NOVA

University of Newcastle Research Online

nova.newcastle.edu.au

Paterson, M. A.; Smart, C. E. M.; Lopez, P. E.; Howley, P.; McElduff, P.; Attia, J.; Morbey, C.; King, B. R. "Increasing the protein quantity in a meal results in dose-dependent effects on postprandial glucose levels in individuals with Type 1 diabetes mellitus", Published in *Diabetic Medicine* Vol. 34, Issue 6, p. 851-854. (2017)

Available from: <http://dx.doi.org/10.1111/dme.13347>

This is the peer reviewed version of the above article, which has been published in final form at <http://dx.doi.org/10.1111/dme.13347>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

Accessed from: <http://hdl.handle.net/1959.13/1352871>

Title: Increasing the protein quantity in a meal results in dose dependant effects on postprandial glucose levels in individuals with Type 1 Diabetes Mellitus.

Running Title:

‘Dose dependant effect of dietary protein in type 1 diabetes mellitus’

Authors and affiliations:

M.A. Paterson, RN CDE^{1,3}

C.E.M. Smart, RD, PhD^{1,3}

P.E. Lopez, MD^{1,3}

P. Howley, PhD²

P. McElduff, PhD²

J. Attia PhD¹

C. Morbey, MD MBChB, MRCP, FRACP⁴

B.R. King, MD, PhD^{1,3}

1. Hunter Medical Research Institute, School of Medicine and Public Health, University of Newcastle, Rankin Park, New South Wales, Australia.
2. Hunter Medical Research Institute, Clinical research Design and Statistical Support, University of Newcastle, Rankin Park, New South Wales, Australia.

3. Department of Paediatric Endocrinology and Diabetes, John Hunter Children's Hospital, Newcastle, New South Wales, Australia.
4. Faculty of Health, School of Medicine, University of Newcastle, New South Wales, Australia.
5. Aim Diabetes Management Centre, Newcastle, New South Wales, Australia.

Corresponding Author:

Bruce R. King

Department of Paediatric Endocrinology and Diabetes, John Hunter Children's Hospital. Lookout Rd, New Lambton Heights Australia. Locked Bag 1, HMRC, 2310.

Bruce.King@hnehealth.nsw.gov.au Ph: +612 49855634 Fax: +612 4921 3599

Manuscript word count:

Abstract: 240 words, Text: 1632 words.

Declarations:

There are no conflicts of interest relative to this article to declare.

Novelty statement:

- This research looks at the postprandial glycaemic impact of dietary protein when consumed with carbohydrate and minimal fat
- Results indicate that increasing amounts of protein result in dose dependant changes in glycaemia
- This impact has an inverse relationship in the early and late postprandial period

- This is the first study to demonstrate the effect of protein on postprandial glycaemic control is dose dependant and bi-phasic
- This study has significant implications for insulin dosing for meals containing protein.

Abstract:

Aim

To determine the glycaemic impact of increasing protein quantities when consumed with consistent amounts of carbohydrate in individuals with Type 1 diabetes using intensive insulin therapy.

Methods

Participants with Type 1 diabetes ages 10-40 years, HbA1c ≤ 64 mmol/mol [≤ 8 mmol/L], BMI $\leq 91^{\text{st}}$ percentile received a 30g carbohydrate (negligible fat) test drink daily over 5 days in randomised order. Protein (whey isolate 0g/kg carbohydrate, 0g/kg lipid) was added in amounts of 0g (control), 12.5, 25, 50 and 75g. A standardised dose of insulin was given for the carbohydrate. Postprandial glycaemia was assessed by 5 hours of continuous glucose monitoring.

Results

Data were collected from 27 participants (15 male). A dose response was found with increasing grams of protein. A significant negative relationship between protein dose and mean excursion was seen at 30 and 60min time-points ($p=0.007$ and $p=0.002$ respectively). No significant relationship was seen at the 90 and 120min time-points. Thereafter, the dose-response relationship inverted, such that there was a significant positive relationship for each of the 150-300min time-points respectively ($p<0.004$).

Mean glycaemic excursions were significantly greater for all protein-added test drinks from 150-300min ($p<0.005$) with the 75g protein load resulting in a mean excursion 5 mmol/l higher when compared with the control test drink ($p<0.001$).

Conclusions

Increasing protein quantity in a low-fat meal containing consistent amounts of carbohydrate decreases glucose excursions in early (0-60min) postprandial period and then increases in the later postprandial period in a dose dependant manner.

Introduction

Dietary protein has previously been shown to cause delayed and sustained postprandial glycaemic excursions and increased insulin requirements in individuals with Type 1 diabetes [1-4]. Postprandial hyperglycaemia is a common problem for individuals living with Type 1 diabetes, often despite intensive management and careful mealtime insulin calculation [5, 6] and has been identified as an independent risk factor for the development of long-term complications, including cardiovascular disease [7]. Previously, we examined the glycaemic effect of protein consumed without carbohydrate or fat in individuals with Type 1 diabetes and demonstrated that $\geq 75\text{g}$ protein alone resulted in significantly delayed and sustained postprandial glycaemic excursions between 3-5hrs when compared with a control drink (water) [3].

The objective of this study was to determine the postprandial blood glucose response to protein when consumed in a low-fat, carbohydrate test drink.

Research Design and methods

We conducted a within-subject, repeated measures, randomised trial involving the provision of test drinks of varying protein quantities, each with a fixed amount of carbohydrate (30g) and low fat ($<0.3\text{g}$). Participants aged 7-40 years, diagnosed with Type 1 diabetes for more than 1 year and using intensive insulin therapy (insulin pump or multiple daily injections) were recruited from the John Hunter Children's Hospital and the Hunter Diabetes Centre (Newcastle, NSW, Australia).

Inclusion criteria included HbA1c ≤ 64 mmol/mol ($\leq 8\%$), BMI $\leq 91^{\text{st}}$ percentile, with no complications of diabetes or any other major medical conditions present.

Ethics approval was obtained from Hunter New England Research Ethics committee. Written informed consent was obtained from all participants and caregivers. The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12614000471684).

Participants blood glucose levels and insulin doses were reviewed daily by a member of the research team for one week prior to the study, ensuring 24hour glycemic targets were met.

Test drinks were administered in randomised order over 5 consecutive days, 4 hours after a standardised evening meal. Randomisation was achieved using proc Plan in the SAS statistical software package (SAS institute Inc., Cary, NC, USA). Participants fasted (water only) for the following 5 hours, unless required to treat an episode of hypoglycaemia. If fasting glucose levels were >13 or <3.6 mmol/l prior to consuming a test meal, participants were instructed to treat as per usual management and the study day excluded and repeated. In the event of hypoglycaemia during the 5 hour study period the participant consumed 15g of carbohydrate and the analysis was stopped at that point.

Each test drink contained carbohydrate (30g) and fat (0.3g) with protein added in amounts ranging from 0-75g. The largest protein drink would be equivalent to consuming a 300g rump steak with 20 hot chips. Test meals were provided to participants in sealed bags containing pre-measured protein powder in amounts of 0g (0kcal-control), 12.5g (50kcal) 25g (100kcal), 50g (200kcal) and 75g (300kcal) (Isolyze 100% whey isolate protein powder [27g protein, 0g lipid, 0g carbohydrate per 30g] Species Nutrition, Westbury, NY, USA), with 17g of glucose powder (Glucose polymer carbohydrate powder [Carb Plus, 0g protein, 0g lipid, 9.3g glucose per 10g serve] Flavour Creations Pty Ltd, QLD, Australia). The powder was weighed using salter kitchen scales (model 323; Salter, Tonbridge, UK) (error $\pm$ 1g).

Participants individual insulin:carbohydrate ratios were used to determine insulin doses for the 30g carbohydrate in the test drinks and delivered either by subcutaneous injection or a normal bolus through an insulin pump 15 minutes prior to consumption. Pump infusion sites were changed second daily.

Blinded CGM (Dexcom G4 Platinum, Inc., San Diego, CA, USA) was used to measure interstitial glucose. The sensor was inserted on the afternoon prior to study commencement and worn for the study period. Participants were instructed to calibrate using two finger-stick blood glucose measurements as per manufacturers instruction. As the CGM was blinded, participants were also asked to continue with usual self-blood glucose monitoring and were provided with a diary to record these values. In order to ensure integrity of the data, CGM traces during the study periods were carefully reviewed by an experienced member of the research team. If there were any potential sensor inaccuracies or failures the data from that study period was excluded, a new sensor inserted and the study day repeated. Participants were contacted daily by a member of the research team to ensure adherence to the protocol.

Statistical analysis

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 2.0mmol/l at 5 hrs between the standard test drink (control) and protein-added drinks, assuming a within-person standard deviation of differences in blood glucose levels of 3.1mmol/l.

The primary outcome measure was the mean postprandial glucose excursions over 30 minute intervals from 0-300 min. The secondary outcome was the mean dose response with increasing grams of protein. Excursions were calculated as the difference in blood glucose between each postprandial time point and the blood glucose at time zero. Differences in mean glucose excursions over each 30-min interval and dose-response were tested using generalised linear mixed models to allow for the correlation of repeated measures on the same subject. Testing was conducted at the 5% significance level.

Results

Thirty-two participants were recruited. 27 participants (15 male) with a mean age 20.7 (± 10.3) years and a mean HbA1c of 54mmol/mol (± 3.1) / 7.1% (± 0.95) completed the protocol. All other clinical characteristics of participants are presented in table 1. Three participants withdrew and data from 2 subjects were excluded due to failure to complete the protocol. Incomplete days for hypoglycaemia, illness and lost sensor were excluded and repeated where possible. Two subjects experienced hypoglycaemia during the 5 hour study period with analysis stopped at 120 and 180 mins respectively. Clinical accuracy of the sensor was demonstrated, with the Mean Absolute Relative Difference (MARD) being 13%.

Postprandial glucose excursions

Mean postprandial glucose excursions (0-300mins) following the 5 test meals are presented in Figure 1. The postprandial glycaemic excursion for control (no added protein) peaked by 60min, returning to baseline by 120 min. Compared with control, 75g-added protein produced a significantly lower glycaemic excursion at 30 and 60min ($p=0.039$ and 0.046 ; 95%CI for difference (-1.52,-0.04) and (-2.56,-0.02). From 90min, the glycemic rise from all protein-added test drinks became higher than control. Significant differences for 12.5 and 75g protein became apparent by 120min, with mean excursions of 1.71 mmol/l (95% CI -0.380-3.04) and 1.28 mmol/l (95% CI -0.132-2.69) ($p<0.05$ for both). From 150 min all protein-added test meals resulted in significantly higher postprandial glycaemic excursions than control ($p<0.05$). This was sustained for the remainder of the study period, resulting in a mean maximal glycaemic excursion of 5mmol/l (95% CI 2.59-7.46) higher for the 75g protein-added drink at 300min ($p<0.001$).

Dose response

A dose-response was seen with increasing grams of added protein. At the 30 and 60min time-points, there was a significant negative relationship between protein dose and mean excursion ($p=0.007$ and $p=0.002$ respectively) with mean excursions decreasing by 0.1 and 0.18 mmol/l respectively per 10g increase in protein dose. Thereafter the dose-response relationship inverted, such that mean excursions increased, on average, by 0.27 up to 0.64mmol/l (for each of the 150 to 300 min time-points respectively) per 10g increase in protein.

Conclusions

This study has shown that in individuals using IIT, the addition of protein to a carbohydrate meal decreases the postprandial glucose from 30-60 minutes and then increases the postprandial glucose excursions between 150-300 minutes' post-meal in a dose dependant manner.

Previously we demonstrated that ≥ 75 -100g of protein consumed alone had a significant impact on postprandial glucose levels in the 2-5 hour period [3]. This study extends these findings, demonstrating that when protein is consumed with carbohydrate (low fat), a smaller amount has a significant impact on postprandial glycaemia. In this study, there was an initial reduction in postprandial glycaemic excursions during the 0-60 min postprandial period with the protein-added test drinks when compared no- added protein test drinks. As the amounts of protein increased, the glycaemic excursion beyond 2 hours also increased in a dose dependant manner.

A limitation of this study was that we did not examine the glycaemic effect beyond 5 hours. Data from this and previous published studies [1-3] demonstrate that the postprandial glycaemic impact of protein does continue beyond 5 hours and therefore a longer study period should be considered. Another limitation may be the fact that it is unlikely that protein and carbohydrate are consumed in isolation of fat. However, we conducted this study with the intention to better inform us of the glycaemic impact of protein when consumed in a low fat meal. It is also possible that the gastric emptying time of the test drink would be faster than if a solid meal of equal macronutrient values was consumed [8], however the glycaemic excursion seen in this study is similar to the shape of the curve of the glycaemic excursions seen in previous research using similar amounts of protein and carbohydrate [1]. We chose to use a liquid test meal for this study to allow us to precisely titrate the amounts of protein given.

Whilst carbohydrate is the predominant macronutrient to impact on postprandial blood glucose, there is now a growing body of evidence that protein can also significantly impact postprandial glucose levels. It is thought that this occurs via the stimulation of glucagon secretion [9] and, the conversion of amino acids to glucose via gluconeogenic pathways[10, 11]. Consistent with these mechanisms, the blood glucose rise seen from the protein-added drinks in this study was delayed and slower than the rise seen with the no-added protein carbohydrate test drink. This study suggests that insulin dosing for the protein component of a meal should be considered. Further research is required to determine how to safely and accurately calculate these additional doses and importantly, the optimal method of insulin delivery.

Acknowledgments

The authors would like to thank the Paediatric diabetes team at the John Hunter Children's Hospital for assistance with subject recruitment. Parts of this study were presented at the 9th International Conference on Advanced Technologies and Treatments in Diabetes, Milan, Italy, 3-6th February 2016.

Author-Contributions

C.S. and B.K. conceived and designed the study, analysed data and contributed to writing the manuscript; M.P. conducted the study, recruited subjects, collected the data and wrote the manuscript; P.L. and C.M. recruited subjects, collected data and contributed to discussions; P.M. and P.H. analysed the data and contributed to writing the manuscript; J.A. contributed to discussions and reviewed and edited the manuscript. BK. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

1. Smart C, E.M., O'Connell S, McElduff P, Lopez P, Jones T, Davis E, King B. , *Both Dietary Protein and Fat Increase Postprandial Glucose Excursions in Children With Type 1 Diabetes, and the Effect Is Additive*. Diabetes Care, 2013. **36**(12): p. 3897.
2. Peters, A.L. and M.B. Davidson, *Protein and fat effects on glucose responses and insulin requirements in subjects with insulin-dependent diabetes mellitus*. The American Journal of Clinical Nutrition, 1993. **58**(4): p. 555-60.
3. A., P.M., et al., *Influence of dietary protein on postprandial blood glucose levels in individuals with Type 1 diabetes mellitus using intensive insulin therapy*. Diabetic Medicine, 2016. **33**(5): p. 592-598.
4. Neu, A., et al., *Higher glucose concentrations following protein- and fat-rich meals – the Tuebingen Grill Study: a pilot study in adolescents with type 1 diabetes*. Pediatric Diabetes, 2014: p. n/a-n/a.
5. Boland, E., et al., *Limitations of Conventional Methods of Self-Monitoring of Blood Glucose: Lessons learned from 3 days of continuous glucose sensing in pediatric patients with type 1 diabetes*. Diabetes Care, 2001. **24**(11): p. 1858-1862.
6. Choleau, C., et al., *High day-to-day glucose variability: a frequent phenomenon in children and adolescents with type 1 diabetes attending summer camp*. Diabetes Metab, 2008. **34**(1): p. 46-51.
7. Nathan, D.M., et al., *Risk factors for Cardiovascular Disease in Type 1 Diabetes*. Diabetes, 2016.
8. Hellmig S, Gadow C, Katsoulis S, Hedderich J, Folsch UR et al. *Gastric emptying time of fluids and solids in healthy subjects determined by ¹³C breath tests: Influence of age, sex and body mass index*. The Journal of Gastroenterology and Hepatology, 2006. **21**: p. 1832-1838.
9. Brown, R.J., N. Sinaii, and K.I. Rother, *Too Much Glucagon, Too Little Insulin: Time course of pancreatic islet dysfunction in new-onset type 1 diabetes*. Diabetes Care, 2008. **31**(7): p. 1403-1404.
10. Linn, T., et al., *Effect of dietary protein intake on insulin secretion and glucose metabolism in insulin-dependent diabetes mellitus*. J Clin Endocrinol Metab, 1996. **81**(11): p. 3938-43.
11. Felig, P., et al., *Amino acid and protein metabolism in diabetes mellitus*. Arch Intern Med, 1977. **137**(4): p. 507-13.