Cover Page

Official title of the study:

Effect of Protein Intake on Post Prandial Hyperglycemia in Children and Adolescents with Type1 Diabetes Mellitus

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Title: Effect of protein intake on post prandial glycemia in children and adolescents with Type 1 Diabetes Mellitus.

Background:

In target glycemic control in children with type 1 diabetes (T1D) continues to be a challenge despite advances in methods of insulin delivery and medical knowledge in this area. One of the major aspects is controlling postprandial glycemia (PPG). The relationship between dietary intake of carbohydrates and PPG is well established, and the use of insulin coverage for carbohydrate intake is standard of care. Insulin dose for carbohydrate coverage increases with body weight and progression through puberty. Multiple researchers have attempted to study the effect of dietary intake of protein and fat on PPG as well, but this relationship is not well established in the pediatric age group and there are not clear guidelines for patients on when and how to give insulin for protein intake.

Meals with high protein content have been shown to cause higher glucose excursions in patients with T1D [(1)], and lower glycemic response in healthy individuals [(7), (8), (9), (10)], which suggests that physiologic response to protein intake involves higher insulin secretion. This has also been demonstrated by Sun et al [(9)], where they showed an increase in insulinemic index in healthy individuals when consuming chicken with rice compared to rice alone.

The effect of dietary protein in individuals with T1D has been studied in mixed meals several times. Smart et al [(1)] demonstrated that the greatest glucose excursions after high protein low fat meal occurred most significantly form min 150 to 300 after the meal, when insulin is given to cover carbohydrates only. In 2013, Borie-Swinburne et al [(4)] measured interstitial glucose levels by CGM in 28 c-peptide negative T1D patients, on two consecutive nights, with and without addition of 21.5 grams of protein to dinner (40 g vs 61.5 g). They concluded that no additional insulin is needed to cover for the added protein. Neu et al (6) studied 15 adolescents with T1D on two consecutive nights. They used CGM monitoring for 12 hours, and they compared the area under the curve (AUC) between regular meals and fat/ protein rich meal. They found a significant difference and they recommended additional insulin for fat /protein rich meals.

Other studies attempted to characterize the ideal insulin coverage for protein rich meals in individuals with T1D. In 2008, Pankowska et al [(12)] studied 499 children with T1D on insulin pumps and found that HbA1C is better controlled in patients who use dual wave boluses or square wave boluses to cover protein intake on a daily basis compared to patients who use these modes less frequently. In another study [(5),] they also studied a group of 26 patients with TID and looked at the efficacy of applying a dual wave bolus (covering for carbohydrates, protein and fat) to help reduce the post prandial hyperglycemia after consuming a high fat-protein diet and it was deemed effective in preventing post-prandial hyperglycemia. They proposed that the insulin dose for 10 g of CHO should be equivalent to the insulin needed for every 100 kcal of fat/protein.

Piechowiak et al [11] studied 58 children with T1D, and they observed a significantly lower glucose levels 3 hours after the meal (low fat, high protein with carbohydrates) when using a dual wave bolus compared to standard bolus. Insulin in dual wave bolus (consisting of normal bolus plus square bolus) was calculated based on the number of CHO exchanges (normal bolus) and protein-fat exchanges (square bolus). One CHO exchange was defined as 40 kcal of carbohydrates. One protein-fat exchange was defined as a 100 kcal from protein and fat. The same Insulin-ratio was used for one CHO exchange and one protein-fat exchange.

Kordonouri et al[13] studied 42 patients with T1D and compared carbohydrate counting with carbohydrate- fat- protein counting (CFP) and found out that regardless of the type of bolus (normal vs dual wave), the CFP counting reduced the post prandial glucose levels but they had increased incidence of post prandial hypoglycemia. In a systematic review, Bell et al [16] recommended to increase insulin dose 15-20% for meals containing at least 40 grams of protein and 30 grams of carbohydrates. They also noted that insulin may not need to be adjusted for protein only meals containing less than 75 grams of protein.

Investigating the effect of protein-only intake is also an area of research focus. Paterson et al [14] studied 27 patients with TID , aged 7-40 yrs, where they were given 6 test meals of varying amounts (0g, 12.5g, 25g, 50g, 75g and 100g) of pure protein without giving insulin. Postprandial glycemia was found to be significantly higher only for 75 and 100 grams of protein compared to the lower quantities. Glucose levels were slower to rise when compared to consumption of 20 grams of carbohydrates. Paterson et al also conducted another study with slightly different design: 27 participants with T1D [aged 10-40 years, $HbA_{1c} \leq 64$ mmol/mol (8%), BMI ≤ 91st percentile] received a 30-g carbohydrate (negligible fat) test drink with a variable amount of protein daily over 5 days in randomized order. Protein (whey isolate 0 g/kg carbohydrate, 0 g/kg lipid) was added in amounts of 0 (control), 12.5, 25, 50 and 75 g. A standardized dose of insulin was given for the carbohydrate. PPG was assessed by 5 hours of continuous glucose monitoring. Increasing protein quantity in a low-fat meal containing consistent amounts of carbohydrate decreases glucose excursions in the early (0–60-min) postprandial period and then increases in the later postprandial period in a dose-dependent manner. In summary, Paterson et al concluded that there was a threshold for dietary protein intake (75 grams), and only protein intake above this threshold regardless of body weight would result in post prandial hyperglycemia. However, these studies included a wide range of ages and did not adjust for body weight in their analysis.

The purpose of this study is to explore the role of weight in the relationship between protein intake and post prandial glucose (PPG) levels. The study design (43 children each receiving 6 increasing nominal doses of protein) allows for the relationship to be studied both across patients

of varying weights within each nominal dose, and across patients (whose weights remain the same) across the increasing doses.

Our aims:

Aim 1: To describe the relationship of weight (in kg), and mg of protein per kg body weight, to PPG, graphically and statistically, at each nominal dose. The heaviest children will receive the lowest mg/kg amount of protein at each nominal dose, so these relationships with PPG will be inverse. Additionally, children with different weights and receiving different nominal doses, may be receiving the same mg/kg protein. Observing all nominal doses together will allow us to determine whether the relationship, if any, is linear, demonstrates a threshold, or exhibits a dose-response curve, as examples.

Aim 2: To describe graphically and statistically the relationship of dose of protein to PPG by patient across increasing doses. Since the weight remains constant (or approximately constant) within a patient, adjustment by weight would yield the same results. The expectation is that these results will confirm those from Aim 1.

Aim 3: To construct a multivariate mixed model where any observed relationships can be controlled for other demographic and clinical characteristics possibly associated with blood glucose levels. The type of model will depend on the results of Aims 1 and 2.

Study Design:

Children with T1D will be given a bedtime snack of varying amounts of isolated protein (Protein powder) on 6 different nights and the glycemic response will be measured with the child's personal continuous glucose monitor (CGM).

- Design: Crossover, Prospective; each participant will try all protein amounts (0, 12.5, 25, 37.5,50, and 62.5 grams of protein)
- Population: 43 children with T1D age ≥ 5 and <18 years
- Outcome: postprandial glucose excursions up to 7 hrs post prandially (measured by continuous glucose monitoring and home glucometer)

Vulnerable population:

Prior studies had investigated the effect of dietary protein on post prandial glycemia in diabetic patients regardless of their ages and weights. I hypothesize that this effect is different in children and adolescents given their different weights. Therefore, I believe that this effect needs to be addressed in this specific population to be able to find meaningful information. W will include illiterate and non-English speaking subjects. We will use interpreters for different languages and read the instructions for illiterate subjects.

For illiterate persons who understand English and individuals who are seeing-impaired, the consent document will be read to the participant and the process will be documented in the research file. For illiterate participants, the consent will be subsequently signed by the participant "making their mark" on the signature section of the consent document, in order to document their understanding. An impartial third party witness will be present to confirm the consent process has taken place.
Both the witness and the person obtaining informed consent or interview to obtain permission will sign and date the consent document. The Principal Investigator will be the only study personnel who conducts the interview with illiterate or non-English speaking subjects and obtains consent.

Inclusion Criteria:

- T1D with diabetes duration ≥ 1 year
- On insulin pump or multiple daily injection regimen
- Uses a personal Dexcom CGM
- Age: 5- 17 years
- HbA1C range: ≤ 9%

Exclusion criteria:

- Hyperlipidemia
- Diabetic gastroparesis
- Dietary restrictions
- Celiac disease and other malabsorption syndromes

- Uncontrolled hypothyroidism
- Chronic use of steroids or antipsychotics
- Metabolic disorders of gluconeogenesis
- Use of oral hypoglycemic agents
- Participating in another clinical trial

Data to be collected:

- Patient's age
- Race/ethnicity
- BMI, waist/hip circumference
- Sex
- Last HbA1c
- Duration of DM
- TDD
- POC glucose on home glucometer following protein intake (at 0, 3 and 5 hours)
- Data from CGM: poc glucose values for 7 hrs each intervention night.
- Self-rated pubertal tanner stage

Study Timeline:

Study procedures and data analysis : Between March 2018- February 2019.

Methods:

1. Study investigators will recruit participants from the diabetes clinics at RB&C, University Hospitals Cleveland Medical Center. If they are interested, study investigators will obtain

informed consent/assent in person. If a participant is interested but a study investigator is not present, the participant can inform their physician/ nurse who then can inform the primary investigator by email. The study investigator will call family over the phone to discuss and they will be asked to come in person to the nearest clinic location available to sign the consent/assent.

2. The face-to face time in clinic is about 20 minutes and that include obtaining informed consent/ assent, discussing the instructions to be followed, filling out the questionnaire, providing the protein product and measuring waist/hip circumference.

3. One week before family begins intervention, study investigators will review recent blood glucose levels and optimize insulin dosing with a focus on overnight control.

4. Study investigators will review the step-by-step protocol with participants/guardians. Participants will be asked to complete the intervention on 6 nights while wearing their personal continuous glucose monitor (does not have to be consecutive nights) within 3 weeks in a set order (0, 12.5, 25, 37.5, 50, 62.5 grams of protein). They can participate on a given night if the following conditions are met:

   a- Insulin pump infusion set has been in place for <48 hours and appears to be working.
   b- Bed time glucose 100 - 200 mg/dL and they are not having a carbohydrate containing bedtime snack. Participants/guardians will be asked not to give a dose of rapid acting insulin at bedtime, even if blood glucose is above target (if target is < 200 mg/dL).

      (Younger kids with diabetes usually have bed time glucose targets around 200 mg/dL but older kids sometimes have their targets around 150 mg/dL. Glucose targets are usually well-rounded numbers set to make calculations easy rather than actual cutoffs that separate normal glucose levels from pathological glucose levels. If glucose targets are set < 200 mg/dl , and glucose level at the night of participation is between target and 200 mg/dl, we are withholding rapid acting insulin dose to reduce risk of hypoglycemia).

   c- They have not participated in strenuous physical activity that day.
   d- Negative ketones and patient is not having nausea/ vomiting.
   e- They are wearing their personal Dexcom CGM.
   f- Their dinner was typical for them and was at least 2 hours before bedtime.

5. We will give a protein-only product that is commercially available: 1 scoop is equivalent to 25 grams of protein, 0 carbs, 0 fat. Participants will consume 0, 12.5, 25, 37,5, 50, or 62.5 grams of protein on 6 different nights. They can add the protein powder to 8 oz of water or electrolyte drink that has no carbs and no fat (like Powerade Zero).

Night 1	- No protein drink will be given, but all other procedures should still be followed
Night 2	- ½ scoop of protein powder in the drink (this is 12.5 grams protein)
Night 3	- 1 scoop of protein powder in the drink (this is 25 grams of protein)
Night 4	- 1 and ½ scoops of protein powder in the drink (this is 37.5 grams of protein)
Night 5	- 2 scoops of protein powder in the drink (this is 50 grams of protein)
Night 6	-2 and ½ scoops of protein powder in the drink (this is 62.5 grams of protein)

6. They will be asked not to participate on a given night if they have participated in strenuous physical activity that day, or if they have positive ketones or have nausea/ vomiting.
7. Protein will be consumed at time of bedtime snack (8-9 pm), level of activity for 5 hours after the consumption should be low.
8. Participants/guardians will be instructed to start from the smallest to the largest amount of protein.
9. Participants will be asked to check postprandial glucose levels using home glucometer before the protein intake, at hour 3 and hour 5 after consumption.
10. Participants will be asked to check postprandial glucose levels using home glucometer at any time if they have concerns, patient has symptoms and/or as directed by us when they call us for questions.
11. If glucose is > 300 mg/dL, we will ask for ketones check. If patient is symptomatic and/or having moderate to large ketones and/or has glucose >300 mg/dL for more than one hour, they are instructed to call 216-207-7244 and enter the pager number 31460 * followed by their call back number. They should not administer insulin before calling the research investigator.

    For a given amount of protein, if participation was cancelled after ingestion, they may repeat it on a different night. (Participation should be cancelled on a given night if insulin was administered any time after dinner until 5 hrs after consuming the protein product). Patient/parents may choose to cancel for personal preferences. If cancellation was due to hyperglycemia requiring insulin administration, we will discuss with the family the need to proceed with the higher protein doses.

12. They will be asked to fill out the attached log sheet and the questionnaire.

13. They will be asked to call us when they have the log sheet filled out, if they have questions or if they wish to leave the study.

14. They will be asked to send us the log sheet and we will assist them in downloading the meter and CGM.

15. A chart review will be performed to obtain the following information: Age, weight, gender, Body mass index, race, date of Diabetes diagnosis, insulin total daily dose, last HgBA1C, and past medical history. Redcap will be used to store and analyze the data.

16. Phone Calls:

- We will call them within a week prior to starting participation to review blood glucose levels and optimize glucose control.
- Once they start the intervention, we will call once a week during the 3 week study period to review instructions and answer questions.
- Phone calls will be about 5-10 minutes in length. It may be longer if they have more questions. We will call during office hours 8 am- 5pm. If they have preferred time of day, we will try our best to call then.

16. Addresses of clinic locations where CGM data can be downloaded:

UH Rainbow Babies & Children's Hospital	11100 Euclid Avenue ,Cleveland, OH 44106 , phone **216-844-3911**
UH Landerbrook Health Center	5850 Landerbrook Drive, Mayfield Heights, OH 44124, phone **440-646-2626**
UH Westlake Health Center	960 Clague Road, Westlake, OH 44145, phone **440-250-2100**
UH Parma Medical Center	6115 Powers Blv , Ste 201, Parma, OH 44129
UH Medina Health Center	4001 Carrick Rd, Suite 220, Medina, OH 44256

17. Information learned about how protein snacks at night affect morning glucose readings will be shared with subjects. This information will be provided as a discussion of results with the participants and their families and a summary hand out will be provided.

Risks:

1- High glucose level might happen after protein intake, symptoms related to that might be headache, feeling thirsty, increased urination. This should not cause development of ketones or diabetic ketoacidosis (and we will be monitoring for ketones if needed).

2- Risk of not giving insulin for glucose >200 mg/dL: risk is minimized by frequent monitoring of glucose levels, monitoring of ketones and having a set plan for managing adverse events.
3- Risk of developing ketones: We do not anticipate that protein intake will result in ketone formation, and the risk is minimized by frequent monitoring and having a set plan for managing adverse events.
4- Some children (older than 10 years old) will be asked to assess their own pubertal development using a validated survey and they may find this embarrassing.
5- Risk of breach of Confidentiality: The risk of release of any private information is carefully guarded against by using a study ID not the participant's name on all records, and by keeping data in secure locations that only the study team can access.

Benefits:

Being in this study will help the participant's know how big a protein snack they can have before bed without making their blood glucose high.

We hope that the information from this study will help us to know the right amount of protein in a bedtime snack for other children with diabetes.

The individual participant's results will be discussed with them and a summary hand out will be provided.

A 25 $ worth gift card will be provided to participants upon completion of study procedures.

Alternatives to Study Participation

Participation is completely voluntary. If patients choose not to participate, they can continue their diabetes care as instructed by their endocrinologist. The only alternative to participating is not to participate.

Financial Information

There is no cost to them for participation in the study except phone calls when we communicate as detailed above. If there is cost to parking (depending on the clinic location they use), and if returning to the clinic is needed to turn in study documents, the cost of parking will be covered by the study. A complementary gift card (25$) will be provided to participants upon completion of study procedures.

Confidentiality

All patient information will be used for research purposes only. The consent form will be scanned to the electronic medical records and therefore, the medical team will be aware that they are in this study. Study data will be kept in a secure and locked location without any identifiable information.

Data and Safety Monitoring:

Primary investigator (PI) is responsible for monitoring for adverse events and documenting them on source documents. Parents/Guardians will be instructed to call the PI if at any time during the intervention they have any of the following:
- Glucose >300
- Ketones are positive
- Having symptoms: nausea/ vomiting/ abdominal pain/ headaches.

Based on the above mentioned factors, the PI may consider terminating the participation at a given night and provide a dose of rapid acting insulin based on the participant's prescribed insulin doses. The PI will document the date and time, the symptoms, the glucose level, the urinary ketones, the insulin dose given and its timing. The PI will later discuss adverse events with the faculty advisor. They will be categorized as study related vs. non-study related, and they will be graded (mild, moderate or severe).

Data Monitoring Plan:

If adverse events are moderate or severe (including moderate to large ketones) , the PI will contact faculty advisor immediately. Otherwise, discussion of other adverse events with the faculty advisor will happen within 1-2 days, and discussion of other data will happen on weekly basis. The PI will monitor all data for accuracy and adherence to protocol every month during active study conduct.

Summary of Participants' Rights:

The participation in this research study is voluntary. Refusing to participate will not alter their usual health care or involve any penalty or loss of benefits to which you are otherwise entitled. If they decide to join the study, they may withdraw at any time and for any reason without penalty or loss of benefits. If information generated from this study is published or presented, your identity will not be revealed. In the event new information becomes available that may affect the risks or benefits associated with this study or their willingness to participate in it, they will be notified so that you can decide whether or not to continue participating. If they experience physical injury or illness as a result of participating in this research study, medical care is available at University Hospitals Cleveland Medical Center (UHCMC) or elsewhere; however, UHCMC has no plans to provide free care or compensation for lost wages.

Statistical Analysis:

Data analysis:

Descriptive: Nominal variables will be described using frequencies and per cents. Continuous variables will be described using means and standard deviations for normally distributed variables and medians and ranges for non-normally distributed variables. Demographic variables will be describe overall (n=43). Additionally, weight (in kg) and mg/kg of protein will be described by nominal dose. At each nominal dose, weight and mg/kg protein separately (on the x axis) will be plotted against PPG (on the y axis) and examined visually. Further curve fitting measures may be used to determine the shape of the relationship between the two variables.

Additionally we will plot each patient's PPG at each nominal dose. We expect to provide plots for each individual separately and a composite ("spaghetti") plot for all patients together to assess consistency of results across individuals.

Inferential: Ideally we will construct an unadjusted mixed model to assess the strength of a linear relationship, if one appears to exist. *This model is capable of testing both the slopes and the differences in means between nominal doses*. The use of such models is data-dependent and can't be fully described at this point.

Finally, if the mixed model is appropriate we will construct a similar model adjusted for other demographic and clinical characteristics possibly associated with blood glucose levels. Again, the type of model is data-dependent and can't be fully described in advance.

Sample Size Considerations:

We based our sample size of 43 on the work of Paterson et Al [17] on which the design and analysis plan of our study was based. We expect the assumptions of this analysis to hold for our study.

"A sample size of 32 was determined to provide 80% power at the 5% significance level, to detect a potential mean difference in glucose of 2.0 mmol/L at 5 h between the standard test drink (control) and protein-added drinks, assuming a within-person standard deviation of differences in blood glucose levels of 3.1 mmol/L."

In the referenced study [17], 27/32 completed, so we increased the sample size to 43 to allow for non-completers. If all complete, our power will be greater than 80%. Based on these assumptions, we did not perform and independent sample size calculation.