# Beating Prediabetes Study Protocol

1. **Background**

Background: People with prediabetes, an intermediate state of hyperglycaemia1, are at high-risk of developing type 2 diabetes mellitus (T2DM)2. In New Zealand there are over 300,000 people with T2DM3 and currently the estimated annual conversion rate of prediabetes to T2DM is 5-10%2,4. Prediabetes has been recognised as being the key period during which interventions may lead to preventing development of T2DM1,5,6.

Polyphenol-rich foods have been investigated for their ability to modulate blood glucose levels7-14 and reduce postprandial glucose responses in both healthy subjects and in those with T2DM. Our research group has focused on using foods with bioactives containing polyphenols and nitrates (e.g beetroot (BR); antioxidant-rich foods and food extracts), to improve modifiable risk factors such as blood pressure15,16 and postprandial blood glucose response in healthy subjects7, 17,18  and in people with prediabetes19.

BR and blackcurrant (Bk) are foods both rich in polyphenols and other bioactives, but their effectiveness in improving glycaemic control in the prediabetes state has not yet been investigated. BR is high in both dietary nitrate and phytochemicals (e.g. betalains, polyphenols)20,21. Nitrate is the precursor to the bioactive nitric oxide (NO), which plays a pivotal role in controlling vascular tone and blood pressure22 and also mediates glucose uptake from the intestines and skeletal muscle23,24. Bk is high in polyphenols including anthocyanins which are proposed to modify postprandial glycaemia25. We suggest BR and Bk have the potential to be natural, dietary interventions that could have a significant impact on glycaemic control in people with prediabetes. Furthermore, chronic supplementation with dietary nitrate has been shown to improve insulin sensitivity and glucose control in a rat model, however we are unaware of any robust studies being conducted in a chronic setting in humans.

This study looks to establish the efficacy of chronic supplementation with BR and Bk on measures on metabolic syndrome and glucose metabolism such as glycated haemoglobin A1c (HbA1c), fasting blood sugar and lipid profiles in people with prediabetes.

1. **Hypothesis**

12 weeks of supplementation of blackcurrant anthocyanins or beetroot compounds, or both, will improve HbA1c and measures of metabolic syndrome compared to placebo.

1. **Experimental design**

The study will be a double-blind, randomized, placebo-controlled, parallel study with four testing arms (placebo, blackcurrant juice, beetroot juice, beetroot and blackcurrant mix). Participants will receive one of four drinks to consume daily for 12 consecutive weeks with study visits taking place pre- and post-intervention. The participants will be randomized by age and gender by an independent randomization officer, and placebo drinks will be matched in colour to the beetroot/blackcurrant drinks. Drinks bottles will be labelled A, B, C or D (coding to be determined by randomization officer).

Measures will be taken for fasting blood glucose, HbA1c, lipid profile, blood pressure and body composition pre and post intervention. Participants will measure their own fasting blood glucose from home and will complete a compliance diary once per week. A feasibility questionnaire will also be completed during study visit 2, at the end of the 12-week intervention. Food frequency questionnaires will be assessed to ensure participants have not significantly changed their dietary intakes from baseline to 12 weeks.

**Study Drinks**

Participants will be given study drinks at their first visit.

The beetroot juice drink will contain 600mg of nitrate per day, using ~67g of concentrate. The participants will receive the concentrate diluted to 100ml servings to ease the burden of diluting the drink themselves.

The blackcurrant drink will contain 300mg anthocyanins per day, using ~9g of concentrate. The participants will receive the concentrate diluted to 20ml servings to ease the burden of diluting the drinks themselves.

The blackcurrant and beetroot mix will contain both 600mg of nitrate, and 300mg of anthocyanins, using ~67g of beetroot concentrate and ~9g of blackcurrant concentrate. The participants will receive the concentrate mixed and diluted to 100ml servings to ease the burden of diluting the drinks themselves.

The placebo will be made up using a red/purple food colorant and sweetener. It will contain negligible nitrate and anthocyanins.

The drinks are not isocaloric. The placebo will contain sweetener rather than be sugar-matched as the cohort is prediabetic and it may not be advised to add sucrose to their regular diet if avoidable.

**Whole Study at a Glance**

Screening Visit/ Study Visit 1

Study Visit 2

Measurements:

* HbA1c
* Fasting Glucose
* Lipid Profile
* Blood Pressure
* FFQ
* Body Composition

Interval (12 weeks)

Daily consumption of intervention beverage at breakfast (300mg anthocyanin BC, 600mg nitrate BR, mix, placebo)

Measurements:

* Weekly fasting glucose from home
* Weekly compliance diaries

Measurements:

* HbA1c
* Fasting Glucose
* Lipid Profile
* Blood Pressure
* FFQ
* Body Composition
* Feasibility Questionnaire

**Screening visit (Visit 1)**

During the screening and first study visit, we will assess whether the participant meets the study inclusion criteria. Participants will arrive at the Massey University Human Nutrition Research Unit ONE morning to have a screening fasted blood sample collected. The session should take approximately one hour. Measures will be taken for fasting blood sugar, lipid profile and HbA1c levels through a finger prick blood sample. The participant will be invited to participate in the study visits if screening indicates they are eligible for the study.

In addition, body mass index (BMI), waist and hip circumference, and body composition (using a bioelectrical impedance scale) will be measured. Blood pressure and heart rate will also be measured. The participant will also be asked about relevant medical history, and any medication use. Finally, the participant will complete a food frequency questionnaire online which will provide us information on eating habits over the previous 30 days.

If eligible to participate in the study, the participant will be issued with a handheld glucometer and all materials required to test their fasting blood glucose at weekly intervals for 12 weeks. A trained researcher will also show them how to do this safely at home. If the participant already owns and uses a handheld glucometer, they may use their own device.

**Study Interval (12 weeks)**

Between study visits 1 and 2 there will be a 12-week interval during which time the participant will be asked to consume a beverage daily, before breakfast. This beverage will be either placebo, blackcurrant, beetroot or a blackcurrant/beetroot mix. During this time, they will be asked to complete a weekly fasting glucose measurement using the handheld glucometer issued during first visit (or their own). The participant will be asked to complete a compliance diary each week including questions about how they are feeling and how they are finding the intervention drink. The participants will also be asked to return any unconsumed intervention products to corroborate the compliance diaries. This should take no longer than 5-10 minutes to complete.

**Study Visit 2**

Study visit 2 will be identical to study visit 1. The participant will be required to come to the lab fasted (10 hour fast) and the same measurements that were taken at visit 1, will be taken by the researcher, to determine if the intervention has influenced any of the measures stated above. The participant will also complete a feasibility questionnaire to assess the long-term viability of the intervention drink.

1. **Methods and procedures**
	1. **Sample size**

The study will recruit 40 people into each group (160 participants total, both male and female). As there is no robust previous literature using chronic supplementation of either BR or Bk in prediabetes, the study is a pilot study, and the sample size has been chosen based on our current knowledge of the expected changes in primary outcome measures over the 84-day intervention period. We anticipate that 40 participants per group will be sufficient to see changes in HbA1c and other outcome measures over this time period.

* 1. **Inclusion criteria**
* 30-65 years of age
* BMI 18.5 - 40.0 kg/ m2
* HbA1c 41-49 mmol/ mol
* Fasting glucose > 5.6 mmol/ L
* Not taking any medications that include blood glucose/sugar lowering prescriptions
* Not pregnant or breastfeeding
* Not allergic to beetroot or blackcurrant
* Non-smoker
* Able to communicate well in English
	1. **Exclusion criteria**
* Use insulin
* Chronic kidney disease
* Significant weight loss in previous 12 months
* Regularly participating in >5hrs exercise weekly
* On strict dietary restrictions
* Other medical factors that may affect study
	1. **Blood sampling**

Blood samples will be collected using finger-prick blood sampling methods. A trained researcher will take blood samples and collect samples into HbA1c and lipid cartridges for analysis on a Cobas101+ machine. Fasting glucose will be measured via the same finger-prick sample using glucometer test strips on a Caresens® glucometer. Samples will be immediately discarded in biohazard waste bags using standard aseptic procedures.

* 1. **Other Methods**

Body composition, waist and hip circumference and blood pressure will be measured according to Massey University’s Standard Operating Procedures for these measures. These measures will be carried out by a trained researcher.

* 1. **Statistical Analysis**

Paired t-test analyses will be used to compare HbA1c, lipids, body composition and blood pressure before and after the twelve-week intervention. Fasting glucose samples will be compared using a one-way ANOVA with a repeated measures design. A p-value <0.05 will be considered statistically significant.

**5 References**

1. Initiating interventions in people with intermediate hyperglycaemia (“pre-diabetes”)(2012) *NZ Best Practice Journal* , Issue 48. <http://www.bpac.org.nz/BPJ/2012/november/hyperglycaemia.aspxI> accessed 13/9/2016
2. Tabák, A.G., Herder, C., Rathmann, W., Brunner, E.J., & Kivimaki, M. (2012) Prediabetes: a high-risk state for diabetes development. *The Lancet*, 379(9833):2279–90.
3. Ministry of Health. 2014. Annual Update of Key Results 2013/14: New Zealand Health Survey. Wellington: Ministry of Health.
4. American Diabetes Association (2013) Diagnosis and Classification of Diabetes Mellitus; *Diabetes Care,* 36(1): S67-S74
5. Bansal, N. (2015). Prediabetes diagnosis and treatment: A review. *World Journal of Diabetes*, 6(2), 296-303
6. Gillies, C.L., Abrams, K.R., Lambert, P.C., Cooper, N.J., Sutton, A.J., Hsu, R.T. & Khunti, K. (2007) Pharmacological and lifestyle interventions to prevent or delay type 2 diabetes in people with impaired glucose tolerance: systematic review and meta-analysis. *British Medical Journal*, 334(7588):299
7. Chepulis, L., Al-Aubaidy, H., **Page, R.** (2016) Effect of selected antioxidant food extracts on postprandial glucose responses in healthy individuals. *Functional Foods in Health and Disease,* 6(8): 493-505
8. Deng, R. (2012). A review of the hypoglycemic effects of five commonly used herbal food supplements. *Recent patents on food, nutrition & agriculture*, *4*(1), 50.
9. Bernardo, M. A., Silva, M. L., Santos, E., Moncada, M. M., Brito, J., Proença, L., Singh, J. & de Mesquita, M. F. (2015). Effect of Cinnamon Tea on Postprandial Glucose Concentration. *Journal of Diabetes Research*, 2015.
10. Williamson, G. (2013). Possible effects of dietary polyphenols on sugar absorption and digestion. *Mol. Nutr. Food. Res*. 57: 48-57
11. Zheng, X. X., Xu, Y. L., Li, S. H., Hui, R., Wu, Y. J., & Huang, X. H. (2013). Effects of green tea catechins with or without caffeine on glycemic control in adults: a meta-analysis of randomized controlled trials. *The American journal of clinical nutrition*, *97*(4), 750-762.
12. Pandey, K.B. & Rizi, S.I. (2009), Polyphenols as dietary antioxidants in human health and disease, *Ox Med and Cellular Longevity*, 2, 270-278
13. Nasri, H., Shirzad, H., Baradaran, A. & Rafieian-kopaei, M. (2015) Antioxidant plants and diabetes mellitus, *J Res Med Sc*, 20(5), 491-502
14. Törrönen, R., Sarkkinen, E., Niskanen, T., Tapola, N., Kilpi, K. & Niskanen L. (2012) Postprandial glucose, insulin and glucagon-like peptide 1 responses to sucrose ingested with berries in healthy subjects. *British Journal of Nutrition.*107(10):1445-1451.
15. Stanaway, L., **Rutherfurd-Markwick, K.J.**, **Page, R. A.,** Wong, M., Jirangrat, W., The, K.H. & Ali, A. (2019). Acute Supplementation with Nitrate-rich Beetroot Juice Causes a Greater Increase in Plasma Nitrite and Reduction in Blood Pressure of Older Compared to Younger Adults. *Nutrients.* 11(7), 1683; <https://doi.org/10.3390/nu11071683>
16. Stanaway, L., **Rutherfurd-Markwick, K.J.**, **Page, R. A.,** Wong, M., Jirangrat, W., The, K.H. & Ali, A. (2019). Does Acute Supplementation with Nitrate-Rich Beetroot Juice Benefit Older Adults More than Younger Adults? *Nutrients, Proceedings* 2019, 8(1): 26; <https://doi.org/10.3390/proceedings2019008026>
17. Lim, W.X.J., Chepulis, L., von Hurst P.R., Gammon, C.S. & **Page, R.** (2019) An acute, placebo-controlled, crossover study to assess the effects of New Zealand pine bark extract on glycaemic responses in healthy participants. *Nutrients 2019 – Nutritional Advances in the Prevention and Management of Chronic Disease, Barcelona, September 25-27 2019*
18. Lim, W.X.J., Chepulis, L., von Hurst P.R., Gammon, C.S. & **Page, R.** (2020) An acute, placebo-controlled, single-blind, crossover, dose-response, exploratory study to assess the effects of New Zealand pine bark extract (Exogenol®) on glycaemic responses in healthy participants. *Nutrients.* 2020, 497; doi:10.3390/nu12020497
19. Lim, W.X.J., Chepulis, L., von Hurst P.R., Gammon, C.S. & **Page, R.** (2019) Investigating the hypoglycaemic potential of antioxidant-rich plant extracts (GLARE study). Poster presented at the 13th Federation of European Nutrition Societies (FENS) European Nutrition Conference- Malnutrition in an Obese World: European Perspectives 15 – 18 October 2019, Dublin, Ireland. Reference number 533
20. Wootton-Beard, P.C., Brandt, K., Fell, D., Warner, S., & Ryan, L. (2014) Effects of a beetroot juice with high neobetanin content on the early-phase insulin response in healthy volunteers. 2014;3.
21. Nemzer, B., Pietrzkowski, Z., Spórna, A., Stalica, P., Thresher, W., Michałowski, T. & Wybraniec, S. (2011) Betalainic and nutritional profiles of pigment-enriched red beet root (Beta vulgaris L.) dried extracts. *Food Chemistry.* 127(1):42-53.
22. Larsen, F.J., Ekblom, B., Sahlin, K., Lundberg, J.O.& Weitzberg, E. (2006) Effects of Dietary Nitrate on Blood Pressure in Healthy Volunteers. *New England Journal of Medicine.* 355(26):2792-3.
23. Gheibi, S., Jeddi, S., Carlström, M., Gholami, H., Ghasemi, A. (2018) Effects of long-term nitrate supplementation on carbohydrate metabolism, lipid profiles, oxidative stress, and inflammation in male obese type 2 diabetic rats. *Nitric Oxide*. 75:27-41.
24. Avogaro, A., Toffolo, G., Kiwanuka, E., De Kreutzenberg, S.V., Tessari. P. & Cobelli, C. (2003) L-Arginine-Nitric Oxide Kinetics in Normal and Type 2 Diabetic Subjects: A Stable-Labelled 15N Arginine Approach. *Diabetes* 52(3):795-802.
25. Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E. & Prior, R.L. (2006) Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *Journal of agricultural and food chemistry*. 54(11):4069-75.