0.000

Protein*	11.87651	-66.93855 90.69157	0.768						
Pre-Meal Glucose	-89.01624	-99.18542 -78.84706	0.000	-88.97225	-99.132 -78.8125	0.000	-72.67961	-82.7174 -62.64181	0.000
Random effects									
	Estimate	**95% CI**	**Std. Err**	**Estimate**	**95% CI**	**Std. Err**			
Participant	1.36e+07	7172664 2.59e+07	4463368	1.36e+07	7140717 2.58e+07	4445282			

Model 1 used a mixed effects model. Model 2 used a mixed effects model. Fixed effects with *p>0.05* were considered insignificant and excluded. Model 3 used a simple linear regression.
* = The total percentage of calories per meal from carbohydrates, protein, and fat.
Pre-Meal Glucose is defined as the glucose reading at the start of the meal.

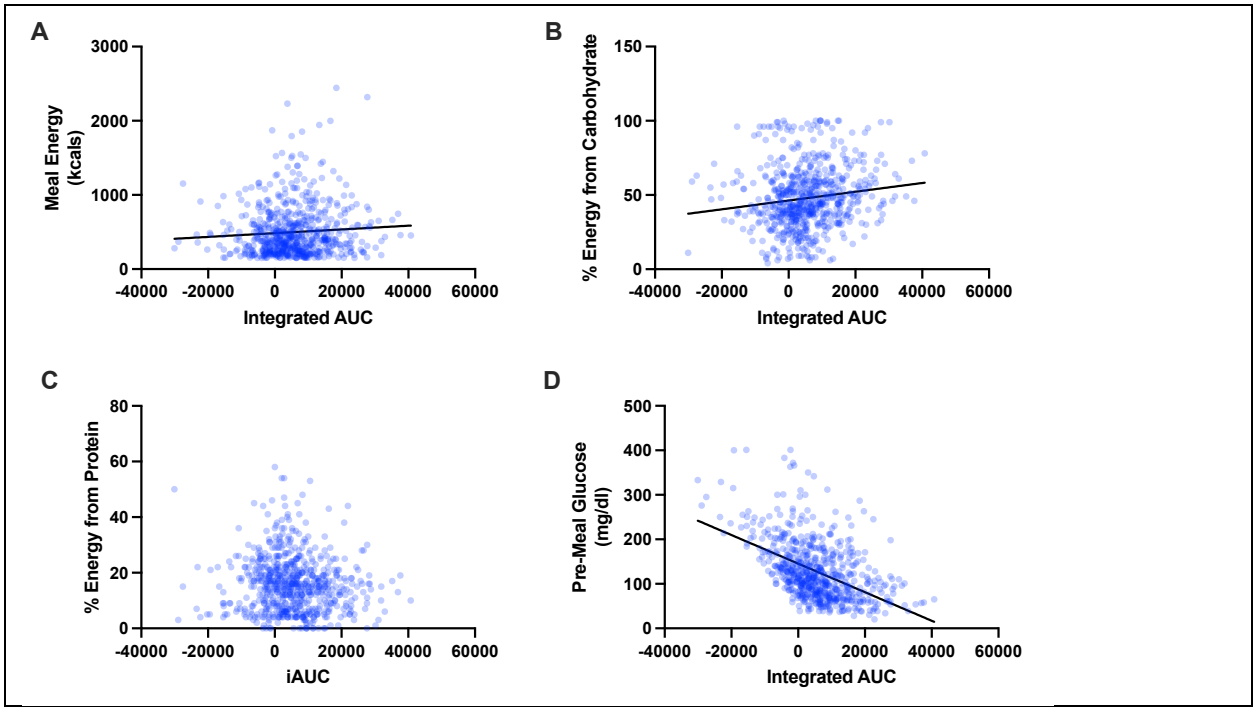


Figure 4. Scatterplot of incremental AUC of the fixed effects as described in Table 4. A) iAUC and meal energy B) iAUC and % energy from carbohydrates C) iAUC and % energy from protein, D) iAUC and pre-meal glucose. The carbohydrates and protein are expressed as the percent of total calories. The strongest correlation was between iAUC and pre-meal glucose.

In the previous model using incremental AUC, higher pre-meal glucose was associated with lower glucose excursion. This initially seemed counter-intuitive so we further explored the relationship of pre-meal glucose to total AUC. We ran another mixed-effects model using total

AUC with the following fixed effects: total calories consumed, protein (% total calories), carbohydrate (% total calories), and pre-meal glucose. The random effect was the participant (Table 5 model 1). A reduced model that removed protein (% total calories), a non-significant effect, was run (model 2). A simple multiple-linear regression model was used for model 3. The R^2 of the simple multiple-linear model was 0.41, which indicated that 41% of the change in PPG was explained by the percent of total calories, carbohydrates of the meal, and pre-meal glucose concentrations. The protein of the meals did not have a significant effect on PPG in the mixed effect models. However, not surprisingly, higher pre-meal glucose is associated with higher total AUC after the meal. Figure 5 illustrates the linear relationships between total AUC and A) total calories consumed, B) carbohydrates (% total calories), C) protein (% total calories), and D) pre-meal glucose (glucose reading at the start of the meal).

Table 5: Total AUC mixed effects models using percent

	Mixed Effects Model						Simple Linear Regression		
	Model 1			Model 2			Model 3		
	Coef.	95% CI	p>|t|	Coef.	95% CI	p>|t|	Coef.	95% CI	p>|t|
Fixed effects									
Calories Consumed	3.719669	1.779735 5.659602	0.000	3.735493	1.799625 5.67136	0.000	3.628491	1.637123 5.619859	0.000
Carbohydrate*	50.79333	12.44948 89.13718	0.009	47.78314	15.13358 80.43271	0.004	65.66605	32.59586 98.73624	0.000
Protein*	11.87651	-66.93855 90.69157	0.768						
Pre-Meal Glucose	90.98376	80.81458 101.1529	0.000	91.02775	80.868 101.1875	0.000	107.3204	97.2826 117.3582	0.000
Random effects									
	Estimate	95% CI	Std. Err	Estimate	95% CI	Std. Err			
Participant	1.36e+07	7172664 2.59e+07	4463368	1.36e+07	7140717 2.58e+07	4445282			

Model 1 used a mixed effects model. Model 2 used a mixed effects model. Fixed effects with *p>0.05* were considered insignificant and excluded. Model 3 used a simple linear regression.
* = The total percentage of calories per meal from carbohydrates, protein, and fat.
Pre-Meal Glucose is defined as the glucose reading at the start of the meal.

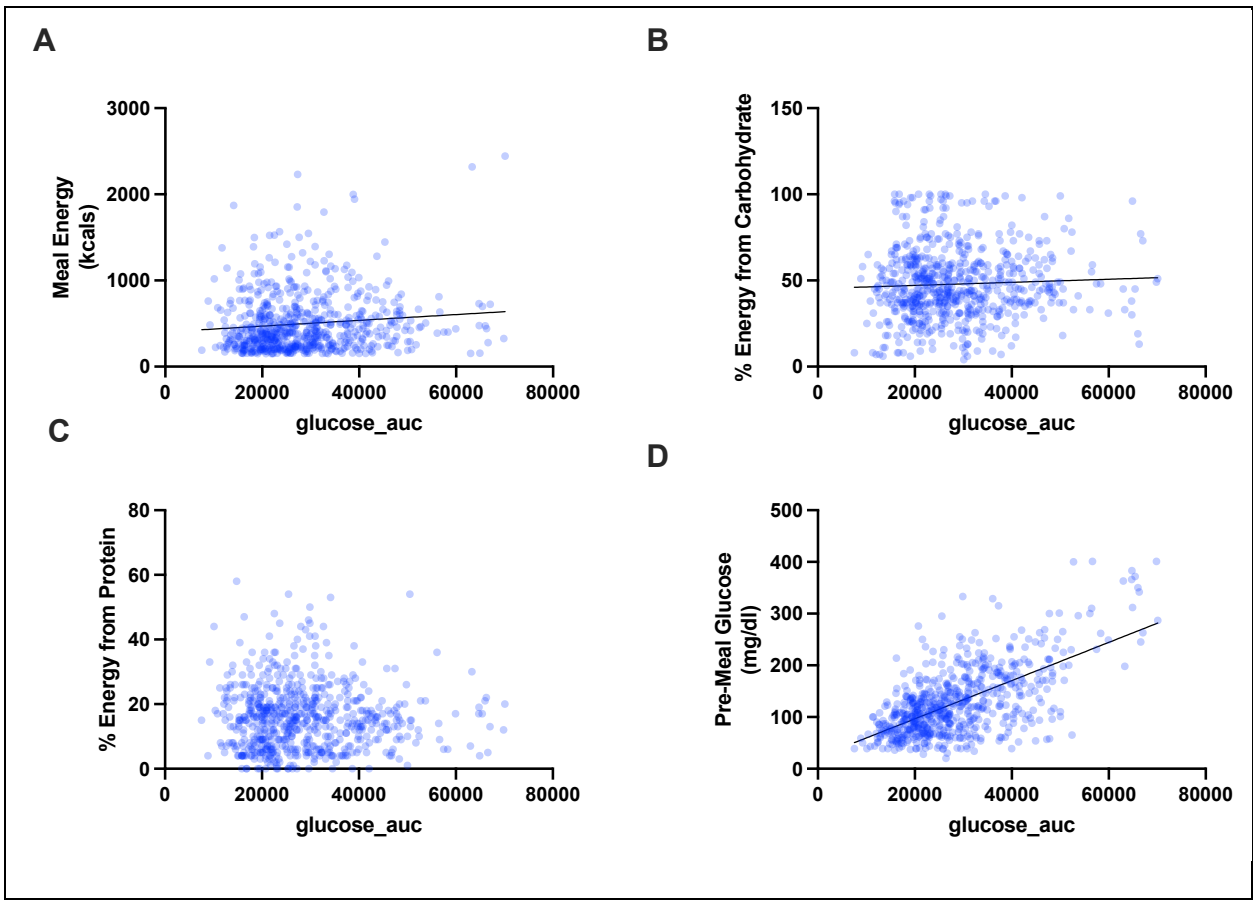


Figure 5. Scatterplot of the total AUC (variable named glucose_auc) of the fixed effects as described in Table 5. A) total AUC and meal energy B) total AUC and % energy from carbohydrates C) total AUC and % energy from protein, D) total AUC and pre-meal glucose. The carbohydrates and protein are expressed as the percent of total calories. The strongest correlation was between total AUC and pre-meal glucose.

4.6: Post prandial glucose excursion as a percent of total calories

To visualize the change in post prandial glucose, we graphed the mean and 95% confidence intervals of PPG grouped by protein (Figure 6A) and fat content of the meals (Figure 6B). Mean pre-meal glucose concentrations were similar across groups. While there appears to be a subtle difference to the shape of the PPG excursion, there is a great deal of overlap between the high, medium and low protein groups and high, medium and low fat groups as a percentage of total energy. It is notable that protein as a percent of total calories was not a

significant factor in the models shown in Tables 4 and 5. Fat was not included in the models due to the high correlation with carbohydrates. The differences in PPG curve we observed in this figure were not reflected in our mixed model effects

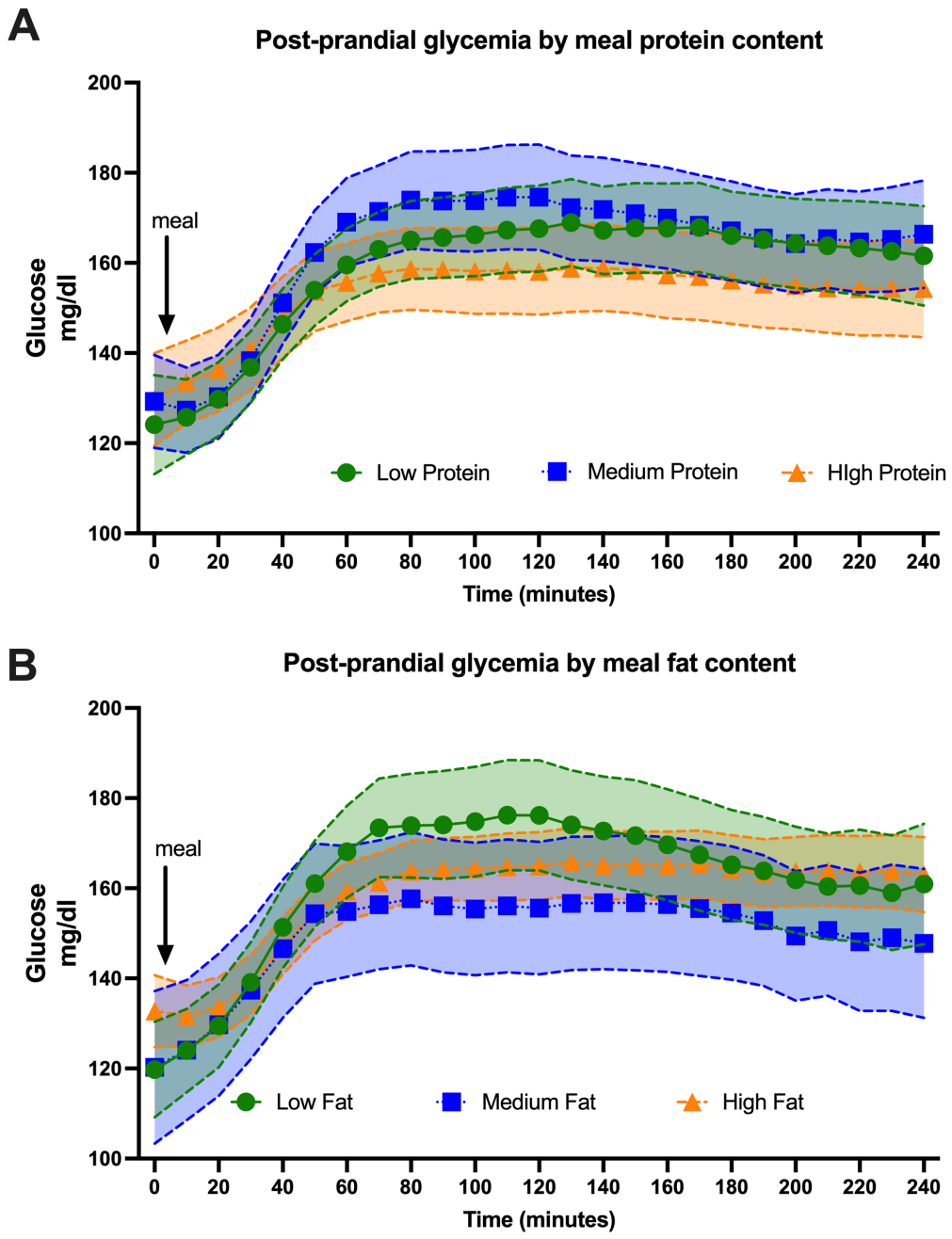


Figure 6: Post-prandial glucose curves A) mean and 95% confidence interval of low, medium, high protein meals. Green represents low protein (<13%, n=262). Blue represents medium

protein (14-18%, n=183). Orange represents high protein (>19%, n=209). B) mean and 95% confidence interval of low, medium, high fat meals. Green represents low fat (<25%, n=166). Blue represents medium fat (26-32%, n=89). Orange represents high fat (>33%, n=399). Dots and lines represent the mean of the meals at each 10-minute interval. Shaded area represents the 95% confidence interval of the PPG excursion.

4.7: Does protein or fat content of the meal increase post-prandial glucose excursion when expressed in grams?

Previous literature described the effects of protein and fat on PPG based on grams of protein[28,29] and fat.[7,11] Were some of the relationships observed in our models based on expressing protein and fat as a % of the total energy? To test the impact of different units, we re-ran the analysis using grams and found slightly different results. A mixed effects model was run with grams of carbohydrate, protein, and fat in a meal. The random effect was the participant (Table 6 model 1). In this model, total calories, pre-meal glucose, protein and carbohydrates were significant, but fat was not a significant factor. A reduced model that removed fat (grams fat in the meal), a non-significant effect, was run (model 2). A simple multiple-linear regression model was used for model 3. The R^2 of the simple linear-model was 0.27, which indicated that 27% of the change in PPG was explained by the percent of total calories, grams of protein and carbohydrates in a meal, and pre-meal glucose. In the mixed effects model, grams of fat did not have a significant effect on PPG. Protein did a have positive effect on AUC, similar to what has been seen in the literature. However, calories consumed has a negative impact on PPG in this model. This association seems counter intuitive perhaps suggesting other factors may be influencing PPG that we have not controlled for in this model.

Table 6: Incremental AUC mixed effects models using grams

	Mixed Effects Model						Simple Linear Regression		
	Model 1			Model 2			Model 3		
	Coef.	95% CI	p>|t|	Coef.	95% CI	p>|t|	Coef.	95% CI	p>|t|

Fixed effects									
Calories Consumed	-20.16604	-37.21511 -3.116973	0.020	-6.225744	-11.75697 -.6945169	0.027	-5.846911	-11.6443 -.0495257	0.048
Carbohydrate*	124.8275	56.63899 193.016	0.000	72.53312	41.0953 103.9709	0.000	76.01453	43.85596 108.1731	0.000
Protein*	104.6252	18.56655 190.6839	0.017	49.03011	-8.276581 106.3368	0.094	27.41818	-31.7996 86.63595	0.364
Fat*	135.7521	-21.32946 292.8336	0.090						
Pre-Meal Glucose	-88.09878	-98.16903 -78.02854	0.000	-88.69364	-98.75006 -78.63722	0.000	-72.42743	-82.39681 -62.45805	0.000
Random effects									
	Estimate	**95% CI**	**Std. Err**	**Estimate**	**95% CI**	**Std. Err**			
Participant	1.39e+07	7389233 2.62e+07	4500101	1.38e+07	7305503 2.60e+07	4462902			

Model 1 used a mixed effects model. Model 2 used a mixed effects model. Fixed effects with *p>0.05* were considered insignificant and excluded. Model 3 used a simple linear regression.
* = The total grams per meal from carbohydrates, protein, and fat.
Pre-Meal Glucose is defined as the glucose reading at the start of the meal.

Again, we observed high pre-meal glucose concentrations was associated with a lower glucose excursion after the meal. We looked at the effects of pre-meal glucose and on total AUC as described above. A mixed effects model with the dependent variable total AUC included pre-meal glucose, total calories, grams of carbohydrate, protein and fat in a meal. The random effect was the participant (Table 7 model 1). In this model, total calories, pre-meal glucose, protein and carbohydrates were significant, but fat was not a significant factor. A reduced model that removed fat (grams fat in the meal), a non-significant effect, was run (model 2). A simple multiple-linear regression model was used for model 3. The R^2 of the simple linear-model was 0.42, which indicated that 42% of the change in PPG was explained by the percent of total calories, grams of protein and carbohydrates in a meal, and pre-meal glucose. In this model, pre-meal glucose, carbohydrates, and protein had a positive effect on AUC.

In both models represented in Tables 6 and 7, we did observe that the coefficient for calories consumed is a negative value. The relationship of protein and fat with calories

consumed when expressed as a percent of total calories demonstrated a positive relationship whereas protein and fats expressed in grams demonstrated a negative relationship. The change in direction of the coefficients are counter-intuitive.

Table 7: Total AUC mixed effects models using grams

	Mixed Effects Model						Simple Linear Regression		
	Model 1			Model 2			Model 3		
	Coef.	95% CI	p>|t|	Coef.	95% CI	p>|t|	Coef.	95% CI	p>|t|
Fixed effects									
Calories Consumed	-20.16604	-37.21511 -3.116973	0.020	-6.225744	-11.75697 -.6945169	0.027	-5.846911	-11.6443 -.0495257	0.048
Carbohydrate*	124.8275	56.63899 193.016	0.000	72.53312	41.0953 103.9709	0.000	76.01453	43.85596 108.1731	0.000
Protein*	104.6252	18.56655 190.6839	0.017	49.03011	-8.276581 106.3368	0.094	27.41818	-31.7996 86.63595	0.364
Fat*	135.7521	-21.32946 292.8336	0.090						
Pre-Meal Glucose	91.90122	81.83097 101.9715	0.000	91.30636	81.24994 101.3628	0.000	107.5726	97.60319 117.5419	0.000
Random effects									
	Estimate	**95% CI**	**Std. Err**	**Estimate**	**95% CI**	**Std. Err**			
Participant	1.39e+07	7389233 2.62e+07	4500101	1.38e+07	7305503 2.60e+07	4462902			

Model 1 used a mixed effects model. Model 2 used a mixed effects model. Fixed effects with *p>0.05* were considered insignificant and excluded. Model 3 used a simple linear regression.
* = The total grams per meal from carbohydrates, protein, and fat.
Pre-Meal Glucose is defined as the glucose reading at the start of the meal.

4.8: The variability between participants

The coefficient for participant, the random effect, in all of our models was small but significant. Could the difference in our mixed effects models between the models that expressed macronutrient content as a % of calories versus grams be related to variability within participants? To understand the variability of meal composition by individual participants, we looked at the histogram distribution for each participant by % of total energy compared to grams in the meal. Figure 7A provides a visual histogram of the distribution of fat consumption as a percent of total calories for each participant. In this figure, many individual participants' fat

consumption typically falls within 25 to 50% of total calories. Consumption of ranged from 0 to 75% of total kcals. However, in Figure 7B, we see the distribution of fat consumption by grams was more condensed around 0 to 25 grams at a meal Some larger meals contained as much as 125 grams. The difference in units, % of total calories or grams of fat in a meal, changed the shape and distribution of the data within participants.

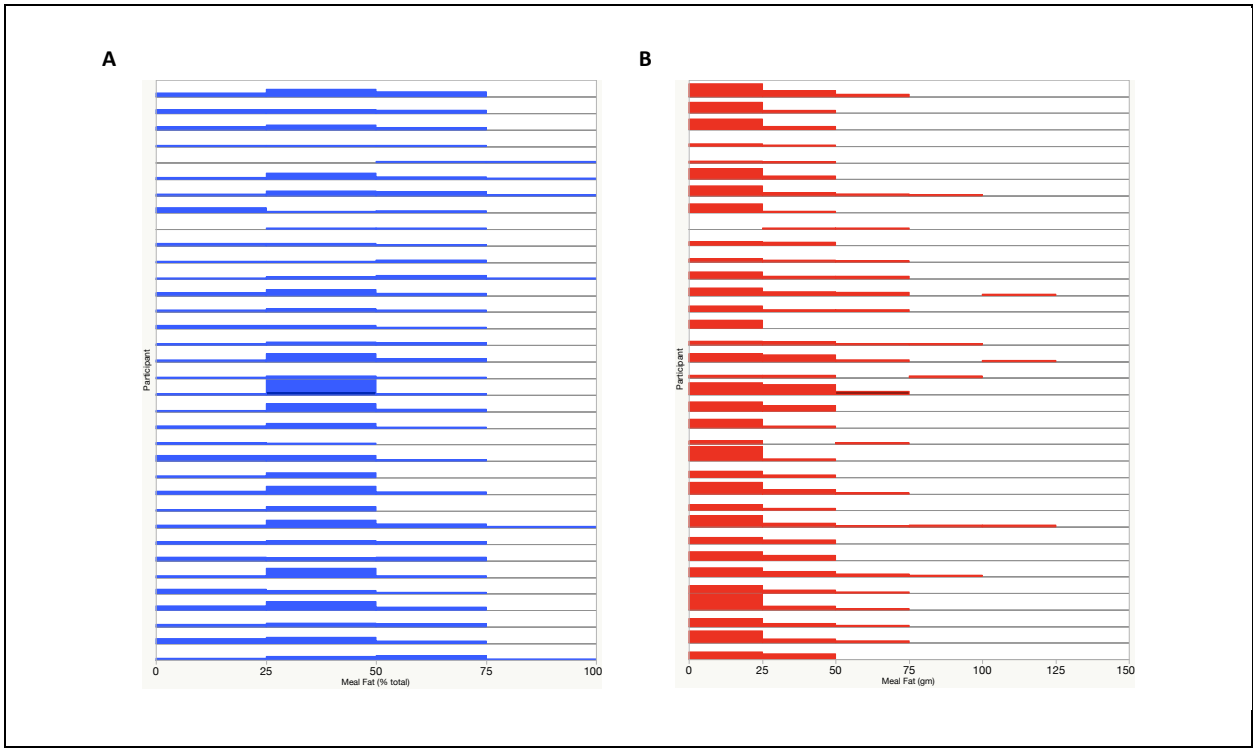


Figure 7: Distribution of fat consumption by participant, (n=35) A) Meal fat by % of total calories. Each blue line represents the distribution of the individual participant's fat consumption. B) Meal fat by gram. Each red line represents the individual participant's fat consumption.

A similar graph of the protein content of the meals is provided in Figure 8. In Figure 8A, we see that for many individuals, the percent of calories from protein in the meals consolidates heavily between 10 to 20% of total calories from protein. In Figure 8B, there is a larger range of grams of protein in a meal from 0 to 240 gm, with most meals containing 0 to 15 gm.

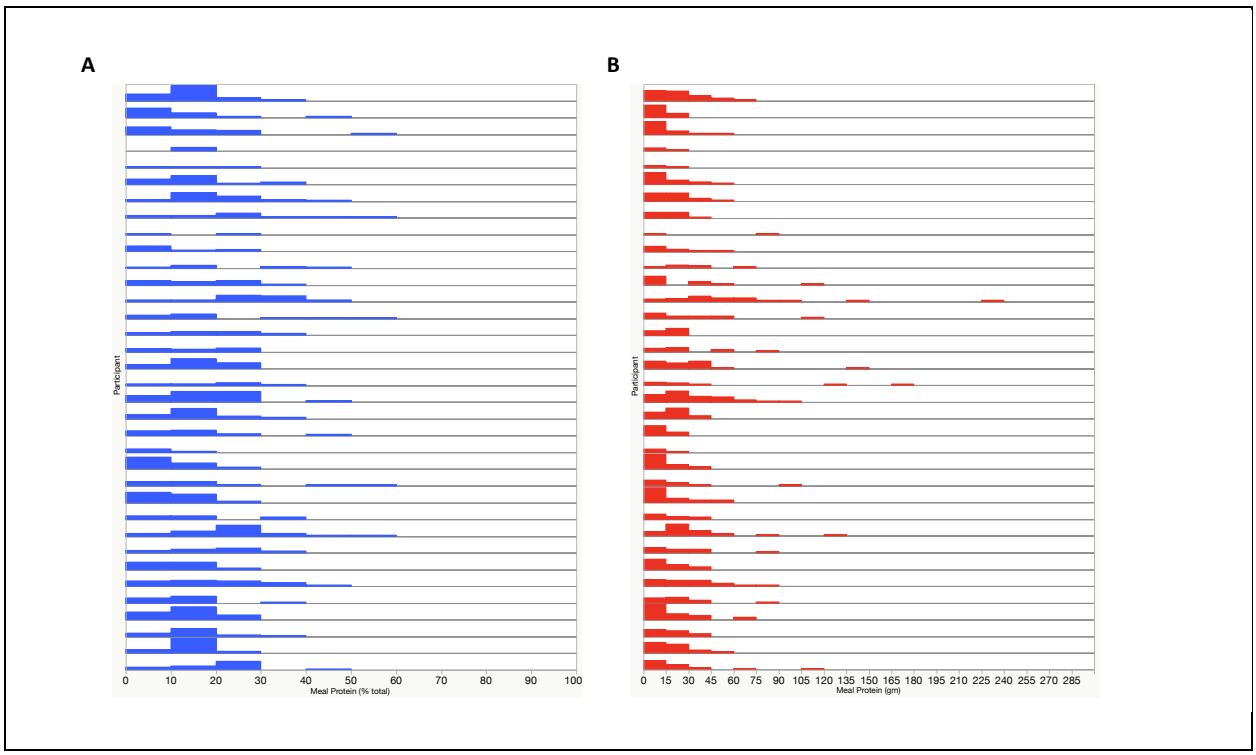


Figure 8: Distribution of protein consumption by participant (n=35) A) Meal protein by % of total calories. Each blue line represents the individual participant's protein consumption. B) Meal protein by gram. Each red line represents the individual participant's protein consumption.

4.9: Effect of Pre-Meal Glucose on AUC

The effect of pre-meal glucose varied between the dependent variables iAUC and total AUC. The results suggested high pre-meal AUC was associated with a decrease in glucose excursion after the meal but that total AUC was in fact higher due to the elevated pre-meal glucose concentrations. In assessing the data, 27% of the meals (n=176) had a negative incremental AUC, thus suggesting that the AUC decreased after the meal. These meals had a mean pre-meal glucose of 174.8 ± 70.6 mg/dL. There was 73% of meals (n=487) that had a positive incremental AUC. These meals had a mean pre-meal glucose of 110.7±54.7 mg/dL. We could speculate that the negative incremental AUC may be related to individuals taking a

correction insulin dose or higher bolus dose at the start of the meal to correct for the high pre-meal glucose.

4.10: Are our models underpowered to detect differences in PPG?

We considered that our models may be underpowered to detect changes in PPG AUC and ran a post-hoc power analysis of grams of protein and fat for the model shown in Table 6. For protein content of the meal, we had only 35% power to detect a 550 mg/dL/min change in iAUC with 600 meals. Similarly, for fat content of the meal we had only 25% power to detect a 450 mg/dL/min change in iAUC with 600 meals. Our analysis is most likely underpowered despite including 654 meals.

Chapter 5: Discussion

In this cohort of free-living participants with type 1 diabetes, percent of energy from protein and fat had minor effects on the time to peak post-prandial glucose and the total post-prandial glucose excursion. We initially hypothesized that high fat and protein meals would delay the time to peak to later and extend the PPG excursion increasing the total area under the curve. However, we saw that there was no difference in time to peak PPG when comparing low, medium and high protein or fat meals expressed as a % of the total energy. The percent of % of carbohydrate increased PPG AUC and by inference % fat lowered PPG AUC. During the assessment for AUC, we observed that high carbohydrate percent was reciprocally correlated with low-fat percent and represented the same value in the model (Table 4). In our models, the tight correlation of high carbohydrate and low-fat percent of the meal showed that

carbohydrates raised glucose excursions but fats lowered it. However, the effect of fats lowering AUC has not been observed in previous literature.

We reviewed the previous literature regarding protein and fat and the PPG response. In most of these studies, carbohydrates were kept constant and protein and fat was added in addition to the carbohydrate in the meals. Protein and fat were expressed as gram amounts rather than % of total energy. To address this difference, we repeated the mixed linear models using grams of carbohydrate, protein and fat in the meal. In this model grams of protein did significantly increase PPG AUC; grams of fat were not a significant factor in PPG AUC.

Why was there a difference in the mixed models between % of total energy from fat and protein and grams of fat and protein in the meal? The two measures are related; grams of fat and protein are used to calculate the macronutrient distribution. On possible explanation could be the spread of the data is greater when expressed in grams versus % of total energy. The histogram distributions graphs of participant variability by grams and % of total energy (Figure 7 and Figure 8) illustrate the difference in scale. Another possible explanation could be biological. Previous studies found no effect of less than 12 grams of protein but an additive effect with 12 or more grams in combination with carbohydrate. Likewise, protein alone did not change PPG with small amounts of protein was consumed but more than 70 gm elicit a PPG excursion. Regardless of the % of total calories, perhaps an intake of at least 12 grams is needed to impact the gastric emptying, alter hormone concentrations such as cortisol and increase gluconeogenesis in the liver. It is possible to have a “high protein” meal with 25% of the energy from protein containing 150 calories that is in fact less than 12 grams of protein. It is also

possible to have a “low protein” meal with 10% energy from protein containing 1500 calories that has more than 37 grams of protein.

Previous literature has shown that increasing protein in a meal can lead to an increase in PPG levels by 48mg/dl at 5 hours post-prandially[25] and glycemic excursions between 150 and 300 minutes post-prandially.[28] These previous studies have suggested that protein increased the duration of blood glucose elevation to 5 hours rather than 2-3 and delayed time to peak glucose. Our results are not consistent with these results; although we did see that increased protein content (gm) increased PPG AUC, we did not see an impact on time to peak PPG. It is notable that these feeding studies were conducted under very tightly controlled feeding environments, whereas this current study is a free-living situation. The meals in this study varied in size (total kcals from 150-2444), varied in carbohydrate content (2.7-328.9 gm/meal; 3.6-100% of the meal) and varied in protein and fat. Other important differences in free-living data is that meals are consumed at variable times throughout the day and time between the current meal and the previous meal is not controlled.

In all of our models, pre-meal glucose concentrations, carbohydrate intake and the size of the meal impacted PPG AUC. Participants with pre-meal glucose concentrations within treatment range had lower PPG excursions. Larger meals (higher kcals) and more carbohydrate (high % of energy from carbohydrate) induced a greater PPG excursion. For clinical application, discussions with patients around meal size and total calories consumed could help with blood glucose management. In this study, we saw a wide range of meal sizes from 150 calories to 2444 calories with the larger meals leading to increases in PPG curves. Regular moderate-sized meals rather than less frequent large-sized meals (high calories) may improve PPG. The percent

of carbohydrates in a meal had significant effects on time to peak and AUC. Continuing to educate those with diabetes about carbohydrate counting can also be beneficial for improving glycemia.

There were many strengths to our study. They study's sample size was large with a total of 35 eligible participants and 654 meals with nutrient analysis. The diets of the participants were captured using remote food photography. Remote food photography improves dietary reporting and estimation of meal nutrient composition when compared to the traditional dietary assessment tools like the 24-hour recall and diet food frequency questionnaires. Lastly, the mixed effects model controlled for the variability between participants enrolled in the study. We recognize that the meals eaten by one particular participant are not independent observations; the participant's response to the meal are not independent events but rather a cluster of responses within an individual.

Limitations of this study includes the following: non-diverse population being assessed, free-living situation, and missing GCM data. The population being assessed all identified as white and had very controlled diabetes, as seen in the range of HgA1c. The information reported does not accurately represent all peoples and may not be applicable to all individuals with diabetes. A free-living situation is ideal for assessing unaltered eating patterns of the participants. However, this free-living environment made it difficult to tightly control for and evaluate the impacts of the food groups of interest (fat and protein) as eating occurrences and meal components where highly variable. A post-hoc power analysis also indicated our mixed effects models were underpowered to detect differences in iAUC from changes in protein and fat content of meals. Lastly, missing CGM data values impacted the analysis. Although CGM

values were to be recorded in 5-minute intervals, many meals had missing values which lead to either the exclusion from the analysis or possible alteration to the AUC curve being observed.

Future directions for this dataset include controlling for additional effects and assessing AUC by increments of time. It is possible that controlling for insulin dose and insulin sensitivity could help to explain some of the unexpected associations we observed in our mixed models. For instance, the high fasting pre-meal glucose could have been considered by the participant by including a higher insulin bolus dose at the start of the meal. A higher insulin dose could change the relationship of the pre-meal glucose to the area under the curve. Previous literature, has seen the impact of protein on glucose excursions several hours after the meal in the late postprandial period.[7,28,29] In our analysis, including total AUC may have caused us to miss the effect of protein and fat during the early and late phase postprandial periods. It is possible that analyzing the data by separate phases of early and late PPG excursion may help explain the relationship of increased protein and fat in the meal.

In conclusion, the protein and fat content of the meal did not demonstrate a substantial influence on time to peak post-prandial glucose, but protein did have a small but significant impact on total post-prandial glucose excursion (AUC). This study does confirm that meal size, pre-meal glucose, and carbohydrates are important factors to monitor for the daily management of blood glucose among patients with type 1 diabetes.

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