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HYPOGLYCAEMIC POTENTIAL OF BEETROOT AND BLACKCURRANT JUICE

A thesis presented in partial fulfilment of

the requirements for the degree of

Doctor of Philosophy

in

Health Sciences

Massey University, Albany

Aotearoa New Zealand

Cameron Haswell

2025

MASSEY UNIVERSITY
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

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Student Name:	Cameron Haswell	ID Number:	[redacted]
Supervisor Name:	A/Prof Rachel Page	Date:	7-04-2025
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There were several factors that had an impact on Cameron's research and delayed completion and what could be done during his PhD. The first was COVID-19, the second was movement of laboratory spaces from the Oteha Rohe to the main Albany campus which took 1 month and then third was the time it took for the new lab space to be up and running with all facilities functioning (8 months delay)

The COVID-19 pandemic had considerable impact on Cameron's PhD causing major disruptions and delays and altering the content of studies, trial design and participant numbers. Beetroot juice has been the main intervention product under investigation and beetroot received for juicing during COVID lockdowns had to be stored for longer than usual and once juiced the product was unfortunately not safe for consumption and had to be discarded and an alternative source of juice found. The process of juicing and subsequent analysis of the beetroot juice took 6 weeks to complete and resulted in little output for the thesis.

The COVID-19 lockdowns in 2020 and 2021 [Lockdown 1: March 25th-May 13th 2020; Lockdown 2: August 12th – August 30th 2020 (Auckland Only); Lockdown 3: September 23rd – October 7th2020 (Auckland Only); Lockdown 4: August 17th – December 3rd 2021] and closing of the University during these times caused significant disruption to both participant recruitment and data collection (for Study 1 and 2 --Chapters 4 and 5). While the university was closed, participants were not allowed on campus for data collection and once the University was open again there were a number of restrictions in place limiting person-to-person contact. Hence limiting the number of participants that could be seen on any one day. Post lockdowns, recruitment also became more difficult with people reticent to commit to participation. Due to the lockdowns, the final chronic 12-week human study (Study 3 – Chapter 7) was redesigned to make it more resilient should further lockdowns occur. This resulted in major changes to the trial design and multiple changes to the ethics application as the NZ government changed from an Alert Level System to the COVID-19 Protection Framework (Traffic Light System) in December 2021
The University laboratories which Cameron used also moved location towards the end of his Study 2 period (move date August 31st 2022). The new laboratories and specific required pieces of equipment and induction processes were not installed or fully operational for varying lengths of time ranging from 2-6 months. This resulted in delays to ongoing studies. The most serious delay was of approximately 8 months to produce the drinks required for the final study (Study 3 - Chapter 7). This was due to delays in the installation and commissioning of the food grade hood which was out of operation from August 31st 2022 until 5th May 2023. This delay seriously impacted on the time available for completion of the final 12-week chronic human study, and a final date was put in place for recruitment, resulting in low participant numbers for this study. Due to these delays the focus of Study 3 became investigation of best design for this chronic study. We still had to put an end date for the last participant to be recruited to enable Cameron to be able to complete his PhD and not prolong it further.

Research Suspension – COVID-19: 1 January 2021 – 30 June 2021

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Cameron Haswell **Student**	*Associate Professor Rachel Page* **Supervisor**	**Deputy Head of Academic Unit (or nominee)** *A/Professor Wyatt Page*

Abstract

The global rise in type 2 diabetes mellitus (T2DM) and its precursor, prediabetes, have created an urgent need for accessible and effective interventions targeting early glycaemic and metabolic dysregulation. Metabolic syndrome (MetS), comprising central adiposity, hypertension, dyslipidaemia, and impaired glucose tolerance, significantly increases T2DM and cardiovascular disease (CVD) risk. Functional foods rich in dietary polyphenols and nitrates may offer a low-risk, scalable approach to improving glucose regulation and vascular health, yet data on their efficacy in at-risk populations remain limited.

This thesis investigated the acute and short-term metabolic effects of beetroot (BR), rich in dietary nitrate and polyphenols, and blackcurrant (BC), rich in anthocyanins, on glycaemic control as well as other MetS components including insulin dynamics, vascular resistance, and lipid profiles. A final aim was to evaluate the feasibility of a longer-term functional food intervention in individuals with prediabetes.

A narrative review established that BR and BC have mechanistically plausible effects on glucose and lipid metabolism via enhanced nitric oxide (NO) bioavailability, α-glucosidase inhibition, increased insulin sensitivity, and antioxidant activity. However, human trials often lack robust outcome measures or clearly defined bioactive content in interventions and frequently exclude at-risk groups such as those with prediabetes or MetS.

Due to observed methodological discrepancies between different intervention trials investigating acute glycaemic control, we aimed to determine optimal methodology for capturing intervention effects when targeting peripheral glucose uptake. A repeated-measures, crossover trial using an oral glucose tolerance test (OGTT) compared capillary, venous whole blood, and plasma glucose after acute beetroot juice intervention (BRI) ingestion and placebo (PL). Capillary sampling revealed significantly reduced 30 min postprandial glucose (BRI = 2.51 ± 1.29 mmol/L vs. PL = 3.40 ± 1.47 mmol/L; $p = 0.002$) and incremental area under the curve ($iAUC_{0-30}$: BRI = 52.2 mmol·min/L, PL = 66.5 ± 27.2 mmol·min/L; $p = 0.017$) in the BRI group vs PL. These effects were not detected in plasma or venous samples. The data suggested enhanced peripheral glucose uptake, likely via glucose transporter type 4 (GLUT4) translocation in skeletal muscle stimulated by NO, is best captured via capillary measures.

A randomised crossover trial in healthy adults assessed the acute effects of BRI (600 mg nitrate) and the blackcurrant juice intervention (BCI; 120 mg anthocyanins) on postprandial glucose and insulin responses. Compared with PL, BRI significantly reduced capillary glucose early-phase $iAUC_{0-30}$ (BRI = 52.24 mmol·min/L, PL = 66.50 mmol·min/L; p = 0.017) and $iAUC_{0-60}$ (BRI = 95.3

mmol·min/L, PL = 122.9 mmol·min/L; p = 0.010) and insulin $iAUC_{0-60}$ (BRI = 836 ± 480 vs. PL = 848 ± 446 µU·min/mL). In contrast, BCI did not significantly reduce blood glucose concentrations but showed a trend toward lower insulin iAUC (BCI = 772 ± 365 µU·min/mL), suggesting improved insulin sensitivity. These findings support complementary mechanisms: BRI enhances glucose uptake via NO-dependent vasodilation or increased GLUT4 translocation; BCI may modulate insulin signalling or incretin response.

To isolate the effects of betalains from nitrate in BR, a 6-day intervention compared two beetroot juice formulations with differing phytochemical profiles: UKBR (1280 mg nitrate, 294 mg betalains) and NZBR (1120 mg nitrate, 506 mg betalains). Plasma nitrate and nitrite concentration, systolic and diastolic blood pressure (SBP, DBP), systemic vascular resistance (SVR), fasting glucose and lipid profiles were assessed in a fasted state (overnight) after 6 days of supplementation and acutely at 2 h and 5 h following ingestion on day 7. Baseline plasma nitrate concentrations after 6 days were 267.3 µmol/L for UKBR, 163.5 µmol/L for NZBR, and 47.1 µmol/L for PL. Plasma nitrite increased significantly with UKBR (Δ +96 ± 94 nM; $p < 0.001$), but not with NZBR, suggesting differential NO bioavailability. Reductions in SBP were observed after 6 days in NZBR vs PL (-5.3 mmHg for NZBR vs. PL, p = 0.038) and at 2 h post-ingestion for both UKBR (-5.7 mmHg, p = 0.003) and NZBR (-5.6 mmHg, p = 0.029) compared with PL. The NZBR formulation elicited a greater reduction in systemic vascular resistance relative to baseline after 6 days' intake compared with PL (NZBR = –219 dyne·sec·cm^{-5}, PL = –25 dyne·sec·cm^{-5}; p = 0.024). Both PL and UKBR showed modest reductions in high density lipoprotein cholesterol (HDL-C) compared with baseline (PL -0.13 mmol/L, p = 0.047; UKBR -0.13 mmol/L, p = 0.011) whilst NZBR trended lower (NZBR, -0.13 mmol/L, p = 0.082). These results indicate that betalains may exert independent vascular effects beyond those attributable to nitrate.

A 12-week feasibility trial evaluated the practicality of long-term beetroot juice (BRJ) and blackcurrant juice (BCJ) supplementation in adults with prediabetes (n = 12 enrolled; n = 8 completed), as well as establish common challenges with recruitment and retention to longer-term nutritional intervention studies in individuals with prediabetes. Despite strong interest during recruitment (45 screened), only 12 participants were randomised primarily due to having a HbA1c result at screening outside the prediabetes range (41 – 49 mmol/mol). Reasons for participant attrition included time constraints and lack of immediate benefit from the intervention. Nevertheless, compliance among completers exceeded 90% and participant feedback favoured encapsulated or powdered formats, with gastrointestinal issues reported by 3 participants resulting from the intervention. Although statistical power was insufficient to

detect significant changes in fasting glucose, lipids, or blood pressure, metabolic outcomes varied among individuals and further investigation with larger sample sizes is recommended.

Collectively, these studies demonstrate that both BRJ and BCJ intake exert beneficial effects on early-phase postprandial glucose regulation and vascular function. Acute BRJ ingestion significantly reduces postprandial glycaemia in healthy adults, effects likely mediated by NO-induced improvements in skeletal muscle perfusion and glucose uptake. Acute BCJ intake may enhance insulin sensitivity, though higher anthocyanin doses may be required to achieve statistical significance. Importantly, the vascular effects of BRJ intake are not solely nitrate-dependent; betalains appear to play a previously understudied role in modulating SVR, potentially via redox-sensitive mechanisms affecting endothelial function.

These findings also highlight critical methodological considerations for future trials. Capillary blood glucose sampling provides enhanced sensitivity for detecting peripheral glucose clearance, a key parameter in early insulin resistance. Bioactive content should be quantified and standardised, and intervention formats adapted to population preferences to improve long-term compliance. Recruitment of at-risk individuals requires culturally appropriate strategies and simplified intervention protocols.

In conclusion, this thesis supports the inclusion of nitrate-, anthocyanin-, and betalain-rich functional foods as part of early dietary strategies for reducing T2DM risk. Future work should prioritise longer-term trials in prediabetic cohorts, with mechanistic endpoints to clarify bioactive action, and optimised delivery systems to enhance adherence. These data contribute to the growing evidence base supporting functional foods as viable tools in metabolic disease prevention.

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Abbreviations

ADA	American Diabetes Association
AHA	American Heart Association
AHEI	Alternative Healthy Eating Index
ALE	Advanced Lipoxidation End Products
AMPK	AMP-activated protein kinase
ARIC	Atherosclerosis Risk in Communities
BC	Blackcurrant
BCJ	Blackcurrant Juice (literature)
BCI*	Blackcurrant Juice Intervention (this thesis)
BCE	Blackcurrant Extract
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
BOP	Beetroot and Blackcurrant on Postprandial Glucose
BP	Blood Pressure
BR	Beetroot
BRJ	Beetroot Juice (literature)
BRI*	Beetroot Juice Intervention (this thesis)
CHD	Coronary Heart Disease
CHO	Carbohydrate
CRP	C-reactive protein
CVD	Cardiovascular Disease
DASH	Dietary Approaches to Stop Hypertension
DPP	Diabetes Prevention Program
DPP4	Dipeptidyl Peptidase-4
DPS	Diabetes Prevention Study
EASD	European Association for the Study of Diabetes
EDTA	Ethylenediaminetetraacetic Acid
EGIR	European Group for the Study of Insulin Resistance
FAD	Flavin Adenine Dinucleotide
FAM	Familiarisation
FFA	Free Fatty Acids
FFQ	Food Frequency Questionnaire

FPG	Fasting Plasma Glucose
GIP	Gastric Inhibitory Polypeptide
GLP-1	Glucagon-like Peptide-1
GLUT4	Glucose Transporter Type 4
H_2O_2	Hydrogen Peroxide
HDEC	Health and Disability Ethics Committee
HDL-C	High-density Lipoprotein Cholesterol
HOMA2	Homoeostasis Model Assessment 2
HPLC	High Performance Liquid Chromatography
IDF	International Diabetes Federation
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IL-10	Interleukin-10
IL-6	Interleukin-6
LDL-C	Low-density Lipoprotein Cholesterol
LDLR	LDL Receptor
MIX*	Beetroot and Blackcurrant Juice Mix (this thesis)
MUFA	Monounsaturated Fatty Acids
NCEP	National Cholesterol Education Program
NDBR	Nitrate-depleted Beetroot Juice
NO	Nitric Oxide
NRF2	Nuclear Factor Erythroid 2-related Factor 2
NZBR*	New Zealand Beetroot Juice (Chapter 6 intervention)
NZMA	New Zealand Medical Association
NZSSD	New Zealand Society for the Study of Diabetes
OGTT	Oral Glucose Tolerance Test
PAD	Peripheral Artery Disease
PDX-1	Pancreatic Duodenal Homeobox-1
PI3K	Phosphatidylinositol 3-kinase
PL	Placebo Intervention (this thesis)
POC	Point-of-care
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RR	Risk Ratio

SBP	Systolic Blood Pressure
SGLT-1	Sodium-Glucose Transport Protein 1
SGLT-2	Sodium-Glucose Co-transporter 2
SV	Stroke Volume
SVR	Systemic Vascular Resistance
T2DM	Type 2 Diabetes Mellitus
TC	Total Cholesterol
TG	Triglycerides
UKBR*	United Kingdom Beetroot Juice (Chapter 6 intervention)
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organisation

Explanatory Note

* In this thesis, BCI, BRI, MIX, and PL are reserved exclusively for intervention groups. BC and BR always refer to the whole foods, while BCJ and BRJ are used for juices in the literature. BCE and NDBR appear only in literature contexts. UKBR and NZBR denote specific beetroot juice formulations tested in the Chapter 6 intervention study.

Chapter 1 – Introduction

This chapter introduces the PhD study and its relevance to the field of research. The chapter outlines 5 specific research questions and concludes with a discussion of the structure of the thesis and contributions of each researcher involved in this work.

1.1 Background

1.1.1 Prevalence and Burden of Diabetes

Modern-day lifestyle factors, such as obesity and long periods of sedentary behaviour, are significant contributors to the development of insulin resistance, prediabetes and type 2 diabetes mellitus (T2DM). Global prevalence of T2DM has risen from 108 million in 1980 to 422 million in 2014, a 9% yearly rise, with current projections estimating that 592 million people will have T2DM worldwide by 2035 (1, 2). In Aotearoa New Zealand (NZ), T2DM rates have also increased in recent years, rising by 7% per year over the past 8 years (3), with 292,400 people, or 4.15% of the population, estimated to be living with T2DM as of 2021 (4), and expected to increase to 400,000 people by 2040 (5).

Of great concern is that the largest relative increase in diabetes in NZ is in younger people aged 25-44 years (6), with 16% of under 45-year olds having prediabetes (7). Early onset of T2DM is associated with increased mortality, with data indicating that a 10 year earlier onset of T2DM results in 1.6 times increased death rate from cardiovascular disease (CVD) (7). Prediabetes is also becoming increasingly common in NZ adolescents and children, with 12% of NZ adolescents classified as obese (BMI ≥ 30 kg/m^2) (8), of which over half (53%) are living with prediabetes. T2DM rates are increasing by 5% per year among children aged <15 years (9), with 16% of 8-11 year olds now living with prediabetes in NZ (10).

The prevalence of diabetes has also increased in older age groups, with approximately 15-20% of people aged over 65 years having T2DM, and diabetes is more prevalent in men (8.3%) than women (5.8%) (4, 11). Furthermore, the greatest area of growth in incidence of T2DM between now and 2040 is expected in the 60-79 years age group, with the prevalence predicted to increase from 12.9% to 15.6-16.4% (5). In addition, the absolute number of people in this age group with T2DM will increase due to Aotearoa New Zealand's aging population (12).

The incidence of both prediabetes and T2DM in NZ differs with ethnicity, being higher in Māori (30.4%, prediabetes; 4.6% T2DM) and Pacific peoples (29.8% prediabetes; 9.6% T2DM) than NZ Europeans (24.6%, prediabetes; 4.2% T2DM) (5, 11). In Auckland, over 40% of the Māori, Pacific and Indian populations aged between 35-39 years have been diagnosed with prediabetes, and this rises to over 50% in 45-49-year olds (6). New Zealand also has a growing Asian population, and at the 2018 census, 28% of Aucklanders identified with Asian ethnicity, up from 23% in 2013 (13). Therefore, it is of concern that age-standardised T2DM rates are similar in Asian populations

to Māori and Pacific peoples (Asian: 8%, Māori: 8%, Pacific: 10%, NZ European: 3%), with South Asians having higher incidence rates than other Asian populations (South Asian: 14%, all Asian: 8%) (14). This is also reflected in the paediatric population (aged 8-11 years), with South Asian (30%), Pacific peoples (27%), and Māori (18%) having higher rates of T2DM than NZ Europeans (6%) (10).

Risk factors for the development of T2DM include central adiposity, hypertension, dyslipidaemia and smoking (15), the first three of which are all components of metabolic syndrome (MetS) (16); a cluster of metabolic risk factors including increased waist circumference, hypertension, hyperglycaemia, dyslipidaemia and lowered HDL-C (16) associated with CVD and T2DM. The presence of MetS is associated with a relative risk of diabetes incidence of 3.5-5.2 (17), highlighting that a change in MetS status also leads to a change in T2DM risk. Furthermore, the comorbidities and disorders associated with hyperglycaemia can be compounded by the presence of hypertension and dyslipidaemia in T2DM. The macrovascular and microvascular complications associated with T2DM and metabolic syndrome can be debilitating, and include neuropathy (loss of nerve sensations leading to increased amputation risk), retinopathy (loss of vision), nephropathy (kidney disorder) and cardiovascular disease (CVD), which in turn leads to strokes, heart attacks and cardiac arrest (18). In addition, people with T2DM suffer from an increased prevalence of anxiety and depression (19), imposing a significant burden on both patients and whānau (family).

As well as the societal burden that comorbidities and mental health issues present, diabetes-related healthcare incurs a significant economic burden for NZ, as healthcare costs increased from NZD $247 million (USD $153.8 million) in 2001, to NZD $600 million (USD $374 million) in 2008 (20) and is currently NZD $2.1 billion (USD $1.3billion) per annum (5), representing 0.67% of GDP in New Zealand. Current estimates project an increase in economic cost to NZD $3.5 billion (USD $2.18 billion) by 2040, in part due to the increased cost associated with earlier diagnosis, and Aotearoa New Zealand's trend of increased diagnosis at earlier ages (5).

1.1.2 Type 2 Diabetes Mellitus and Metabolic Syndrome

Type 2 diabetes mellitus pathophysiology is characterised by a state of hyperglycaemia where blood glucose levels are chronically raised beyond normal parameters due to a combination of insulin deficiency, poor insulin sensitivity and pancreatic β-cell dysfunction (21). Chronic hyperglycaemia causes the production of advanced glycation end products (AGEs); formed when glucose molecules react with proteins and lipids in glycoxidation reactions in the blood vessel

walls (22, 23). These AGEs cause the cross-linking of proteins and stiffening of blood vessels, increasing susceptibility to narrowing and damage (22), the consequence of which is accelerated progression to microvascular and macrovascular complications such as atherosclerosis, nephropathy and retinopathy (22-24). Chronic hyperglycaemia also leads to the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (25, 26), which can cause oxidative and nitrosative stress and inflammation, further exacerbating blood vessel dysfunction and nerve damage (27). Macrovascular complications which stem from this damage include CVD (28), stroke (29) and peripheral artery disease (PAD) (30), whilst microvascular outcomes include retinopathy (31), nephropathy (32) and neuropathy (33).

As previously mentioned, MetS and T2DM are strongly related, and the presence of MetS is associated with a fivefold increased risk for development of T2DM (17, 34). MetS in childhood more than triples the likelihood of developing T2DM in adulthood compared with those who do not develop MetS during childhood (35). This is because many of the pathophysiological mechanisms which are involved in the development of MetS also contribute to increased insulin resistance through the direct impairment of insulin signalling pathways as well as chronic low-grade inflammation and oxidative stress through lipotoxicity (36, 37). Hyperglycaemia and insulin resistance are key components of MetS, with hyperglycaemia leading to the pancreatic beta cell failure seen in people living with T2DM (37).

Whilst elevated blood glucose levels increase AGE production, increased fatty acid levels cause impaired insulin secretion, inhibition of insulin gene expression and apoptosis of beta cells in the pancreas (38-40). Furthermore, lipid peroxidation reactions decrease vascular endothelial growth factor (VEGF) expression which inhibits angiogenesis and vascular control (41), leading to increased blood pressure. These lipid peroxidation reactions, increase in the presence of dyslipidaemia, produce advanced lipoxidation end products (ALE) which act similarly to AGE. The presence of both ALE and AGE significantly contributes to T2DM pathophysiology (42, 43).

1.1.3 Prediabetes and Prevention of Type 2 Diabetes Mellitus

Prediabetes is a state of high-risk for T2DM development, characterised by either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT), or a combination of both. The latter condition, IGT, is caused by muscle insulin resistance and mild hepatic insulin resistance, which leads to reduced glucose transporter-4 (GLUT-4) translocation and uptake of glucose into skeletal muscle. In contrast, IFG is caused by increased hepatic insulin resistance and more normal skeletal muscle insulin sensitivity (44). Both IGT and IFG result in hyperglycaemia following carbohydrate ingestion, however their definitions differ slightly (Table 1.1).

Table 1.1 Definitions for impaired fasting glucose vs impaired glucose tolerance (45)

Diagnostic Test	Impaired Fasting Glucose	Impaired Glucose Tolerance
2-hr 75 g oral glucose tolerance test value (mmol/L)	N/A	7.8 – 11.0
Fasting plasma glucose value (mmol/L)	6.1 – 6.9	N/A

As impairments in glucose regulation become more severe, a person progresses towards T2DM. Both IGT and IFG are independent risk factors for T2DM development, and those with both IGT and IFG are at particularly high risk (46). Moreover, IGT and IFG both result in increased glycosylated haemoglobin (HbA1c) levels, although HbA1c measurement does not distinguish between the two (IGT and IFG), which makes it a less sensitive measure than the oral glucose tolerance test (OGTT) (47). HbA1c levels are used for diagnosis of T2DM and predict diabetes-related CVD risk (48), with reductions in HbA1c scores indicating a reduction in development and progression of macrovascular and microvascular complications and risk (49).

Prediabetes is recognised as the key period during which interventions can provide great opportunity to slow or prevent the development of T2DM. Current guidelines (45) suggest more emphasis should be placed on prevention of diabetes rather than treatment and management, reflecting the fact that, without intervention, up to 70% of people with prediabetes will develop T2DM during their lifetime (1). Early, effective interventions may also delay or prevent the need for pharmacological management, including drugs such as metformin, which is the primary drug for treating T2DM and people with prediabetes, which has a range of side effects including diarrhoea, nausea/vomiting, abdominal pain and physical weakness.

Dietary intervention has been shown to reverse insulin resistance and T2DM (50). Several lifestyle-intervention studies such as the Finnish Diabetes Prevention Study (DPS) (51-53) and the Diabetes Prevention Program (DPP) (54, 55) have been shown to improve diabetes-related health outcomes by reducing both high blood pressure and elevated HbA1c levels in people with prediabetes. The DPP study involved six key features such as individual goal setting, workshops and designated “health coaches” to provide support throughout the intervention, which resulted in a 58% decrease in the incidence of T2DM at the 2.8-year follow-up (54, 55). The DPP study showed that early lifestyle modification in prediabetes can lead to a return to normoglycaemia and delays in the onset of T2DM, as well as a reduction in CVD risk at follow-up. Furthermore, a prospective cohort study including 820 people living with prediabetes, found that returning to normoglycaemia reduced the incidence rate of CVD events by 50% (56). Clearly, intervention

during prediabetes can be an effective method to reduce T2DM and CVD incidence, however, holistic lifestyle interventions are costly and intensive for providers and patients.

In recent years, a number of studies have investigated the impact of specific foods including those high in soluble fibre, such as oats (57), whole grains (58) and rice bran (59), those high in phytochemicals, including honey (60, 61), tea (62) and cinnamon (63), and those high in dietary nitrate, such as spinach (64) and beetroot (65) as potential interventions to normalise blood glucose levels and help delay the onset of T2DM. Single-food interventions may provide a 'magic bullet' effect, allowing a tangible change in health outcomes at a manageable intervention level for participants. The potential of foods such as beetroot and blackcurrant to improve glucose homeostasis due to their high anthocyanin (blackcurrants), betalains and nitrate (beetroot) levels will be investigated in this PhD project. This is of particular interest in New Zealand where cultivars of blackcurrants are particularly high in active ingredients (66, 67).

1.1.4 Potential Hypoglycaemic Effects of Beetroot and Blackcurrant Juice

Beetroot juice (BR) is naturally high in both dietary nitrate and phytochemicals (e.g. betalains, polyphenols), whilst blackcurrant juice (BC) is high in anthocyanins, a class of polyphenols, and hence these foods may present a unique opportunity to impact on glycaemic control via multiple mechanisms of action (68). Nitric oxide (NO), which can be derived from dietary nitrate through the action of L-arginine-dependent NO synthases, is a potent vasodilator and plays a pivotal role in controlling vascular tone and blood pressure (69). Nitric oxide also mediates glucose uptake from the intestines and skeletal muscle and could play an important role in regulation of blood glucose levels (70). Previous studies have shown that patients with T2DM generate less NO from L-arginine compared with healthy controls (71), due to the inhibition of NO synthases by AGEs (72). Current evidence suggests BR could improve early phase glucose responses (0-30 min post-OGTT) through inhibition of salivary enzymes, delayed uptake of glucose across the intestinal wall and increased GLUT-4 translocation (68), however the mechanisms are not yet clear. This may lead to a decrease in AGEs production and oxidative damage associated with hyperglycaemic episodes, potentially leading to improvement in glucose management over a chronic supplementation period, however this has yet to be established.

The first study to highlight the potential effects of BR juice supplementation on postprandial glucose in a human population was conducted by Wootton-Beard and colleagues (2014) and used healthy adults consuming 225 mL BR containing 990 mg of nitrate vs a sugar-matched placebo, and saw a decrease in early-phase insulin response and glucose peak during an OGTT

(65). Since then, other studies have corroborated these results in healthy populations in an acute setting, showing reductions in blood glucose following carbohydrate ingestion after consumption with 250 mL BR (300 g carbohydrate plus juice; nitrate not stated) (73) and 270 mL BR (50 g carbohydrate from white bread plus juice, nitrate not stated) (74). More recently, chronic studies have investigated the effect of BR on glucose metabolism in people living with T2DM. Aliahmadi and colleagues (75) showed that daily consumption of 100 g raw red beetroot for 8 weeks resulted in a decrease in fasting blood glucose (-0.75 mmol/L) and HbA1c (-3.7 mmol/mol) compared with baseline. In contrast, another study involving people living with T2DM consuming 24 mL concentrated BR juice (180 mg nitrate) for 12 weeks saw no significant changes in glycaemic parameters (76). To date, no chronic studies have been published relating to people living with prediabetes using BR as an intervention for improving glucose control.

Studies to date suggest that BC juice consumption decreases the postprandial glucose peak during an OGTT and this may take place through the inhibition of carbohydrate absorption during the early phase (0-30 min) as the proanthocyanidins in blackcurrant inhibit alpha amylase (77) and alpha-glucosidase (discussed in detail in Section 2.3 and Chapter 3) (68). Previous studies have investigated the acute effects of blackcurrant supplementation on postprandial blood glucose responses in healthy participants (78) and overweight, inactive adults (79). Acute supplementation with BC extract (600 mg anthocyanins) in healthy adults resulted in a significant decrease in early phase (0-30 min) glucose and insulin responses (78). In overweight, sedentary individuals, BC extract appears to have no effect in an acute setting, however short-term (8 days) supplementation improved insulin sensitivity and postprandial glucose responses (79). Chronic studies of more than 8 days have not been conducted to date using BC as an intervention to aid blood glucose control in any population.

1.1.5 Effects of Beetroot and Blackcurrant Juices on other Metabolic Syndrome Risk Factors

Consumption of both BR and BC have also been shown to improve other MetS factors (dyslipidaemia and hypertension) which play a role in incidence of T2DM and CVD risk. The increased NO production associated with BR consumption has been shown to reduce hypertension (80-89) due to increased synthesis of the vasodilator nitric oxide, and improve dyslipidaemia (73, 90-92), possibly through inhibition of lipid peroxidation, reducing ALEs production and oxidative damage (93), although the mechanisms are not comprehensively understood.

Consumption of BC has also been shown to lower blood pressure (94, 95), possibly through inhibition of vasoconstriction-promoting molecules such as angiotensin II and thromboxanes (96), or through increased NO bioavailability (97, 98). Lipid profiles have also been shown to improve with BC anthocyanin consumption (99-102), although mechanisms have yet to be established and the evidence is currently inconclusive.

1.2 Justification for PhD Study

Although there is some evidence from acute clinical trials and animal model studies suggesting that supplementation with BR and BC have potential to positively impact on blood glucose levels and therefore may be suitable interventions to help delay the onset of T2DM, however intervention trials to date have produced mixed outcomes. Therefore, it is important to undertake a thorough review of the literature to identify research studies which have appropriately investigated the potential of BR and BC juices to improve MetS markers and glycaemic control (Chapter 3). A narrative review was selected for Chapter 3 rather than a formal systematic review or meta-analysis since the evidence concerning BR and BC supplementation spans multiple distinct outcome domains (fasting and postprandial glycaemia, blood pressure, lipid profile etc), several bioactive compounds (dietary nitrate, betalains, anthocyanins), and multiple intervention formats (whole vegetable/fruit, juice, extract), tested in heterogenous populations. This heterogeneity precludes meaningful quantitative pooling of effect sizes within a single PICO framework and reduces the appropriateness of a meta-analysis. This review will also highlight the countries of origin of the interventions, particularly those grown in NZ, since NZ blackcurrants could be particularly high in the bioactive ingredients purported to elicit these beneficial results, i.e., anthocyanins (66, 67), compared with other cultivars (103).

Another important consideration is that previous studies reporting conflicting results have often used different blood glucose sampling methodologies, most commonly plasma glucose or capillary glucose, to establish the effects of their interventions on glucose control. These methods are not directly interchangeable, making comparison between study results challenging, and can yield different values and subsequently different apparent intervention effects. This is particularly true in the early postprandial phase when localised peripheral glucose uptake may be detected in capillary but not plasma or venous samples. It is therefore necessary to establish methodological best practice for glucose measurement before assessing intervention efficacy. Chapter 4 addresses this by directly comparing plasma, venous whole blood, and capillary glucose responses during OGTTs under placebo and beetroot juice

conditions, providing an evidence base for selecting the most appropriate method for subsequent trials as well as context to previous research findings in the literature.

Given the shared pathophysiology between MetS and T2DM, improving MetS biomarkers could help delay or prevent T2DM progression. Both BR and BC contain bioactives (dietary nitrate, betalains, anthocyanins) that have been shown to modulate glycaemic control, blood pressure, and lipid profiles. Thus, evaluating the impact of ingesting these juices in this thesis is relevant, particularly as a simple daily dietary addition such as BR or BC juice is more feasible to deliver at scale and easier for participants to maintain over time than comprehensive lifestyle modification programmes, which, while effective, are often challenging to implement widely and sustain at the individual level.

As mentioned, previous BC intervention studies have often used extracts, whilst BR studies use either whole beetroot or BR juice. To establish proof of concept and acute efficacy under controlled conditions and at known nitrate and anthocyanin concentrations, Chapter 5 of this PhD will investigate the acute effect of concentrated juices on postprandial glycaemia in healthy participants, to establish proof of concept. Given the evidence that BC can improve glucose and insulin responses following just 1 week of daily supplementation (79), Chapter 6 in this PhD will investigate whether BR juice could improve fasting blood glucose as well as lipid profiles and blood pressure following 7 days of supplementation in a healthy population. This study will test different BR formulations, allowing comparison of the individual and combined contributions of nitrate and betalains on MetS markers like blood pressure and lipid profiles.

Despite current evidence suggesting that BR and BC juices may positively impact postprandial glycaemia and MetS outcomes, few studies have explored the hypoglycaemic potential of these juices in a prediabetes cohort. Given the clear evidence that intervention at the prediabetic stage can slow or halt the progression from prediabetes to T2DM and even improve glucose tolerance, the third study for this PhD will investigate a longer-term (12 wk) intervention in a cohort living with prediabetes. This study will investigate the efficacy of BR and BC juices on fasting glucose, HbA1c, lipid profile and blood pressure during prediabetes.

Together, these studies form a logical progression from acute proof-of-concept to short-term mechanistic analysis to longer-term efficacy evaluation. This PhD is therefore well-justified in addressing several gaps in the literature regarding the potential role of BR and BC supplementation to improve MetS components and early-stage T2DM prevention.

1.3 Research Questions

1.3.1 Research Question 1

Does beetroot and/or blackcurrant supplementation have the potential to improve metabolic syndrome risk factors and glucose control?

Beetroot are high in nitrate and betalains, whilst blackcurrants contain high levels of anthocyanins; these compounds have shown the potential to improve individual markers of metabolic syndrome, such as hyperglycaemia (78, 79, 102, 104-106), hypertension (76, 80, 84, 89, 94, 107-110) and dyslipidaemia (73, 76, 111). Dietary nitrate enhances NO production, promoting vasodilation, increased blood flow and improved glucose uptake (112). Similarly, anthocyanins have been reported to inhibit carbohydrate-ingesting enzymes, enhance insulin sensitivity, and modulate inflammatory pathways associated with impaired glucose regulation (102, 113). However, study design, doses and intervention formats remain inconsistent. A narrative review investigating BR and BC as whole-food interventions with the potential to improve glycaemic control and MetS risk factors in humans will be performed.

1.3.2 Research Question 2

Are we using the correct site and method of blood collection to observe differences in acute blood glucose control following interventions targeting glucose uptake at the skeletal muscle?

Interventions such as BR and BC juice are hypothesised to enhance skeletal muscle glucose uptake, possibly through NO-mediated vasodilation (BR) (114), anthocyanin-driven improvements in insulin sensitivity (BC) (79) or increased GLUT4 translocation (BR) (99, 114). Given GLUT4 translocation and NO-mediated dilation primarily affect peripheral tissues, they may be more readily detected in capillary blood samples, reflecting the metabolic status of perfused skeletal muscle, than venous blood, which represents a systemic mix of return circulation (115). Establishing the optimal blood sampling method is essential to avoid underestimation of the efficacy of interventions that act primarily through peripheral glucose clearance.

1.3.3 Research Question 3

Does acute beetroot juice or blackcurrant juice consumption improve postprandial blood glucose responses in healthy individuals?

Postprandial glucose spikes are a known contributor to oxidative stress, endothelial dysfunction, and longer-term cardiometabolic risk, even in non-diabetic individuals (116). Foods that can moderate postprandial glycaemia are therefore of interest due to

their potential to mitigate this damage and subsequently reduce the long-term consequences of repeated postprandial glucose spikes. Blackcurrants are rich in anthocyanins that can inhibit α-glucosidase and α-amylase, slowing carbohydrate digestion and delaying glucose absorption (102). Beetroot-derived inorganic nitrate may enhance glucose uptake via nitric oxide-induced vasodilation and insulin-independent increases in GLUT-4 translocation, as observed in animal and early-phase human studies (112, 114). While some clinical trial evidence shows attenuated glycaemic responses with these two interventions, standardisation of dosages and strength of evidence is somewhat limited. Investigating the acute glycaemic impact of BR and BC juices in healthy adults will clarify their functional potential and inform dosages for trials in at-risk population groups.

1.3.4. Research Question 4

Do the betalains in beetroot juice contribute to previously observed improvements in blood pressure, lipid profile and/or blood glucose control following supplementation in healthy individuals?

While the efficacy of BR is often attributed to dietary nitrate, betalains are potent antioxidant pigments which may provide additional or synergistic benefits. Structurally similar to anthocyanins, betalains have been shown to scavenge free radicals, inhibit oxidative stress pathways, and inhibit enzymes involved in carbohydrate digestion (93, 117). Evidence also suggests that betalains may enhance NO bioavailability, improve lipid homeostasis and improve endothelial function (118). However, most studies involving beetroot juice supplementation focus solely on the nitrate content of the juice to determine efficacious dosages. Evaluating BR juices with varying betalain and nitrate contents may inform whether betalains contribute meaningfully to BR's cardiometabolic effects.

1.3.5 Research Question 5

Can chronic consumption of beetroot and blackcurrant juices improve blood glucose control, blood pressure and/or lipid profiles in people living with prediabetes?

Prediabetes represents a critical window for intervention, where modest improvements in metabolic indices including glucose control, blood pressure and lipid profiles can significantly reduce the risk of progression to T2DM (119, 120). Beetroot and BC juices, due to their high nitrate/betalain and anthocyanin contents, respectively, have the potential to act as nutraceutical interventions during prediabetes. While previous trials

have shown promise in an acute or short-term (6 days) setting for reducing post prandial glycaemia and blood pressure (79) little is known about their sustained impact on fasting glucose levels, HbA1c and lipid profiles in people living with prediabetes. If these juices can provide clinically meaningful improvements in HbA1c, with minimal side effects and good adherence, they may serve as scalable dietary strategies to reduce the public health burden of T2DM and its associated comorbidities.

1.4 Thesis Structure

This thesis follows the format of a PhD by publication. The chapters are organised to address the overarching aim of evaluating the glycaemic and cardiometabolic potential of BR and BC juice supplementation in individuals with varying degrees of metabolic risk. Chapters that are published or prepared for publication are indicated accordingly.

Chapter 1 introduces the background, rationale, and objectives of the PhD research. It outlines the increasing global and NZ-specific burden of prediabetes and type 2 diabetes mellitus (T2DM), and the urgent need for accessible, nutritional interventions. This chapter introduces the five research questions that frame the thesis chapters and discusses the justification for evaluating beetroot and blackcurrant juice as candidate functional foods for improving glycaemic control and metabolic health.

Chapter 2 provides a comprehensive review of the literature on the prevalence and pathophysiology of prediabetes and T2DM, with a specific focus on the relationship with MetS. It reviews current dietary strategies for T2DM prevention and introduces the potential role of nitrate- and polyphenol-rich foods in modulating MetS and T2DM risk factors. The chapter sets the foundation for the specific focus on beetroot and blackcurrant bioactives.

Chapter 3 addresses Research Question 1 by reviewing the evidence for the effects of BR- and BC-derived bioactives, namely dietary nitrate, betalains, and anthocyanins, on three modifiable components of MetS and risk factors for T2DM development; blood pressure, blood glucose, and lipid profiles. The chapter has been published as a narrative review in *Metabolites*.

Chapter 4 explores Research Question 2 by comparing capillary, venous plasma, and venous whole blood glucose measurements during OGTT. This methodological investigation assesses which sampling strategy best captures early changes in blood glucose following dietary interventions that act on peripheral glucose uptake. This chapter is prepared for submission to a journal for review.

Chapter 5 addresses Research Question 3 through findings from the Beetroot and Blackcurrant on Postprandial Glucose (BOP) study, a randomised, controlled crossover trial examining the acute effects of BR and BC juice supplementation on postprandial glucose and insulin responses in healthy individuals. This chapter has been presented at the UK Nutrition Society conference (Summer 2022) and is formatted as a manuscript for publication.

Chapter 6 investigates Research Question 4 through a 7-day crossover study evaluating the vascular and metabolic effects of two BR juices differing in nitrate and betalain composition. This chapter explores the potential contribution of betalains to observed effects on blood pressure, systemic vascular resistance, and fasting metabolic biomarkers. This study has been prepared as a manuscript.

Chapter 7 was originally intended to address Research Question 5 by evaluating whether chronic supplementation with BR and/or BC juice or a mix of the two could improve fasting glucose, HbA1c, blood pressure, and lipid profiles in individuals with prediabetes. However, due to significant time constraints, as well as recruitment challenges and the high resource demands of delivering a long-term intervention in this at-risk population, the study was shortened. Therefore, this chapter presents an evaluation of the feasibility and cost of conducting such a trial, including analysis of recruitment barriers, retention issues, and logistical constraints. In addition to these operational considerations, the chapter reports data on the acceptability of the intervention itself—based on participant feedback from preliminary testing—and explores potential transitions to alternative delivery formats (e.g. encapsulated or powdered extracts) that may improve compliance and enable the intervention to be delivered more widely at a population scale. While it no longer directly answers the original research question, this chapter provides valuable insights into the real-world considerations to inform future chronic nutrition trials in prediabetic populations.

Chapter 8 provides an overall discussion and synthesis of the key findings from the PhD research. It considers the implications of these findings for nutritional interventions in prediabetes, discusses the novel findings and limitations of the work, and outlines recommendations for future research and translation into public health strategies.

Appendices include participant materials (e.g., information sheets and consent forms), ethics approvals, detailed protocols, and supplementary data relevant to the studies conducted as part of this PhD.

1.5 Researcher Contributions

Table 1.2 outlines the researchers' contributions to this PhD thesis.

Table 1.2. Contributions by each researcher involved in this PhD study.

Researcher	Contribution
Cameron Haswell **PhD Researcher**	Responsible for all aspects of three human clinical trials (Chapters 4, 5 and 7) involving study design, human ethics application, participant recruitment, data collection, analysis of plasma and juice samples, statistical analysis and interpretation of data. Responsible for study design, participant recruitment, data collection, statistical analysis and interpretation of data for the UK vs NZ trial (Chapter 6). Responsible for all aspects of the manuscripts involving conceptualisation and design of manuscripts, literature search, data extraction and analysis, drafting, editing and submission of manuscripts. Contributed to funding acquisition for the BOP study described in Chapter 5 and the Prediabetes trial described in Chapter 7.
Associate Professor Rachel Page **Primary Supervisor**	Conceptualisation and design of the BOP study (Chapter 5) and the Prediabetes study (Chapter 7), study design and analysis of the UK vs NZ study (Chapter 6) and the mechanistic study (Chapter 4), funding acquisition for all clinical trials, reviewing of thesis and all manuscripts.
Associate Professor Kay Rutherfurd-Markwick	Conceptualisation and design of the BOP study (Chapter 5), the UK vs NZ study (Chapter

Co-Supervisor	6) and the Prediabetes study (Chapter 7), design of the mechanistic study (Chapter 4), funding acquisition for all clinical trials, supervision of trials, analysis of plasma and juice samples, reviewing of thesis and all manuscripts.
Professor Ajmol Ali **Co-Supervisor**	Conceptualisation and design of the BOP study (Chapter 5), the UK vs NZ study (Chapter 6) and the Prediabetes study (Chapter 7), design of the mechanistic study (Chapter 4), supervision of trials, reviewing of thesis and all manuscripts.
Dr Roger Hurst **External Supervisor**	Conceptualisation and design of the BOP study (Chapter 5), the UK vs NZ study (Chapter 6) and the Prediabetes study (Chapter 7), design of the mechanistic study (Chapter 4), reviewing of thesis and all manuscripts.

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Chapter 2 – Review of the Literature

This chapter provides an overview of the current literature on prediabetes and type 2 diabetes mellitus, including their prevalence, pathophysiology, and dietary management. It also summarises the mechanistic and clinical evidence supporting the potential role of beetroot and blackcurrant in improving glucose regulation.

2.1 Diabetes and Prediabetes Prevalence and Burden

Type 2 diabetes mellitus (T2DM) has become one of the most prevalent chronic diseases worldwide, causing debilitating complications, reducing lifespans, and costing billions of dollars each year in funding for preventative actions and treatment as well as lost productivity {Heald, 2020 #1}. Type 2 diabetes incidence worldwide has increased in recent decades due to a combination of population aging and increased prevalence of lifestyle risk factors such as obesity and sedentary behaviour, particularly in developed countries {Chan, 2020 #2}.

2.1.1 Diabetes as a global epidemic

Globally, T2DM rates have increased dramatically over the past 25 years, and in 2021, 536.6 million people, comprising 10.5% of the 20-79 year-old population worldwide, were estimated to have diabetes (3), of which approximately 90-95% were living with T2DM and 5-10% have type-1 diabetes (4). Current estimates project an increase to 783.2 million cases (prevalence of 12.2%) by 2045 (3), however previous estimates have often been exceeded long before the estimated date. For example, a 1998 World Health Organisation (WHO) report estimated that diabetes prevalence would rise from 135 million (4%) in 1995, to 299 million (5.4%) in 2025 (5); however, this estimate was surpassed by 2011 (2011 International Diabetes Federation (IDF) diabetes atlas report), when the global prevalence was estimated at 366.2 million (8.3%) (6). The 2011 report predicted 551.9 million (9.9%) cases of T2DM globally by 2030, which in 2021 was nearly surpassed in terms of cases (536.6 million), but did in fact surpass the prevalence (10.5%) (3). Given the current trend of underestimation, where prevalence has reached future estimates in approximately 50% of the predicted timeframe, it would not be surprising for cases to increase to or surpass 783 million by 2033.

Whilst some of the increase in T2DM cases can be attributed to aging populations and declining death rates among individuals with diabetes, the age-standardised diabetes prevalence in working age adults (20 – 64 years) increased from 3.2% to 7.8% in men, and from 3.9% to 6.8% in women between 1980 and 2014 (7). This indicates that more people are being diagnosed with diabetes at a younger age, increasing the burden and cost of lifetime treatment and lost productivity.

Despite the upward global trend, some higher income countries such as Sweden (8), Switzerland (9) and Norway (10) have exhibited decreases or stabilisations in their diabetes prevalence since the mid-2000s (11). This is likely due to increased awareness and education, as well as increased access to walking and cycling through improved city planning reducing sedentary behaviour and obesity rates (12-14). Furthermore, increased uptake of screening

programmes, which comes with increased awareness and healthcare funding, has been shown to decrease the incidence of T2DM (15), as more cases are prevented through diagnosis and intervention at the prediabetic stage or through diabetes awareness programmes. Furthermore, the initiation of lifestyle intervention programmes for targeted prevention has been shown to decrease incidence of T2DM in higher income countries, including the USA (16) and Finland (17).

In lower-middle income countries however, prevalence of T2DM is expected to continue to rise, alongside increasing overall population numbers (18, 19). Many of these populations, including those of East Asian, African and Mexican ethnicities are known to have a higher susceptibility to T2DM due to insufficient insulin response in relation to weight gain (20). Furthermore, migrant populations living in higher income countries generally have a higher prevalence of T2DM than Caucasians (21). The genetic predisposition of migrant populations in higher income countries is exacerbated by a poorer socioeconomic status, with greater food insecurity, poor affordability of healthy foods and high consumption of low-quality calories (22, 23), as well as decreased access to organised leisure activities (24). Migrants in higher income countries also have more barriers to healthcare including financial barriers, difficulty with communication and reduced knowledge and education about the host country's healthcare system, which reduces their likelihood of engaging with healthcare services (25). Improvements to social and care disparities for migrant, minor ethnicities and underserved populations in higher income countries may reduce diabetes prevalence in the coming years (2), and previous education programmes in these groups have been shown to be cost-effective (26). Lower-middle income countries present a greater challenge to global T2DM rates, as increased migration from rural to urban areas leads to longer working hours, poor sleep hygiene and greater disposable income, accompanied by poor health literacy (27). Current United Nations (UN) projections estimate the world's urban population to increase from 4.2 billion in 2018 to 6.7 billion by 2050, with Asia and Africa, where populations are at higher risk of T2DM development than European and Europid North American populations, accounting for 90% of this increase (28).

Alongside the increase in prevalence of T2DM, trends towards earlier-onset of T2DM has meant global funds dedicated to care and prevention have now increased to over USD $1 trillion, approximately 1/10th of the global healthcare expenditure (2). Of this USD $1 trillion, 90% is spent in higher income countries, despite 70% of people living with T2DM residing in lower-middle income countries (2). In the USA, diabetes care accounts for 25% of the national cost

of healthcare, and T2DM is associated with 2.3-fold higher health expenditure than those living without T2DM (29). As well as the direct healthcare costs of diabetes, there are also associated indirect costs, such as reduced work performance due to missed workdays for care and disability resulting from diabetes complications. With the prevalence of diabetes increasing significantly in working-age people, there is an increased cost to national economies, leading to a cycle of poverty which worsens care and productivity as funding is shifted towards treatment instead of prevention.

The comorbidities associated with T2DM also have a significant impact on the quality of life of individuals living with T2DM. Diabetic retinopathy is present in 34.6% of individuals living with T2DM, with 10.2% of these classed as 'vision-threatening' (30) and the risk of a lower extremity amputation is 20-fold higher for an individual with T2DM than individuals without T2DM (31). Individuals living with T2DM are twice as likely to suffer from depression than their healthy counterparts (32) due to the clustering of environmental stressors which may lead to diabetes being shared with those which cause depression (e.g., low socioeconomic status, smoking and malnutrition), as well as the increased mental burden of lifestyle change, medication use and anxiety which comes with diagnosis.

Clearly, global T2DM prevalence is rising, particularly in lower-middle income countries and underserved populations within higher income countries. Trends in Europid populations in higher income countries provide encouragement that greater screening and improved health literacy, as well as lifestyle intervention may help to curb this global trend in at-risk populations, but more needs to be done to prevent the repeated breaking of global predictions of T2DM prevalence to reduce the economic and societal burdens of this disease.

2.1.2 Diabetes and Prediabetes within Aotearoa New Zealand

Diabetes prevalence rates have also risen in Aotearoa New Zealand (NZ) over the past decade and the rate of T2DM incidence is increasing, unlike the slowing rates of T2DM development in other higher income countries. Global T2DM rates rose by 4.67% annually between 2011 and 2021 (3, 6), however, Diabetes New Zealand estimates that diabetes rates will increase by 3.28 - 3.87% annually over the next 20 years (33), despite a lower yearly rise (1.92%) than global rates over the past decade. Aotearoa New Zealand also has a high prevalence of prediabetes, with 18.6% of NZers living with prediabetes and approximately 5-10% of those will go on to develop T2DM (34). Over the next 20 years the prevalence of T2DM is expected to rise from 4.7% to around 7%. In that same time period, the projected annual cost of diabetes in NZ will rise from NZD $2.1 billion to $3.5 billion (63% increase) (33).

Alongside the increases in T2DM and prediabetes rates, there are also significant increases in prediabetes rates in younger people aged 25-44 years (35), with some estimates indicating that 16% of 18-45-year-olds have prediabetes (36). This is also the case for even younger groups, including adolescents and children. Of NZ adolescents, 12% are now classified as obese (BMI ≥ 30 kg/m^2) (37), of which over half (53%) are living with prediabetes and T2DM rates are increasing by 5% per year among children aged <15 years (38), with 16% of 8-11-year-olds now living with prediabetes (39). As previously stated, this earlier onset of prediabetes leads to increased societal and economic costs, and data indicates that a 10-year earlier onset of T2DM results in a 1.6 times increased death rate from cardiovascular disease (CVD) (36). Whilst there has been an increase in both T2DM and prediabetes in the youth and younger adults' populations, the greatest area of growth in T2DM in NZ will be in the 60-79-year-old population. Prevalence in this population is expected to increase from 12.9% (2018) to 15.6 - 16.4% by 2040 (33). Alongside an aging NZ population (40), this will lead to an increase in the prediabetic population to between 390,000 - 430,000 by 2040, a 70-90% increase compared with today (228,000 people in 2025).

In terms of ethnicity, NZ Pacific, Asian and Māori populations are disproportionately affected by T2DM. Both prediabetes and T2DM prevalence are higher in Māori (30.4% prediabetes; 4.6% T2DM) and Pacific peoples (29.8% prediabetes; 9.6% T2DM) than NZ Europeans (24.6% prediabetes; 4.2% T2DM) (33, 34). This trend is predicted to continue, with the gap in prevalence expected to rise even further by 2040. The gap in prevalence becomes even greater when considering the age-standardised prevalence of T2DM across these populations, as the median age of all NZ Europeans, both with and without T2DM or prediabetes, is significantly higher (41.4 years) than that of all Asian (31.3 years), Māori (25.4 years) and Pacific peoples (23.4 years) (41). This results in an age-standardised prevalence of 15.1% for Pacific peoples, 8.2% for Asian and 7.5% for Māori, compared with 4.2% for other groups (33). This trend is also reflected in the prediabetic working-age adult population and in Auckland, over 40% of the Māori, Pacific and Indian populations aged between 35-39 years have been diagnosed with prediabetes, rising to over 50% in 45-49-year-olds (35). Aotearoa New Zealand also has a growing Asian population, and at the 2018 census, 28% of Aucklanders identified with Asian ethnicity, up from 23% in 2013 (42). Therefore, it is of concern that age-standardised T2DM rates are similar in Asian populations to Māori (Asian: 8.2%, Māori: 7.5%, Pacific: 15.1%, NZ European: 3%) (33), with South Asians having higher incidence rates than other Asian populations (South Asian: 14%, all Asian: 8%) (43). This is also reflected in the paediatric population (aged 8-11 years), with South Asian (30%), Pacific peoples (27%), and Māori (18%)

having higher rates of prediabetes than NZ Europeans (6%) (39). Clearly, without extensive, targeted interventions, Pacific, Asian and Māori populations will continue to be disproportionately affected by T2DM into the future.

Diabetes-related healthcare expenditure incurs a significant economic burden for NZ, as healthcare costs increased from $247 million in 2001, to $600 million in 2008 (44) and now sit at $2.1 billion per annum representing 0.67% of gross domestic product (GDP) in NZ. Current estimates project a 63% increase in health cost to $3.5 billion by 2040, in part due to the increased cost associated with earlier diagnosis, and NZ's trend of increased diagnosis at earlier ages (45). For example, if diagnosed with T2DM at age 75 years, the average lifetime healthcare cost is $44k NZD, compared with someone diagnosed at 25 years of age, where the lifetime cost is $521k NZD. The most significant part of this cost is healthcare-related costs, of which 57% are secondary care costs associated with diabetes-related complications such as retinopathy, neuropathy or nephropathy (33). There are also costs in terms of workdays lost and reduced productivity, and this is particularly relevant given the increase in people diagnosed with T2DM during their working age (33). Given the current trajectory in NZ, accessible and effective intervention strategies need to be established urgently to reduce T2DM and prediabetes prevalence to reduce its social and economic burden and achieve health equity for all populations.

2.2 Pathophysiology of Diabetes

The pathophysiology of T2DM includes hepatic, peripheral (skeletal) and adipose tissue insulin resistance combined with pancreatic beta (β)-cell and alpha (α)-cell dysfunction, leading to insulin deficiency and hyperglucagonaemia, respectively (46). Whilst insulin resistance is present in many healthy individuals (47), a reduction in pancreatic cell mass and function is the primary driver for progression towards to T2DM (46).

2.2.1 Metabolic Syndrome and Prediabetes

Metabolic syndrome (MetS) is a term which describes a clustering of metabolic abnormalities seen as risk factors for the development of T2DM, cardiovascular disease (CVD) and coronary heart disease (CHD) (48, 49). These metabolic abnormalities include hypertension, dyslipidaemia, hyperglycaemia and central obesity (50), and each contribute to or characterise the development of insulin resistance, prediabetes and subsequent T2DM.

Unlike type 1 diabetes, T2DM is manifested after a period of prediabetes, a stage characterised by increased blood glucose levels (fasting glucose levels 6.1-6.9 mmol/L or two hours postprandial blood glucose levels between 7.8-11 mmol/L), brought about by peripheral insulin resistance, but at a lower level than T2DM (51, 52). Initially, this insulin resistance is accompanied by hyperinsulinaemia, due to increased insulin secretion and an increase in β-cell mass (53), to compensate the reduced utilisation of insulin in the peripheral tissues, allowing maintenance of glucose levels within a manageable range. For this reason, most people with prediabetes are asymptomatic (51).

The direct mechanism causing the initial insulin resistance is still not clear, although high visceral adipose tissue, which releases cytokines and free fatty acids (FFAs) (54), is associated with increased insulin resistance and T2DM prevalence. Increased FFA levels cause impaired insulin secretion, inhibition of insulin gene expression and apoptosis of beta cells in the pancreas (55-57). Visceral adipose tissue mass is generally associated with overweight and obesity, although the cause-and-effect interaction between these two characteristics is not clear, as many relatives of T2DM patients exhibit increased insulin resistance and hyperinsulinaemia before the onset of overweight or obesity (58, 59). These relatives also exhibit higher levels of circulating FFAs and intramuscular lipids than healthy individuals of the same adiposity (60). As a result, it is thought that the distribution of adipose tissue is more important than its volume, when considering the development of insulin resistance and T2DM (54).

Another component of metabolic syndrome, dyslipidaemia, can contribute to increased insulin resistance and studies have shown that triglyceride (TG)/high density lipoprotein (HDL-c) ratio can predict insulin resistance (61, 62). Dyslipidaemia leads to lipotoxicity, which can cause an increase in insulin resistance as well as a reduction in islet β-cell function (63), particularly when coinciding with hyperglycaemia. Furthermore, hypertension is thought to be derived from an increase in low density lipoprotein (LDL-c), which contributes to atherosclerotic plaque production, narrowing blood vessels and raising blood pressure (64). Taken together, this relationship of metabolic syndrome abnormalities leads to increased insulin resistance, and insulin resistance exacerbates the characteristics of metabolic syndrome.

2.2.2 Impaired Fasting Glucose or Impaired Glucose Tolerance

In NZ, glycated haemoglobin A1c (HbA1c) is the diagnostic screening test recommended for use by the New Zealand Society for the Study of Diabetes (NZSSD) to diagnose prediabetes

(HbA1c 41-49 mmol/mol) and T2DM (HbA1c ≥ 50 mmol/mol) (65). HbA1c is the result of the covalent bonding of glucose to haemoglobin in a reaction known as glycation. The amount of glycation of haemoglobin is directly proportional to concentrations of blood glucose across the lifespan of an erythrocyte (approx. 90-120 days) (66, 67), making HbA1c a measure of mean blood glucose levels over a 12-week period.

Insulin resistance and β-cell dysfunction contribute to the development of the early stages of T2DM, which include states of impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) (68). Prediabetes is often characterised by one of these conditions, or a combination of both. Whilst both conditions are indicative of disturbed glucose homeostasis, they exhibit differing pathophysiological mechanisms. IGT is caused by muscle insulin resistance and mild hepatic insulin resistance, which leads to reduced glucose transporter-4 (GLUT-4) translocation and uptake of glucose into skeletal muscle. In contrast, IFG is caused by increased hepatic insulin resistance and more normal skeletal muscle insulin sensitivity (68). These distinctions in pathophysiology between IGT and IFG result in different responses to glucose challenges (Table 2.1).

Table 2.1 Differences between Impaired Fasting Glucose (IGF) and Impaired Glucose Tolerance (IGT).

IFG	Parameter	IGT
Elevated	Fasting blood glucose levels	Normal
Elevated	Hepatic Insulin Resistance	Normal
Normal	Skeletal Muscle Insulin Resistance	Elevated
Increased	AUC for OGTT	Increased
High	OGTT blood glucose peak	High
Reduced	Insulin Secretion at 0-30min OGTT	Reduced
Normal	Insulin secretion at 60-120min OGTT	Reduced
Normal	2hr OGTT Blood Glucose Concentration	Elevated

IFG: Impaired fasting glucose. IGT: Impaired glucose tolerance. AUC: Area under the curve. OGTT: Oral glucose tolerance test. Adapted from Nathan *et al.*[69].

The causes and risk factors for IGT and IFG appear to differ, with IFG being caused primarily by genetic factors and smoking, whereas IGT is predominantly caused by lifestyle factors such as poor diet and sedentary behaviour (70, 71). Both conditions cause an increase in circulating blood glucose concentrations beyond normal parameters, resulting in a raised HbA1c value which may be in the prediabetic range according to the NZSSD guidelines (72). However, as

HbA1c is a proxy for mean blood glucose concentrations over a 3-month period, it cannot be used as a diagnostic tool to distinguish between IGF and IGT, and people living with prediabetes may have either IGT, IFG or a combination of the two.

Despite NZ guidelines using HbA1c as a diagnostic tool for prediabetes since 2012, the World Health Organisation (WHO) (73, 74) and American Diabetes Associations (75) recommend a 2hr oral glucose tolerance test (OGTT) alongside fasting blood glucose measurements to distinguish between IFG and IGT in prediabetes given HbA1c alone does not allow differentiation between IFG or IGT (see Table 2.2 for further detail).

Table 2.2 Diagnostic criteria for normal glucose tolerance, IFG, IGT and T2DM.

Blood test	Normal	IFG	IGT	IFG/IGT	T2DM
World Health Organisation (73, 74)					
Fasting blood glucose (mmol/L)	<6.1	6.1-6.9	<7.0	6.1-6.9	≥7.0
2-h postprandial glucose (mmol/L) *	<7.8	<7.8	7.8-11.0	7.8-11.0	>11.1
HbA1c (mmol/mol)	Not considered sensitive enough for intermediate hyperglycaemia				≥48 ‡
American Diabetes Association (75)					
Fasting blood glucose (mmol/L) †	<5.6	5.6-6.9	<5.6	5.6-6.9	≥7.0
2-h postprandial glucose (mmol/L) *	<7.8	<11.1	7.8-11.0	7.8-11.0	>11.1
HbA1c (mmol/mol)	<39	39-47			≥48

IFG: Impaired fasting glucose. IGT: Impaired glucose tolerance. T2DM: Type 2 diabetes mellitus. HbA1c. Glycated haemoglobin A1c. * 2h postprandial glucose is determined by a standard 2h oral glucose tolerance test (OGTT) with 75 g of carbohydrates. † A random plasma glucose ≥11.1 mmol/L (≥200 mg/dL) alone may be used to diagnose type 2 diabetes mellitus (T2DM) according to American Diabetes Association (ADA) ‡ A glycated haemoglobin A1c (HbA1c) value of <48 mmol/mol does not exclude diabetes diagnosed using glucose tests.

In NZ, HbA1c has been used for the diagnosis of T2DM since 2012 as it has several advantages over 2-h OGTT and fasting plasma glucose levels. HbA1c does not require the need for fasting and is less affected by day-to-day variation due to other factors such as exercise, medicines and dietary patterns (76). Furthermore, HbA1c has easier sampling and analysis requirements, as samples can be collected during consultation and remain stable, whereas samples

collected for glucose analysis are unstable and require anti-glycolytic fixatives applied to the vacutainer (77). Using point-of-care blood glucose analysis measured using a glucometer may overcome some of these issues, as it is more convenient, cheaper and quicker than laboratory analysis of plasma samples. Previous studies however have shown significant differences between plasma and capillary glucose levels, both in a fasted and postprandial state (78). Capillary sampling has been proposed as a screening tool followed by an OGTT with plasma glucose sampling to diagnose prediabetes and T2DM, however this is costly and time consuming (78). Finding a method to correlate capillary glucose levels during an OGTT to plasma glucose levels may create a more convenient method for diagnosing prediabetes into its constituent components, IGT and IFG.

2.2.3 Glycaemic Measures and Their Associations with Long-Term Health Outcomes

The choice of glycaemic measure determines what can be detected and how findings should be interpreted, with each available measure capturing different windows of glucose exposure and carrying different strengths of association with longer-term cardiometabolic outcomes. Section 2.2.2 introduced the three measures most commonly used in clinical practice (fasting plasma glucose (FPG), 2-h post-load plasma glucose, and HbA1c). Emerging clinical research draws on a wider toolkit including alternative glycation markers (fructosamine, glycated albumin), glycaemic variability and continuous glucose monitoring (CGM)-derived metrics. The choice between these is consequential for both diagnosis and intervention research.

Fasting plasma glucose is simple, cheap and accessible but misses a substantial portion of dysglycaemia. In the Diabetes Epidemiology Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) pooled analysis of 14 European cohorts, 2-h post-OGTT glucose predicted cardiovascular and all-cause mortality independently of FPG, whilst FPG added little beyond 2-h glucose (79). A continuous relationship between 2-h post-load glucose and cardiovascular death was evident even below the diabetes diagnostic threshold (79), and across 38 prospective studies a linear association between 2-h post-load glucose and fatal and non-fatal cardiovascular events has been established (80). The 1-h post-load value has more recently been identified as particularly relevant and informative. The Israel Study of Glucose Intolerance, Obesity and Hypertension showed a 1-h glucose >8.6 mmol/L but a normal 2-h value (<7.8mmol/L) had a 28% higher mortality hazard than those with normal glucose at both timepoints (1-h ≤8.6 mmol/L; 2-h <7.8 mmol/L). This hazard rose to 1.76 in participants with elevations at both 1-h (>8.6 mmol/L) and 2-h (7.8–11.1 mmol/L, i.e. impaired glucose

tolerance) (81). These observations are consistent with work showing that repeated postprandial glucose spikes drive endothelial dysfunction and accelerated atherogenesis even when FPG and HbA1c remain normal (82).

Alternatively, HbA1c integrates glucose exposure over approximately 90-120 days (the erythrocyte lifespan) and remains the standard measure of glycaemic exposure. Each 1% absolute reduction in mean HbA1c in newly diagnosed T2DM is associated with a 21% reduction in any diabetes-related endpoint, 14% reduction in myocardial infarction and 37% reduction in microvascular complication (83). The limitations of HbA1c are, however, considerable. Conditions altering erythrocyte lifespan or glycation kinetics distorts HbA1c-mean glucose relationship including deficiencies in iron, B12 and folate, chronic alcohol use, blood loss and pregnancy (84). Furthermore, HbA1c is known to be systematically higher in African-American, Hispanic and Asian populations compared with European populations at the same mean glucose concentrations (85).

Alternative glycation markers such as fructosamine and glycated albumin reflect glycation of plasma proteins and indicate glucose exposure over approximately 2-3 weeks given the shorter half-life of albumin. In the Atherosclerosis Risk in Communities (ARIC) Study (n = 11,104), baseline fructosamine and glycated albumin were associated with coronary heart disease, ischaemic stroke, heart failure and all-cause mortality over more than two decades of follow-up, comparable to HbA1c (86). These biomarkers also predicted incident diabetes, retinopathy and chronic kidney disease in other analyses from the ARIC study (87, 88). These markers seem particularly useful during shorter-term interventions lasting 2-8 weeks, whereas HbA1c is useful in studies greater than 12 weeks.

Further, within-individual glucose fluctuations have been proposed as a determinant of vascular complications independent of mean glycaemia, since acute spikes in blood glucose drive endothelial dysfunction (82). Mean amplitude of glycaemic excursions (MAGE) is a key metric for measuring glycaemic variability and represents the average height of glucose spikes and dips that exceed one standard deviation of an individual's mean daily glucose. Higher MAGE results are associated with higher risk of cardiovascular disease events over 12 months in non-diabetic patients (89) as well as major adverse cardiac events post myocardial infarction (90). Continuous glucose monitoring (CGM) devices sample interstitial glucose every 1-5 min and enable calculation of Time in Range (TIR; % time between 3.9 and 10.0 mmol/L), with each 10% absolute increase in TIR corresponding to approximately a 0.5% (5.5 mmol/mol) decrease in HbA1c (91). In the INDIGO prospective cohort (n = 6,225 T2DM, median 6.9 years'

follow-up), participants with TIR ≤50% had hazard ratios of 1.83 and 1.85 for all-cause and cardiovascular mortality respectively compared with TIR >85%, independent of HbA1c (92). CGM has not yet been widely deployed in dietary intervention trials in prediabetes, with cost and the lack of validated cut-off points for non-diabetic populations remaining barriers to interpretation.

The evidence summarised above has direct implications for the design of the trials in this thesis, and most pointedly for the methodological investigation reported in Chapter 4. Postprandial glucose excursions carry prognostic information beyond that captured by FPG or HbA1c, and interventions hypothesised to act in the early postprandial phase through peripheral glucose uptake, are by their nature undetectable with integrated measures such as HbA1c, glycated albumin and fructosamine. Glucose uptake mediated by GLUT4 translocation and increased capillary perfusion is expected to be most evident at the capillary bed, before mixing with systemic venous return. Despite this, BR and BC trials to date (Chapter 3) have used capillary and venous sampling interchangeably, but more attention should be given to whether the choice of sampling method yields useful conclusions as to the efficacy of these interventions.

2.2.4 Progression of Prediabetes to Type 2 Diabetes Mellitus

Around 5-10% of people with prediabetes develop T2DM each year (69), however progression rates vary depending on whether HbA1c levels are in the prediabetic range due to isolated IGT (4-6%), isolated IFG (6-9%) or a combination of both (15-19%) (93). According to the American Diabetes Association, up to 70% of individuals with prediabetes will eventually develop diabetes (52), and this is the result of increasing failure of pancreatic β-cells to respond to the increased demand for insulin secretion caused by insulin resistance either hepatically or peripherally, or both. The British Whitehall II study described the trajectories of fasting and postprandial glucose concentrations, in conjunction with insulin sensitivity and insulin secretion to produce a model of T2DM progression from prediabetes (94). In this model (Figure 2.1), multiple stages of development of T2DM can be identified and are described in greater detail in Weir and colleagues' 5-stage model of diabetes progression (95). The five stages are compensation, stable adaptation, unstable early decomposition, stable decomposition, and severe decomposition.

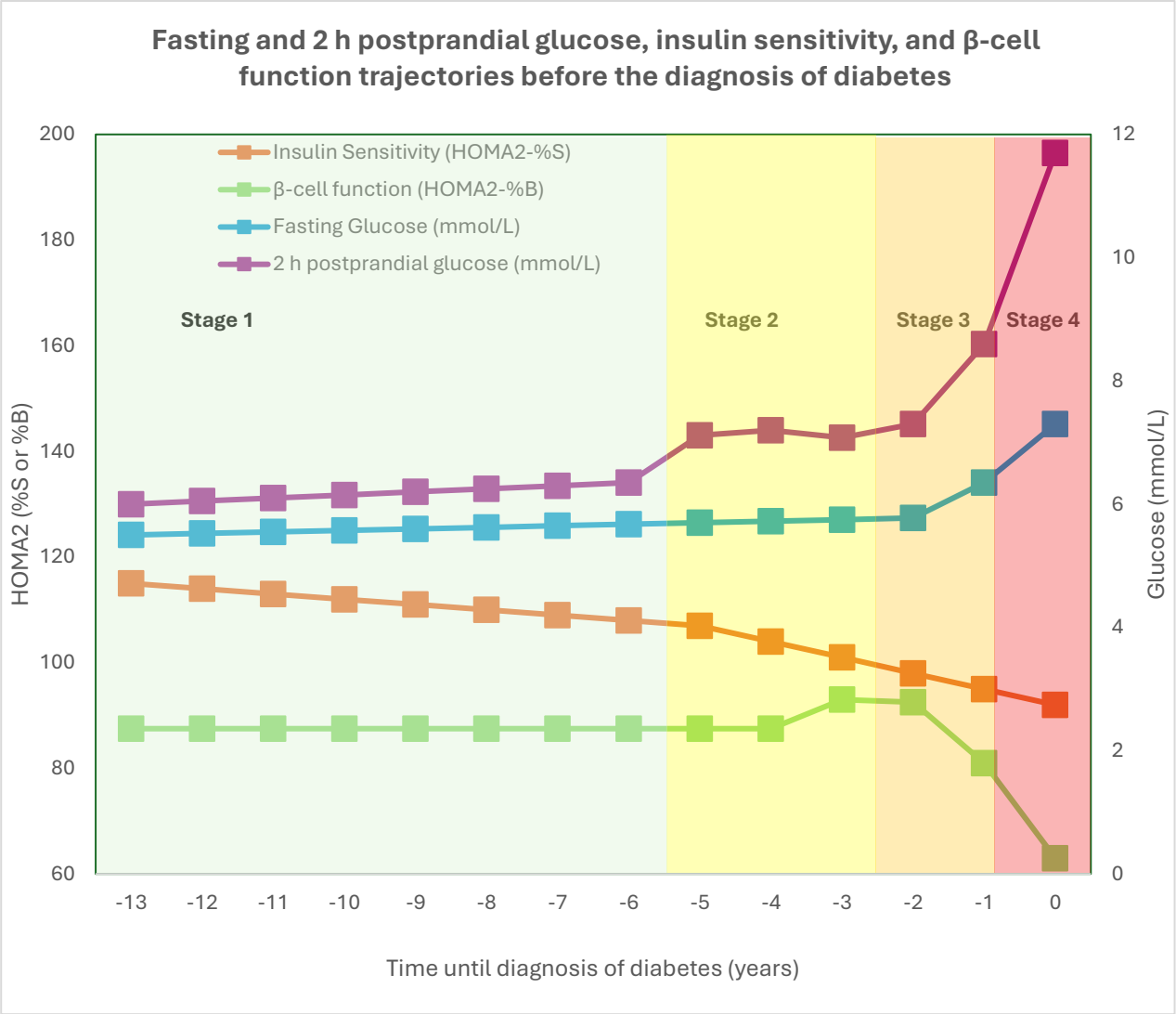

Figure 2.1. Time 0 years is diagnosis of diabetes. The updated homoeostasis model assessment (HOMA2) was used to calculate insulin sensitivity (HOMA2-%S) and β-cell function (HOMA2-%B) trajectories. Adapted from Tabak and colleagues (94). Stages 1-4 of Weir and colleagues' 5-stage model of diabetes progression are represented in the figure by shaded colour. Green represents stage 1, yellow represents stage 2, orange represents stage 3 and red represents stage 4. Stage 5 occurs after the diagnosis of T2DM and therefore is not represented in the figure.

The first stage of Weir and colleagues' model is 'compensation' which is defined by low-level peripheral or hepatic insulin resistance compensated by increased β-cell mass and insulin secretion which keeps fasting and postprandial glucose levels tightly regulated. In stage 2, 'stable adaptation', occurs in which β-cells can no longer fully compensate for reduced insulin sensitivity, and glucose levels begin to rise. Both stage 1 and stage 2 are thought to occur before HbA1c levels rise above prediabetic thresholds (52, 94, 95). At some point during stage 2, β-cells are no longer able to maintain glucose levels within a manageable range, due to a decline in β-cell mass and/or further increase in insulin resistance caused by a combination of

the glucotoxicity and lipotoxicity associated with dietary and genetic risk factors (96, 97). This leads to the third stage of diabetes progression, 'unstable early decomposition'. This stage normally signifies the start of elevated HbA1c levels in the prediabetic or T2DM range, particularly if dietary or lifestyle management of glucose levels is absent (95).

In stage 4, 'stable decomposition', glucose levels rise quickly due to the now rapid apoptosis of β-cells, and T2DM patients have around 50% of the β-cell mass of healthy controls (98). Furthermore, glucotoxicity leads to decreased insulin signalling through interruption of insulin mRNA signalling (99), decreasing the capacity for insulin secretion further. The continued hyperglycaemia results in the production of advanced glycation end products (AGEs) (100), reactive oxygen species (101) and reactive nitrogen species (RNS) (102). The AGEs cause protein cross-linking, stiffening blood vessels and increasing their susceptibility to narrowing and damage (103), the consequence of which is accelerated progression to macrovascular complications, such as atherosclerosis, and microvascular complications such as nephropathy and retinopathy that are associated with T2DM (100, 103, 104). Reactive oxygen species and RNS cause oxidative and nitrative stress and drive inflammatory signalling. These processes damage lipids, proteins and DNA, causing the blood vessel dysfunction and nerve injury implicated in diabetic vasculopathy and neuropathy (101, 102, 105). These macrovascular changes lead to increased risk of CVD (106), stroke (107) and peripheral artery disease (PAD) (108), whilst microvascular outcomes include blindness or vision impairment from retinopathy (109), chronic kidney disease and eventual kidney failure from nephropathy (110) and pain, peripheral numbness and increased infection and subsequent necrosis and amputation from neuropathy (111). Whilst β-cell mass and function are severely reduced in stage 4, medication and lifestyle management mean most T2DM patients likely stay in this stage for the rest of their lives, because although insulin signalling declines, apoptosis rarely progresses to near-complete loss, and only 27% of T2DM patients receive insulin therapy over the disease course (112).

In stage 5, 'severe decomposition', the loss of β-cell mass and function becomes so pronounced that people become ketotic and insulin treatment is necessary for survival.

2.2.5 Current Management of Prediabetes and Type 2 Diabetes Mellitus

Prediabetes is recognised as the key period during which interventions may lead to a slowing or prevention of development of T2DM and dietary and lifestyle intervention at both the prediabetes and T2DM levels can help manage glucose levels and return HbA1c to below prediabetic levels (113). Current NZ guidelines suggest lifestyle intervention including

education, support, healthy eating and exercise, as well as weight management in patients who are overweight (BMI 25-30) or obese (BMI >30) (114) as the first line of defence in prediabetes. Intervention with either metformin or vildagliptin, or a combination of both, is recommended if patients with prediabetes do not respond to lifestyle intervention, or if HbA1c passes 45mmol/mol (72).

For people with T2DM, current guidelines are aimed at preventing or delaying the complications of T2DM and maintaining quality of life (115). Currently, management of glycaemic control and other CVD risk factors such as lipid levels and blood pressure are prioritised (116). Glucose-lowering medications currently registered and funded in NZ include metformin, sodium-glucose co-transporter 2 (SGLT-2) inhibitors, glucagon-like peptide-1 receptor (GLP-1) agonists, dipeptidyl peptidase-4 (DPP4) inhibitors, α-glucosidase inhibitors and insulin (117). For people with T2DM, intervention, both lifestyle and pharmaceutical, should aim to maintain HbA1c levels between 50-55mmol/mol but targets should be negotiated with patients using shared decision-making processes (116).

2.3 Dietary Intervention and the Potential Prevention of the Progression to Prediabetes

Dietary intervention has been shown to reverse insulin resistance and T2DM (113). Several lifestyle-intervention studies such as the Finnish Diabetes Prevention Study (DPS) (118-120) and the Diabetes Prevention Program (DPP) (121, 122) have been shown to improve diabetes-related health outcomes by reducing both high blood pressure and elevated HbA1c levels in people with prediabetes. The DPP study involved six key features such as individual goal setting, workshops and designated health coaches to provide support throughout the intervention, which resulted in a 58% decrease in the incidence of T2DM at the 2.8-year follow-up (121, 122). The DPP study showed that early lifestyle modification in individuals with prediabetes can lead to a return to normoglycaemia and delays in the onset of T2DM, as well as a reduction in CVD risk at follow-up. Furthermore, a prospective cohort study including 820 people living with prediabetes, found that returning to normoglycaemia reduced the incidence rate of CVD events by 50% (123). Clearly, intervention during prediabetes can be an effective method to reduce T2DM and CVD incidence, however, holistic lifestyle interventions are costly and intensive for providers and patients.

Specific dietary patterns have been linked with reductions in T2DM prevalence and risk. Several meta-analyses have shown that adherence to a Mediterranean diet, characterised by high

intakes of monounsaturated fatty acids (MUFA), vegetables, fruits, plant proteins, whole grains, fish and low-fat dairy products alongside lower intakes of alcohol and red meat (124), is associated with a reduced risk ratio (RR) of incidence of T2DM between 0.44 and 0.87, depending on adherence relative to low Mediterranean diet adherence (125-127). Other dietary patterns such as the Dietary Approaches to Stop Hypertension (DASH) (RR: 0.81) and Alternative Health Eating Index (AHEI) (RR: 0.79) have also been associated with significant reductions in diabetes incidence (127).

Specific foods have also been investigated for their potential to normalise blood glucose levels and help delay the onset of T2DM, including foods high in soluble fibre such as oats (128), whole grains (129) and rice bran (130), phytochemical rich foods, including honey (131, 132), tea (133) and cinnamon (134), and those high in dietary nitrate, such as spinach (135) and beetroot (136). Rather than a considerable transformation of an individual's dietary pattern, interventions involving single-food supplementation may provide the opportunity to improve glucose control whilst requiring only a limited amount of time and effort on the part of participants. Discovering a range of foods with bioactive compounds capable of improving blood glucose regulation, reduce development of prediabetes and improve MetS outcomes would therefore be beneficial for improving population-level health outcomes.

2.3.1 Potential Bioactive Compounds in Beetroot

Beetroot (*beta vulgaris;* BR) contains many micronutrients including thiamine (Vit. B1; 0.27 mg/100g, 26% RDI), riboflavin (Vit. B2; 0.27 mg/100 g, 20% RDI), folate (Vit. B9; 80 µg/100 g, 20% RDI) and iron (0.79 mg/100 g, 9.8% RDI) (137, 138). Beetroot has primarily gained attention for its high levels of dietary nitrate which is primarily contained in their roots, and beetroot is one of the most abundant sources of nitrate alongside rocket, radish and celery. The nitrate content of beetroot has been recorded at over 2500 mg/kg (139, 140), however, different cultivars yield different concentrations (141, 142). Other factors including sowing date (142), length of growth period (141) and nitrogen supply (142) can also have a significant impact on the nitrate content of the beetroot. Furthermore, the processing of BR into juice can change the nitrate yield in the juice, and there is large variability in nitrate content between different BR juice brands (139), as well as within different batches of the same product (143).

Dietary nitrate is suggested to have potent effects on human health due to its capacity to be reduced into nitric oxide (NO), a key regulator of blood vessel function (144, 145), lipid peroxidation (146) and glucose metabolism (147). Dietary nitrate is reduced to NO through the

enterosalivary circuit, as nitrate is absorbed in the gut, then re-enters the oral cavity via salivary glands through the sialin transporter where it is reduced to nitrite through nitrate reducing bacteria, such as *Staphylococcus sciuri* (148). This nitrite is then swallowed and absorbed across the gut wall, and converted to NO by nitrite reductase enzymes in the circulation (149). Studies have shown that plasma nitrate and nitrite concentrations increase 2 h post-consumption of BR juice and remain elevated at 6 h (150, 151), whilst NO metabolites (152) and total nitroso species (153) are also elevated following ingestion of BR juice. These nitroso species include S-nitrothiols, which carry and store NO in the blood, and are resistant to scavenging by ROS due to their relative redox inertness compared with other NO-related species (154).

Beetroot is also a rich source of betalains, polyphenol pigments which give BR its distinct purple colouration. Betalains are subdivided into betacyanins (red pigments) and betaxanthins (yellow pigments). Similar to nitrate, betalain content in BR can vary according to growing conditions, cultivar, and length of growth period (139, 141), with commercial juices containing between 700 and 1300mg/L of betalains (139). Most betalains are readily absorbed and metabolised following ingestion, since urine concentrations are only 0.3-0.9% of that ingested (155, 156). Furthermore, chronic consumption of BR juice causes an increase in betalain metabolism, with plasma betalain levels dropping after 2 weeks of consumption to 10% of that observed in the first week (157). Sawicki and colleagues showed high interindividual variability in plasma betalain concentration following ingestion between 24 individuals (coefficient of variation 5% to 78% concentration) however following 4 weeks of chronic ingestion, this variation was less than 10%, suggesting all participants more efficiently metabolised the betalains after 4 weeks, possibly through adaptation of the gut microbiome (157). It is thought the betalains are decarboxylated and dehydrogenated in the body, firstly in the acidic environment of the stomach and through the higher temperature exposure of the body (158), and then through bacteria in the gut microbiome (157), as is the case with other polyphenols (159).

2.3.2 Beetroot Bioactive Compounds for Glucose Management

Beetroot may have the potential to improve blood glucose management in people living with prediabetes through increases in NO and betalain bioavailability. Patients with T2DM exhibit lower levels of NO due to inhibition of NO synthase by AGEs (160). Furthermore, animal studies have shown increased circulating NO in animals with impaired NO production following dietary nitrate supplementation (161, 162). Beetroot juice may be even more effective in increasing NO

bioavailability than dietary nitrate supplementation due to the polyphenols present in BR juice. Polyphenols enhance the reduction of nitrite into NO in the gut (163, 164), and BR juice supplementation increases nitroso species production more than alternative nitrate-matched supplementation methods (153). As nitroso species are protected from scavenging by ROS, compounds seen in elevated levels in T2DM and MetS (101), this provides a promising avenue for intervention which may be effective in T2DM or prediabetes.

Previous studies indicate that BR juice supplementation may improve glucose management through a number of mechanisms. Gheibi et al. (162) showed increased glucose transporter-4 (GLUT4) protein expression in T2DM rats following chronic dietary nitrate supplementation for 2 months. This was due to increased GLUT4 mRNA expression through AMP-activated protein kinase (AMPK). These pathways are the same as those currently targeted by metformin (165). Increased NO production may also lead to improved glucose control, as NO production increases terminal arteriole dilation, increasing capillary recruitment, as well as relaxation of large blood vessels, increasing peripheral blood flow (166, 167). Approximately 25-40% of insulin action resulting in glucose uptake can be attributed to increases in blood flow to peripheral muscles (168), and BR juice supplementation may counteract the downregulation of NO production in T2DM and prediabetes caused by oxidative stress and insulin resistance (169, 170).

Further detail regarding the current evidence for BR juice supplementation to improve blood glucose management can be found in Chapter 3, 'Potential of Beetroot and Blackcurrant Compounds to Improve Metabolic Syndrome Risk Factors'.

2.3.3 Potential Bioactive Compounds in Blackcurrants

Blackcurrants (BC) also contain many bioactive ingredients and are well known for their vitamin C content, with consumption of 25 g of BC sufficient to meet the recommended daily intake (45 mg) for vitamin C in Australia and NZ (171). Blackcurrants also contain notable quantities of potassium (322 mg; 8.5% RDI), iron (1.54 mg; 19.3% RDI), manganese (0.256 mg; 4.7% RDI) and phosphorous (59 mg; 5.9% RDI)/100 g (172).

Anthocyanins, the pigments responsible for the purple/red colour of BC, are present in high quantities in BC compared with other berries, with BC containing up to 476 mg/100 g of anthocyanins, compared with 386 mg/100 g, 140 mg/100 g and 122 mg/100 g in blueberries, cranberries and cherries, respectively (173). Of note is that BC cultivars grown in NZ , in particular *Ben rua* and *Ben ard*, are especially high in anthocyanins compared with other BC

cultivars from other countries (174). The anthocyanins present in BC are primarily cyanidin 3-O-rutinoside, delphinidin 3-O-rutinoside and delphinidin 3-O-glucoside (175), which varies slightly to the common anthocyanins found in other edible berries/plants (see Chapter 3, Table 2).

In terms of bioavailability, anthocyanins are susceptible to breakdown by heat and pH variation, making pharmacokinetics studies challenging. Röhrig et al. (176) found that plasma and urine concentrations of delphinidin-3-O-rutinoside and cyanidin-3-O-rutinoside degradation products peaked 2 h following ingestion, with only 0.040% and 0.048% of ingested delphinidin-3-O-rutinoside and cyanidin-3-O-rutinoside present in the plasma, respectively. As the degradation products were higher in concentration than the ingested compounds, it appears these anthocyanins are readily broken down within two hours of ingestion.

In terms of bioactivity, BC anthocyanins have been shown to regulate or suppress several different inflammatory and antioxidant cellular signalling pathways. Blackcurrant anthocyanins activate the nuclear factor erythroid 2-related factor 2 (NRF2) pathway which regulates cellular oxidant resistance. Elevated NRF2 expression has been observed in rats, which leads to reduced intracellular ROS levels (177). Cyanidin-3-glucosidase supplementation has also been shown to inhibit interleukin-6 (IL-6) secretion, a common and important pro-inflammatory marker (178). Blackcurrant anthocyanins also induce increased LDL receptor (LDLR) expression in the liver (179, 180), which may increase LDL resorption and breakdown, therefore decreasing circulating LDL cholesterol levels.

2.3.4 Blackcurrant Bioactive Compounds for Glucose Management

Some of the earliest evidence supporting the glycaemic-modulating effects of BC comes from the work of Törrönen et al. (181), who conducted OGTTs with 14 participants following the ingestion of BC juice and BC juice fortified with crowberry powder. In the early phase (0-30 min), BC containing crowberry extract enhanced glucose and insulin responses, but had no significant effect on glucose area under the curve (AUC; 0-120 min). These results may be explained by studies examining anthocyanins including those in BC (182, 183) indicating that acute supplementation with BC might cause a delay in glucose absorption through inhibition of α-amylase and α-glucosidase. These results have been corroborated by Castro-Acosta and colleagues (184) who showed decreased plasma glucose levels within 0-30 min of ingestion of a high-carbohydrate meal when supplemented with beverages containing 600 mg of anthocyanins vs control. They also showed that BC juice consumption decreased plasma insulin, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-

1) after 120 min and 90 min, respectively, compared with placebo (184). Rat model studies have also shown an antidiabetic effect of BC anthocyanins through increased AMPK activation (185-187), a key compound responsible for GLUT-4 translocation at the plasma membrane (188).

Nolan et al. (189) investigated the effects of short-term (8 days) supplementation with BC extract (300 mg anthocyanins at breakfast and dinner, 600 mg daily) on postprandial glucose regulation using continuous glucose monitoring in overweight and obese participants. Compared with the placebo group, insulin sensitivity increased by 22% in the intervention group, while postprandial glucose responses to breakfast and lunch decreased by 9% and 8%, respectively (189). To date, no studies have been conducted showing the effect of BC anthocyanins on glucose metabolism in a chronic setting greater than 8 days. Further, human trials are limited and much of the mechanistic evidence for the hypoglycaemic effect of BC anthocyanins is derived from studies in animal models.

More details regarding studies investigating the effects of BC anthocyanins on glucose management can be found in Chapter 3.

2.3.5 Limitations and Future Directions of Beetroot and Blackcurrant Research in Metabolic Health Studies

While evidence supports the potential of beetroot and blackcurrant juice to improve glycaemic control and other MetS risk factors, several limitations in the current literature require further investigation. Many trials have been acute or short-term (<8 days) in design (151, 189-196), with limited sample sizes and a predominant focus on healthy volunteers (136, 145, 181, 190, 194, 197-205). Further, bioactive content is often underreported, particularly in the case of betalains, or highly variable between studies, limiting comparability (172), whilst most BC trials use extracts rather than juices (180, 184, 185, 189, 194, 206-209). Blood glucose sampling locations vary between studies, with both capillary sampling and intravenous sampling used, despite them not being interchangeable (210), which limits detection of early postprandial effects. Future studies should standardise bioactive quantification and reporting, match sampling methods to hypothesised mechanisms, explore both acute and longer-term effects, and include diverse metabolic risk groups.

2.4 Conclusion

The prevalence of T2DM and prediabetes is rising at unsustainable levels in NZ, becoming a major social and economic issue. Multiple strategies need to be employed to mitigate this epidemic, including lifestyle and nutritional intervention. Targeting intervention during prediabetes appears to be an effective way of delaying or preventing the onset of T2DM and finding appropriate interventions which reduce the time and cost burden on participants is essential. The naturally occurring bioactive compounds found in BR and BC juices may have the potential to improve markers of metabolic syndrome and positively impact various mechanisms of oxidant activity, glycaemic control and inflammation related to the pathophysiology of T2DM. Whilst the current evidence indicates that BR and BC juices may be promising interventions, the efficacy of these foods is yet to be established when taken chronically, and the mechanisms of action are yet to be fully understood. This is particularly important given the evidence that metabolism of polyphenols appears to increase when taken over a longer duration, meaning acute or short-term ingestion may not utilise the full bioactive potential of the betalains or anthocyanins present in BR and BC.

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In which chapter is the manuscript/published work?	Chapter 3

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Cameron Haswell (C.H.), Kay Rutherfurd Markwick (K.R.M.), Ajmol Ali (A.A.), Rachel Page (R.P.) and Roger Hurst (R.H.) contributed to the conceptualization of the manuscript; C.H., performed the literature search and paper selection for the review; C.H., completed the original draft preparation; C.H., K.R.-M., A.A., R.P. and R.H. reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Chapter 3 – Potential of Beetroot and Blackcurrant Compounds to Improve Metabolic Syndrome Risk Factors

This chapter addresses Research Question 1 (see Section 1.3.4) and discusses randomised controlled trials which have investigated the impact of beetroot and blackcurrant bioactive ingredients (dietary nitrate, betalains, anthocyanins) on three key modifiable components of the metabolic syndrome: hyperglycaemia, hypertension, and dyslipidaemia. This review has been presented in manuscript format and has been published in the journal *Metabolites*.

Haswell C, Ali A, Page R, Hurst R, Rutherfurd-Markwick K. Potential of beetroot and blackcurrant compounds to improve metabolic syndrome risk factors. *Metabolites*. 2021;11(6):338. (Appendix 1)

Abstract

Metabolic syndrome (MetS) is a group of metabolic abnormalities which together lead to increased risk of coronary heart disease (CHD) and type 2 diabetes mellitus (T2DM), as well as reduced quality of life. Dietary nitrate, betalains and anthocyanins may improve risk factors for MetS and reduce the risk of development of CHD and T2DM. Beetroot is a rich source of dietary nitrate and anthocyanins are present in high concentrations in blackcurrants. This narrative review considers the efficacy of beetroot and blackcurrant compounds as potential agents to improve MetS risk factors, which could lead to decreased risk of CHD and T2DM. Further research is needed to establish the mechanisms through which these outcomes may be taking place, and chronic supplementation studies in humans may corroborate promising findings from animal models and acute human trials.

Keywords: anthocyanins; dietary nitrate; betalains; hyperglycaemia; hypertension; dyslipidaemia; diabetes; glucose control.

3.1 Introduction

The term metabolic syndrome (MetS) is used to describe a group of metabolic abnormalities associated with an increased risk of coronary heart disease (CHD), cardiovascular disease (CVD), stroke, and type 2 diabetes mellitus (T2DM) (1, 2). These metabolic abnormalities include glucose intolerance, insulin resistance, central obesity, dyslipidaemia and hypertension (3). The three definitions for MetS currently in use are from the World Health Organisation (WHO) (4), the Adult Treatment Panel (ATP III) (5), and the European Group for the Study of Insulin Resistance (EGIR) (6). Each organisation's definition and measures differ slightly, but all agree on basic components which include glucose intolerance, obesity, hypertension and dyslipidaemia (Table 3.1).

Table 3.1 Comparison of definitions of Metabolic Syndrome from WHO, EGIR and ATP III.

WHO, 1999	EGIR, 1999	ATP III, 2001
Diabetes, impaired fasting glucose or insulin resistance: euglycaemic clamp; glucose uptake in lowest 25%	**Insulin resistance:** top 25% of the fasting insulin values from non-diabetic population.	
Plus 2 or more of the following:	*Plus 2 or more of the following:*	*Plus 3 or more of the following:*
Obesity: Body Mass Index > 30 or waist-to-hip ratio >0.9 (M) or >0.85 (F)	**Central Obesity:** Waist circumference ≥94 cm (M) or ≥80 cm (F)	**Central Obesity:** Waist circumference >102 cm (M) or >88 cm (F)
Microalbuminuria: Excretion >20 µg/min	**Impaired Fasting Glucose:** Fasting plasma glucose ≥6.1 mmol/L	**Impaired Fasting Glucose:** Fasting plasma glucose ≥6.1 mmol/L
Hypertension: Blood pressure >140/90 mm Hg	**Hypertension:** Blood pressure ≥140/90 mm Hg and/or medication	**Hypertension:** Blood pressure ≥135/85 mm Hg or medication
Dyslipidaemia: Triglycerides ≥1.7 mmol/L or HDL-C <0.9 (M) or <1.0 (F) mmol/L	**Dyslipidaemia:** Triglycerides >2.0 mmol/L or HDL-C <1.0 mmol/L	**Dyslipidaemia:** Triglycerides ≥1.7 mmol/L or HDL-C <0.9 (M) or <1.0 (F) mmol/L

Abbreviations: WHO, World Health Organisation; EGIR, European Group for the Study of Insulin Resistance; ATP III, Adult Treatment Panel III; M, Male; F, Female; HDL-C, High-density lipoprotein cholesterol.

As a result of the differences in the definitions for MetS, it is difficult to establish and compare prevalence data, as different studies employ different measures. Despite this, the International Diabetes Federation estimates that around one-quarter of the worldwide adult population (1.9 billion people) has MetS, with further increases expected due to increased obesity in developed and developing nations (7). Furthermore, a 2007 study of MetS prevalence (using the 2001 ATP III definition) in Auckland (NZ) including 1006 Māori, 996 Pacific people, and 2020 of other ethnicity (mostly Europeans) showed that 16% of people aged 35-74 years had MetS, with higher rates in Māori (32%) and Pacific peoples (39%) (8), showing a clear difference in MetS risk between ethnic groups. Disparities between ethnicities are also shown in the US population, with non-Hispanic black women 1.2 times more likely to have MetS as non-Hispanic white women (9). This study by Moore and colleagues (9) also showed an association between education level and MetS risk. These differences may also be explained through education and deprivation levels, with Māori and Pacific populations in NZ experiencing greater deprivation than NZ Europeans (10), and non-Hispanic black populations having a lower mean education level than non-Hispanic white populations in the US (11).

Predictably, several studies have indicated that the presence of MetS is a significant indicator for future diabetes (2, 12, 13), with a 2013 study (14) finding a Cox proportional hazard ratio of 2.71 (95% CI 2.30–3.18). Global prevalence of T2DM rose from 108 million in 1980 to 422 million in 2014 and current projections estimate that worldwide 591.9 million people will have T2DM by 2035 (15, 16). Early onset of T2DM is associated with increased mortality, with data indicating that a 10 year earlier onset of T2DM, results in a 1.6 times increased death rate from CVD (17). Moreover, MetS itself is associated with a higher relative risk (RR) for mortality from CVD (RR 1.74) (18). Clearly, reducing the incidence of MetS is needed to curb mortality from both T2DM and CVD.

Dietary intervention has been shown to reverse insulin resistance and T2DM (19), improve lipid profiles (20, 21) and reduce cardiovascular risk (22, 23). Recent evidence suggests a role for 'functional' foods and beverages containing flavonoids in reducing MetS risk (24). Foods high in dietary nitrate – such as beetroot – may be effective in reducing T2DM risk, and improving blood glucose control (25), blood pressure (26-28) and lipid profiles (29). Interest has also been shown in the consumption of the anthocyanin subclass of flavonoids, which is associated with reduced T2DM and CVD risk (30-32).

Recently, both *Beta vulgaris* (beetroot) and *Ribes nigrum* (blackcurrant) have been studied for their bioactive potential in improving postprandial blood glucose (25, 33-41) and other risk

factors associated with MetS (42-44). Beetroot has gained renown as a source of dietary nitrate, however it also contains high quantities of betalain pigments, as well as other antioxidants such as carotenoids, ascorbic acid, flavonoids and phenolic acids (45). Blackcurrant is a rich source of anthocyanins, a type of flavonoid responsible for the red/purple colour of many berries, which have been shown to improve blood glucose control and other risk factors associated with MetS (39). Whilst a range of anthocyanins are present in many common berries, blackcurrants have been shown to be a particularly good source of these bioactive compounds (46). This review will explore the current evidence for bioactive compounds in beetroot and blackcurrant and their impact on MetS risk factors. Particular focus will be given to the compounds nitrate and betalains in beetroot, and anthocyanins in blackcurrant.

3.2 Bioactive Compounds in Beetroot

Beetroot contains a number of micronutrients in varying abundance. Beetroot contains notable quantities of thiamine (Vit. B_1; 0.27 mg, 26% RDI), riboflavin (Vit. B_2; 0.27 mg, 20% RDI), folate (Vit. B_9; 80 µg**,** 20% RDI) and iron (0.79 mg, 9.8% RDI) per 100 g beetroot (47, 48). Whilst these compounds are involved in many important metabolic processes, the focus of this review is on the bioactive effects of nitrates and betalains as these compounds may have a significant effect on MetS risk factors.

3.2.1. Dietary Nitrate

Beetroot store nitrate in their roots and along with rocket, radish and celery are one of the foods most abundant in nitrate. The nitrate content of beetroot has been consistently recorded at over 2500 mg/kg (49, 50), with values varying depending on the beetroot cultivar and growing conditions. For instance, beetroot juices prepared from 7 different cultivars showed nitrate content of between 564 ± 129 mg/L and 4626 ± 658 mg/L (50). Furthermore, beetroot harvested in the summer contain lower nitrate concentrations than those harvested in autumn, as light causes a reduction in nitrate accumulation (51). The nitrate content of the surrounding soil is also a key variable affecting the nitrate content of the beetroot (52), with low-soil nitrate resulting in reduced uptake into the vegetable.

Dietary nitrate may be a key bioactive within beetroot, as nitrate can be broken down into nitric oxide (NO), which plays a pivotal role in regulating vascular tone, blood pressure (27, 53, 54) glucose metabolism (55), and lipid peroxidation (29). Previous studies have shown that patients with T2DM generate less NO from L-arginine compared with healthy controls (56), due

to the inhibition of NO synthase by advanced glycation end products (AGEs) (57), resulting in poorer vascular tone regulation, increased blood pressure and impaired glucose metabolism.

The enterosalivary circuit acts as the pathway through which dietary nitrate is reduced into NO. Once ingested, nitrate is absorbed readily across the gut wall and transported to the blood plasma. Around 60-75% of this nitrate is then lost to excretion within 48 h of consumption (58). Nevertheless, around 25% of nitrate in the circulation is actively concentrated by the sialin transporter to the salivary glands, where it re-enters the oral cavity (59). Here, nitrate is reduced to nitrite through nitrite-producing bacteria such as *Staphylococcus sciuri* (60) and then swallowed, before being absorbed across the gut wall to increase circulatory nitrite levels (61). Once in circulation, nitrite is further reduced to NO by nitrite reductase (62) to function as a potent vasodilator, controlling vascular tone and blood pressure (27). Nitric oxide also mediates glucose uptake from the intestines and skeletal muscle and could play an important role in regulation of blood glucose levels (55).

Previous research in animal models shows that dietary nitrate supplementation increases circulating NO in animals with impaired NO production brought about by MetS (25, 33). Therefore, the high nitrate content of beetroot may offer a viable pathway to increase circulating NO in humans, particularly in groups who may have impaired NO production, such as people living with diabetes and/or MetS (57).

3.2.2. Betalains

Betalains are divided into two subclasses, betacyanins, which are red pigments and betaxanthines, which are yellow pigments. Beetroot and prickly pear cacti are the only edible food products currently known to contain betalains (45). Beetroot contain around 120 mg/100 g dry weight of betalains (63) and beetroot juice contains between 700-1300 mg/L of betalains depending on the cultivar and growing methods (50).

Structurally, betalains are similar to anthocyanins (Figure 3.1); pigments which give foods such as berries their bright purple, blue and/or red colours, and within plants betalains perform analogous functions to anthocyanins, meaning the two never naturally coexist (64). Anthocyanins have been shown to have strong antioxidant effects, and given the similarity in structure, it seems likely that betalains will have equivalent effects. In support of this, both betacyanins and betaxanthines have been shown to have a radical-scavenging capacity three to four-fold greater than ascorbic acid, catechin and rutin (65), with other work demonstrating

that the antioxidant capacity of beetroot is positively associated with betalain concentration (66).

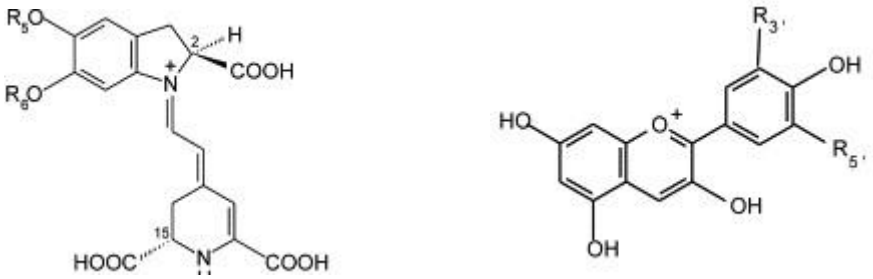

Figure 3.1 Typical structure of betalains (left - betacyanin) and anthocyanins (right - anthocyanidin).

Following beetroot juice consumption betalain concentrations in urine are only 0.3-0.9% of that ingested (67, 68), suggesting a high absorption and an alternative method of elimination, such as metabolism. Furthermore, at least 12 betalain derivatives (5 native betalains and 7 betalain metabolites) have been identified in blood plasma and urine after beetroot juice consumption (66). Interestingly, whilst the betalain concentrations in blood plasma increase with beetroot consumption, after 2 weeks of chronic supplementation, the process of adaptation favouring betalain metabolism takes place, so that plasma betalain levels drop to less than 10% of that observed during the first week of supplementation (66). This suggests that as supplementation continues, the breakdown and utilisation of betalains and their metabolites increases.

3.3 Bioactive Compounds of Blackcurrant Juice

Blackcurrants contain a number of bioactive ingredients, and they are particularly potent sources of vitamin C, with just 25 g containing 100% of the Australia and New Zealand recommended daily intake (45 mg) (69). In addition, blackcurrants contain notable quantities of potassium (322 mg; 8.5% RDI), iron (1.54 mg; 19.3% RDI), manganese (0.256 mg; 4.7% RDI) and phosphorous (59 mg; 5.9% RDI)/100 g. This review, however, focuses on the anthocyanins present in blackcurrants and their effects on markers of MetS, as these pigments are found in greater quantities in blackcurrants than most other berries and edible plants (Table 3.2)

Whilst the anthocyanins listed in Table 3.2 are not unique to blackcurrants, they are present at higher levels than in other berries and plants, with blackcurrants containing 476 mg/100 g of

anthocyanins, compared with 386 mg/100 g, 140 mg/100 g and 122 mg/100 g in blueberries, cranberries and cherries, respectively (46). Furthermore, certain New Zealand cultivars are particularly high in anthocyanins, with the total anthocyanin content in New Zealand blackcurrant juices ranging between 346-850 mg/100 mL (70, 71), compared with 179-310 mg/100 mL for non-New Zealand cultivars (72).

Table 3.2 Content of edible berries/plants for the anthocyanins delphinidin 3-O-glucoside, cyanidin 3-O-glucoside, cyanidin 3-O-rutinoside and delphinidin 3-O-rutinoside

Anthocyanin	Plant/Fruit	Mean Content (mg/100g FW)	Reference
Delphinidin 3-O-glucoside	Bilberry	136.0	(73)
	Blackcurrant	86.68	(74)
	Black Grape	16.61	(75)
	Blueberry	15.17	(76)
	Black Bean	14.50	(77)
Cyanidin 3-O-glucoside	Elderberry	794.13	(78)
	Blackberry	138.72	(75)
	Blackcurrant	25.07	(74)
	Sweet Cherry	18.73	(79)
	Black Olive	10.62	(80)
Cyanidin 3-O-rutinoside	**Blackcurrant**	160.78	(74)
	Sweet Cherry	143.27	(79)
	Black Olive	72.35	(80)
	Plum	33.85	(81)
	Raspberry	5.20	(79)
Delphinidin 3-O-rutinoside	**Blackcurrant**	304.91	(74)
	Eggplant Peel	37.80	(82)
	Tamarillo	5.26	(83)

Abbreviations: FW, fresh weight. Anthocyanin contents are ordered from highest to lowest concentrations per 100g fresh weight for each of the 4 anthocyanins.

The absorption and bioavailability of anthocyanins is complex, since they are highly susceptible to degradation by heat and pH variation (84). Anthocyanins may undergo several transformations before excretion through liver microsomes and epithelial gut bacteria and via enzymatic degradation (85). The effects of anthocyanins on health may be due to both the bioactivity of their metabolites (86) as well as their absorbed intact structures. The most abundant anthocyanins present in blackcurrants are delphinidin-3-O-rutinoside and cyanidin-3-O-rutinoside (Table 3.2). Röhrig et al. (87) investigated the bioavailability of these anthocyanins and their degradation products gallic acid and protocatechuic acid and found

that plasma and urine concentrations peaked 2 h following ingestion, with recoveries of 0.040% and 0.048% for delphinidin-3-O-rutinoside and cyanidin-3-O-rutinoside, respectively (87). In this study, significant quantities of bioactive degradation products were present in the plasma and urine following ingestion of blackcurrant, indicating an abundance of breakdown pathways for anthocyanins and their degradation products (87).

3.4. Effects of Dietary Nitrate and Beetroot Juice on MetS

3.4.1 Glucose Homeostasis

Whilst investigations into the potential of beetroot juice supplementation to improve glucose control are limited (Table 3.3), previous studies using animal models have shown that nitrate supplementation is effective in improving blood glucose metabolism. In a placebo-controlled study, Khalifi et al. (25) divided 32 rats into 4 groups (n=8): control (C), control + nitrate (CN), diabetes (D), and diabetes + nitrate (DN), and supplemented them with either sodium nitrate (CN and DN) or tap water (C and D) for 8 weeks. Prior to intervention, all diabetic rats had lower serum nitrite and nitrate levels, as well as raised systolic blood pressure, compared with controls. Following 8 weeks of supplementation, in the group of diabetic rats supplemented with nitrate (DN), serum nitrite and nitrate and systolic blood pressure returned to values similar to both control groups (C and D) following 8 weeks of supplementation. Furthermore, increases in serum glucose during intravenous glucose tolerance tests for the diabetic, nitrate-supplemented rats were significantly lower than those in the diabetes control group (24.1% vs 90.2%)(25).

Gheibi et al. (33) used a similar methodology to supplement diabetic and non-diabetic rats, and demonstrated improved glucose tolerance, lipid profiles and insulin resistance during glucose tolerance tests, fasting lipids tests and insulin tolerance tests, respectively, in T2DM rats following *ad libitum* nitrate supplementation with sodium nitrate in drinking water. These effects were associated with decreased gluconeogenesis, inflammation and oxidative stress, and most importantly, increased expression of GLUT4 transporter proteins in insulin-sensitive tissues due to increased activation of AMP-activated protein kinase (AMPK). Prior to supplementation, mRNA expression and protein levels of GLUT4 were significantly lower in the soleus muscle (54% and 34%, respectively) and adipose tissue (67% and 41%, respectively) of the diabetic rats vs. controls. Supplementation increased mRNA expression and protein levels of GLUT4 translocators in the soleus (215% and 17%, respectively) and adipose tissue (344%

and 22%, respectively). This is interesting, as the AMPK and GLUT4 translocation pathways are similar to those currently targeted by common antidiabetic drugs including metformin (88).

Insulin release also causes increases in NO production, which in turn leads to dilation of terminal arterioles to increase capillary recruitment (the number of perfused capillaries), as well as relaxation of larger blood vessels to increase peripheral blood flow (89, 90). It is thought that between 25-40% of glucose uptake caused by insulin release can be attributed to NO-dependent increases in blood flow to skeletal muscles (91). As previously stated, oxidative stress from insulin resistance and glucose spikes can downregulate this response (92), however supplementation with dietary nitrate through beetroot juice has been shown to counteract the impairment of endothelial function typically associated with ingestion of a mixed macronutrient meal (93).

Whilst these studies show that dietary nitrate can improve glucose metabolism in diabetic animal models, it is yet to be established if these effects can be translated to humans. Furthermore, an effective method of delivery of dietary nitrate in humans other than drinking water would be of greater significance given the lifestyle nature of MetS and T2DM. Beetroot may provide a viable and effective method of delivering dietary nitrate through lifestyle intervention.

Whilst both beetroot juice and whole beetroot contain dietary nitrate and betalains, beetroot juice has been the preferred method of intervention in trials to date. Beetroot juice contains less dietary fibre than whole beetroot, although dietary fibre may have beneficial effects on glucose metabolism itself (94), however, beetroot juice does not need to be cooked, which may degrade the betalain due to heat treatment (95, 96).

Five studies have investigated the effects of beetroot juice supplementation on glucose control during an acute supplementation trial (34, 36-38, 97) (Table 3.3). Wootton-Beard et al. (34) recruited 16 healthy volunteers to complete an oral glucose tolerance test (OGTT) with either placebo or beetroot juice (990 mg nitrate) intake, in a repeated-measures design. In this study early postprandial insulin (0-60 min) and glucose responses (0-30 min) were lowered, with a lower peak in glucose response in the beetroot supplemented (22.0 mmol/L/min) vs. the matched control (28.3 mmol/L/min). Fuchs et al. (35) recruited 16 obese, insulin-dependent patients to consume 100 mL beetroot juice (300 mg nitrate) or water but found no effect on postprandial glucose and insulin responses (35). It is worth noting that Fuchs et al. (35) used far lower levels of nitrate supplementation (100 mL of beetroot juice containing 300 mg nitrate)

than used by Wootton-Beard and colleagues (34) (225 mL beetroot juice containing 990 mg nitrate). Furthermore, Fuchs and colleagues (35) only took measures of glucose every 30 min, hence they may not have had the sensitivity to detect differences in early-phase glucose response such as those found by Wootton-Beard et al. (34), who took measures at baseline, 5, 15, 30, 45, 60, 90, 120 and 150 min. Shepherd et al. (36) recruited 31 healthy participants to complete a 3-h OGTT with either beetroot juice (738 mg nitrate) or nitrate-depleted beetroot juice and found no differences in plasma glucose, C-peptide, or incretin levels, however these measures were taken hourly, which may also have led to the same sensitivity issues as Fuchs and colleagues 35]. Also of note is that the nitrate-depleted juice acted as a control, and whilst depleted of the bioactive compound nitrate, this juice likely contained high betalain concentrations and therefore both the intervention and control drinks may have had a hypoglycaemic effect on glucose metabolism due to the presence of betalains.

Chang and colleagues (38) recruited 10 healthy volunteers in a cross-over trial and administered either 270 ml beetroot juice (nitrate values not available) or a sugar-matched control with white bread to make 50 g of total carbohydrate. The beetroot juice intervention reduced blood glucose levels at 15, 30, 90 and 180 min compared with control, showing a reduction in early phase and peak glucose, as well as a delay in glycaemic response (38). Furthermore, Holy and colleagues (37) administered 300 g of carbohydrate in a meal with either 250 ml of beetroot juice (nitrate not specified) or 250 ml of water and found that blood glucose levels at 2 h post-meal were significantly lowered in the test condition than in the control group.

Data from a recent longer-term supplementation study involving 30 healthy participants showed that daily consumption of a 10% beetroot juice beverage (9808 mgGAE/100 ml; volume and nitrate concentration not supplied) resulted in a 34.5% decrease in plasma glucose following 4 weeks of supplementation (97). Whilst these results are promising, the lack of detail in the methods for this study mean comparison is not possible and it is difficult to draw concrete conclusions based on this research. Further research should be conducted to corroborate these findings, and interventions lasting more than 90 days would be particularly useful to determine how long-term supplementation may affect HbA1c, the gold standard measure of long-term diabetes status.

Currently, there is evidence from human trial data to suggest that beetroot juice may improve acute blood glucose responses through inhibition of salivary enzymes, delayed uptake of glucose across the intestinal wall and increased GLUT-4 translocation. Whilst this may not lead to an improvement in incremental area under the curve during OGTT tests, there does

appear to be a decreased peak in blood glucose, which may limit the production of AGEs and oxidative damage associated with hyperglycaemic episodes. Future studies should analyse incretins such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) to establish mechanisms of action or utilise interstitial glucose measurement to give a clearer picture of how these compounds are affecting glucose responses. In a chronic setting, evidence is limited to rat models (25, 33) and one 4-week intervention study (97), however results show promise in both settings (Table 3.3). Chronic studies should investigate changes in HbA1C levels for examining impact on glyceamic control and currently dosing requirements are unclear, since studies are limited and lacking detail. Dietary nitrate appears to have long-term benefits in an animal model, however the effect of betalains is difficult to observe independently of nitrate. Studies investigating nitrate-depleted beetroot juice vs placebo may give insight into the action of betalains on blood glucose responses and insulin sensitivity, particularly given the findings of Shepherd and colleagues (36), who found no difference between nitrate-depleted beetroot juice and normal beetroot juice on OGTT outcomes, although, as previously discussed, the sensitivity of these findings may be lower than those of Wooton-Beard and colleagues (34).

Table 3.3 Details of beetroot and dietary nitrate studies investigating glucose homeostasis.

Study Author	MetS Measures	Trial Type	Subjects (n)	Subject Characteristics	Duration	Intervention Dosage	Placebo/Control	Outcome
Khalifi *et al.,* 2015 (25)	Hyperglycaemia, glucose tolerance	Within-groups crossover	8, 8/8	Diabetic male Wistar rats	8 wk	100 mg/L sodium nitrate, *ad libitum*	Water	↓ serum glucose ↑ glucose tolerance
Gheibi *et al.,* 2018 (33)	Hyperglycaemia, glucose tolerance	Within-groups crossover	10, 10/10	Diabetic male Wistar rats	8 wk	100 mg/L sodium nitrate, *ad libitum*	Water	↓ serum glucose ↑ GLUT4 translocation ↑ glucose tolerance
Wootton-Beard *et al.,* 2014 (34)	Insulin and glucose responses	Within-groups crossover	16, 16/16	Healthy adults	Acute	225 mL BR, 990 mg nitrate	Sugar-matched placebo drink	↓ postprandial insulin (0-60 min) ↓ glucose response (0-30 min), ↓ glucose peak
Fuchs *et al.,* 2016 (35)	Vascular resistance and glucose response	Within-groups crossover	16, 16/16	Obese, insulin-dependent males	Acute	100 mL BR, 300 mg nitrate	Sugar-matched placebo	↓ postprandial vascular resistance, no effect on glucose homeostasis
Shepherd *et al.,* 2016 (36)	Plasma glucose, C-peptide, incretins	Within-groups crossover	31, 31/31	16 Healthy young (18-35 yrs), 15 healthy older (50-75 yrs)	Acute	140 mL BR, 738 mg nitrate	Nitrate-depleted beetroot juice	No significant differences between groups
Holy *et al.,* 2017 (37)	Blood glucose, lipid profile	Between groups, Placebo Controlled	50, 30/20	Healthy adults	Acute	250 mL BR (nitrate not specified)	Water	↓ blood glucose
Chang *et al.,* 2018 (38)	Blood glucose	Within-groups crossover	10, 10/10	Healthy, 20-24 yrs	Acute	270 mL BR (nitrate not specified)	Sugar-matched placebo	↓ blood glucose after 15, 30, 90 and 180 min
Olumese and Obah, 2016 (97)	Blood Glucose	Within-groups, before-after	30	Healthy young, (aged 18-29) (18 m, 12 f)	6 wk	10% BR (volume and nitrate not specified)	No control	↓ plasma glucose

Abbreviations: ↓, decreased; ↑, increased; wk, weeks; GLUT4, glucose transport protein-4; BR, Beetroot juice; m, male; f, female. Subjects are listed by total subject number then number in each intervention group.

3.4.2 Hypertension

To date, several studies have investigated the effects of beetroot juice supplementation on blood pressure (Table 3.4), with most studies finding a significant reduction in blood pressure following both acute (28, 98, 99) and longer-term (1 – 8 weeks) supplementation (44, 100-103); however, other studies have shown no positive effects (104-106). Several factors may have influenced the outcomes from these studies, including participants' age, gender, BMI and/or beetroot dosage.

Most studies have shown that supplementation with beetroot juice has a greater effect on blood pressure in males (105, 107), since premenopausal females tend to have lower initial blood pressure than males of the same age and BMI (108). One study showed a decrease in systolic blood pressure in males of 4-5 mmHg compared with 2-3 mmHg in females, 6 hours after supplementation 500 g of beetroot and apple juice (465 mg nitrate) (107). Furthermore, a recent meta-analysis (109) showed that subjects with a BMI >25 had greater decreases in blood pressure following beetroot juice consumption compared with subjects with a normal BMI.

The mechanisms through which beetroot supplementation may reduce blood pressure are well described, with dietary nitrate potentially increasing NO content in the vasculature through nitrate and nitrite-reducing enzyme activity. NO then stimulates cyclic 3', 5'-guanosine monophosphate in the vascular smooth muscles which initiates Ca^{2+} release, resulting in increased vasodilation and decreased blood pressure (52). Interestingly, Bahadoran et al. (109), in a meta-analysis of 27 studies using nitrate-depleted beetroot juice as a control, showed that beetroot juice seems to have blood pressure-lowering effects (SBP, -2.91 mmHg: DBP -0.91 mmHG) independent of nitrate, which may suggest a role for betalains in the responses observed in previous trials.

To date, findings suggest that beetroot juice supplementation improves blood pressure regulation through increased NO content in the vasculature. Furthermore, evidence suggests there are hypotensive effects of beetroot juice independent of nitrate, meaning betalains may have an important role to play. Further investigation is needed to understand the hypotensive effect of betalains, since studies to date have tended to focus on the nitrate content of their interventions, and do not report betalain content. Also, promising data from studies using anthocyanins (42, 110), the structurally similar counterparts of betalains found in berries and other plants, suggests betalains may work through similar mechanisms as anthocyanins, although these mechanisms are not presently fully understood.

Table 3.4 Details of beetroot trials investigating hypertension.

Study Author	MetS Measures	Trial Type	Subjects (n)	Subject Characteristics	Duration	Intervention Dosage	Placebo/Control	Outcome
Coles & Clifton, 2012 (107)	Blood pressure	Within-groups crossover	30, 30/30	Healthy adults (15 m, 15 f)	Acute	500 g beetroot and apple juice (465 mg nitrate)	Apple juice concentrate	↓ SBP in males
Hobbs *et al.*, 2013 (98)	Blood pressure, vasodilation	Within-groups crossover	24, 24/24	Healthy adults	Acute	200 g beetroot bread (68.2 mg nitrate)	200 g control white bread	↓ DBP ↑ vasodilation
Raubenheimer *et al.*, 2017 (99)	Blood pressure	Within-groups crossover	12, 12/12	Older adults (57-71 yrs) (5 m, 7 f)	Acute	140 mL BR (800 mg nitrate)	Nitrate-depleted beetroot juice	↓ DBP ↓ SBP
McDonagh *et al.*, 2018 (100)	Blood pressure	Within-groups crossover	10, 10/10	Healthy males	Acute	55 mL BR conc (357 mg nitrate)	70 mL deionised water	↓ DBP ↓ mean arterial BP
Stanaway *et al.*, 2019 (28)	Blood pressure	Within-groups crossover	24, 24/24	13 healthy young (18-30 yrs), 11 healthy older (50-70 yrs)	Acute	150 mL BR (651 mg nitrate)	Beetroot juice concentrate (1 mmol/150 mL nitrate)	↓ DBP in older adults, ↓ SBP in both groups
Bondonno *et al.*, 2015 (105)	Blood pressure	Within-groups crossover	27, 27/27	Hypertensive adults (53-70 yrs, SBP 120-160 mmHg)	1 wk	2x70 mL BR daily (nitrate not specified)	Nitrate-depleted beetroot juice	No significant differences between groups
Ormesher *et al.*, 2018 (104)	Blood pressure	Between groups, Placebo Controlled	41, 20/21	Hypertensive, pregnant females (SBP 130-144 mmHg and/or DBP 80-94 mmHg)	8d	70 mL BR (400 mg nitrate)	70 mL nitrate-depleted beetroot juice	No significant differences between groups
Asgary *et al.*, 2016 (101)	Blood Pressure, systemic inflammation	Within-groups crossover	24, 24/24	Hypertensive adults (25-68 yrs)	2 wk	250 mL BR or 250 g cooked beetroot daily (nitrate not specified)	No control	↓ SBP ↓ DBP, ↑ FMD, ↓ inflammatory markers
Ashor *et al.*, 2015 (102)	Blood pressure	Between groups, Placebo Controlled	21, 10/11	Overweight older adults (55-70 yrs, BMI 25-40 kg/m^2)	3 wk	70 mL BR conc (300-400 mg nitrate)	Blackcurrant juice (<5 mg nitrate)	No significant differences between groups
Capper *et al.*, 2020 (44)	Blood pressure, gut microbiota	Between groups, Placebo Controlled	36, 19/17	Healthy older adults	8 wk	150 g whole beetroot & banana every other day (590 mg nitrate)	Banana every other day	↓ SBP

Abbreviations: m, male; f, female; ↓, decreased; ↑, increased; SBP, systolic blood pressure; DBP, diastolic blood pressure; BR, Beetroot juice; BP, blood pressure; wk, weeks; FMD, flow-mediated dilation; BMI, body mass index. Subjects are listed by total subject numbers then number in each intervention group.

3.4.3 Dyslipidaemia

Different aspects of lipid profiles have been investigated following supplementation with beetroot (Table 3.5) in healthy individuals (37), physically fit soldiers (111) and in hypercholesterolaemic rats (112). Early *in vivo* studies by Al-Dosari and colleagues (113) involving rats with hypercholesterolaemia showed that administration of 250 mg/kg body weight of freeze-dried beetroot extract (anthocyanin data not given) significantly decreased total cholesterol and triglycerides in the intervention group vs control. Al-Dosari and colleagues (113) also found a significant increase in high-density lipoprotein cholesterol (HDL-C) in the intervention group vs control.

In human trials, Holy et al. (37) showed that in healthy subjects, an acute dose of beetroot juice (250 ml, nitrate and betalains not specified) with a carbohydrate meal (300 g) lowers blood triglycerides, total cholesterol and low-density lipoprotein cholesterol (LDL-C) (37). Research by Singh et al. (111) involved daily supplementation of 30 soldiers with 400 ml of beetroot (nitrate and betalains not specified) juice for 15 days in a before-after study. HDL-C was significantly increased (1.109 ± 0.214 mmol/L to 1.298 ± 0.253 mmol/L) and LDL-C was significantly reduced (3.354 ± 2.128 mmol/L to 3.090 ± 2.048 mmol/L) from baseline to 15 days (111). However, Singh et al. [106] did not include a control group, meaning conclusions may be difficult to draw as other variables such as training status in their military rotation cycle and changes in diet due to the intervention may have affected these results. Taken together, the results from these studies (37, 111, 113) show that beetroot juice may be beneficial in improving dyslipidaemia both acutely and following longer-term consumption, however more robust methodology, such as randomised control trials, is needed in longer-term trials to corroborate the work of Singh and colleagues (111).

The mechanism(s) by which beetroot supplementation improves lipid profiles is not yet clear, and it may be that both the increased NO production from nitrate and the potential antioxidant effects of the betalains from beetroot consumption are leading to improvements in the lipid profile. Previous work has shown that isolated betanin inhibits lipid peroxidation, leading to fewer reactive oxygen species and less oxidative damage (68), whilst others (25) have shown improved lipid profiles following dietary nitrate supplementation in the absence of betalains. Combined, this may show independent effects of both betalains and nitrate in beetroot in improving lipid profiles.

Table 3.5 Details of beetroot and dietary nitrate trials investigating dyslipidaemia

Study Author	MetS Measures	Trial Type	Subjects (n)	Subject Characteristics	Duration	Intervention Dosage	Placebo/Control	Outcome
Rabeh & Ibrahim, 2014 (112)	Lipid profile	Between groups, Placebo Controlled	35, 7/7/7/7	Hypercholersterolaemic Sprague Dawley rats	4 wk	Beetroot waste extract, 200, 400 or 600 mg/kg/day	Basal diet only	↓ TC ↓ LDL-C ↓ TG ↑ HDL-C
Khalifi *et al.*, 2015 (25)	Hyperglycaemia Lipid profile	Between groups, Placebo Controlled	8, 8/8	Diabetic male Wistar rats	8 wk	100 mg/L sodium nitrate *ad libitum*	Water	↓ LDL-C ↓ TG ↑ HDL-C
Al-Dosari *et al.*, 2011 (113)	Lipid Profile, Free radical generation	Between groups, Placebo Controlled	30, 6/6/6/6	Hypercholersterolaemic Wistar rats	10 wk	Beetroot, 250 or 500 mg/kg/day (nitrate not specified)	Basal diet only	↓ TC ↓ TG ↑ HDL-C
Holy *et al.*, 2017 (37)	Lipid profile	Between groups, Placebo Controlled	50, 30/20	Healthy adults	Acute	250 mL BR (Nitrate not specified)	Water	↓TC ↓TG ↓LDL-C
Singh *et al.*, 2015 (111)	Lipid profile	Within groups, Before - After Study	30, 30/0	Physically active soldiers	15 days	2x400 mL BR daily (Nitrate not specified)	No Placebo Group	↓ LDL-C ↑ HDL-C

Abbreviations: ↓, decreased; ↑, increased; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; BR, beetroot juice. Subjects are listed by total subject numbers then number in each intervention group

It is possible that a 'functional food' high in both dietary nitrate and betalains may have a marked effect on dyslipidaemia in people living with MetS. Future research should focus on establishing mechanisms of action and establishing the efficacy of intervention in a chronic trial, using a placebo-controlled crossover design. A Latin square design including nitrate-depleted beetroot juice may also help clarify the effects of betalains in this complex equation. Furthermore, dosing studies using different concentrations of both nitrate and betalains may aid in our understanding of the mechanisms of action through which beetroot may improve dyslipidaemia.

3.5. Effects of Blackcurrant Anthocyanins on MetS

3.5.1. Glucose Homeostasis

The effects of blackcurrant anthocyanins (mostly delivered in studies as juice) on blood glucose responses have been extensively researched in recent years (Table 3.6), with randomised crossover and controlled trials conducted in both healthy (39, 40) and overweight adults (41). Following consumption of blackcurrant juice and blackcurrant juice fortified with crowberry powder, Törrönen et al. (114) conducted OGTT trials with 14 participants and found that whereas glucose and insulin responses in the early phase (0-30 min) were improved in the blackcurrant with crowberry extract group, there was no significant difference in overall AUC (area under the curve) for glucose (0-120 min). This is similar to findings in beetroot juice trials (34, 38), and may indicate that carbohydrate absorption is delayed, rather than reduced, following acute supplementation.

Castro-Acosta and colleagues (39) showed that drinks containing blackcurrant extract providing 600 mg of anthocyanins were effective in reducing plasma glucose responses to a high-carbohydrate meal at 0-30 min post-meal. Furthermore, plasma insulin, plasma GIP and GLP-1 were all significantly reduced following blackcurrant extract consumption compared with control. In a subsequent study (40), 26 healthy participants were supplemented with placebo, apple extract, or apple extract and blackcurrant extract, in association with a starch and sucrose meal. The iAUC (incremental area under the curve; 0-30 min) improved in the apple vs. placebo trials, whilst apple and blackcurrant improved iAUC vs both placebo and apple extract (40). As apple extract has been shown to inhibit sodium-glucose transport protein-1 (SGLT-1) action (115) and proanthocyanidins in blackcurrant inhibit alpha amylase (116) and alpha-glucosidase (117), combining different polyphenol-rich foods may result in a

synergistic effect, acting on multiple pathways, to impact on postprandial glucose responses; however further research is needed to corroborate these findings.

Watson et al. (118) took glucose measurements at 60 and 150 min following consumption of blackcurrant fruit juice (525 mg polyphenols/ 60 kg BW; 0.91 g carbohydrate/ kg BW) following an overnight fast and observed elevated blood glucose at both time points in the intervention vs control. Whilst these results appear to show increased blood glucose following blackcurrant supplementation, this supports the findings of Castro-Acosta (39, 40) and Törrönen (119), where the early-phase blood glucose response was inhibited, with elevated glucose readings occurring at 60 and 150 min. It is possible that if glucose measurements had been taken in the early phase (0-30 min), a blunted blood glucose would have been seen at this time point, however this can only be speculated upon. Furthermore, in this trial (118), the intervention drinks were not consumed as part of a meal, but on their own, which may have affected the speed of absorption of the carbohydrates.

Several chronic supplementation studies using blackcurrant anthocyanins to investigate glucose regulation have also been conducted. Nolan et al. (41) conducted a two-experiment intervention including an acute supplementation and a short-term (8 days) supplementation trial. They demonstrated improvements in free-living individuals' glucose control using continuous glucose monitoring over 8 days of supplementation with a New Zealand blackcurrant extract (300 mg ingested prior to breakfast, 300 mg ingested prior to dinner; 600 mg anthocyanins per day) in overweight and obese adults (41). Compared with placebo, insulin sensitivity was improved by 22% and postprandial glucose responses to breakfast and lunch were reduced by 9% and 8%, respectively, in the intervention group (41). In the acute supplementation trial, they found significant reductions in postprandial blood glucose at 45 min, 60 min and 90 min, as well as decreased AUC for glucose and insulin following acute ingestion of blackcurrant extract containing 105 mg anthocyanins with a mixed macronutrient meal (41). This data suggests that even short-term supplementation with New Zealand blackcurrant extract can improve insulin sensitivity. This has been shown previously with blueberries (120), however this is the first study of its kind conducted using an anthocyanin-rich blackcurrant extract.

From the current evidence, it is clear that blackcurrant supplementation has an acute effect on blood glucose levels, delaying blood glucose responses and decreasing peak glucose during OGTTs through the inhibition of carbohydrate absorption during the early phase (0-30 min). Further work needs to be undertaken to understand the appropriate doses for achieving

long-term improvements in blood glucose responses, as previous work has utilised anthocyanin levels ranging from 105 mg (41) to 1200 mg (40), and have been consumed at different times of the day.

Table 3.6 Details of trials investigating blackcurrant juice and extracts on glucose homeostasis

Study Author	MetS Measures	Trial Type	Subjects (n)	Subject Characteristics	Duration	Intervention Dosage	Placebo/Control	Outcome
Park *et al.,* 2015 (43)	Glucose tolerance	Between groups, Placebo Controlled	30, 10/10/10	Sprague-Dawley mice with metabolic syndrome	4 wk	100 mg/kg/day or 300 mg/kg/day of BCE	Basal diet only	↓ iAUC glucose ↓ IRS-1 ↓ p-AMPK in muscle
Benn *et al.,* 2015 [115]	Glucose tolerance	Between groups, Placebo Controlled	24, 13/11	Male hypercholesterolaemic rats	12 wk	Equivalent 540mg BCE in humans (Actual mg unclear)	Basal diet only	↓ plasma glucose
Törrönen *et al*., 2012 (114)	Blood glucose	Within-groups, crossover	20, 20/20	Healthy participants (3 m, 11 f)	Acute	300mL BC juice and 300mL BC juice with crowberry powder (159 and 293 mg/100 mL polyphenols)	Water	↓ glucose response (0-30 min) ↓ insulin response (0-30 min)
Watson *et al.,* 2015 (118)	Blood glucose	Within-groups, crossover	36, 36/36	Healthy Participants (18-34 yrs, BMI <35)	Acute	anthocyanin-enriched BCE extract or 142 mL BC juice (each containing 525mg polyphenols)	Placebo drink (0 mg polyphenols)	↑ blood glucose (60 and 150 min)
Castro-Acosta *et al.,* 2016 (39)	Blood glucose Insulin Incretins	Between groups, Placebo Controlled	23, 23/23/23/23	Healthy participants (14 m, 9 f)	Acute	BCE, (150, 300 and 600 mg anthocyanins)	No extract	↓ plasma glucose ↓ plasma insulin ↓ plasma GIP ↓ plasma GLP-1
Castro-Acosta *et al.,* 2017 (40)	Blood glucose insulin incretins	Between groups, Placebo Controlled	25 25/25/25	Healthy participants (20 m, 5 f)	Acute	1200 mg apple polyphenols or 600 mg apple polyphenols + 600 mg blackcurrant anthocyanins	Placebo drink (0 mg polyphenols)	↓ iAUC glucose ↓ plasma insulin ↓ C-peptide ↓ plasma GIP
Nolan *et al.,* 2020 (41)	Insulin sensitivity, free-living glucose, inflammation	Within-groups crossover	13 13/13	Overweight, inactive adults (BMI 28.8 ± 3.9 kg/m-2, <1h structured PA/week	8 days	2 x 300 mg BC extract daily (pre -breakfast and dinner)	Placebo capsule	↑ insulin sensitivity ↓ C-reactive protein ↓ postprandial glucose responses

Abbreviations: wk, week; BCE, blackcurrant extract; ↓, decreased; iAUC, incremental area under the curve; IRS-1, insulin receptor substrate 1; p-AMPK, 5' adenosine monophosphate-activated protein kinase; m, male; f, female; BMI, body mass index; ↑, increased; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide -1; PA, physical activity. Subjects are listed by total subject numbers then number in each intervention group.

3.5.2 Hypertension

The potential effects of blackcurrant anthocyanins on hypertension are currently not well established (Table 3.7), however a recent study (42) showed that ingestion of 600 mg/day of anthocyanins from a NZ blackcurrant extract over a 7-day intervention period resulted in decreases in systolic (6 mmHg) and diastolic (6 mmHg) blood pressure in a cohort of 14 healthy older adults. Such changes are sufficient to improve health outcomes, and reduction in systolic blood pressure of 6 mmHg contribute about 35-40% less stroke and 20-25% less coronary heart disease mortality (121) after just one week of supplementation.

Anthocyanins are proposed to lower blood pressure through three main mechanisms. Firstly, anthocyanins increase endothelial-derived NO through modulation of endothelial NO synthase (eNOS) expression (122). As previously stated, NO contributes to endothelium-dependent vasorelaxation through increased stimulation of cyclic 3', 5'-guanosine monophosphate, in turn leading to increased blood vessel vasodilation. Secondly, due to their high antioxidant activity, anthocyanins are able to protect against reactive oxygen species which normally interact rapidly with NO to form other radical intermediates, resulting in lower NO availability, leading to vasoconstriction and hypertension. The protective effect of anthocyanins reduces this NO conversion, thus preventing oxidative damage (123). Thirdly, anthocyanins are proposed to inhibit the production of vasoconstriction-promoting molecules such as angiotensin II and thromboxanes through inhibition of the enzymes (angiotensin-converting enzyme and cyclooxygenase respectively) which catalyse the production of these vasocontrictors (124). Although yet to be confirmed in human trials, Park (43) also showed that blackcurrant downregulated endothelin-1 expression, another vasoconstrictor following blackcurrant supplementation in rats, and this could be another contributing mechanism to the effects observed in humans in previous trials.

Whilst current crossover studies are limited (Table 3.7), the mechanisms through which blackcurrant supplementation may regulate vascular tone are well understood. More studies need to be conducted in human populations, both in an acute and chronic setting, to corroborate the findings of Cook et al. (42), as well as establish ideal dosage for chronic studies.

3.5.3 Dyslipidaemia

As well as improving both glucose tolerance and blood pressure, consumption of blackcurrant extract has also been shown to have beneficial effects on lipid profiles in both rat (43) and

human trials (119) (Table 3.7). Park et al. (43) induced MetS in Sprague-Dawley rats and supplemented them with placebo, 100 mg/kg/day or 300 mg/kg/day of blackcurrant extract for 4 weeks to investigate lipid profile responses. Following supplementation, rats ingesting 100 mg/kg/day and 300mg/kg/day of blackcurrant showed significant decreases in triglyceride and LDL-C levels, and a significant increase in HDL-C levels vs control.

More recently, Nanashima et al. fed ovariectomised rats *ad libitum* either a regular diet or one containing 3% blackcurrant extract and showed a decrease in body weight, visceral fat weight, serum triglycerides, total cholesterol and LDL-C in the extract-treated rats (125). The findings of this study (125) show promise for the efficacy of blackcurrant anthocyanins to decrease dyslipidaemia in a menopausal animal model. Postmenopausal women with low oestrogen activity show increased risk of dyslipidaemia (126), however blackcurrant anthocyanins may attenuate this response through phytoestrogen signalling in estrogen receptors α (127) and β (128).

In human trials, Törrönen et al. [119] investigated blood glucose and free fatty acid (FFA) responses in 20 healthy females (aged 25-69 yrs) in a randomised, crossover, acute meal study, consuming blackcurrants or ligonberries as a puree with 35 g sucrose added or 35 g sucrose with water. Ingestion of the berry meals decreased the concentration of FFAs compared with the control meal, despite there being an overall higher carbohydrate load in the berry meal (119).

Whilst there is evidence that blackcurrant extract supplementation can improve blood lipid profiles in an acute setting and mechanisms including phytoestrogen signaling through receptors, chronic studies have yet to be conducted in humans and dosages are yet to be established.

Table 3.7 Details of trials investigating blackcurrant juice and extracts on hypertension and dyslipidaemia.

Study Author	MetS Measures	Trial Type	Subjects (n)	Subject Characteristics	Duration	Intervention Dosage	Placebo/Control	Outcome
Hypertension								
Cook et al., 2020 (42)	Blood Pressure	Within-groups crossover	14, 14/14	Older adults (69 ± 4 yrs)	1 wk	2 x 300 mg BC extract daily (210 mg anthocyanins)	300 mg microcrystalline cellulose	↓ DBP ↓ SBP
Okamoto et al., 2020 (110)	Arterial function, central blood pressure	Within-groups crossover	14, 14/14	Older adults (73.3 ± 1.7 yrs)	1 wk	2 x 300 mg BC extract daily (210 mg anthocyanins)	300 mg microcrystalline cellulose	↓ Central blood pressure ↓ Carotid-femoral pulse-wave velocity
Dyslipidaemia								
Park *et al.*, 2015 (43)	Lipid profile	Between groups, Placebo Controlled	30, 10/10/10	Sprague-Dawley mice with metabolic syndrome	8 wk	100 mg/kg/day or 300mg/kg/day of BC	Basal diet only	↓ TC ↓ TG ↓ LDL-C
Nanashima *et al.*, 2020 (125)	Lipid Profile	Between groups, placebo controlled	20, 10/10	Ovariectomised female Sprague-Dawley rats	12 wk	3% blackcurrant extract (ad libitum)	Basal diet only	↓ TC ↓ TG ↓ LDL-C
Benn *et al.*, 2015 (129)	Lipid profile	Between groups, Placebo Controlled	24, 13/11	Male hypercholesterolaemic rats	12 wk	Equivalent 540mg BC extract in humans (Actual mg unclear)	Basal diet only	↓ TC
Törrönen *et al.*, 2012 (119)	Glucose, insulin, free fatty acids	Within-groups crossover	20, 20/20	Healthy females	Acute	150 g blackcurrant puree with 35 g sucrose (anthocyanins not specified)	Water	↓ FFA rebound following meal

Abbreviations: wk, week; BC, blackcurrant; ↓, decreased; ↑, increased; DBP, diastolic blood pressure; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; FFA, free fatty acids. Subjects are listed by total subject numbers then number in each intervention group.

3.6 Future Perspectives

This review has focused on the efficacy of beetroot and blackcurrants to improve risk factors for MetS. Whilst there is currently strong evidence to suggest the compounds present within these foods may have positive effects on MetS and its risk factors, there remain some key gaps in the literature that need to be addressed before it can be concluded that blackcurrant and beetroot compounds improve MetS risk factors.

Firstly, current literature investigating beetroot and MetS risk factors focusses on dietary nitrate as the active ingredient, despite evidence to suggest that betalains may be having an additive or synergistic effect through other, currently unclear, pathways. It is important that future studies report both the betalain content as well as the nitrate content of interventions to ensure results can be compared between studies, and to enable the sources of any observed effects to be established. Following this, studies investigating nitrate-depleted beetroot may be useful in determining the potential of betalains to affect MetS outcomes. Nitrate-depleted beetroot juice has already been suggested to improve hypertension (109), however further work needs to be conducted to investigate its effects on hyperglycaemia and dyslipidaemia.

Interestingly, research by Sawicki and colleagues (130), who supplemented volunteers with red beetroot juice for 6 weeks, found that betalain concentrations were highest in the plasma after 1 week, and urine after 2 weeks, however the lowest levels for plasma and urine were found in weeks 3 and 4, respectively. The authors suggest that this results from a process of adaptation towards betalain metabolism, as has been shown for other phytochemicals (131). This may mean that to elicit changes in MetS risk factors, chronic supplementation with beetroot juice is more effective than acute supplementation, as the shift in metabolism can only take place after 2 weeks of exposure to betalains. Trials investigating chronic supplementation should be conducted in the future, measuring plasma and urine betalain concentrations as well as MetS risk factors to establish whether this is the case.

Furthermore, in both blackcurrant and beetroot trials, there is little information regarding the appropriate doses of bioactive ingredients to elicit changes, with anthocyanin levels in blackcurrant studies ranging from 105 mg (41) to 1200 mg (40) and nitrate content administration in beetroot juice studies ranging between 68.2 mg (98) and 990 mg (34). Future studies, particularly in areas such as hypertension, where considerable evidence currently exists should look to establish appropriate minimum dosages for eliciting the improvements in MetS risk factors. This would allow more appropriate administration of any lifestyle intervention, which may increase compliance within an at-risk population.

The majority of the studies evaluating the effects of blackcurrant anthocyanins on biomarkers of MetS have used blackcurrants and blackcurrant extracts from New Zealand which have reported higher anthocyanin levels (132) than non-New Zealand fruit/juice (46). It is speculative to suggest that New Zealand blackcurrant could be more beneficial in managing MetS and its risk factors, but more research focused on this possibility in well-designed RCTs is required.

3.7 Conclusions

This review aimed to collect and highlight scientific evidence regarding the role of beetroot and blackcurrant consumption in the possible prevention of MetS and its associated risk factors. The current evidence demonstrates that beetroot and blackcurrant have a positive effect on managing several MetS risk factors including dyslipidaemia, hyperglycaemia and hypertension. Beetroot and blackcurrant may improve glucose uptake by activating AMPK, GLUT-4 and SGLT-1 whilst also inhibiting breakdown of glucose in the intestines and saliva, leading to overall improvements in glucose control. Beetroot exhibits hypotensive effects through increasing NO availability through nitrate, but betalains may also have a role, as has been shown for structurally similar blackcurrant anthocyanins, through modulation of endothelial NO synthase (eNOS) expression and inhibition of the production of vasoconstriction-promoting molecules, although more research is required to demonstrate this conclusively. Finally, beetroot and blackcurrants may improve lipid profiles, however the mechanisms through which this takes place are not clear, and more mechanistic as well as RCTs are needed to strengthen the currently limited evidence.

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Cameron Haswell (C.H.), Kay Rutherfurd Markwick (K.R.M.), Ajmol Ali (A.A.), Rachel Page (R.P.) and Roger Hurst (R.H.) contributed to the conceptualization of the manuscript; CH., K.R.M., A.A., R.P., and RH contributed to the methodology; C.H., Hajar Mazahery (H.M.), performed formal analysis; C.H., conducted data collection and sample analysis; C.H., performed writing — original draft preparation; C.H., K.R.M., A.A., R.P., and R.H., contributed to writing — review and editing.

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Chapter 4 – Comparison of Plasma, Venous Whole Blood, and Capillary Blood Glucose Measurements during Oral Glucose Tolerance Tests: Implications for Dietary Interventions and Methodological Design

This chapter addresses Research Question 2 (see Section 1.3.4) and presents findings from a methodological study investigating the differences between blood glucose testing from plasma, venous whole blood and capillary blood during oral glucose tolerance tests under different conditions. This chapter has been prepared in manuscript format.

Abstract

Introduction: Accurate blood glucose measurement is vital in clinical and research settings to evaluate metabolic function, diagnose type 2 diabetes mellitus (T2DM), and assess intervention efficacy. The choice of blood glucose measurement site can influence the outcomes of trials. This study aimed to compare plasma, venous whole blood, and capillary blood glucose measurements during oral glucose tolerance tests (OGTT) and assess these measurements under both placebo and acute dietary nitrate (beetroot juice) intervention conditions.

Methods: Fifteen healthy adults (aged 18–60 years, BMI 18–30 kg/m^2) completed a randomised, crossover trial with two test conditions (placebo or beetroot juice containing 600 mg nitrate), each administered as part of a 75 g carbohydrate meal. Blood glucose was measured at baseline, 5, 15, 30, 45, 60, 90, 120 and 150 min postprandially using three methods: plasma glucose via glucose oxidase-peroxidase (GOD-PAP) assay (PLAS), venous whole blood via HemoCue 201+ (VWBH), and capillary blood via HemoCue 201+ (CAPH). Repeated-measures ANOVA was used to assess differences between sampling methods across timepoints, with further analyses exploring potential method-dependent treatment effects.

Results: Significant differences between measurements from the sampling methods were observed across both interventions ($p < 0.001$). Under placebo conditions, VWBH glucose values were consistently lower than PLAS, by 8.2–18.2% across timepoints ($p < 0.05$). CAPH values under placebo were lower than PLAS at baseline and 150 min ($p < 0.05$), but higher than PLAS at 30, 45, and 60 min ($p < 0.05$). In the beetroot juice condition (BCI), VWBH values were 9–15.3% lower than PLAS at most timepoints ($p < 0.05$), and CAPH was lower than PLAS at baseline but exceeded PLAS at 60, 90, and 120 min ($p < 0.05$). A significant early-phase (15 and 30 min) reduction in glucose with BCI versus placebo ($p = 0.012$) was detected in CAPH, suggesting a heightened sensitivity of capillary sampling to localised peripheral glucose uptake effects. No significant treatment effects were detected in PLAS measurements ($p = 0.327$).

Conclusions: This study demonstrates that plasma, venous whole blood, and capillary blood glucose measurements are not interchangeable during OGTTs, with each method capturing distinct aspects of postprandial glucose dynamics. Plasma glucose remains the standard for assessing systemic glucose control, particularly in diagnostic contexts. However, capillary blood sampling is more sensitive to localised and dynamic metabolic changes, such as peripheral glucose uptake. These findings underscore the importance of selecting the correct blood sampling method to align with the specific, metabolic mechanistic target being investigated.

Keywords: plasma glucose, venous whole blood glucose, glucose oxidase – peroxidase, postprandial glycaemia, sampling methodology, trial design.

4.1 Introduction

4.1.1 Background

Accurate and reliable measurement of blood glucose levels is essential in both clinical and research settings to evaluate metabolic function, diagnose conditions such as type 2 diabetes mellitus (T2DM), and assess treatment and intervention efficacy. In the context of research trials, selecting the appropriate blood collection method for glucose measurement is important for accurate analysis and interpretation of results. The method selected can affect the precision of blood glucose measures and the overall reliability of the data. It may also impact the feasibility of the study, particularly in trials with large cohorts that require cost-effective testing. Additionally, the blood collection method can influence participant adherence, both in terms of their willingness to participate and their ability to accurately follow protocols. Some methods and measurement devices are also capable of recording changes in blood glucose at different tissues or body sites. This is particularly relevant in randomised controlled trials (RCTs) that seek to understand the efficacy of acute interventions targeting glucose metabolism to develop effective methods of managing postprandial blood glucose levels.

Type 2 diabetes mellitus can be diagnosed through blood glucose measurements based on criteria established by major health organisations such as the American Diabetes Association (1). Key diagnostic biomarkers and methods include fasting plasma glucose (FPG) (2), oral glucose tolerance tests (OGTT) (3), and glycated haemoglobin (HbA1c) levels (2, 4). HbA1c is the gold standard for diagnosing T2DM due to its accuracy and stability against acute fluctuations in blood glucose levels. However, HbA1c measurement is unable to detect acute changes in glucose tolerance as it is a proxy for mean blood glucose levels over 3 months, making OGTT more appropriate for RCTs investigating acute/short-term (1-2 weeks) intervention strategies.

During OGTTs, blood glucose concentrations are measured following the ingestion of a glucose-rich drink or meal, typically containing 75 g of carbohydrate (CHO). Blood glucose concentrations are measured at baseline and at intervals of 15, 30 or 60 min, for 2 to 3 h, with more frequent intervals giving more accurate information of when blood glucose control is altered. The OGTT reveals the speed at which blood glucose levels rise and subsequently fall (i.e., how quickly glucose is utilised by tissues and cleared from the blood stream), providing a dynamic view of glucose metabolism and insulin response (5).

Blood glucose concentrations can be measured using serum or plasma, and blood collected either intravenously or from capillaries via finger or ear pricks. The method of blood glucose

analysis and the location of sample collection are important when considering trial design for RCTs. A summary of the differences between each sampling method can be found in Table 4.1

Plasma glucose samples are more stable than whole blood samples, as they are less influenced by changes in haematocrit and red blood cell glucose metabolism. Plasma glucose collected intravenously is less impacted by changes in peripheral perfusion and local glucose dynamics than capillary samples (6). Furthermore, plasma glucose measurements may not capture localised glucose changes, such as those occurring in skeletal muscle or peripheral tissues during postprandial periods, limiting their utility in intervention studies targeting tissue-specific glucose uptake (7).

Plasma samples are typically analysed for glucose using enzymatic methods such as glucose oxidase - peroxidase (GOD-POD) or hexokinase (6, 8, 9). Both methods use spectrophotometry and are considered the gold standard methods for determining glucose concentrations (8). The GOD-POD method uses a two-step enzymatic process whereby glucose oxidase catalyses the oxidation of β-D-glucose, producing hydrogen peroxidase (H_2O_2) as a byproduct, which is then detected spectrophotometrically (using peroxidase-mediated oxidation of chromogenic dyes) (10). Various chromogens can be used depending on the assay type, and one of the most frequently used is a quinonimine dye formed from phenol reacting with + 4-aminophenazone (PAP) which can be detected at a wavelength of ~500 nm (10). This assay method is known commonly as GOD-PAP. The cost per test is significantly higher than point-of-care (POC) devices, may strain research study budgets and is impractical for large scale studies involving multiple testing days. Furthermore, post-collection sample analysis from frozen plasma can introduce delays to data analysis and subsequent dissemination of findings from trials compared with POC devices (11).

Like plasma sampling, venous whole blood glucose testing involves intravenous blood sampling by trained phlebotomists. It provides a practical alternative to plasma glucose analysis in settings where centrifugation and analysis equipment are unavailable. However, whole blood samples contain both plasma and cellular components (RBC and haemoglobin), which influence glucose readings. Glucose concentrations in whole blood are typically 10-15% lower than plasma glucose due to intracellular metabolism of glucose by RBCs (12). Whole blood samples can be affected by atypical haematocrit levels, which alter the ratio of plasma to RBC, influencing glucose results. Other factors such as haemolysis, sample handling time, and temperature can affect the accuracy of whole blood glucose measurements, as glucose may degrade with heat or diffuse into red blood cells if samples are not analysed promptly (8).

Table 4.1. Comparison of the characteristics of venous plasma, venous whole blood and capillary whole blood sampling and analysis methods for

Characteristic	Blood Sample		
	Venous Plasma	**Venous Whole Blood**	**Capillary Whole Blood**
Collection Method	Intravenous blood draw. Centrifugation to separate plasma from RBCs.	Intravenous blood draw. Analysed as whole blood without centrifugation.	Finger or ear prick. Collected as whole blood.
Vacutainers	NaF & KF/EDTA to inhibit glycolysis and prevent coagulation, respectively.	No glycolysis inhibitors. Glucose may degrade over time if not processed quickly (8).	Typically, no anticoagulants. Used directly in POC devices.
Glucose Measurement Method	Enzymatic methods (GOD-POD, hexokinase) with spectrophotometry as gold standard (10). End-point assay.	GDH method in POC devices (e.g., HemoCue 201+). End-point assay.	GDH method in POC devices (e.g., HemoCue 201+ or handheld glucometers with electrochemical biosensors). End-point assay
Stability & Accuracy	Most stable. Minimal influence from haematocrit & RBC metabolism (12). Typical CV of 1 - 2% (8).	Lower stability than plasma. Influenced by haematocrit and RBC glucose metabolism. ~10-15% lower than plasma glucose (12). Typical CV of 1.6 - 1.8% (13, 14).	Least stable. Influenced by haematocrit, temperature, peripheral perfusion, and interstitial fluid contamination. Typical CV of 1.6 - 1.8% in HemoCue (13, 14) and 2.3 - 2.6% in handheld Glucometer (15).
Advantages	Highly accurate, reliable for diagnostics (e.g., OGTT). Removes RBC interference. Systemic glucose measure.	Much lower cost than plasma testing. Can be used when centrifugation is unavailable. Systemic glucose measure.	Least expensive. Convenient, less invasive, allows at-home testing. Captures microvascular glucose changes.
Limitations	Requires specialised lab equipment, centrifugation, trained phlebotomists and trained laboratory staff. Most expensive.	Glucose degrades over time. Affected by haematocrit variation. Venous sampling is invasive and required trained phlebotomists.	Affected by peripheral perfusion, haematocrit variation, and temperature. First blood drop contamination risk. Limited QC for handheld glucometers.
Best Use Case	Gold standard for research and clinical diagnostics (e.g. OGTT, systemic glucose monitoring).	Alternative when plasma testing is impractical, or specialist equipment is not available.	Ideal for large-scale, participant-driven studies and localised glucose dynamics research.
Cost Per Sample (NZD)	$10 - 15	$4 - 5	$3 - 4 via Hemocue. $1 - 2 via handheld glucometer.

RBC, Red Blood Cell; NaF, Sodium Fluoride; KF, Potassium Fluoride; EDTA, Ethylenediaminetetraacetic Acid; POC, Point-of-care; GOD-POD, Glucose Oxidase – Peroxidase; CV, Coefficient of Variation; GDH, Glucose Dehydrogenase; OGTT, Oral Glucose Tolerance Test; QC, Quality Control; NZD, New Zealand Dollars.

Instruments such as the HemoCue 201+ glucose analyser (HemoCue, Ängelholm, Sweden) provide rapid glucose measurement in venous whole blood or capillary glucose samples using the enzymatic glucose dehydrogenase (GDH) method for determining glucose levels. With this method, GDH oxidises β-D-glucose into gluconolactone, and nicotinamide adenine dinucleotide (NAD^+) is reduced to NADH proportionally to the rate of gluconolactone formation. NADH then reacts with a chromogen in the microcuvette which causes a colour change which can be detected by a dual-wavelength photometer. The blood glucose results are accurate within the normal haematocrit range (20–60%) but are less accurate than plasma glucose analysis (13, 14). Further, high haematocrit levels (e.g., in polycythaemia) can lead to underestimation of glucose, while low haematocrit levels (e.g., in anaemia) may cause overestimation (16, 17) as haemoglobin absorbs light across the 500-600 nm range, and the photometer measures at 520-550 nm, the most suitable wavelength for the chromogen (18). Venous sampling is still invasive, which can reduce participant compliance in studies with frequent sampling as in OGTTs.

Capillary blood, collected via finger or ear pricks, offers a convenient and low-cost alternative to venous sampling (19). Capillary blood for measurement of glucose levels provides insights into microvascular glucose levels, which is particularly useful in studies targeting localised changes in blood glucose dynamics at the tissue, such as through increased GLUT4 translocation (20, 21) or peripheral tissue perfusion (22-24). Capillary blood can also be collected at home using lancets and handheld glucometers such as the Caresens Dual Glucometer (i-Sens, Seoul, South Korea). These glucometers use the GDH method for determining blood glucose concentrations whereby blood is applied to a test strip containing GDH which oxidises β-D-glucose to produce gluconolactone. The coenzyme flavin adenine dinucleotide (FAD) acts as an electron acceptor, forming $FADH_2$. This creates an electrical current proportional to the glucose concentration, which is detected by an electrochemical biosensor. Glucometers are advantageous for at-home testing by research participants, reducing the burden on participants to visit laboratories for testing and allowing more frequent testing of capillary blood glucose levels for intervention periods lasting several days/weeks.

Capillary samples are whole blood samples and contamination from interstitial fluid during the collection of the first blood drop can introduce variability, emphasising the need to follow best practice during collection by discarding the first drop (7). Despite this, capillary glucose testing remains an important sampling method in T2DM research and is particularly suited for large-scale, participant-driven studies that prioritise participant convenience and at-home testing.

Understanding the relationships between blood glucose concentrations obtained from plasma, whole blood, and point-of-care capillary testing is crucial. Such comparisons are vital for designing targeted study protocols, particularly those involving OGTTs. As each method measures glucose levels from a different blood matrix or from a different site, different blood glucose concentrations are likely due to variations in glucose distribution following a meal/glucose load. These differences are particularly relevant during dynamic changes in blood glucose levels, such as postprandial periods or in individuals with altered microvascular function, which is commonly seen in T2DM (25).

The choice of blood collection method for glucose measurements can affect the interpretation of trial results and, consequently, the translation of research findings into clinical practice or research into exercise and nutritional interventions. Glucose measured from plasma using the GOD-PAP method provides the most precise diagnostic information, which is critical in studies focused on T2DM screening or early detection. However, capillary glucose measurements may be better suited for ongoing monitoring in T2DM and glucose management studies where participants are supplemented for long periods of time, or where differences in glucose levels between interventions may only be seen when testing at the capillary site. Firstly, monitoring of blood glucose levels at home via finger prick and glucometers is useful for reducing trial visits and increasing convenience for participants. Secondly, when detecting changes in glucose uptake at the skeletal muscle or peripheral tissues, capillary blood glucose measurements may provide a better insight into blood glucose changes in the microvasculature, the site targeted by many nutraceutical interventions, either through increased GLUT4 translocation (20, 21) or enhanced peripheral tissue perfusion (22-24) or both. In comparison, plasma glucose measurement may not detect these more subtle changes, as it is a measure of systemic glucose control rather than localised or transient glucose dynamics.

Beetroot juice has previously been shown to alter early-phase (30-60 min) blood glucose levels during OGTTs (26). This has been attributed to its high levels of dietary nitrate which increases nitric oxide (NO) production (27-29), which leads to increased GLUT4 translocation at the skeletal muscle (30, 31). Beetroot juice therefore provides a potential intervention which causes changes in glucose levels at the vascular periphery (compared with placebo), but possibly not at a systemic level.

This study aimed to compare plasma glucose levels analysed via glucose oxidase-peroxidase-aminophenazone phenol (GOD-PAP) method (PLAS), venous whole blood analysed via HemoCue (VWBH), and capillary blood glucose analysed via HemoCue (CAPH during an OGTT.

We also aimed to compare how beetroot juice, an intervention known to affect peripheral tissue glucose uptake (26, 32, 33), impacts these measures, given the differences in assessment site provided by plasma and capillary measurements. By comparing the relationships between the results generated from each of these methods, we sought to provide an analysis of their utility and implications for study design in a research context. This will aid researchers in selecting the most appropriate collection method for blood glucose based on the specific aims of their RCTs and anticipated outcome measures, ensuring that quality data collection aligns with research goals.

4.2 Methods

The data for this study were collected as part of a larger project comparing blood glucose responses after ingestion of a meal containing 75 g of carbohydrate, including either a beetroot beverage, blackcurrant beverage or placebo beverage. The data presented in this chapter present the comparison of both blood sampling method and resultant glucose measurement from the sample site. The data comparing blood glucose responses across the three beverage conditions are presented in Chapter 5. Full trial reporting details including randomisation, blinding, adverse events, and registration are provided in Chapter 5.

4.2.1 Study Population

Participants were recruited using a combination of poster advertising, social media outreach and word-of-mouth. Fifteen healthy participants completed data collection at all visits (Figure 4.1). Inclusion criteria for the study were: (i) aged 18 - 60 years, (ii) BMI of 18 - 30 kg/m^2, (iii) HbA1c < 41 mmol/mol and, (iv) fasting glucose < 5.7 mmol/L. Participants were excluded from the study if they were; (i) diagnosed with diabetes or any other chronic illness which might have affected the study results, (ii) smokers, (iii) taking medications which may affect glucose control or interfere with the intervention.

As this study comprised secondary analysis of data collected as part of another trial (Chapter 5), no a priori sample size calculation was conducted. A post-hoc power analysis using the observed means and standard deviations of the paired differences between PLAS and VWBH from the present dataset indicated the sample (n = 15) provided greater than 80% power to detect between-method differences at all timepoints across both conditions ($\alpha = 0.05$), with Cohen's d values ranging from 0.78 to 2.66.

Ethics approval for this study was granted by the Massey University Human Ethics Committee (Southern A, Application 20/34). As this study involved a secondary analysis of data collected as

part of a previously conducted trial (Chapter 5), independent clinical registration was not required. All participants were required to give written informed consent prior to participation in the study.

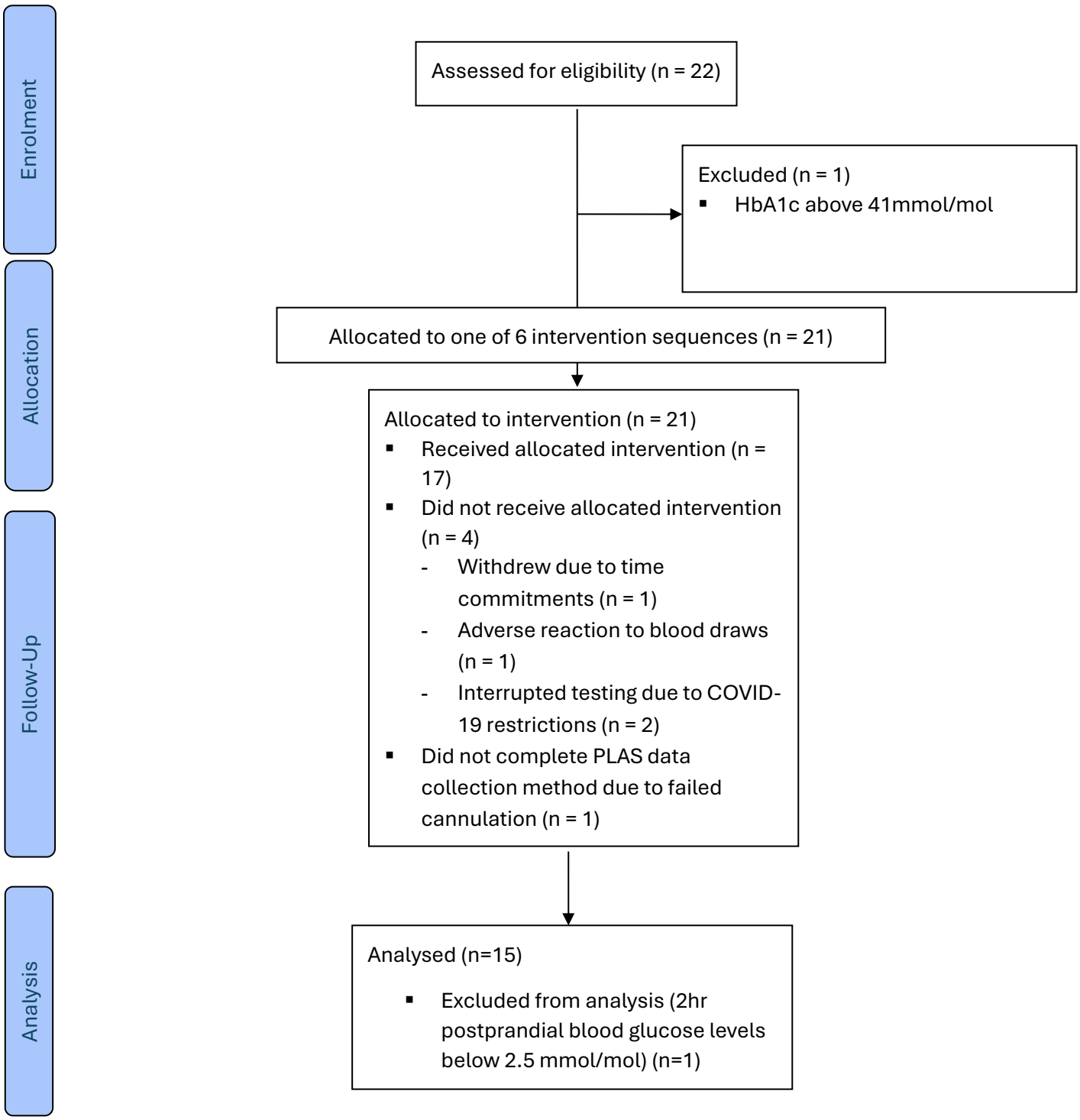

Figure 4.1. Study CONSORT flow diagram. A total of 22 participants were screened with 16 participants completing data collection at all timepoints. HbA1c, glycated haemoglobin A1c.

4.2.2 Study Design

This study used data from two testing arms (beetroot juice and placebo) of a larger, repeated measure, randomised, crossover trial. Participants attended an initial screening visit to determine their eligibility for the study. Researchers gathered information on medical and familial health history, demographic details, body measurements (waist and hip circumference ratio), height, weight, body mass index (BMI), body fat, and skeletal muscle mass using bioelectrical impedance analysis (BIA, InBody 270, Inbody, Seoul, South Korea). Additional measurements taken during screening were blood pressure (Omron HEM-7121, Omron, Kyoto, Japan), resting heart rate (Omron HEM-7121, Omron, Kyoto, Japan), HbA1c (Cobas® b 101 system, Roche Diagnostics, Basel, Switzerland), and fasting blood glucose (HemoCue Glucose 201+ System, HemoCue, Ängelholm, Sweden).

Participants arrived at the Massey University Sport and Exercise Science Laboratory (Auckland, New Zealand) for research visits 1, 2, and 3 after fasting for at least 10 h, and a cannula was inserted at the antecubital fossa using aseptic techniques. Fasting capillary (finger prick) and venous whole blood (cannula) samples were taken prior to ingestion of the test meal which comprised of toast (Nature's Fresh White Bread, 49.6 g) and jam (Pam's Apricot Jam, 12.5 g) with either 77.3 g of nitrate-rich beetroot juice concentrate (made up to 200 g with 122.7 g of water) containing 600 mg nitrate or a placebo beverage containing 42.9 g of sucrose and 1 g of red food colourant (made up to 200 g with 156.1 g of water). Each meal contained a total of 75 g of carbohydrate including the beverage, which was served in a pink glass to disguise the intervention. Following ingestion of the test meal, venous and capillary blood samples were collected over a further 150 min period (Figure 4.2). Blood samples were collected from both venous and capillary sources at baseline (-20 min) and timepoints 5, 15, 30, 45, 60, 90, 120 and 150 min post meal consumption. Participants were asked to consume the test meal and drink within a 10-min period and subsequent blood sample timings were taken from the end of meal consumption (Figure 4.2). Cannulas were flushed with saline between each sample collected.

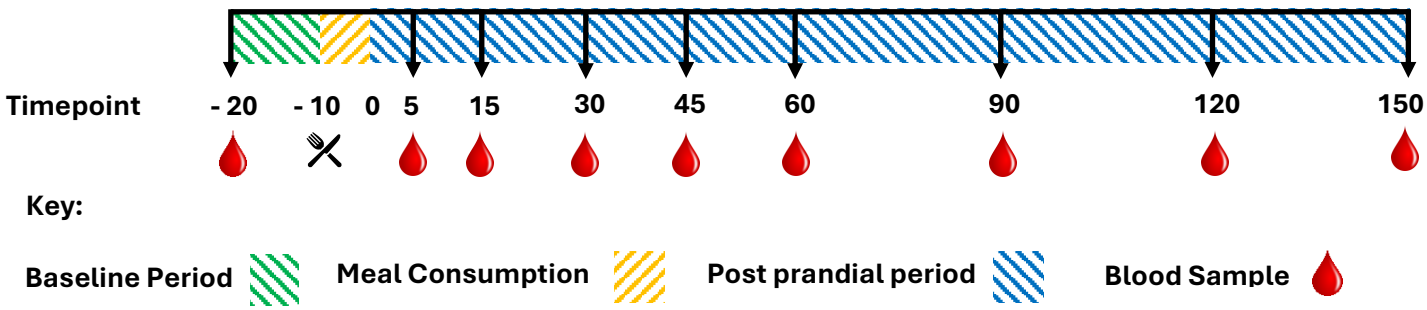

Figure 4.2. Timeline of study visits.

4.2.3 Sample Handling and Analysis

Venous blood samples were collected into fluoride/EDTA vacutainer tubes (glucose) from which 10 µl was used immediately for point-of-care analysis using the HemoCue Glucose 201+ System (HemoCue, Ängelholm, Sweden). The vacutainers were then centrifuged at 1,500 x *g* for 10 min then 500 µl of separated plasma was aliquoted into 2-ml Eppendorf tubes and stored at -80 °C for later analysis. Frozen plasma samples were transported to the Massey University Nutrition Laboratory (Palmerston North, New Zealand) on dry ice for analysis of glucose (via GOD-PAP method). Concurrent capillary blood samples (5 µl) were obtained using a sterile lancet prick to the finger, with blood applied directly to the HemoCue reagent cuvette. Samples were analysed immediately using the HemoCue Glucose 201+ System (HemoCue, Ängelholm, Sweden). A schematic of blood sampling and analysis is presented in Figure 4.3.

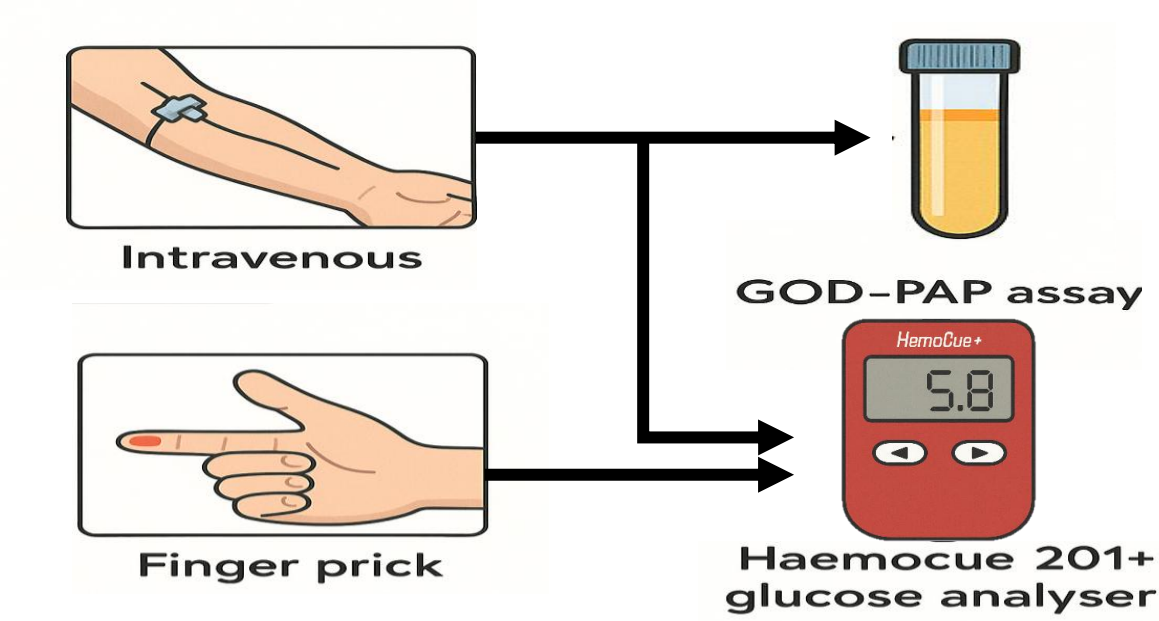

Figure 4.3. Schematic of blood glucose sampling and handling techniques. Samples from intravenous blood draws were analysed via Hemocue 201+ analyser (VWBH) and GOD-PAP method (PLAS). Finger prick samples were analysed via Hemocue 201+ analyser (CAPH). GOD-PAP, Glucose Oxidase-Peroxidase Phenol + 4-Aminophenazone. Component illustrations generated via ChatGPT (OpenAI DALL·E, 2025) (34).

4.2.3 Statistical Analysis

Blood glucose results measured from plasma samples using the GOD-PAP method (PLAS) , capillary samples via HemoCue 201+ (CAPH) and venous whole blood via HemoCue 201+ (VWBH) were compared using repeated-measures two-way ANOVA at each time point with 'method' and 'time' as the two factors, with post-hoc t-tests using LSD adjustment used to compare PLAS to VWBH and CAPH at each timepoint under the condition of a treatment*time

main effect. Separate ANOVAs were used to compare the results from placebo and beetroot conditions with the CAPH method and PLAS method to establish the importance of the sampling method based on experimental design and hypothesis, with post-hoc t-tests using Bonferroni adjustment to compare beetroot and placebo conditions. A *p*-value of less than 0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics (SPSS Inc., Chicago, IL, USA) for Windows version 24.0.

4.3 Results

Fifteen participants completed data collection using all three blood glucose collection methods in both the placebo and beetroot juice condition.

4.3.1 Comparison of Blood Glucose Collection Methods

There was a significant effect of time ($p < 0.001$), method ($p < 0.001$) and a method*time interaction ($p < 0.001$) on blood glucose levels in the placebo condition. Blood glucose concentrations for each sampling method across all timepoints are presented in Tables 4.2 and 4.3. Blood glucose values for VWBH were lower than PLAS at all timepoints ($p < 0.05$). Glucose levels in VWBH ranged from 8.2 - 18.2% lower than PLAS, with the smallest differences at 5, 15 and 30 min ($p < 0.05$; Figure 4.4). CAPH was lower than PLAS at baseline and 150 min but was higher than PLAS during the mid-phase of the OGTT (at 30 min, 45 min and 60 min; Figure 4.5).

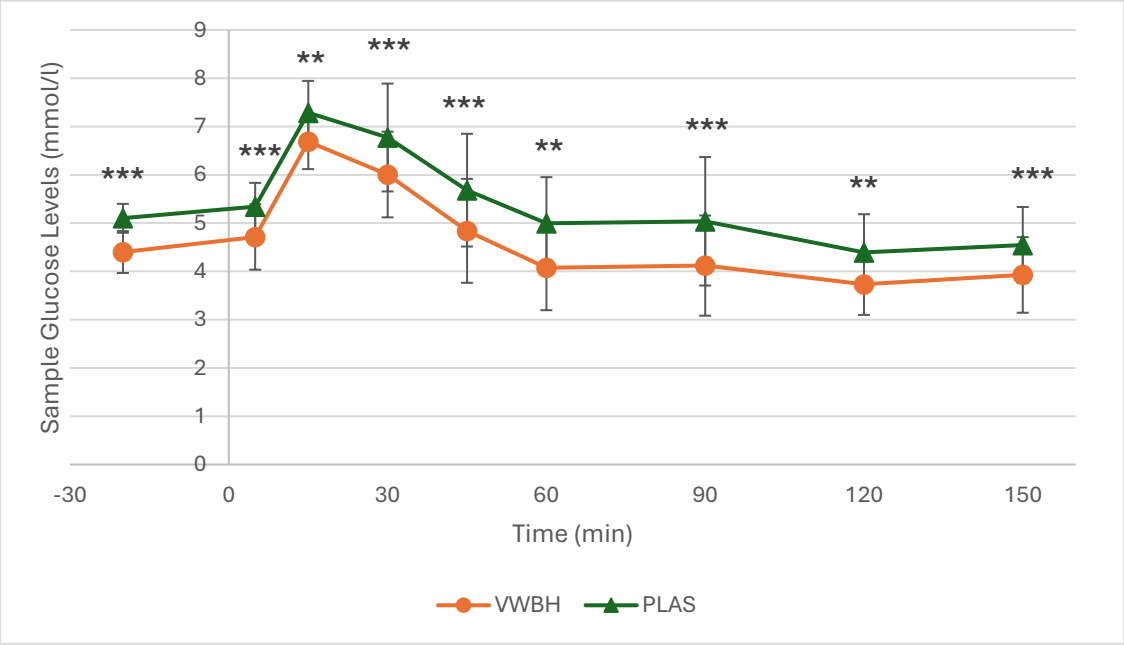

Figure 4.4. Comparison of blood glucose concentrations measured using plasma (PLAS) and venous whole blood (VWBH) methods across timepoints following consumption of the placebo beverage (n=15). -20 min represents baseline measurement timepoint. * indicates a significantly lower VWBH result than PLAS., * p < 0.05, ** p < 0.01, *** p < 0.001.

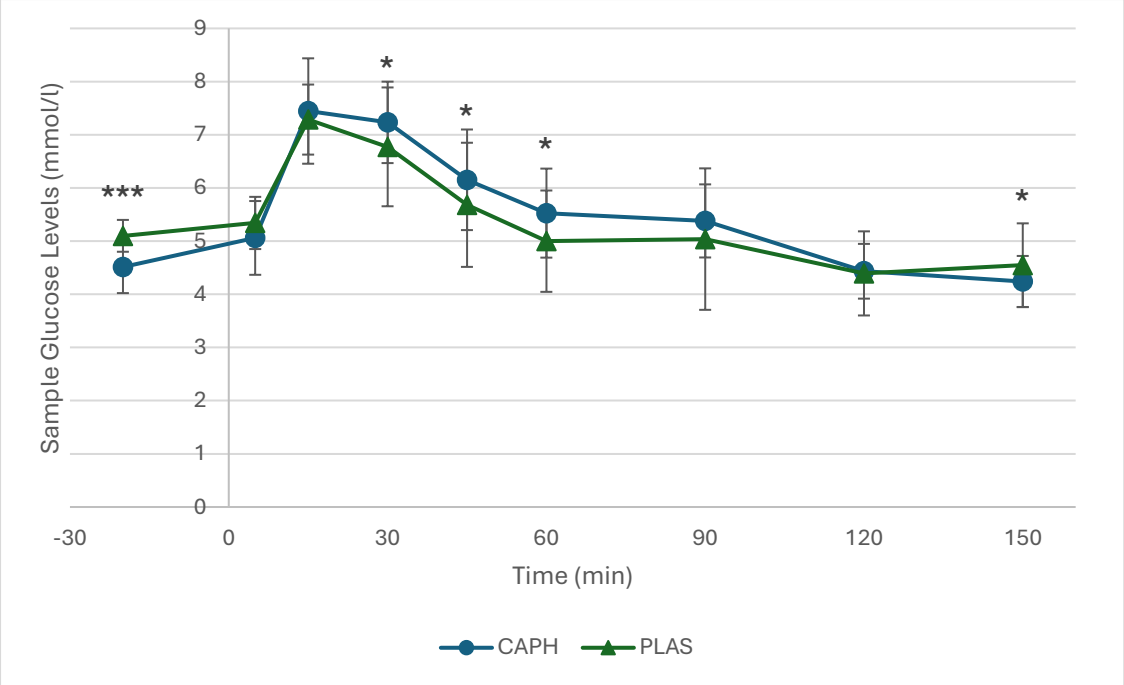

Figure 4.5. Comparison of blood glucose concentrations measured using plasma (PLAS) and capillary blood (CAPH) methods across timepoints during the placebo condition (n=15). -20 min represents baseline measurement timepoint. * indicates a significant difference between CAPH and PLAS., *, $p < 0.05$, *** $p < 0.001$.

Table 4.2. Blood glucose concentrations (mmol/L) by sampling method and timepoint for placebo conditions.

Placebo condition						
Time (min)	**Blood glucose concentration (mmol/L)**			**Between-method difference (mmol/L)**		
	CAPH Mean ± SD (95% CI)	**VWBH Mean ± SD (95% CI)**	**PLAS Mean ± SD (95% CI)**	**PLAS – VWBH Mean (95% CI)**	**CAPH – PLAS Mean (95% CI)**	**CAPH – VWBH Mean (95% CI)**
-20	4.51 ± 0.49 *(4.24, 4.78)*	4.40 ± 0.43 *(4.16, 4.64)*	5.10 ± 0.30 *(4.93, 5.26)*	0.70 *(0.45, 0.95)*	-0.59 *(-0.91, -0.26)*	0.11 *(-0.18, 0.40)*
5	5.06 ± 0.69 *(4.68, 5.44)*	4.71 ± 0.68 *(4.34, 5.09)*	5.34 ± 0.49 *(5.07, 5.61)*	0.63 *(0.32, 0.93)*	-0.28 *(-0.73, 0.17)*	0.35 *(-0.15, 0.84)*
15	7.45 ± 0.99 *(6.90, 8.00)*	6.69 ± 0.57 *(6.37, 7.00)*	7.29 ± 0.66 *(6.92, 7.65)*	0.60 *(0.17, 1.02)*	0.16 *(-0.28, 0.60)*	0.76 *(0.21, 1.31)*
30	7.23 ± 0.77 *(6.81, 7.66)*	6.01 ± 0.89 *(5.52, 6.50)*	6.77 ± 1.12 *(6.15, 7.39)*	0.77 *(0.45, 1.08)*	0.46 *(0.05, 0.87)*	1.23 *(0.87, 1.58)*
45	6.15 ± 0.95 *(5.63, 6.68)*	4.84 ± 1.08 *(4.24, 5.44)*	5.68 ± 1.17 *(5.04, 6.33)*	0.84 *(0.50, 1.18)*	0.47 *(0.04, 0.91)*	1.31 *(0.81, 1.82)*
60	5.53 ± 0.84 *(5.06, 5.99)*	4.07 ± 0.88 *(3.59, 4.56)*	5.00 ± 0.95 *(4.47, 5.53)*	0.93 *(0.37, 1.49)*	0.53 *(0.08, 0.98)*	1.45 *(0.74, 2.17)*
90	5.38 ± 0.69 *(5.00, 5.76)*	4.12 ± 1.04 *(3.55, 4.69)*	5.04 ± 1.33 *(4.30, 5.77)*	0.92 *(0.48, 1.35)*	0.34 *(-0.24, 0.93)*	1.26 *(0.81, 1.71)*
120	4.43 ± 0.52 *(4.15, 4.72)*	3.73 ± 0.63 *(3.38, 4.08)*	4.39 ± 0.79 *(3.95, 4.83)*	0.66 *(0.25, 1.07)*	0.04 *(-0.40, 0.48)*	0.70 *(0.41, 0.99)*
150	4.24 ± 0.48 *(3.97, 4.51)*	3.93 ± 0.78 *(3.49, 4.36)*	4.55 ± 0.79 *(4.11, 4.98)*	0.62 *(0.28, 0.96)*	-0.31 *(-0.63, 0.01)*	0.31 *(-0.02, 0.65)*

Values are mean ± SD with 95% confidence intervals in parentheses (n = 15). Differences are unadjusted paired means. CAPH, capillary blood glucose via HemoCue 201+; VWBH, venous whole blood glucose via HemoCue 201+; PLAS, venous plasma glucose via GOD-PAP assay. Positive difference values indicate the first-named method exceeded the second; negative values indicate the reverse.

Table 4.3. Blood glucose concentrations (mmol/L) by sampling method and timepoint for beetroot conditions

Beetroot condition						
Time (min)	**Blood glucose concentration (mmol/L)**			**Between-method difference (mmol/L)**		
	CAPH Mean ± SD (95% CI)	**VWBH Mean ± SD (95% CI)**	**PLAS Mean ± SD (95% CI)**	**PLAS – VWBH Mean (95% CI)**	**CAPH – PLAS Mean (95% CI)**	**CAPH – VWBH Mean (95% CI)**
-20	4.41 ± 0.49 *(4.14, 4.68)*	4.36 ± 0.47 *(4.10, 4.62)*	5.13 ± 0.30 *(4.96, 5.29)*	0.77 *(0.51, 1.03)*	-0.72 *(-0.98, -0.46)*	0.05 *(-0.29, 0.39)*
5	5.01 ± 0.88 *(4.52, 5.49)*	4.72 ± 0.53 *(4.42, 5.02)*	5.30 ± 0.47 *(5.04, 5.56)*	0.58 *(0.29, 0.88)*	-0.30 *(-0.74, 0.14)*	0.29 *(-0.25, 0.83)*
15	6.59 ± 0.86 *(6.11, 7.06)*	6.04 ± 0.91 *(5.54, 6.54)*	7.13 ± 0.92 *(6.62, 7.64)*	1.09 *(0.86, 1.32)*	-0.54 *(-1.18, 0.09)*	0.55 *(-0.13, 1.22)*
30	6.33 ± 1.05 *(5.75, 6.91)*	5.69 ± 0.77 *(5.26, 6.11)*	6.64 ± 1.23 *(5.96, 7.32)*	0.95 *(0.52, 1.38)*	-0.30 *(-0.75, 0.15)*	0.65 *(0.07, 1.23)*
45	5.69 ± 1.01 *(5.12, 6.25)*	4.84 ± 0.96 *(4.31, 5.37)*	5.54 ± 1.09 *(4.94, 6.14)*	0.70 *(0.39, 1.02)*	0.14 *(-0.37, 0.66)*	0.85 *(0.24, 1.45)*
60	5.39 ± 0.83 *(4.93, 5.85)*	4.20 ± 0.86 *(3.73, 4.67)*	4.75 ± 0.87 *(4.27, 5.23)*	0.55 *(0.33, 0.77)*	0.64 *(0.15, 1.12)*	1.19 *(0.71, 1.67)*
90	4.94 ± 0.65 *(4.58, 5.30)*	3.89 ± 0.55 *(3.58, 4.19)*	4.31 ± 0.73 *(3.90, 4.71)*	0.42 *(0.22, 0.62)*	0.63 *(0.33, 0.94)*	1.05 *(0.70, 1.41)*
120	4.75 ± 0.77 *(4.32, 5.17)*	3.83 ± 0.83 *(3.37, 4.30)*	4.29 ± 0.80 *(3.85, 4.73)*	0.46 *(0.18, 0.73)*	0.46 *(0.01, 0.91)*	0.91 *(0.47, 1.36)*
150	4.26 ± 0.57 *(3.95, 4.57)*	3.95 ± 1.18 *(3.30, 4.60)*	4.34 ± 0.58 *(4.02, 4.66)*	0.39 *(-0.06, 0.84)*	-0.0 *(-0.36, 0.21)*	0.31 *(-0.01, 0.63)*

Values are mean ± SD with 95% confidence intervals in parentheses (n = 15). 95%. Differences are unadjusted paired means. CAPH, capillary blood glucose via HemoCue 201+; VWBH, venous whole blood glucose via HemoCue 201+; PLAS, venous plasma glucose via GOD-PAP assay. Positive difference values indicate the first-named method exceeded the second; negative values indicate the reverse.

There was a significant effect of time ($p < 0.001$), method ($p < 0.001$) and a method*time interaction ($p < 0.001$) on blood glucose levels in the beetroot group. Blood glucose values for VWBH ranged from between 9 -15.3% lower than PLAS at all timepoints other than at 45 min and 90 min ($p < 0.05$) (Figure 4.6). The CAPH was lower than PLAS at baseline but was higher than PLAS in the late phases of the OGTT; at 60 min 90 min and 120 min (Figure 4.7).

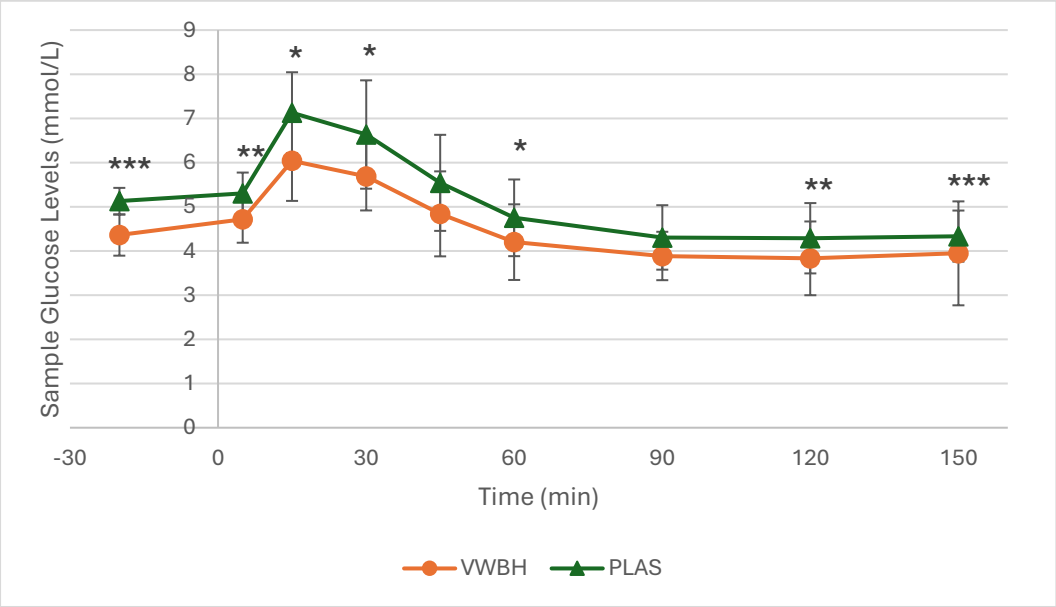

Figure 4.6. Comparison of blood glucose concentrations measured using plasma (PLAS) and venous whole blood (VWBH) methods across timepoints during the beetroot condition (n=15). -20 min represents baseline measurement timepoint. * indicates a significantly lower VWBH result than PLAS. *, p < 0.05, ** p < 0.01, *** p < 0.001.

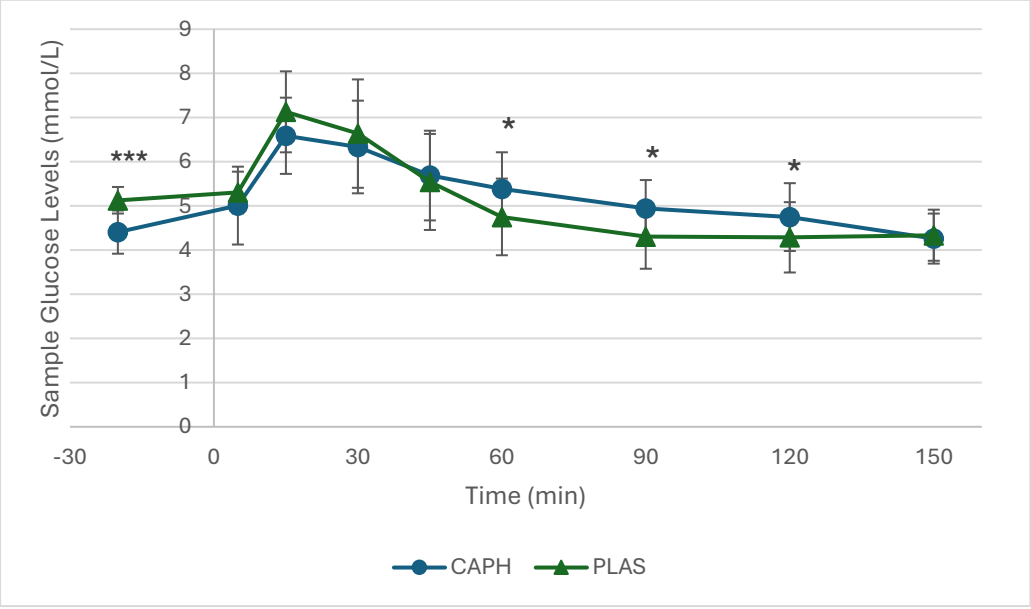

Figure 4.7. Comparison of blood glucose concentrations measured using plasma (PLAS) and capillary blood (CAPH) methods across timepoints during the beetroot condition (n=15). -20 min represents baseline measurement timepoint. * indicates a significant difference between CAPH and PLAS. *, p< 0.05. ***, p < 0.001.

4.3.2 Comparison of Treatments

Capillary blood glucose results showed a significant time*treatment interaction (p = 0.012). Capillary glucose levels were lower in the beetroot than the placebo group in the early phase (timepoints 15 min and 30 min) of the OGTT (Figure 4.8). For plasma glucose levels, there was an effect of time ($p < 0.001$), but no effect of treatment (p = 0.327) or treatment*time (p = 0.917) on blood glucose levels.

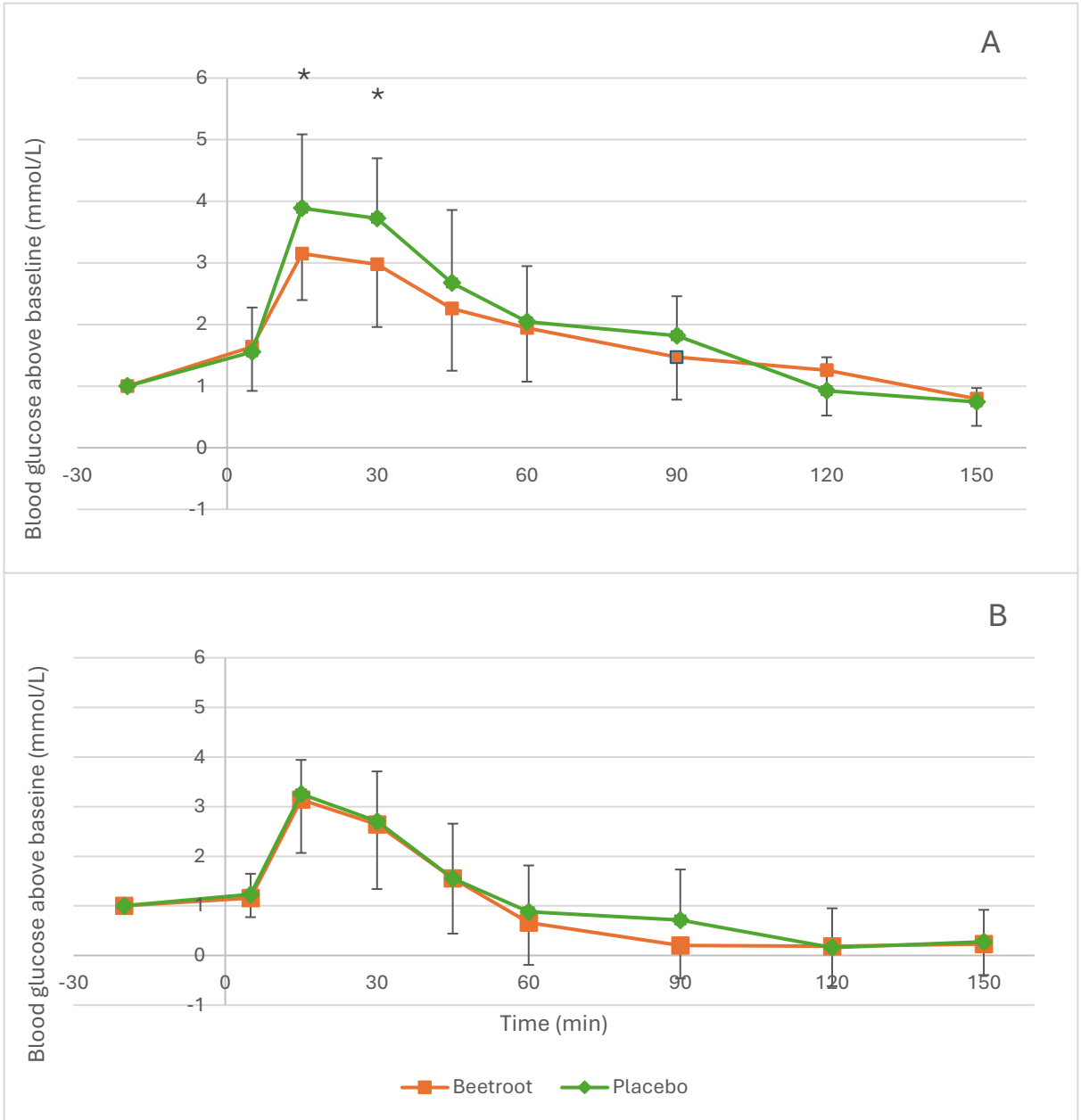

Figure 4.8. Incremental capillary (A) and plasma (B) glucose concentrations above baseline (+1) during OGTT across beetroot and placebo interventions. Values are means (n=15) with standard deviations represented by vertical error bars. For clarity, negative error bars are shown on beetroot and positive error bars presented on placebo. For visual clarity, one was added to each of the glucose mean values to prevent them falling below the x-axis. * indicates a significant difference between beetroot and placebo (A). No significant differences were observed for plasma glucose concentrations between the beetroot and placebo interventions (B).

4.4 Discussion

This study compared three blood collection methods for measuring blood glucose levels, plasma glucose analysed via GOD-PAP assay (PLAS), venous whole blood glucose analysed via HemoCue (VWBH), and capillary blood glucose analysed via HemoCue (CAPH) following a placebo and beetroot beverage intervention, to evaluate their ability to capture dynamic blood glucose responses. The findings revealed significant differences in glucose levels between these methods, and significant differences in the effect of intervention on blood glucose levels, with implications for the interpretation of results in research and clinical contexts.

4.4.1. Venous Plasma Glucose via Glucose Oxidase Peroxidase Spectrophotometry

The PLAS blood glucose results were consistently higher than VWBH across all timepoints in the placebo condition and all timepoints but 45 min and 90 min in the beetroot condition. As previously mentioned, plasma glucose sampling using GOD-PAP assays for analysis is commonly used in laboratories due to its high accuracy, precision and stability. It is unaffected by variability caused by cellular components or post-collection glycolysis making it highly reproducible and precise (35). This method of sample collection and analysis aligns with guidelines from major health organisations, such as the American Diabetes Association, which recommend plasma glucose assessment using GOD-PAP or hexokinase assays for diagnostic purposes (8).

However, intravenous blood sample collection methods, like those used for the PLAS and VWBH samples, measure systemic glucose levels combined from all tissues and organs, and represent the equilibrium state of hepatic glucose production, peripheral glucose uptake and insulin and glucose action, and therefore do not reflect localised metabolic activity at specific tissues. For example, in this study, while PLAS accurately assessed whole body glucose dynamics, it did not capture the early-phase reductions in blood glucose levels detected by CAPH at the tissue during the 0 - 30 min period. This limitation suggests that while the PLAS method is ideal for diagnostic and steady-state measurements, measuring blood glucose from blood collected intravenously may miss critical metabolic nuances in studies targeting postprandial or localised glucose changes.

4.4.2. Venous Whole Blood Glucose via HemoCue 201+

Venous whole blood glucose values measured using the HemoCue 201+ were consistently lower than plasma samples analysed using the GOD-PAP assay, a finding supported by previous

research showing that whole blood glucose concentrations are influenced by haematocrit and red blood cell glucose uptake (17). Measurements of VWBH consistently lower compared with PLAS by 8 - 18% across timepoints. Previous literature has shown venous whole blood glucose to be 11.3% lower than venous plasma glucose (12), which falls within the range seen in this study. The level of difference between PLAS and VWBH glucose measurements varied depending on the timepoint, with those in the early phase (5 – 30 min) tending to be closer in value to each other (8.2 – 11.3% lower than PLAS) than the later timepoints (60 – 150 min; 13.6 – 18.5% lower than PLAS). Blood glucose values measured from venous whole blood tend to be lower than from plasma because RBCs present in the whole blood sample take up glucose through GLUT1 co-transporters on their surface membrane, and the presence of these cells reduces the relative plasma volume and total water content of the sample, diluting the concentration of glucose when compared with plasma, which is composed almost entirely of the aqueous phase where glucose is distributed (29). During the early phase of the OGTT, the venous whole blood glucose measures are more similar to that of the plasma samples as glucose has been rapidly absorbed into the plasma, but not yet into the RBCs in the whole blood (36). The consistency in trends between the PLAS and VWBH results suggests that venous whole blood analysis via HemoCue may be a reliable alternative to PLAS in settings where access to laboratory facilities capable of measuring plasma glucose using either the PLAS or hexokinase methods are unavailable. It is important however, to be mindful of the disparities in magnitude of difference between plasma glucose and whole blood glucose due to the delay in equilibration of glucose within RBCs and plasma in the early phase (0 – 30 min) of OGTTs, so any correction factors used do not overestimate blood glucose levels.

Venous whole blood testing offers a practical balance between the accuracy of plasma analysis via enzymatic assay and the accessibility of capillary sampling using POC devices as it does not need specialised laboratory analysis procedures, particularly for moderately sized studies or fieldwork. However, like the PLAS results, the VWBH results reflect systemic glucose levels and may not capture localised changes that can be observed with CAPH. This makes venous whole blood sample collection less suited for studies investigating interventions that are primarily proposed to affect peripheral glucose metabolism.

4.4.3. Capillary Blood Glucose via HemoCue 201+

Capillary blood glucose measured by HemoCue 201+ (CAPH) detected greater differences in blood glucose between the beetroot and placebo interventions compared with PLAS and VWBH, particularly during the early postprandial phase (0 - 30 min). These changes are consistent with the literature highlighting the influence of peripheral perfusion, capillary blood flow, and tissue

glucose uptake on capillary blood glucose measurements (19, 25). For example, the early-phase reductions in CAPH blood glucose levels following beetroot juice beverage consumption may reflect enhanced glucose uptake in peripheral tissues, a phenomenon that is less apparent in systemic measurements when comparing blood glucose changes during OGTT using plasma or venous whole blood.

Many factors can affect capillary blood glucose measurements, and previous studies have shown capillary blood glucose measures have slightly higher coefficient of variations than venous whole blood samples (1.8% vs 1.6%) (13). Johannis and colleagues (7) showed that using the first blood droplet during capillary sampling results in consistently lower blood glucose readings than subsequent droplets due to the presence of interstitial fluid. Although wiping away the first drop of blood can reduce measurement error, human error in sample collection can still add error to the results, particularly if researchers are not properly trained or are complacent. Despite this, measurement of blood glucose levels from capillary samples is low cost and its ease of use make it particularly suitable for large-scale longitudinal studies, but its limitations in precision must be recognised, accepted and accounted for when interpreting results.

4.4.4. Method-Specific Observations in Response to Intervention

The observed differences in blood glucose results across CAPH, PLAS and VWBH underscore the critical role of blood collection methodology in study design and study quality and robustness. The CAPH results showed that the beetroot intervention reduced early-phase blood glucose responses and peaks compared with the placebo intervention, as has been shown previously (26, 37). We anticipated that the dietary nitrate in beetroot juice would enhance glucose uptake in the skeletal muscle through increased GLUT4 translocation and improved microvascular perfusion. The CAPH method's ability to measure glucose in the microvascular context provides insights that the PLAS and VWBH results, which reflect systemic glucose, could not provide. For instance, had this study relied solely on blood samples collected intravenously, the early-phase reductions in CAPH glucose would not have been detected, potentially leading to incomplete or misleading conclusions about the effects of the dietary intervention.

The results from this study highlight the importance of aligning the choice of blood collection method with the study objectives. Plasma glucose measured using GOD-PAP or hexokinase assay should remain the method of choice for studies prioritising diagnostic precision or systemic glucose dynamics. However, for research focusing on acute interventions or localised metabolic effects, capillary blood offers the unique advantage of measuring glucose uptake at the tissue, which may outweigh its limitations in measurement variability.

Our findings align with previous research demonstrating blood collection site-specific differences in glucose measurements. For example, Shen and colleagues (6) reported that blood glucose levels measured using capillary blood, tend to underestimate systemic glucose during rapid postprandial changes due to uptake of glucose in peripheral tissues via GLUT4 transporters in insulin-sensitive tissues, such as skeletal muscle and adipose tissue. Similarly, studies comparing blood glucose collected as venous whole blood and venous plasma have consistently found lower blood glucose values in venous whole blood of between 10 - 15%, attributed to the difference in glucose concentration between plasma and RBCs, as well as haematocrit-related effects (11, 12). The early-phase reductions observed in CAPH glucose following dietary intervention agrees with research highlighting the sensitivity of capillary measurements for detecting localised metabolic changes, including enhanced tissue perfusion and glucose uptake (25). These findings emphasise CAPH's utility in capturing dynamic glucose responses, which are often too small to detect using samples collected intravenously.

4.4.5. Limitations

All participants in this study were healthy adults aged 18-60 years with BMIs within the normal range and normoglycaemic fasting glucose and HbA1c values. Findings may therefore not be representative of populations with altered metabolic profiles, such as those with insulin resistance, prediabetes, or type 2 diabetes mellitus, or conditions known to influence haematocrit and peripheral perfusion, including anaemia and polycythaemia, all of which may disproportionately affect capillary and venous whole blood glucose measurements. More work needs to be done to establish whether these findings are relevant for clinical or research setting involving such populations.

Haematocrit, peripheral blood flow, and microvascular perfusion were not measured concurrently with blood glucose sampling. Since haematocrit is a known source of variability for both the HemoCue 201+ and capillary glucose measurements (14, 15), its absence prevents direct assessment of the contribution of individual differences in red blood cell volume to between-method discrepancies. The lack of peripheral perfusion data similarly limits the mechanistic interpretation of capillary glucose differences attributed to beetroot juice supplementation. The interpretation that capillary glucose differences following beetroot juice supplementation reflect localised peripheral glucose uptake is mechanistically plausible but remains inferential, as this study did not directly measure tissue glucose uptake, GLUT4 translocation, nitric oxide concentrations, or skeletal muscle metabolism. These conclusions should be confirmed through studies incorporating direct measures of peripheral metabolic activity and vascular function.

This study quantifies proportional differences between sampling methods but does not derive or validate correction factors for converting between them. Given that the magnitude of differences varied across timepoints, especially during the early postprandial phase, a fixed correction factor would be unlikely to adequately account for the bidirectional nature of the differences between sampling methods dependent upon postprandial period. Future work deriving method-specific corrections equations across different glucose challenge conditions would strengthen the applicability of these findings.

4.4.6. Implications and Conclusions

The findings from the current study suggest several key considerations for research design. For research studies investigating localised or acute changes in postprandial blood glucose levels in response to intervention, blood glucose measurement from capillary blood glucose samples is ideal for capturing rapid microvascular glucose changes and comparing interventions effectively. However, for diagnostic purposes, plasma glucose analysis via GOD-PAP or hexokinase remains the most reliable methods for studies requiring high precision and reproducibility, as well as those focused on systemic glucose regulation or diabetes diagnosis. Researchers should therefore consider mixed method approaches to utilise the strengths of each method. For example, research using prolonged interventions to enhance insulin sensitivity or glucose tolerance could combine blood glucose measurement via plasma sampling for systemic assessments of overall changes in glucose dynamics with capillary blood glucose measurements to gain insight into localised changes at the skeletal tissue. This combination may help highlight the location where possible improvements in glucose uptake and insulin sensitivity are occurring due to the intervention.

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Chapter 5 – The Effects of Beetroot and Blackcurrant Juice Supplementation on Postprandial Blood Glucose Responses in Healthy Individuals

This chapter addresses Research Question 3 (see Section 1.3.4) and presents findings for the BOP (Beetroot and Blackcurrant on Postprandial Blood Glucose) study. This chapter has been presented in manuscript format. The preliminary results for this chapter have also been presented at the UK Nutrition Society Summer Conference 2022:

Haswell C, Ali A, Page R, Hurst R, Rutherfurd-Markwick K. Acute supplementation of beetroot but not blackcurrant juice manages postprandial blood glucose responses in healthy adults. *Proceedings of the Nutrition Society*. 2022 Jan;81(OCE5):E228.(Appendix 1)

Abstract

Introduction: Postprandial hyperglycaemia is a key contributor to metabolic dysfunction in the pathogenesis of type 2 diabetes mellitus and metabolic syndrome. Dietary interventions using polyphenol- and nitrate-rich foods have emerged as promising strategies to attenuate glycaemic responses following meals. This study investigated the acute effects of beetroot (BR) and blackcurrant (BC) juice supplementation on postprandial blood glucose and insulin responses in healthy adults.

Methods: In a randomised placebo-controlled crossover trial, 16 healthy adults (aged 18-60 y) consumed a carbohydrate-rich test meal (75 g CHO including beverages) with BR juice (600 mg nitrate), BC juice (120 mg anthocyanins), or a sugar-matched placebo (PL), across three visits separated by ≥7-day washout periods. Capillary and venous bloods were collected at baseline, 5, 15, 30, 45, 60, 90, 120 and 150 min postprandially to assess glucose and insulin levels. Blood pressure was measured at baseline, 60 and 120 min. Primary outcomes included early-phase incremental area under the curve (iAUC) for glucose and insulin, and the glucose peak above baseline.

Results: BR juice supplementation significantly reduced early-phase (0-30 and 0-60 min) capillary glucose iAUC and the glucose peak compared with PL ($p < 0.05$), without altering plasma glucose or insulin iAUC. BC juice supplementation reduced early insulin iAUC (0-30 min) compared with BR and PL, though differences were not statistically significant in pairwise comparisons. Neither intervention significantly affected blood pressure relative to PL.

Conclusion: Acute BR juice consumption attenuates early-phase postprandial glucose responses to carbohydrate loads, potentially via nitric-oxide mediated insulin-independent increases in peripheral glucose uptake. BC juice may modestly influence early insulin dynamics by improving insulin sensitivity. These findings support BR and BC juice as promising functional foods to improve postprandial glucose control, with implications for cardiometabolic disease prevention.

Keywords: Glycaemic control, dietary nitrate, anthocyanins, betalains, insulin sensitivity, postprandial metabolism

5.1 Introduction

Glucose homeostasis, the balance between glucose uptake, storage, and utilisation, is essential for overall health and well-being (1). Impaired glucose homeostasis, also known as insulin resistance, is a key hallmark feature of type 2 diabetes mellitus (T2DM) and is associated with numerous chronic diseases such as metabolic syndrome (MetS) (2), cardiovascular disease (3, 4), and non-alcoholic fatty liver disease (5, 6). Managing glucose homeostasis before the onset of T2DM, either during normal glucose regulation or prediabetes, is associated with a significantly reduced risk of future diabetes incidence (7). Lifestyle changes to improve metabolic status are effective in contributing to healthy glucose management (8-10), as well as pharmaceutical interventions such as metformin intake/supplementation during prediabetes (11). In recent years, functional foods and dietary interventions have received attention as potential strategies to maintain normal glucose homeostasis, or, as alternatives to medication at the prediabetic and T2DM stages for improving glucose homeostasis (12-20).

Meal consumption induces a rise in blood glucose concentrations to levels that trigger oxidative stress tissues in blood vessels (21, 22), and in the case of prolonged hyperglycaemia, as seen in T2DM and prediabetes, the production of harmful advanced glycation end products (AGEs) and reactive oxygen species (ROS) and nitrogen species (RNS). Repeated exposure to ROS, RNS and AGEs promote endothelial dysfunction and impairs insulin signalling pathways, ultimately reducing peripheral glucose uptake in skeletal muscle and adipose tissue (23-25). In healthy individuals, blood glucose levels rise then return to normal levels within two hours of meal consumption due to insulin-stimulated glucose clearance in the skeletal muscle (26). However, individuals with compromised metabolic systems experience greater and longer postprandial blood glucose rises compared with healthy individuals (27), resulting in increased reactive oxygen species (ROS) production and subsequent increases in AGEs. Both ROS and AGEs contribute to decreased pancreatic duodenal homeobox-1 (PDX-1) binding, reducing insulin gene transcription and therefore insulin release from pancreatic β-cells (24, 28). Furthermore, glucotoxicity decreases insulin sensitivity at the liver, skeletal muscle and adipose tissue due to decreased transcription of glucose transporter proteins to the cell membrane (29), impairing the body's capacity to clear circulating glucose. Reducing the impact of postprandial glucose increases may be an effective method to reduce the damage caused by ROS and AGEs in healthy individuals and those with impaired glucose tolerance.

Blackcurrants and blackcurrant juice are high in anthocyanins, a group of antioxidant pigments responsible for their dark purple and red colour (30-34). Blackcurrant anthocyanins have been

shown to improve blood pressure (35, 36) and lipid profiles (37-40), and blackcurrant extract may improve glucose homeostasis (40-42), however evidence for the effects of blackcurrant supplementation on glucose control through juice is equivocal (40, 43, 44).

Beetroot and beetroot juice are high in betalains (45-50) as well as dietary nitrate (51-53). Ingestion of dietary nitrate increases nitric oxide production (54, 55), which in turn lowers blood pressure, and regulates insulin secretion, glucose uptake and hepatic glucose output (56). Elevating levels of oral nitrite through consumption of nitrate-rich beetroot juice increases the pH of the oral cavity, inhibiting salivary amylase activity (57), leading to delayed glucose absorption across the intestinal wall, and increasing glucose transporter type 4 (GLUT4) translocation (20, 58-61). While this may not improve incremental area under the curve during oral glucose tolerance tests (OGTT), there appears to be a lower peak in blood glucose levels, which may lower the generation of AGEs and oxidative damage associated with hyperglycaemic episodes. A similar inhibitory effect has been shown with blackcurrant juice; blackcurrant anthocyanins inhibit the activity of two key enzymes (alpha-glucosidase and alpha-amylase) involved in the breakdown of carbohydrate into glucose (62, 63) resulting in delayed glucose absorption and reduced postprandial glucose peaks (41).

Dietary interventions that reduce postprandial blood glucose peaks may help improve blood glucose control and reduce incidence of prediabetes and progression to T2DM. The present study aimed to establish whether beetroot and/or blackcurrant juices, when taken acutely with a carbohydrate meal, mediate postprandial blood glucose responses. It was hypothesised that BR and BC juice would attenuate early-phase postprandial glucose responses compared with PL.

5.2 Methods

5.2.1 Study Population

Participants were recruited from Auckland, New Zealand using a combination of poster advertising, social media outreach and word-of-mouth. Inclusion criteria for the study were; (i) aged 18-60 years of age, (ii) BMI of 18-30 kg/m^2, (iii) HbA1c < 41 mmol/mol and, (iv) fasting glucose < 5.7 mmol/L. Participants were excluded from the study if they were; (i) diagnosed with diabetes or any other chronic illness which might affect the study results, (ii) smokers, (iii) taking medications which may affect glucose control or interfere with the intervention drinks.

Participant recruitment commenced in January 2020 and data collection was completed in December 2020. A priori sample size calculations indicated that 15 participants were required to

achieve 80% power to detect the primary between-condition difference in capillary glucose iAUC 0–60 min (Cohen's d = 0.74; α = 0.05), based on prior literature reporting early-phase postprandial glucose reductions of approximately 20–30% following acute beetroot juice supplementation (20). Sixteen participants were recruited to allow for potential attrition (Figure 5.1).

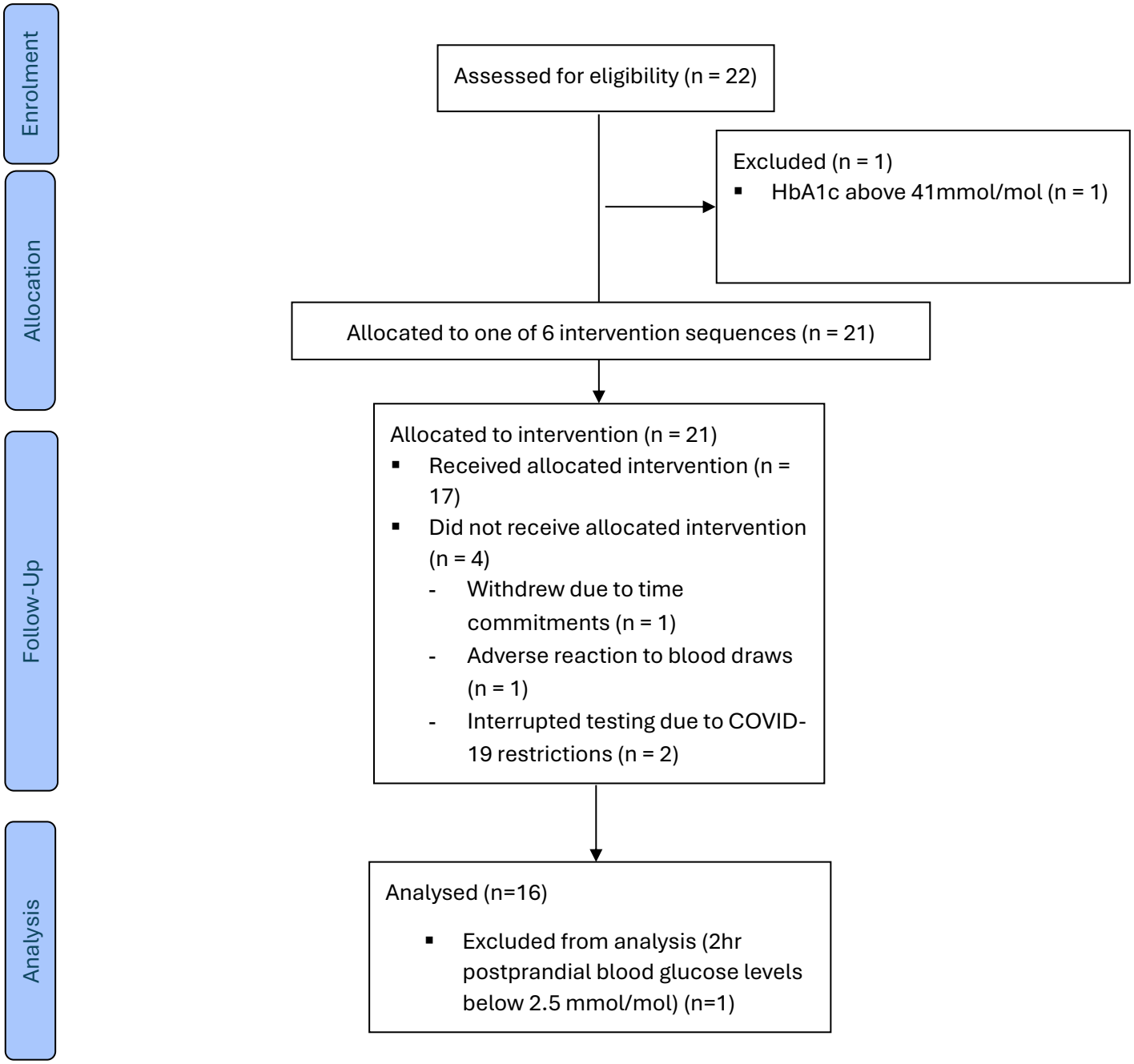


Figure 5.1. Study CONSORT flow diagram. A total of 22 participants were screened with 16 participants completing data collection at all timepoints. HbA1c, glycated haemoglobin A1c.

This study was approved by the Massey University Human Ethics Committee (Southern A, Application 20/34). This trial was not prospectively registered. A formal pre-published protocol was not available for this study. All participants were required to give informed consent prior to participation in the study.

5.2.2 Study Design

The study was conducted as a repeated measure, randomised, crossover trial with 3 treatment arms: beetroot (BR), blackcurrant (BC) and placebo (PL), with a 7-day washout period between study visits. Treatment order was randomised across six possible sequences using a Latin square design, balanced for sex. Participants were asked to refrain from consuming foods high in anthocyanins and dietary nitrate 48 h prior to each study visit as well as alcohol and caffeinated products 24 h prior to each study visit. Fasted (>10 h) participants were invited to a screening visit to assess eligibility for the study during which researchers collected data regarding medical and familial history of health conditions, demographic data, body measurements (waist and hip circumference ratio), height (stadiometer), mass (InBody 270, Inbody, Seoul, South Korea), body mass index (BMI), body fat and skeletal muscle mass via bioelectrical impedance analysis (BIA, InBody 270, Inbody, Seoul, South Korea). Other measurements included during screening were blood pressure (Omron HEM-7121, Omron, Kyoto, Japan), resting heart rate (Omron HEM-7121, Omron, Kyoto, Japan), HbA1c (Cobas® b 101 system, Roche Diagnostics, Basel, Switzerland) and fasting blood glucose (HemoCue Glucose 201+ System, Hemocue, Ängelholm, Sweden).

Study visits 1, 2 and 3 were the main intervention visits; participants arrived at the Massey University Sports Laboratory following an overnight fast (10 h minimum) and a cannula was inserted at the antecubital fossa using aseptic techniques. Fasting capillary (finger prick) and venous blood (cannula) samples were taken prior to ingestion of the test meal (total 75 g carbohydrate), after which venous and capillary blood samples were taken over a duration of 150 min (Fig 5.2). Blood samples were collected by cannulation and finger prick sampling at baseline and time points 5, 15, 30, 45, 60, 90, 120 and 150 min. Participants were asked to consume the test meal and drink within 10 min and blood sample timings were taken from the end of meal consumption. Cannulas were flushed with saline between each sample. Blood pressure was also measured at baseline (pre-supplementation), and 60 and 120 min (post-supplementation).

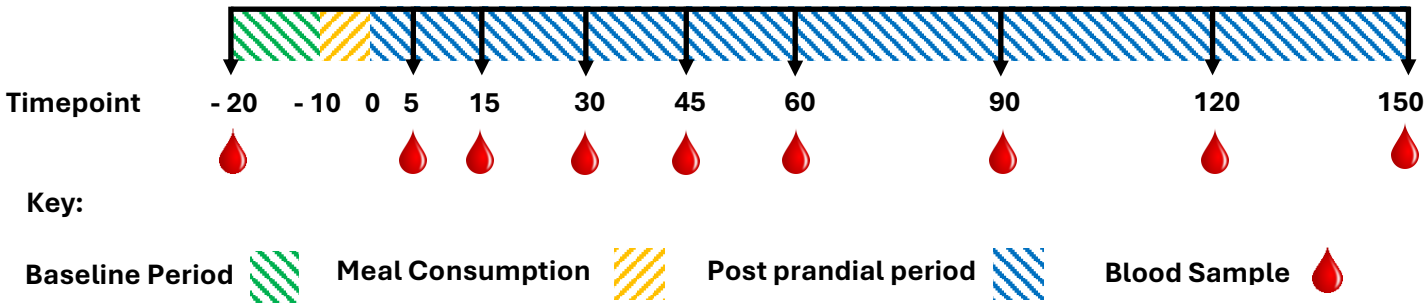


Figure 5.2. Timeline of main study visits. Baseline (-20 min) samples were taken prior to the consumption of the test meal, and participants were asked to consume the meal within 10 min. Time point 0 signals the end of test meal consumption.

5.2.4 Sample Handling

Capillary glucose samples were measured using a point of care glucometer (Hemocue 201+ glucose analyser; Hemocue, Ängelholm, Sweden) and plasma samples were used to measure blood glucose (via hexokinase method) and insulin (via radioimmunoassay). For plasma sample preparation, 6 ml and 4 ml of venous blood were collected into ethylenediaminetetraacetic acid (EDTA) and gel vacutainers for insulin and glucose samples, respectively. Plasma was separated from whole blood by centrifugation (10,000 rpm for 10 min), following which 500 µl of plasma was aliquoted into 2-ml Eppendorf tubes from both the EDTA and Gel vacutainers and stored at -80°C for later analysis at the Nutrition Laboratory, Massey University, Palmerston North.

5.2.5 Treatments

The randomisation officer prepared and labelled all intervention beverages; the primary researcher and participants were blinded to condition assignment throughout.

Beetroot concentrates were analysed for nitrate using HPLC methods modified from Chou and colleagues (64) whilst blackcurrant concentrates were analysed for anthocyanin content using spectrophotometric methods established by Özgen and colleagues (65).

Each participant consumed 75 g of carbohydrate in the form of bread with jam, accompanied by one of the three beverage options: 200 ml of nitrate-rich beetroot juice from concentrate containing 600 mg of nitrate (BR); blackcurrant juice from concentrate with 120 mg of anthocyanins (BC), or a placebo beverage (PL) containing sucrose and food colorant. Beverages were sugar matched to contain 52.9 g of CHO and weigh 200 g total (see Table 5.1). All beverages were served in a pink tumbler to minimise visible colour variation.

Table 5.1 Intervention drink composition.

Drink	*Sugar (g)*	*Juice/Colorant (g)*	*Water (g)*	*Total (g)*
Beetroot	0	77.3	122.7	200
Blackcurrant	38.4	9.1	152.5	200
Placebo	42.9	1	156.1	200

5.2.6 Statistical Analysis

Primary outcomes were early-phase capillary glucose iAUC (0–30 and 0–60 min) and glucose peak above baseline. Secondary outcomes included plasma glucose iAUC, insulin iAUC, blood pressure, Matsuda index and HOMA-IR. Both plasma and capillary whole blood glucose values and insulin indices were compared using repeated-measures ANCOVA at each time point with

'treatment' and 'time' as the two factors, with post-hoc t-tests using Bonferroni adjustment used to compare time points under the condition of a treatment*time main effect. For total incremental area under the curve (iAUC), early-phase iAUC (0-30 min and 0-60 min) and glucose peak above baseline were calculated and compared using repeated-measures ANCOVA, consistent with previous findings in acute beetroot supplementation trials (20). Incremental blood glucose values were calculated by subtracting baseline values (-20 min) from all subsequent time point values. Glucose peak values were calculated by subtracting baseline values from the highest subsequent value across all time points. Insulin sensitivity measured by Matsuda index (66) was calculated from intravenous glucose and treatments were compared using repeated-measures ANCOVA. For glucose and insulin results, the covariates used in the ANCOVA model were HbA1c, fasting glucose, BMI, body fat%, age and treatment order.

Blood pressure indices were compared using repeated-measures ANCOVA at time points 0, 60 and 120 min with 'treatment' and 'time' as the two factors. Covariates for blood pressure analyses included BMI, body fat %, age, treatment order, systolic and diastolic blood pressure at familiarisation.

A *p*-value less than 0.05 was considered statistically significant. Data are presented as means ± standard deviation. Statistical analyses were performed using IBM SPSS Statistics (SPSS Inc., Chicago, IL, USA) for Windows version 24.0.

5.3 Results

5.3.1 Participants

Sixteen participants completed all three treatment arms and were included in the analysis; their physical and baseline characteristics are presented in Table 5.2. All study participants met the inclusion criteria, with HbA1c and fasting glucose concentrations within normal ranges. The mean values for systolic and diastolic blood pressure were lower than the normal range, indicating a generally low blood pressure within this study cohort. Lipid profile parameters were all within a healthy range (see Table 5.2)

Table 5.2. Participants Characteristics. Data are presented as mean values and standard deviations.

Characteristic	Males (n = 4)	Females (n = 12)	All participants (n = 16)
Demographics			
Age (years)	30.2 ± 4.1	27.5 ± 4.0	28.2 ± 4.0
Height (cm)	181.9 ± 3.8	166.1 ± 7.0	170.0 ± 9.4
Body Mass (kg)	77.9 ± 11.9	64.1 ± 10.2	67.5 ± 11.9
BMI (kg/m^2)	23.6 ± 3.8	23.2 ± 2.7	23.3 ± 2.9
Waist circumference (cm)	79.7 ± 8.9	74.8 ± 7.8	76.0 ± 8.1
Hip circumference (cm)	99.0 ± 5.0	97.7 ± 8.1	98.0 ± 7.3
Waist:Hip ratio	0.80 ± 0.06	0.77 ± 0.05	0.78 ± 0.06
Body fat (%)	12.6 ± 3.1	28.5 ± 8.6	24.5 ± 10.3
Blood Pressure			
SBP (mmHg)	113.2 ± 10.1	112.3 ± 11.5	112.5 ± 10.8
DBP (mmHg)	67.5 ± 6.2	76.2 ± 9.6	74.0 ± 9.5
Glycaemic Measures & Lipid Profile			
HbA1c (mmol/mol)	31.2 ± 1.7	31.9 ± 3.7	31.8 ± 3.2
Fasting glucose (mmol/L)	4.2 ± 0.5	4.8 ± 0.4	4.7 ± 0.5
Total cholesterol (mmol/L)	4.18 ± 0.78	4.51 ± 0.65	4.43 ± 0.68
Triglycerides (mmol/L)	0.86 ± 0.17	1.16 ± 0.65	1.08 ± 0.58
HDL-C (mmol/L)	1.64 ± 0.12	1.74 ± 0.31	1.71 ± 0.28
LDL-C (mmol/L)	2.15 ± 0.81	2.27 ± 0.70	2.24 ± 0.70
TC:HDL ratio	2.58 ± 0.61	2.70 ± 0.75	2.67 ± 0.70

Data are presented as mean ± SD. n = 16 participants analysed (4 males, 12 females). SBP, systolic blood pressure. DBP, diastolic blood pressure.

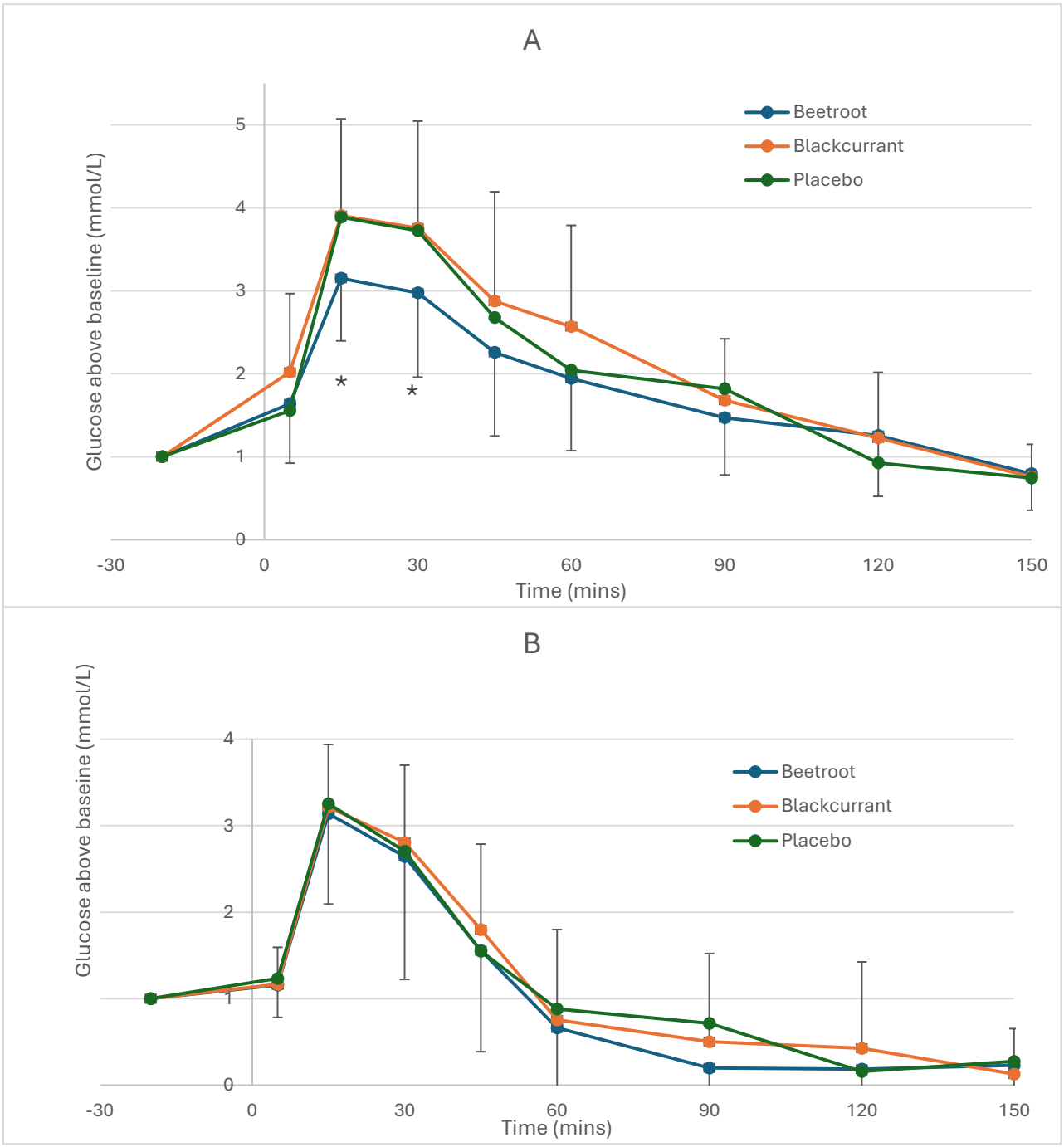
5.3.2 Glycaemic Response

Capillary blood glucose results above baseline showed a treatment*time interaction (p = 0.012). Descriptive statistics and between-condition differences for all capillary glucose outcomes are presented in Table 5.3. BR intervention resulted in lower blood glucose results than the BC intervention (p = 0.012) but not PL (p = 0.058) (Figure 5.3). Capillary glucose levels were lower in BR than PL at 15 min (BR = 3.22 mmol/L, PL = 3.965 mmol/L; p = 0.002) and 30 min (BR = 3.01 mmol/L, PL = 3.676 mmol/L, p = 0.028) post food intake (Figure 5.3). Early phase iAUC was lower in BR than PL at 0-30 min (BR = 52.24 mmol·min/L, PL = 66.50 mmol·min/L; p = 0.017) and 0-60 min (BR = 95.27 mmol·min/L, PL = 122.88 mmol·min/L; p = 0.010) (Figure 5.4). No differences in capillary glucose were detected between BC and PL for early phase iAUC (0-30 min and 0-60 min). The mean capillary glucose peak above baseline was lower in BR than PL (BR = 2.51, PL = 3.40, p = 0.002), but no differences were observed between BC and PL treatments (Figure 5.5).

Table 5.3. Capillary blood glucose responses and plasma insulin responses.

Capillary blood glucose responses (HemoCue 201+)						
	Mean ± SD (mmol·min/L or mmol/L)			**Between-condition difference**		
Outcome	**Beetroot (BR)**	**Blackcurrant (BK)**	**Placebo (PL)**	**PL – BR Mean (95% CI)**	**PL – BK Mean (95% CI)**	**BR – BK Mean (95% CI)**
iAUC 0–30 min (mmol·min/L)	52.24 ± 21.12 *(40.99, 63.49)*	66.93 ± 25.36 *(53.41, 80.44)*	66.51 ± 24.71 *(53.34, 79.67)*	14.27 *(5.65, 22.89)*	-0.42 *(-13.38, 12.54)*	-14.68 *(-25.44, -3.93)*
iAUC 0–60 min (mmol·min/L)	95.28 ± 41.56 *(73.13, 117.43)*	122.98 ± 55.53 *(93.40, 152.57)*	122.88 ± 45.06 *(98.87, 146.89)*	27.60 *(7.11, 48.09)*	-0.10 *(-22.96, 22.75)*	-27.71 *(-44.59, -10.83)*
iAUC 0–150 min (mmol·min/L)	139.90 ± 68.89 *(103.19, 176.61)*	180.04 ± 89.29 *(132.46, 227.62)*	160.71 ± 68.84 *(124.03, 197.39)*	20.81 *(-6.64, 48.27)*	-19.32 *(-57.59, 18.94)*	-40.14 *(-80.52, 0.25)*
Peak above baseline (mmol/L)	2.51 ± 0.78 *(2.09, 2.92)*	3.16 ± 1.08 *(2.58, 3.74)*	3.40 ± 1.21 *(2.75, 4.04)*	0.89 *(0.37, 1.41)*	0.24 *(-0.29, 0.76)*	-0.65 *(-0.98, -0.33)*
Plasma insulin responses (radioimmunoassay)						
iAUC 0–30 min (μU·min/mL)	836.1 ± 389.9 *(628.4, 1043.9)*	772.0 ± 376.7 *(571.3, 972.7)*	847.7 ± 453.7 *(606.0, 1089.5)*	11.6 *(-227.3, 250.5)*	75.7 *(-102.0, 253.5)*	64.1 *(-171.4, 299.7)*
iAUC 0–60 min (μU·min/mL)	1812.8 ± 817.3 *(1377.3, 2248.3)*	1728.6 ± 760.6 *(1323.3, 2133.9)*	1692.0 ± 680.7 *(1329.3, 2054.7)*	-120.8 *(-586.3, 344.7)*	-36.6 *(-401.0, 327.7)*	84.2 *(-331.9, 500.3)*
iAUC 0–150 min (μU·min/mL)	3108.8 ± 1540.0 *(2288.2, 3929.4)*	2935.5 ± 1836.0 *(1957.2, 3913.9)*	2792.9 ± 1168.9 *(2170.0, 3415.8)*	-315.9 *(-964.6, 332.8)*	-142.6 *(-769.3, 484.0)*	173.2 *(-523.6, 870.1)*
Insulin sensitivity indices						
Matsuda Index	6.74 ± 2.23 *(5.55, 7.92)*	6.63 ± 2.10 *(5.51, 7.75)*	6.92 ± 3.38 *(5.12, 8.72)*	0.18 *(-1.34, 1.70)*	0.29 *(-0.80, 1.37)*	0.10 *(-1.11, 1.32)*
HOMA-IR	2.38 ± 0.65 *(2.03, 2.73)*	2.41 ± 0.95 *(1.91, 2.92)*	2.68 ± 1.36 *(1.95, 3.40)*	0.30 *(-0.33, 0.92)*	0.26 *(-0.52, 1.05)*	-0.03 *(-0.52, 0.46)*

Values are mean ± SD with 95% confidence intervals in parentheses (n = 16 per condition). iAUC, incremental area under the curve calculated using the trapezoid method with negative increments set to zero. Peak above baseline = maximum glucose concentration minus fasting (0 min) baseline. Between-condition differences are unadjusted paired means (Condition A − Condition B); positive values indicate Condition A exceeded Condition B. BR, beetroot juice; BK, blackcurrant juice; PL, placebo.



Figures 5.3 A and B. Capillary (A) and plasma (B) blood glucose concentrations during OGTT across different interventions. Values are means (n=16) with standard deviations represented by vertical error bars. For clarity, negative error bars are shown on Beetroot and positive error bars presented on Blackcurrant. Placebo intervention error bars were comparable in magnitude to those of BC and BR interventions. For visual clarity, one was added to each of the glucose mean values to prevent them falling below the x-axis. *, Mean values for mean blood glucose during beetroot intervention were significantly lower than for the placebo intervention ($p < 0.05$) *in capillary blood glucose (A). No significant differences were observed for plasma glucose concentrations between the Beetroot, Blackcurrant and Placebo interventions (B).*

Plasma glucose levels showed a significant effect of time ($p < 0.001$), but no significant effect of treatment ($p = 0.327$) or treatment*time ($p = 0.917$) (Figure 5.3). Plasma blood glucose results

showed no differences between BR, BC and PL for total iAUC, early-phase iAUC (0-30 min and 0-60 min) or glucose peak above baseline.

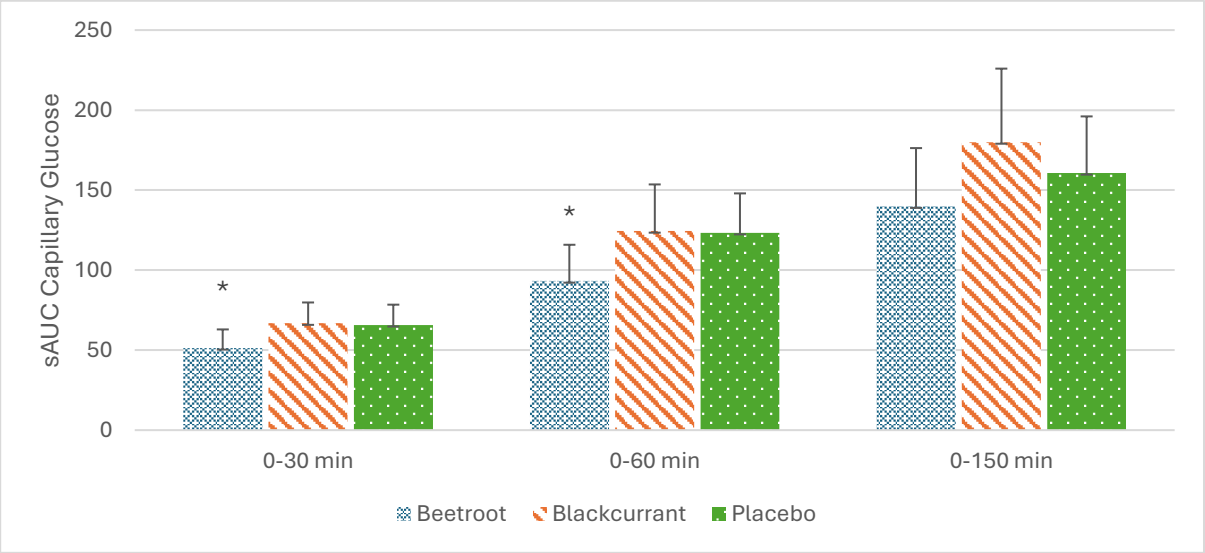


Figure 5.4 Segmental areas under the blood glucose response curve (sAUC). Values are means (n=16) with error bars representing 95% confidence intervals. *, Mean value for beetroot was significantly lower than for placebo (p < 0.05).

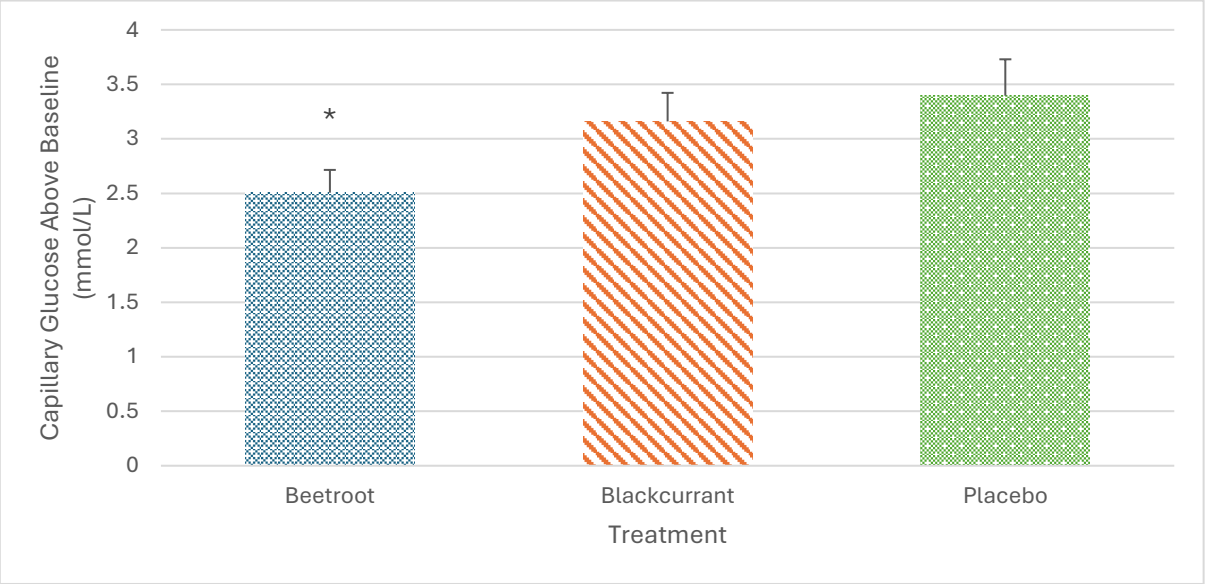


Figure 5.5. Glucose peak above baseline for capillary glucose. Glucose peaks were calculated by subtracting baseline glucose values from the highest subsequent value across all time points. Values are means (n=16) with error bars representing 95% confidence intervals. *, Mean value for beetroot was significantly lower than for placebo (p < 0.05).

5.3.3 Insulin Response

Plasma insulin analysis revealed a time effect ($p < 0.001$) on plasma insulin but no effect of treatment ($p = 0.860$) or treatment*time ($p = 0.888$; Figure 5.6). Descriptive statistics and between-condition differences for all insulin outcomes are presented in Table 5.3. There was a

significant within-subjects effect of intervention on early phase insulin response (0-30 min iAUC) (p = 0.045, partial η^2 = 0.291), indicating that insulin secretion differed across the three experimental conditions. While pairwise comparisons between groups were not statistically significant, the group means suggest that the BC intervention (772 µU·min/mL) elicited a lower early insulin response compared with both BR (836 µU·min/mL) and PL (848 µU·min/mL).

In the 0-60 min analysis, the within-subjects effect approached statistical significance (p = 0.056, partial η^2 = 0.274), again suggesting that intervention may have influenced total insulin exposure over the OGTT. Mean insulin iAUC values over 60 min were non-significantly highest in BR (1812 µU·min/mL), followed by BC (1728 µU·min/mL), and lowest in PL (1692 µU·min/mL).

There was no difference in plasma insulin iAUC from 0-150 min between BR, BC and PL interventions for total iAUC. Comparisons of Matsuda index (p = 0.526) and insulin peak above baseline (p = 0.736) revealed no significant differences between the three interventions.

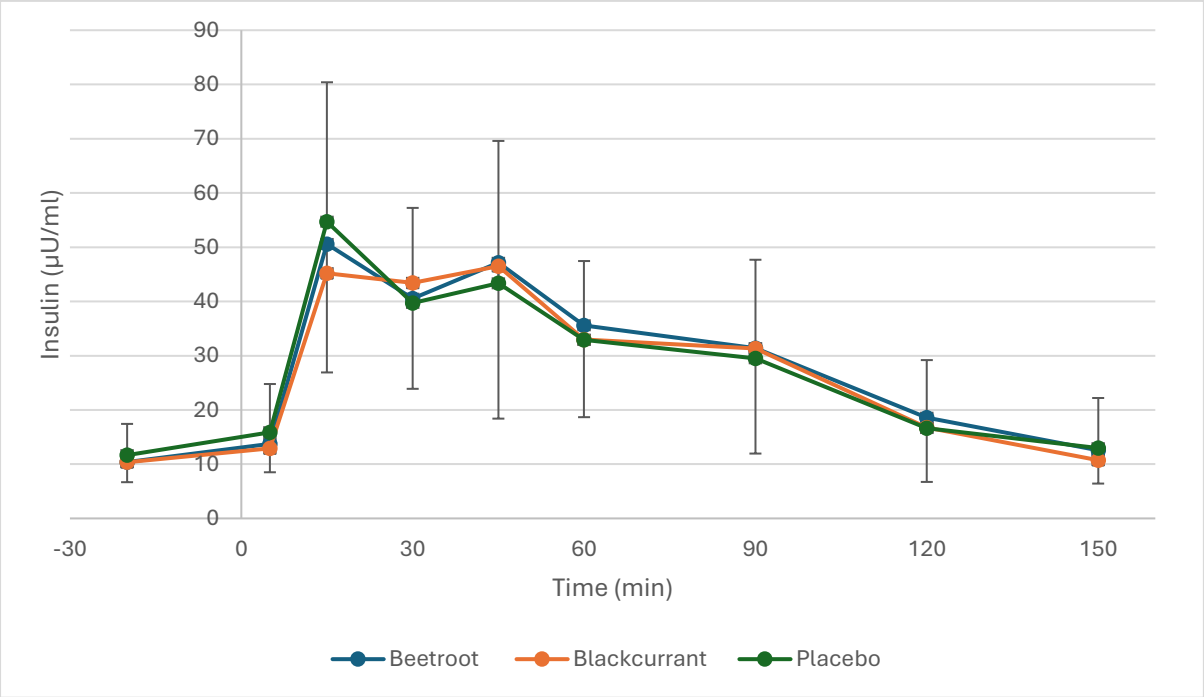


Figure 5.6. Plasma insulin values during OGTT across different interventions. Values are means (n=16) with standard deviations represented by vertical bars. For clarity, negative error bars are shown on Beetroot and positive error bars presented on Placebo. Blackcurrant intervention error bars were comparable in magnitude. No significant differences were observed between interventions.

5.3.4 Blood Pressure

There was no effect of time (p = 0.952), treatment (p = 0.987) or treatment*time (p = 0.829) on systolic blood pressure (SBP) at all time points (baseline, 60 min and 120 min) across the three intervention groups. Descriptive statistics and between-condition differences for all blood pressure outcomes are presented in Table 5.4. No differences were found for change in SBP (p = 0.625) or diastolic blood pressure (p = 0.607) from baseline to 120 min (Figure 5.7).

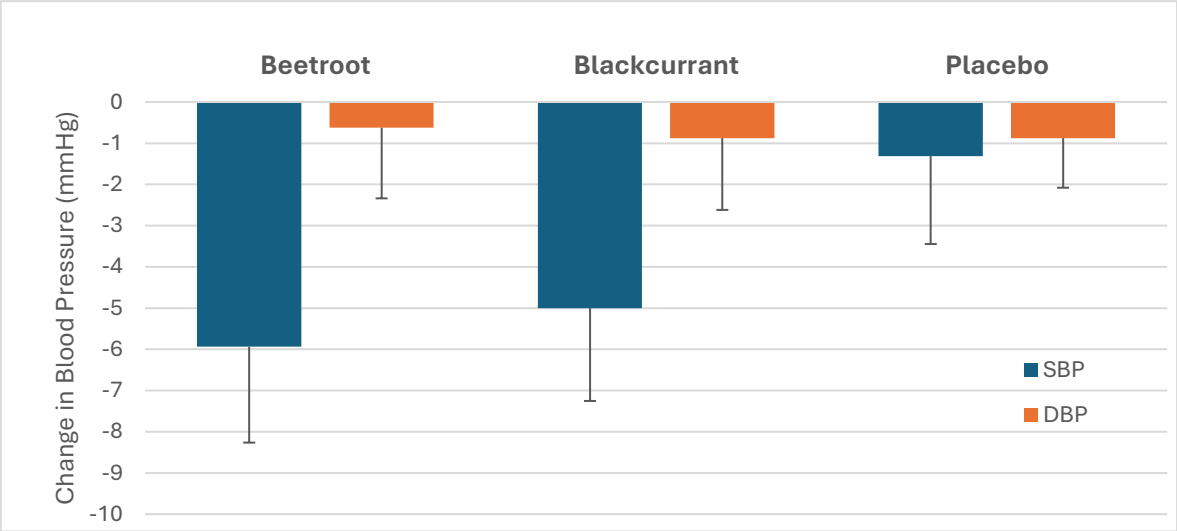


Figure 5.7 Change in blood pressure from baseline to 120 min across different interventions. Values are means (n=16) with standard errors represented by vertical error bars. No significant differences were observed between interventions for both systolic blood pressure and diastolic blood pressure. SBP; Systolic Blood Pressure, DBP: Diastolic Blood Pressure.

No adverse events related to consumption of the intervention or placebo beverages were reported during the trial.

Table 5.4. Blood pressure responses (mmHg)

Blood pressure responses (mmHg)						
	Systolic blood pressure (mmHg)			**SBP Between-condition difference**		
Outcome	**Beetroot (BR)**	**Blackcurrant (BK)**	**Placebo (PL)**	**PL – BR Mean (95% CI)**	**PL – BK Mean (95% CI)**	**BR – BK Mean (95% CI)**
Baseline (-20 min)	117.6 ± 9.7 *(112.4, 122.7)*	115.4 ± 10.4 *(109.9, 121.0)*	110.9 ± 8.8 *(106.2, 115.5)*	-6.7 *(-10.7, -2.6)*	-4.6 *(-8.8, -0.4)*	2.1 *(-3.7, 7.9)*
60 min	113.6 ± 11.9 *(107.2, 119.9)*	112.1 ± 12.6 *(105.4, 118.8)*	112.6 ± 9.7 *(107.4, 117.7)*	-1.0 *(-5.0, 3.0)*	0.4 *(-4.3, 5.2)*	1.5 *(-3.0, 5.9)*
120 min	111.6 ± 13.9 *(104.2, 119.0)*	110.4 ± 11.8 *(104.2, 116.7)*	109.6 ± 10.8 *(103.8, 115.3)*	-2.1 *(-6.6, 2.5)*	-0.9 *(-4.4, 2.7)*	1.2 *(-4.6, 6.9)*
	Diastolic blood pressure (mmHg)			**DBP Between-condition difference**		
Outcome	**Beetroot (BR)**	**Blackcurrant (BK)**	**Placebo (PL)**	**PL – BR Mean (95% CI)**	**PL – BK Mean (95% CI)**	**BR – BK Mean (95% CI)**
Baseline (-20 min)	76.0 ± 8.0 *(71.7, 80.3)*	75.6 ± 9.4 *(70.6, 80.6)*	74.6 ± 7.4 *(70.6, 78.5)*	-1.4 *(-4.8, 1.9)*	-1.1 *(-4.5, 2.4)*	0.4 *(-3.6, 4.4)*
60 min	73.6 ± 10.8 *(67.9, 79.4)*	72.2 ± 10.2 *(66.8, 77.7)*	73.0 ± 8.7 *(68.3, 77.7)*	-0.6 *(-4.7, 3.5)*	0.8 *(-3.5, 5.0)*	1.4 *(-2.5, 5.3)*
120 min	74.4 ± 10.5 *(68.8, 80.0)*	74.8 ± 10.2 *(69.3, 80.2)*	73.7 ± 6.2 *(70.4, 77.0)*	-0.7 *(-5.1, 3.8)*	-1.1 *(-4.7, 2.6)*	-0.4 *(-4.6, 3.8)*

Values are mean ± SD with 95% confidence intervals in parentheses (n = 16 per condition). Between-condition differences are unadjusted paired means (Condition A – Condition B); positive values indicate Condition A exceeded Condition B. Blood pressure measured at baseline (pre-meal), 60 min and 120 min post-meal.

5.4 Discussion

The aim of this study was to investigate the effects of a single dose of nitrate-rich beetroot or anthocyanin-rich blackcurrant juice on postprandial blood glucose and insulin responses as well as blood pressure following a carbohydrate-rich breakfast meal. The results showed that early-phase (0-60 min) postprandial capillary glucose concentrations and peaks above baselines were reduced following BR juice supplementation compared with PL. Further, BC juice supplementation reduced early-phase (0-30 min) insulin iAUC compared with PL.

5.4.1 Glycaemic Response

The study showed, consistent with previous findings using healthy adults (20, 60, 67), early-phase (0-60 min) postprandial capillary glucose concentrations and peaks above baselines were reduced following BR juice supplementation compared with PL. These findings suggest that BR juice may effectively modulate the initial sharp rise in blood glucose levels that occurs following meal consumption, potentially reducing the risk of glucose-induced oxidative stress and the formation of AGEs, and therefore reducing the risk and severity of long-term metabolic damage and T2DM pathophysiology (29).

Differences in blood glucose responses between interventions were observed only at the capillary blood level and not in the plasma, which raises questions about the underlying mechanisms for these observed differences between capillary glucose and venous glucose responses. This discrepancy suggests that the observed differences in capillary blood glucose responses are due to changes in peripheral glucose uptake at the tissues and in the interstitial space, but not overall circulating blood glucose concentrations. Upon ingestion, NO_3^- is ultimately reduced to NO (68), which increases vasodilation and subsequent blood flow to the skeletal muscle and peripheral tissues which could enhance insulin-independent glucose uptake through GLUT4 co-transporters (69, 70), reducing the reliance on insulin for blood glucose management post-meal. Further, nitric oxide increases GLUT4 expression in the skeletal muscle, in turn leading to increased glucose uptake from the blood at the peripheral tissues. Given plasma glucose is reflective of a more systemic balance of glucose absorption and utilisation, if a localised increase in glucose uptake occurred at the peripheral tissues, such changes may not be reflected in the plasma readings as the systemic change may be too negligible to observe.

In this study, the BC juice intervention had no significant effect on glucose indices compared with placebo, which contrasts with prior research where blackcurrant anthocyanins have been shown to modulate glucose metabolism (40-42). Anthocyanins are known to inhibit key digestive

enzymes such as alpha-amylase and alpha-glucosidase (63), which break down starch and disaccharides, respectively, into glucose. Through this inhibition, carbohydrate breakdown into glucose is attenuated, decreasing the rate of glucose absorption. Castro-Acosta and colleagues (41) showed that the modulation of postprandial glucose responses by blackcurrant anthocyanins with 600 mg anthocyanins but not at lower dosages (150 mg and 300 mg) whereas the present study used 120 mg of blackcurrant anthocyanins. An increased dose may have been more effective in reducing postprandial blood glucose peaks. Further, Nolan and colleagues showed that daily short-term (8 days), but not acute blackcurrant anthocyanin ingestion was able to improve insulin sensitivity and postprandial glucose responses to both breakfast and lunch meals (44). Given approximately 12% of ingested anthocyanins are absorbed into the blood (71), repeated doses of anthocyanins may be required to accumulate sufficient bioactive ingredients for a significant change in blood glucose responses.

5.4.2 Insulin Response

The significant overall effect at 0 -30 min points to a potential modulation of first-phase insulin secretion, which is often driven by incretin signalling or rapid glucose sensing (72). Although pairwise comparisons did not yield significant differences, the lower early insulin iAUC observed with blackcurrant compared with beetroot and placebo could indicate modest effects on insulin efficiency or glucose absorption. This is corroborated by Nolan and colleagues (44) who showed improved insulin sensitivity following ingestion of 600 mg of New Zealand BC extract during OGTT. It may be that our lower dose (120 mg) meant between-intervention differences were not large enough to be detected during the pairwise comparisons. Given the lack of statistical separation between individual groups, these results should be interpreted cautiously and warrant further investigation in studies powered specifically to detect these effects.

We also observed no significant differences in any insulin indices between BR and PL, suggesting that the acute glucose-lowering effects of BR juice does not appear to change insulin secretion. Instead, these effects may be due to increased peripheral glucose uptake through non-insulin-dependent pathways. Nitric oxide has been shown to increase phosphatidylinositol 3-kinase (PI3K) dependent GLUT4 translocation at the cell membrane (69), facilitating glucose uptake independently of insulin. Therefore, NO supplementation may be a useful intervention in the presence of impaired insulin signalling and release, as NO may partially compensate for the reduced insulin-dependent GLUT4 translocation by increasing vascular and cell-signalling responses.

5.4.3 Blood Pressure

Despite previous research showing that consumption of both beetroot juice (73-76), through increased NO production, and blackcurrant juice, through reduced arterial stiffness (35, 36) , have the potential to reduce blood pressure, no significant changes in blood pressure were observed in this study. Previous studies have shown that the effects of beetroot juice supplementation on blood pressure responses are more pronounced in older adults (50 – 70 yrs) than younger adults (18 – 30 yrs) (76). The young average age (29.9 yrs) and low resting blood pressure (systolic blood pressure = 112.8 mmHg, diastolic blood pressure = 73.7 mmHg) of the cohort in the present study may have reduced the potential for treatments to modulate BP responses. Blood pressure changes resulting from nitrate ingestion are also most pronounced during the plasma nitrite peak (3-4 h after ingestion), meaning the observational window used in this study may not have captured the peak changes in blood pressure responses in the BR juice group (68). Further, the beneficial effects of BC anthocyanins on blood pressure responses have only been observed following longer-term (1-week) supplementation (35). This study was examining acute responses to blackcurrant ingestion; however, a longer-term consumption such as weeks to months may have resulted in observed changes in BP responses.

5.4.4 Limitations

All participants were healthy, normoglycaemic adults with a mean age of 29.9 years and generally low resting blood pressure, which may have reduced the capacity of either intervention to elicit detectable glycaemic or haemodynamic responses. Findings may not be generalisable to populations with impaired metabolic status such as those with prediabetes, impaired glucose tolerance or elevated blood pressure, for whom the effects of BR and BC juice supplementation may differ substantially.

In the BC juice intervention the BC juice provided 120 mg of anthocyanins per serving, which is considerably lower than doses shown to modulate postprandial glucose responses in previous research (600 mg) (41). Further, this study was powered for the primary capillary glucose comparison. The observed BC effect on early-phase insulin iAUC (0–30 min; $p = 0.045$, partial $\eta^2 = 0.291$) was not significant at pairwise comparison, and the study was estimated to provide 57% power to detect this effect size. Appropriately powered studies are needed to explore and confirm these observations.

Plasma nitrate, nitrite and nitric oxide concentrations were not measured, preventing direct confirmation of the proposed mechanistic pathway linking dietary nitrate ingestion to peripheral

GLUT4-mediated glucose uptake. Similarly, no direct measures of peripheral blood flow, microvascular perfusion, or GLUT4 expression were obtained. The mechanistic interpretation of the capillary-plasma glucose discrepancy remains inferential and should be confirmed in future studies incorporating these measures.

5.4.5 Implications

The present study suggests that the postprandial glucose lowering effects of beetroot juice consumption observed in previous studies may be linked to increases in microvascular NO concentrations from dietary nitrate ingestion, given the observed modulation of postprandial glucose in the capillaries rather than the intravenous plasma. Whilst it has been proposed that the betalains found in beetroot juice and the anthocyanins found in blackcurrant juice may attenuate postprandial glucose excursions, the timeframe of absorption of these polyphenols does not match the observed changes seen in this study. Plasma nitrate and nitrite concentrations have been shown to rise within the first 30 min of beetroot juice ingestion (68), whereas plasma betalain concentrations peak at 3 h post-ingestion, and do not rise significantly until 2 h post-ingestion (77). For blackcurrant anthocyanins, peak plasma concentrations occur between 2-4 h post-ingestion, and antioxidant capacity peaks at 1 hr post-ingestion (78). Whilst beetroot betalains and blackcurrant anthocyanins have been purported to improve blood glucose responses, these effects may need more prolonged, consistent consumption of polyphenol-rich juice to cause changes in glycaemic responses. Future research should explore the chronic effects of beetroot and blackcurrant juice supplementation on glucose metabolism and cardiometabolic risk factors.

Previous studies have shown that the macrovascular effects of dietary nitrate supplementation take place 2-4 h after ingestion (68, 79, 80), meaning that changes in systemic plasma glucose from dietary nitrate may also occur at this time point. Webb and colleagues (68) showed that conversion of nitrate to nitrite and subsequent NO starts in the enterosalivary circulation whereby nitrite enters the stomach following reduction to nitrate by bacterial nitrate reductases on the tongue. Nitrite is then absorbed into the circulation and reduced to NO by red blood cells under hypoxic conditions (81). In this reaction, deoxyhaemoglobin functions as a nitrite reductase, and this activity is greatest at oxygenated haemoglobin (oxyHb) concentrations of 50% (82), making the capillaries a particularly favourable location for nitrite reduction to NO.

Therefore, given that NO tends be produced from nitrite and subsequently utilised at the microvascular level earlier than the macrovascular level due to the capillaries' lower oxygen

tension and lower pH (83, 84), it is not surprising that only the capillary glucose levels, where NO may be acting on GLUT4 cotransporter pathways, showed significant reductions in glucose compared with those in the plasma. Future studies should aim to measure both plasma and capillary nitrate, nitrite and NO concentrations during OGTT testing to establish the differences between plasma and capillary NO production and utilisation.

Furthermore, researching the effects of beetroot supplementation in participants with impaired insulin signalling, such as people living with T2DM and prediabetes, may provide further insight into the insulin-independent effects of dietary nitrate supplementation of GLUT4 cotransporter expression and subsequent glucose uptake at the peripheral tissues. Given that blackcurrant anthocyanins have been shown to enhance NO production (85), blackcurrant should also be investigated further for its potential to improve blood glucose responses in populations with impaired insulin signalling, but over longer-term supplementation periods such as weeks to months using appropriate levels of anthocyanins to elicit an accumulation of plasma anthocyanins and their metabolites.

5.5 Conclusion

Hyperglycaemia and glucotoxicity is a prominent feature in the development of MetS and in T2DM pathophysiology. Beetroot juice supplementation reduces postprandial early-phase blood glucose levels at the peripheral tissues, likely through increased NO production and subsequent insulin-independent GLUT4 translocation. Given that people living with MetS and T2DM have impaired insulin-dependent GLUT4 translocation, beetroot juice supplementation may provide a nutraceutical intervention to compensate for this reduction in glucose disposal capacity. Blackcurrant juice may improve insulin sensitivity following ingestion, reducing postprandial insulin requirements in healthy participants. Further research is needed to establish the efficacy of beetroot and blackcurrant juice supplementation to improve glycaemic control in a chronic setting, particularly in groups with impaired insulin signalling such as those with MetS or T2DM.

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Student name:	Cameron Haswell
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In which chapter is the manuscript/published work?	Chapter 6

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Cameron Haswell (C.H.), Kay Rutherfurd Markwick (K.R.M.), Ajmol Ali (A.A.), Rachel Page (R.P.) and Roger Hurst (R.H.) contributed to the conceptualization of the manuscript; CH., K.R.M., A.A., R.P., .RH., and Marie Wong (M.W.) contributed to the methodology, M.W., performed drink production including food safety assessment; C.H. and Hajar Mazahery (H.M.) performed formal analysis, C.H., conducted data collection, sample analysis; KRM nitrite/nitrate analysis; C.H., performed writing — original draft preparation, C.H., K.R.M., A.A., R.P., and R.H contributed to writing — review and editing.

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Chapter 6 - Effects of Nitrate- and Betalain-Rich Beetroot Juice on Vascular Function and Metabolic Syndrome Risk Factors in Healthy Adults

This chapter addresses Research Question 4 (see Section 1.3.4) and presents results from a 6-day short-term intervention as well as an acute dose with two different beetroot juice beverages containing different levels of nitrate and betalains and their effects on vascular function and metabolic syndrome biomarkers. This chapter has been presented in manuscript form.

Abstract

Introduction: Metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM) are global health challenges linked to obesity, physical inactivity and ageing populations. Beetroot juice (BR), due to its high dietary nitrate and betalain profile, has been proposed as a nutraceutical intervention to mitigate MetS risk factors. This study investigated the effects of two different beetroot juices with different nitrate and betalain compositions, sourced from the UK and New Zealand (UKBR and NZBR respectively), with a placebo beverage (PL), on MetS biomarkers after 6 days of daily ingestion. Blood pressure and vascular resistance biomarkers were also measured acutely following ingestion on day 7.

Methods: Sixteen healthy participants consumed either UKBR (1280 mg nitrate, 147 mg betalains, daily), NZBR (1120 mg nitrate, 506 mg betalains, daily), or PL in a repeated-measures crossover trial. Plasma nitrate and nitrite levels, systolic and diastolic blood pressure (SBP, DBP), systemic vascular resistance (SVR), fasting blood glucose and lipid profiles were assessed in a fasted state (overnight) after 6 days of supplementation and acutely at 2 h and 5 h following ingestion on day 7.

Results: Consumption of both UKBR and NZBR for 6 days resulted in significantly elevated plasma nitrate and nitrite levels compared to PL. Baseline plasma nitrate levels after 6 days were 267.3 µmol/L for UKBR, 163.5 µmol/L for NZBR, and 47.1 µmol/L for PL. On day 7, 2 h post beverage consumption, UKBR achieved higher plasma nitrate (761.5 µmol/L) levels than NZBR (557.3 µmol/L, $p = 0.003$) and PL. At 5 h post-ingestion, nitrate levels remained higher for UKBR (705.8 µmol/L) than NZBR (495.7 µmol/L, $p < 0.001$). Similar trends were observed for plasma nitrite levels. Reductions in SBP were observed after 6 days (-5.3 mmHg for NZBR vs. PL, $p = 0.038$) and at 2 h post-ingestion for both UKBR (-5.7 mmHg, $p = 0.003$) and NZBR (-5.6 mmHg, $p = 0.029$) compared to PL. Reductions in SVR were significant after 6 days for NZBR relative to PL (-219 dyne-sec-cm^{-5} vs. PL, $p = 0.024$). No significant differences were observed for fasting glucose and lipid indices following the intervention period.

Conclusion: Supplementation with BR improves vascular function, with NZBR showing greater SBP and SVR reductions despite its lower nitrate content, suggesting betalains may play an important role. These findings highlight the importance of optimising BR composition to maximise cardiovascular benefits. Further research should evaluate these effects over extended duration of consumption and in at-risk populations.

Key words: Metabolic syndrome, type 2 diabetes, betalains, cardiovascular health, vascular function, systemic vascular resistance, blood pressure

6.1 Introduction

Metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM) represent a growing global health issue which poses significant challenges to public health systems, economies, and population well-being. Metabolic syndrome is a term which describes a cluster of metabolic abnormalities associated with increased risk of T2DM, cardiovascular disease (CVD) and development of coronary heart disease (CHD) (1, 2). Metabolic syndrome comprises five components including hypertension, central obesity, low levels of high density lipoprotein cholesterol (HDL-C), hypertriglyceridemia and impaired fasting glucose (3), and each contribute to or characterise the development of insulin resistance, prediabetes and subsequent T2DM. An individual is diagnosed with MetS if they exhibit three of the five risk factor components. Similar to T2DM, MetS is associated with physical inactivity and central obesity, and an individual with MetS has a fivefold relative risk of developing T2DM (4). Approximately one-quarter of the global adult population has MetS (5), posing a significant risk for an increase in global T2DM rates. With T2DM prevalence increasing worldwide, its global economic and social burden is also growing; in the US for example, currently 25% of the national healthcare cost is accredited to T2DM management and treatment (6), whilst the projected annual cost of T2DM in NZ will rise from NZ$2.1 billion to NZ$3.5 billion (63% increase) between 2022 and 2045 (7). Much of this cost is associated with increased prevalence of T2DM in younger people, with lifetime healthcare costs more than tenfold for someone diagnosed with T2DM at 25 years than someone diagnosed at 75 years (7).

The significant prevalence, healthcare costs and social burden of MetS and T2DM underscores the urgency to find effective interventions to prevent the onset of MetS risk factors and subsequent development of T2DM. Dietary interventions may provide a low-cost, accessible strategy to mitigate these risk factors and beetroot juice supplementation has attracted interest due to its rich composition of bioactives such as nitrate and betalains (8-10). Dietary nitrate, found in high levels in beetroot juice (BR), has the capacity to be reduced into nitric oxide (NO), a regulator of blood vessel function (11, 12), lipid peroxidation (13) and glucose metabolism (14). Previous studies show that BR consumption increases plasma nitrate and nitrite levels 2 h post-ingestion and remain elevated after 6 h (15, 16), with NO metabolites (17) and total nitroso species (18) also elevated. Beetroot juice is also a source of the polyphenolic pigments betalains, and polyphenols further enhance the reduction of nitrate into NO. Betanin, a sub-class of betalains, may also inhibit both α-amylase and α-glucosidase, decreasing the rate of carbohydrate breakdown and glucose absorption into the blood (19).

Previous studies investigating the impact of BR supplementation on improving metabolic syndrome biomarkers have yielded positive results. Several studies have shown BR consumption leads to clinically significant reductions in blood pressure following both acute (16, 20, 21) and longer-term (22-26) supplementation, with most chronic supplementation studies showing reductions in both systolic and diastolic BP between 5-10 mmHg. As dietary nitrate is reduced into nitrite and subsequently to NO, NO bioavailability increases. Nitric oxide activates guanylyl cyclases which catalyse the conversion of guanosine triphosphate into cyclic 3', 5'-guanosine monophosphate in the vascular smooth muscle cells, reducing intracellular Ca^{2+} levels which decreases smooth muscle contraction, leading to vasodilation and decreasing blood pressure (27). Despite this, evidence of the effect of BR intake on systemic vascular resistance (SVR), which unlike systolic blood pressure is regulated primarily by the microvasculature (28), is limited. Systemic vascular resistance, defined as the resistance offered by the systemic vasculature to the flow of blood from the left ventricle into the arterial circulation, reflects the degree of arteriolar vasoconstriction or vasodilation and is a key determinant of mean arterial pressure. Systemic vascular resistance may be a better indicator of early hypertension risk in healthy people (29), as microvascular endothelial dysfunction tends to precede macrovascular endothelial dysfunction (30), making SVR a useful measure in studies predicting the effect of interventions on future hypertension risk in healthy people.

Previous studies have shown that BR and dietary nitrate supplementation may improve glucose metabolism. Gheibi et al. (31) showed increased glucose transporter-4 (GLUT4) protein expression in T2DM rats following dietary nitrate supplementation, due to increased GLUT4 mRNA expression. Nitric oxide also contributes to vasodilation, causing relaxation of large blood vessels, which increases glucose delivery to the peripheral muscles (32, 33). Work by Holy and colleagues (32) showed a reduction in triglycerides (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels in healthy subjects following an acute dose of BR (250 mL, nitrate levels not specified). The betalain pigment betanin has been shown to inhibit lipid peroxidation (34), leading to reduced reactive oxygen species levels, however other work has shown that lipid profiles can be improved from dietary nitrate consumption in the absence of betalains (35).

Based on available evidence, consumption of BR may have the potential to ameliorate MetS risk factors and reduce the overall risk of MetS and onset of T2DM. The exact mechanisms behind these observations are not fully established, and previous studies (16, 20-26, 31) have failed to establish the relative contributions of betalains and dietary nitrate on the observed effects.

This study aimed to investigate the effects of two different beetroot juices with different betalain and nitrate contents, on plasma nitrate and nitrite and MetS risk factors following 6 days of daily consumption By examining acute changes in plasma nitrate and nitrite, blood pressure and SVR following daily ingestion on day 7. We aimed to establish the importance of betalains, as well as nitrates, in BR to improve MetS risk factors.

6.2 Methods

6.2.1 Study Population

The participant group for this study comprised recreational cyclists, as data were collected as part of a larger project investigating the effects of beetroot juice supplementation on cycling time trial performance. The vascular and metabolic data reported in this chapter were collected as secondary outcomes of that trial.

Data for this study was collected as part of a larger project aimed at establishing the efficacy of beetroot juice supplementation to improve cycling time trial performance. A priori sample size calculations were performed based on plasma nitrate data reported by Lansley and colleagues (36). Assuming a standard deviation of 4.5, a minimum sample size of 12 participants was estimated to achieve 80% power at $\alpha = 0.05$. To account for potential participant dropout, 15 participants were recruited. These calculations were conducted in the context of the primary cycling performance outcomes of the parent trial; the vascular and metabolic outcomes reported in this chapter were secondary outcomes for which no independent power calculation was performed. Fourteen participants completed all three conditions and were included in the analysis (Figure 6.1). Treatment order was randomised across six possible sequences using a Latin square design, balanced for sex. Ethics approval was obtained from the Massey University Human Ethics Committee (Southern A, Application 21/36) and was prospectively registered at the Australia New Zealand Clinical Trials Registry (Trial ID: ACTRN12622000714785). All participants were required to give informed consent prior to participation in the study.

Participants commenced from July 2022 in Auckland, New Zealand using a combination of poster advertising, social media outreach and word-of-mouth. Data collection was completed in May 2023. Inclusion criteria for the study were: (i) aged 18-55 years of age (and premenopausal if female) (ii) BMI of 18-30 kg/m^2, (iii) able to cycle at moderate intensity for 120 min. Participants were excluded from the study if they were: (i) diagnosed with diabetes or any other chronic illness which might affect the study results, (ii) smokers, (iii) had a history of diabetes, cardiovascular

disease or hypertension, (iv) allergic to BR intake. Participant characteristics are outlined in Table 6.2.

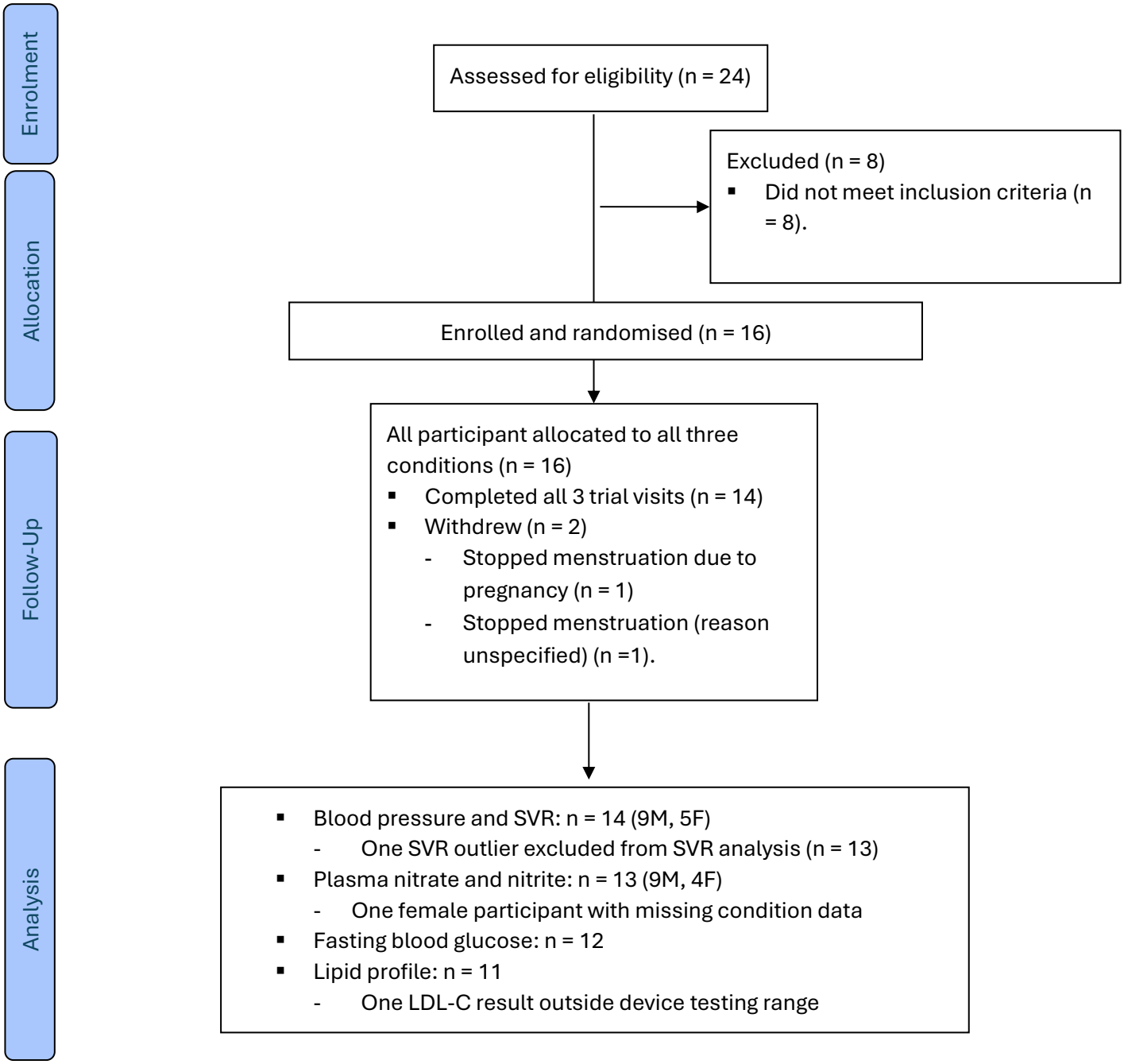

Figure 6.1. Study CONSORT flow diagram. A total of 22 participants were screened with 16 participants completing data collection at all timepoints. M, male. F, female. SVR, systemic vascular resistance. LDL-C, low-density lipoprotein cholesterol.

Study Design

The study was conducted as a repeated measure, randomised, crossover trial with three testing arms: NZ beetroot juice (NZBR), UK Beetroot juice (UKBR), and placebo (PL). Participants were invited to a familiarisation session to provide blood samples using venipuncture (baseline

plasma nitrate/nitrite) and a finger prick for lipid profile (Cobas® b 101 system, Roche Diagnostics, Basel, Switzerland) and fasting capillary blood glucose (HemoCue Glucose 201+ System, Hemocue, Ängelholm, Sweden) as well as providing measures of vascular function (stroke volume (SV) and SVR; USCOM 1A, USCOM Ltd, Sydney, Australia), blood pressure (Omron Smart+ Blood Pressure Cuff, Omron Corporation, Kyoto, Japan), weight (Inbody 270 Body Composition Analyser, Inbody Co Ltd, Seoul, South Korea), height (via stadiometer) and body mass index (BMI).

Study trial visits 1, 2 and 3 took place after participants had completed 6 days' supplementation with one of the three drinks. On day 7, participants arrived at the laboratory, after observing at least a 10-h fast, and provided finger prick blood samples for measures of fasting blood glucose and lipid profile. Measures of SVR, SV, blood pressure, and venous samples for plasma nitrate and plasma nitrite were taken prior to (baseline, time 0-h) and at 2 h and 5 h post-ingestion of a breakfast meal consisting of either peanut butter on toast (energy = 1138 kJ, protein = 14.8 g, carbohydrates = 30.0 g, fats = 10.0 g) or muesli with milk and yoghurt (energy = 1351 kJ, protein = 15.0 g, carbohydrates = 30.0 g, fats = 10.2 g), alongside the intervention drink, all of which participants were asked to consume within a 10 min period. Between the 2 h and 5 h timepoints, participants completed a 2 h cycling protocol followed by a 10 km cycling time trial as part of the larger trial's testing procedures. For males, each trial was followed by a minimum 7-day washout period before the initiation of supplementation with another drink. Females were required to attend their study trial visits during the early follicular phase of the menstrual cycle, or days 3-7 of their contraceptive placebo, resulting in a longer washout period.

6.2.2 Beverage Composition and Dosage

The nitrate concentration of the beverages were measured by high performance liquid chromatography (HPLC) using methods adapted from Chou and colleagues (37). Betalain levels in the beverages were measured by spectrophotometric analysis using the methods and equations from Stintzing et al. (38) and Slatnar et al. (39). Beetroot juice sourced from the UK (Beet-it, James White Drinks, Ipswich, UK) contained 1280 mg of dietary nitrate per dose (2 x 70 mL) and 147 mg betalains per dose whilst the NZBR contained 1120 mg of nitrate and 506 mg of betalains per dose (2 x 100-mL). The betalain and nitrate content of the juices is shown in Table 6.1.

Table 6.1. Betalain and nitrate content of the study beverages.

	UKBR		NZBR	
Daily Dose	**2 x 70-mL**		**2 x 100-mL**	
Compound	**Relative Proportion (%)**	**Total dose (mg)**	**Relative Proportion (%)**	**Total dose (mg)**
Betanin	**27.9**	**41**	**42.5**	**215.2**
Isobetanin	**32.4**	**47.7**	**29.9**	**151.1**
Neobetanin	**36.4**	**4.8**	**26.1**	**7.7**
Unknown	**3.3**	**53.5**	**1.5**	**132**
Total Betalains	**100**	**147**	**100**	**506**
Nitrate		**1280**		**1120**

Individual betalains are presented as a proportion of total betalain content and as absolute amounts per dose (2 bottles). Nitrate is presented as mg per dose (2 bottles). UKBR; United Kingdom Beetroot Juice, NZBR, New Zealand Beetroot Juice

Participants consumed two bottles of either UKBR, NZBR or PL juice per day for 6 days prior to each of the study trial visits. Both participants and the primary researcher were blinded to condition order assignment throughout with bottles labelled A, B and C assigned to the treatment conditions. Participants were allowed to consume the drinks at any time within the 24-h period of each day, however, they were asked to drink the whole content of at least one bottle at any given ingestion time. Participants were asked to return their empty bottles at the end of the 6 days' intervention period to provide a record of compliance to the dosing protocol.

6.2.3 Blood Sampling and Analysis

Blood samples for plasma nitrate and nitrite were collected via venepuncture at the antecubital fossa into lithium heparin vacutainers (6 mL) at baseline (post-6 days, time 0-h), 2 h and 5 h post-meal/beverage consumption. Vacutainers were centrifuged at 1,500 *xg* for 10 min then 500 µl of separated plasma was aliquoted into microcentrifuge tubes and stored at -80°C for later analysis. Nitrate and nitrite plasma samples were then analysed using HPLC methods. Plasma nitrite was analysed in accordance with previous methods adapted from Li and colleagues (40) whilst plasma nitrate was analysed taking 500 µL of filtered plasma prepared during nitrite analysis and analysing according to the method described by Chou et al. (37).

6.2.4 Statistical Analysis

Primary outcomes were plasma nitrate and nitrite concentrations and systolic blood pressure. Secondary outcomes included blood pressure, SVR, fasting blood glucose, and lipid parameters

Plasma nitrate and nitrite levels were compared using repeated-measure 2-way ANOVA at each time point with 'treatment' and 'time' as the two factors, with post-hoc t-tests using Bonferroni adjustment used to compare timepoints under the condition of a treatment*time main effect.

Systolic blood pressure, DBP, SV and SVR measures were compared using repeated-measures 2-way ANOVA at time points 0 h and 2 h with 'treatment' and 'time' as the two factors, with post-hoc t-tests using Bonferroni adjustment used to compare timepoints under the condition of a treatment*time main effect. Given the variability in SBP, DBP, SV and SVR following the exercise component of the methods, the 5 h timepoint was removed from analysis for these parameters.

Glycated haemoglobin A1c, total cholesterol, triglycerides, HDL-C and LDL-C measures were compared using repeated measures ANOVA at time point 0 h. Post-hoc t-tests using Bonferroni adjustment were performed against familiarisation (FAM) results under the condition of a treatment main effect. A *p*-value less than 0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics (SPSS Inc., Chicago, IL, USA) for Windows version 24.0.

Results

6.3.1. Participants

A total of 24 participants were screened, with 16 participants meeting all inclusion criteria. A total of 14 (9M and 5F) participants completed all of the 3 study trial visits between July 2022 and May 2023, with 2 female participants withdrawing from the study before completion of their third visit (Figure 6.1). Baseline characteristics of the participants are shown in Table 6.2.

Table 6.2. Baseline characteristics of study participants stratified by sex.

Variable	Male (n = 9)	Female (n = 5)
Anthropometric characteristics		
Age (y)	27.8 ± 12.4	32.4 ± 15.2
Stature (cm)	179.6 ± 3.2	165.0 ± 5.2
Weight (kg)	80.0 ± 11.3	61.7 ± 9.9
BMI (kg/m^2)	24.8 ± 3.5	22.6 ± 2.8
Vascular function (familiarisation)		
Stroke volume (mL/beat)	79.7 ± 16.5	73.8 ± 7.7
SVR (dyne·sec·cm^{-5})‡	1589.4 ± 355.0	1631.2 ± 236.8
Clinical blood pressure (familiarisation)		
SBP (mmHg)	128.6 ± 11.1	110.8 ± 11.7
DBP (mmHg)	77.2 ± 9.1	70.8 ± 10.2

Data are presented as mean ± standard deviation. Values reflect the 14 participants who completed all three study trial visits (9 males, 5 females). BMI, body mass index; SVR, systemic vascular resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure. ‡ SVR for males calculated excluding one participant with an outlier baseline value of 3309 dyne·sec·cm^{-5} (n = 8).

6.3.2 Plasma Nitrate and Nitrite Analysis

Thirteen participants (9M, 4F) completed all sampling timepoints for plasma nitrate and nitrite.

Plasma nitrate analyses showed a significant treatment*time interaction ($p < 0.001$, Figure 6.2). Descriptive statistics and between-condition differences for all plasma nitrate and nitrite outcomes are presented in Table 6.3. Consumption of both UKBR and NZBR resulted in higher plasma nitrate concentrations than PL at baseline (0 h), 2 h and 5 h post-ingestion ($p < 0.001$). Moreover, the UKBR resulted in higher plasma nitrate concentrations than the NZBR treatment at 2 h (UKBR = 761.5 µmol/L, NZBR = 557.3 µmol/L, $p = 0.003$) and 5 h (UKBR = 705.8 µmol/L, NZBR = 495.7 µmol/L, $p < 0.001$), but not at baseline (UKBR = 267.3 µmol/L, NZBR = 163.5 µmol/L, $p = 0.085$).

Table 6.3. Plasma nitrate and nitrite concentrations, blood pressure and vascular indices at baseline on day 7, and 2h and 5h post beverage ingestion.

Plasma nitrate and nitrite concentrations (n = 13)						
	Mean ± SD (µmol/L or nmol/L)			Between-condition difference Mean (95% CI)		
Outcome	**UKBR**	**NZBR**	**Placebo**	**UKBR – PL (95% CI)**	**NZBR – PL (95% CI)**	**UKBR – NZBR (95% CI)**
Plasma nitrate *Baseline day 7 (µmol/L)*	267.3 ± 165.9 *(167, 367.6)*	163.5 ± 67.3 *(122.8, 204.1)*	60.3 ± 38.6 *(37, 83.6)*	207 *(98.9, 315.1)*	103.2 *(71.2, 135.2)*	103.8 *(-16.8, 224.5)*
Plasma nitrate *2h post-ingestion (µmol/L)*	761.5 ± 151.9 *(669.7, 853.3)*	557.3 ± 96.4 *(499, 615.5)*	78.1 ± 29.2 *(60.4, 95.7)*	683.5 *(590.1, 776.8)*	479.2 *(428.9, 529.5)*	204.2 *(83.7, 324.8)*
Plasma nitrate *5h post-ingestion (µmol/L)*	705.8 ± 136.1 *(623.6, 788)*	495.8 ± 123.9 *(420.9, 570.6)*	112.8 ± 39.9 *(88.7, 136.9)*	593 *(518.7, 667.3)*	383 *(313.2, 452.7)*	210 *(114.9, 305.2)*
Plasma nitrite *Baseline day 7 (nmol/L)*	144.2 ± 61.2 *(107.2, 181.2)*	117.1 ± 46.6 *(89, 145.3)*	102.6 ± 24.4 *(87.8, 117.4)*	41.6 *(13.2, 70)*	14.5 *(-14.3, 43.4)*	27.1 *(-10.3, 64.4)*
Plasma nitrite *2h post-ingestion (nmol/L)*	263.8 ± 126.6 *(187.3, 340.3)*	178.5 ± 88.5 *(125, 232)*	121 ± 39.4 *(97.2, 144.8)*	142.8 *(78.9, 206.7)*	57.5 *(5.1, 109.9)*	85.3 *(0.7, 169.9)*
Plasma nitrite *5h post-ingestion (nmol/L)*	170.7 ± 115.1 *(101.2, 240.3)*	176.5 ± 75.8 *(130.6, 222.3)*	96.9 ± 32.1 *(77.5, 116.3)*	73.8 *(5, 142.6)*	79.6 *(42.3, 116.9)*	-5.7 *(-66.6, 55.2)*
Blood pressure (n = 14) and systemic vascular resistance (n = 12)						
Outcome	**Mean ± SD (mmHg or dyne·sec·cm^{-5})**			**Between-condition difference Mean (95% CI)**		
SBP *Baseline day 7 (mmHg)*	117.9 ± 10 *(112.1, 123.6)*	116.4 ± 9 *(111.2, 121.5)*	121.7 ± 13.3 *(114, 129.4)*	-3.9 *(-10, 2.3)*	-5.4 *(-10.4, -0.3)*	1.5 *(-3.2, 6.2)*
SBP *2h post-ingestion (mmHg)*	117 ± 8.9 *(111.9, 122.1)*	117.1 ± 8.9 *(112, 122.3)*	122.7 ± 10.9 *(116.4, 129)*	-5.7 *(-9.1, -2.3)*	-5.6 *(-10.5, -0.7)*	-0.1 *(-4, 3.8)*
DBP *Baseline day 7 (mmHg)*	76.7 ± 8.2 *(72, 81.4)*	75 ± 7.8 *(70.5, 79.5)*	77.6 ± 8.4 *(72.7, 82.4)*	-0.9 *(-3.9, 2.2)*	-2.6 *(-5.9, 0.7)*	1.7 *(-1.9, 5.4)*
DBP *2h post-ingestion (mmHg)*	73.1 ± 7.3 *(68.9, 77.3)*	72.3 ± 9.1 *(67, 77.5)*	75.4 ± 6.4 *(71.7, 79.1)*	-2.4 *(-5, 0.3)*	-3.1 *(-6.9, 0.6)*	0.8 *(-2.3, 3.9)*
SVR *Baseline day 7* (dyne·sec·cm^{-5})	1523.8 ± 278.4 *(1346.9, 1700.6)*	1379.7 ± 228.5 *(1234.5, 1524.9)*	1538.8 ± 322.9 *(1333.7, 1744)*	-15.1 *(-210.8, 180.7)*	-159.2 *(-303.9, -14.4)*	144.1 *(-7.1, 295.2)*
SVR *2h post-ingestion* (dyne·sec·cm^{-5})	1317.5 ± 233.9 *(1168.9, 1466.1)*	1228 ± 224.4 *(1085.4, 1370.6)*	1321 ± 197.1 *(1195.8, 1446.2)*	-3.5 *(-154, 147)*	-93 *(-250.4, 64.4)*	89.5 *(-55.7, 234.7)*

Values are mean ± SD with 95% confidence intervals in parentheses. n=13 for plasma nitrate/nitrite; n=14 for SBP and DBP; n=12 for SVR (one participant excluded due to outlier baseline value >3000 dyne·sec·cm^{-5}). Positive values indicate first-named condition exceeded second. UKBR, UK beetroot juice (1280 mg nitrate/dose, 147 mg betalains/dose); NZBR, NZ beetroot juice (1120 mg nitrate/dose, 506 mg betalains/dose); PL, placebo; SVR, systemic vascular resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Plasma nitrite analyses showed a significant treatment*time interaction (p = 0.003, Figure 6.3). The UKBR treatment yielded higher plasma nitrite concentrations than PL at baseline (p = 0.008), but no differences were observed between nitrite levels from participants who were consuming the NZBR and PL treatments at baseline (p = 0.295). Post-hoc t-tests revealed the UKBR treatment led to higher plasma nitrite levels at 2 h than the NZBR treatment (UKBR = 264.8 nmol/L, NZBR = 178.5 nmol/L, p = 0.048) and PL treatment (PL = 121.0 nmol/L, p < 0.001), whilst nitrite concentrations at 2 h following the NZBR treatment were also higher than the PL treatment (p = 0.034). At 5 h post-ingestion, no differences in plasma nitrite concentrations were observed between the participants consuming the UKBR and NZBR treatments (P = 0.841), however the UKBR (p = 0.038) and NZBR (p < 0.001) treatments resulted in higher plasma nitrite concentrations than the PL treatment.

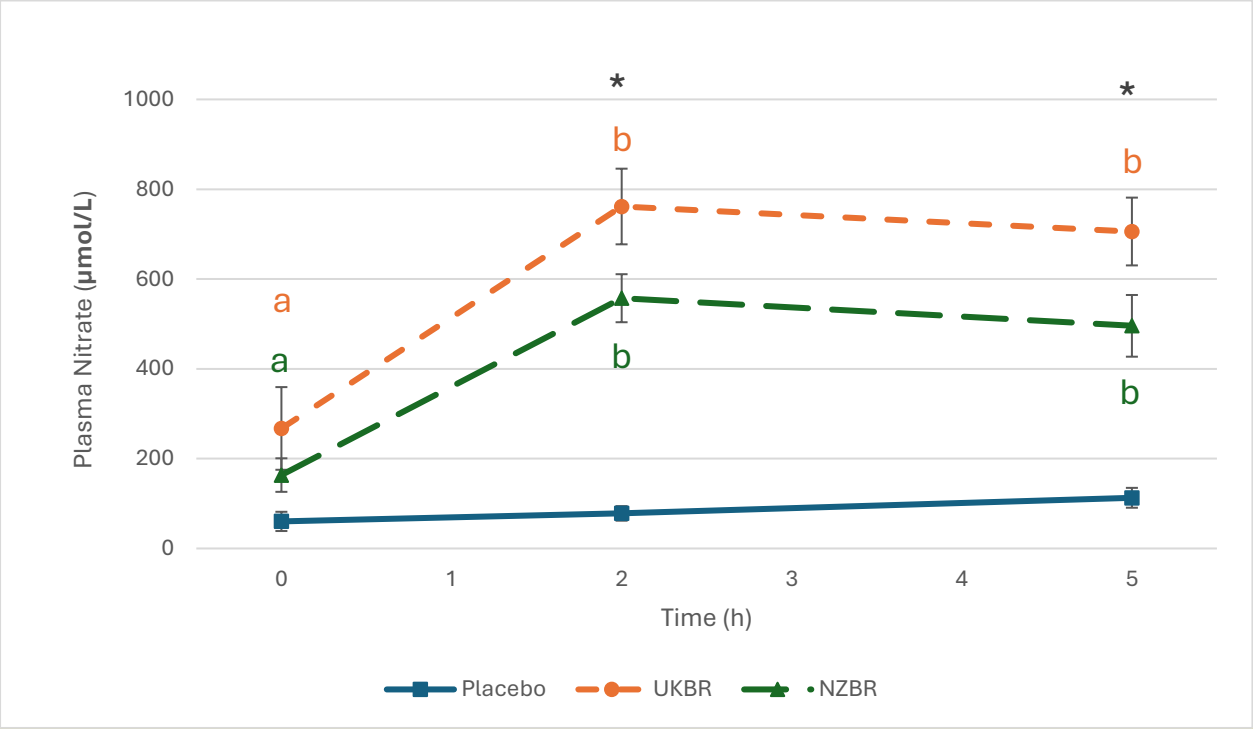

Figure 6.2. Plasma Nitrate concentrations after 6 days of supplementation with either placebo (PL), UK beetroot juice (UKBR) or NZ beetroot juice (NZBR). Baseline represents 0 h on day 7 of supplementation and 2 h represents the 2 h post-meal timepoint on day 7, and 5 h represents the 5 h post-meal timepoint on day 7. Values are mean (n = 13) with error bars representing 95% confidence intervals. Grey letters represent a significant difference between NZBR and PL. Orange letters represent a significant difference between UKBR and PL. * represents a significant difference between NZBR and UKBR. Different lowercase letters (a-b) represent significant differences across time within a given treatment. Significance level of p < 0.05.

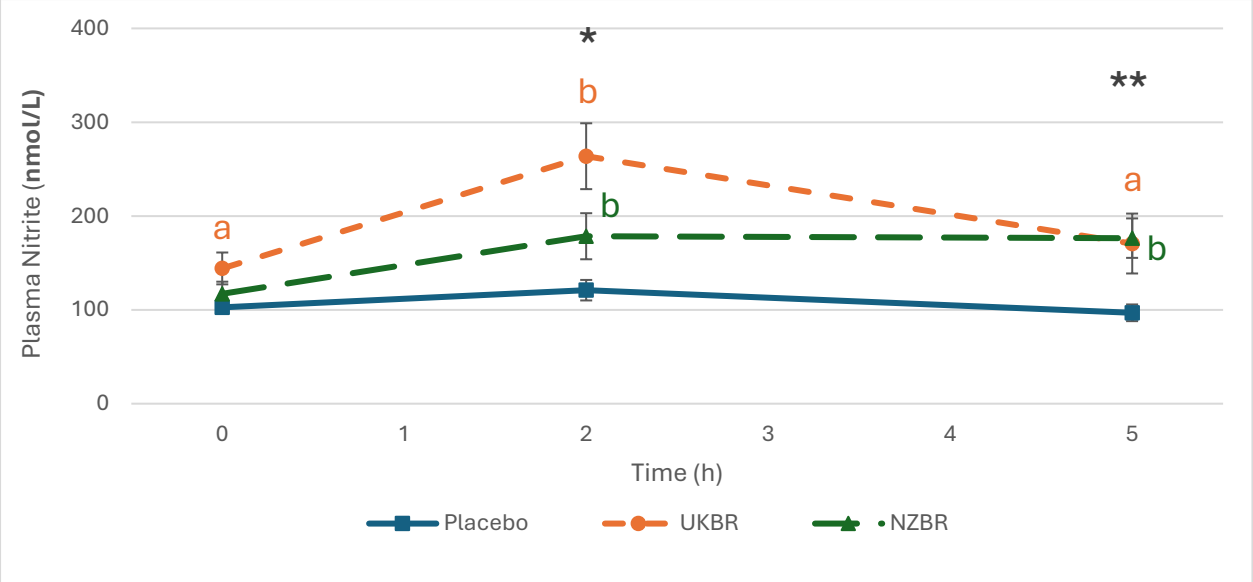

Figure 6.3 Plasma nitrite concentrations after 6 days of supplementation with either Placebo (PL), UK beetroot juice (UKBR) or NZ beetroot juice (NZBR). Baseline represents 0 h on day 7 of supplementation and 2 h represents the 2 h post-meal timepoint on day 7, and 5 h represents the 5 h post-meal timepoint on day 7. Values are mean (n = 13) with error bars representing 95% confidence intervals. Grey letters represent a significant difference between NZBR and PL. Orange letters represent a significant difference between UKBR and PL. * represents a significant difference between all three interventions (NZBR, UKBR and PL). ** represents a significant difference between PL and both NZBR and UKBR. Different lowercase letters (a-b) represent significant differences across time within a given treatment. Significance level of $p < 0.05$.

6.3.3 Blood Pressure and Systemic Vascular Resistance

A total of 14 participants (9 males and 5 females) completed blood pressure indices at all analysed timepoints (baseline on day 7 (after 6 days of ingestion) and 2 h after acute ingestion on day 7).

For SBP, there was an effect of treatment ($p = 0.007$) and time ($p < 001$). Descriptive statistics and between-condition differences for all blood pressure outcomes are presented in Table 6.3. Consumption of the NZBR treatment resulted in lower SBP after 6 days of supplementation (baseline) than PL (-5.3 ± 2.8 mmHg, $p = 0.038$). Further, 2 h post-meal and ingestion of beetroot juice, SBP was lower in the UKBR and NZBR conditions than PL (PL = 122.7 mmHg, UKBR = 117.0 mmHg; $p = 0.003$, NZBR = 117.1 mmHg, $p = 0.029$, Figure 6.4).

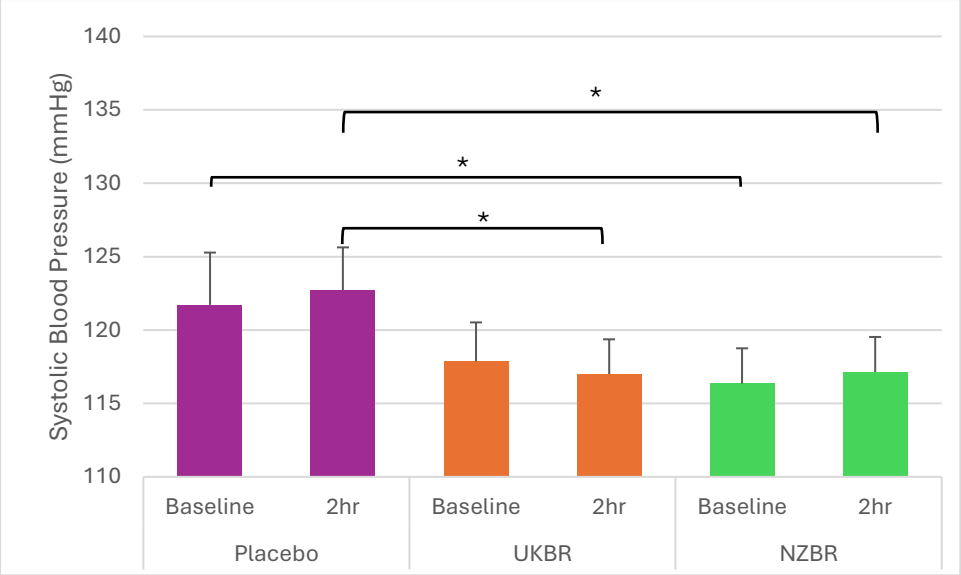

Figure 6.4. Systolic blood pressure after 6 days of supplementation with either Placebo (PL), UK beetroot juice (UKBR) or NZ beetroot juice (NZBR). Baseline represents 0 h on day 7 of supplementation and 2 h represents the 2 h post-meal timepoint on day 7. Values are mean (n = 14) with error bars representing 95% confidence intervals. * indicates a significant difference between treatments ($p < 0.05$).

For DBP there was a significant effect of treatment ($p = 0.020$) as well as trend for time ($p = 0.080$). There were no differences in DBP between treatments after 6 days of supplementation ($p = 0.256$) or 2 h post-meal and ingestion of beetroot juice ($p = 0.107$). There was however a trend that both the NZBR and UKBR interventions reduced DBP 2 h post-consumption compared to the PL intervention (NZ, $p = 0.092$; UK, $p = 0.079$; Figure 6.5). Furthermore, DBP was significantly reduced after 2 h compared to baseline in the UKBR intervention group (Baseline = 76.71 mmHg, 2 h = 73.07 mmHg, $p = 0.024$) but not for the NZBR group.

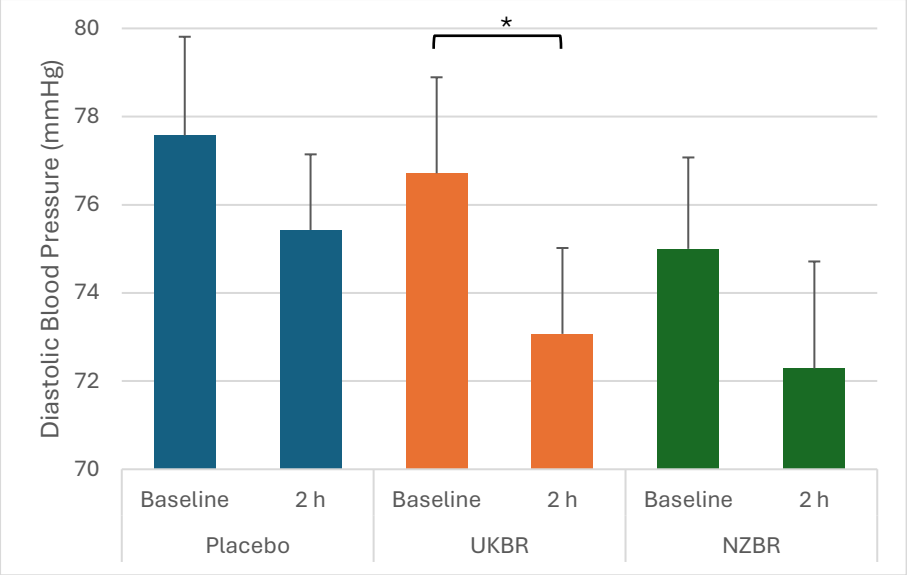

Figure 6.5. Diastolic blood pressure after 6 days of supplementation with either Placebo (PL), UK beetroot juice (UKBR) or NZ beetroot juice (NZBR). Baseline represents 0 h on day 7 of supplementation and 2 h represents the 2 h post-meal timepoint on day 7. Values are mean (n = 14) with error bars representing 95% confidence intervals. No significant differences were observed between groups. * indicates a significant difference between time points (p < 0.05).

There was an effect of time ($p < 0.001$) but not of treatment ($p = 0.162$) or treatment*time ($p = 0.537$) for SV. No significant differences were observed between participant SV at familiarisation and at 6 days' of supplementation in any condition.

One participant was removed from the analysis for SVR as they had an SVR at baseline of 3309 dyne-sec-cm^{-5} (over double the normal range of 800 – 1500 dyne-sec-cm^{-5}) (41). There was an effect of time ($p < 0.001$) and treatment ($p = 0.013$) but no treatment*time ($p = 0.454$) interaction for SVR (Figure 6.6). The NZBR treatment resulted in lower SVR after 6 days of supplementation than the PL treatment (NZBR = 1432 dyne-sec-cm^{-5}, PL = 1651 dyne-sec-cm^{-5}, $p = 0.024$) and the UKBR treatment (NZBR = 1432 dyne-sec-cm^{-5}, UKBR = 1573 dyne-sec-cm^{-5}, $p = 0.046$). No differences in SVR were observed between the UKBR and PL interventions after 6 days of supplementation. There were no differences in participants' SVR between interventions 2 h after meal ingestion, however SVR was reduced from baseline to 2 h post-meal in all conditions (PL, $p = 0.003$; UKBR, $p = 0.001$, NZBR, $p = 0.021$).

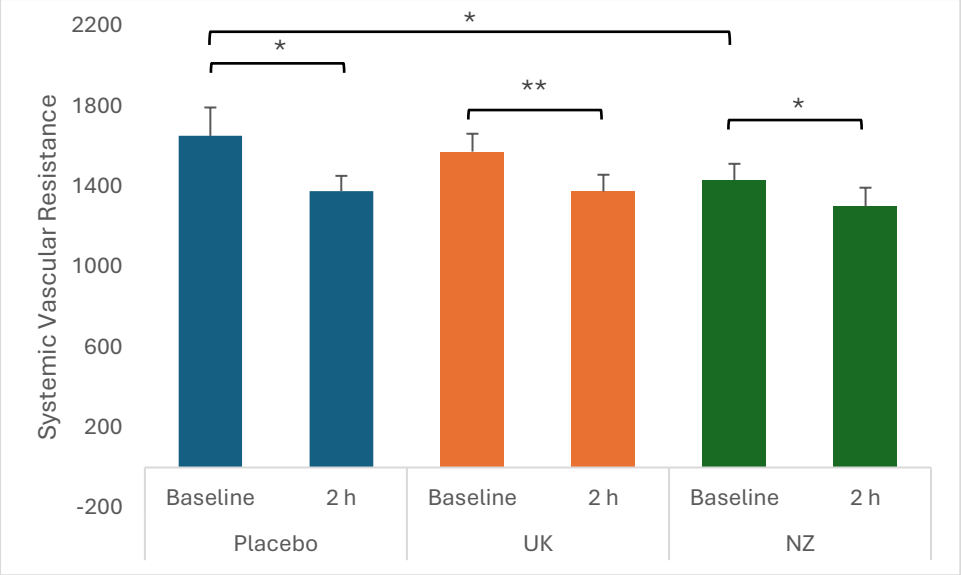

Figure 6.6. Systemic vascular resistance after 6 days of supplementation with either Placebo (PL), UK beetroot juice (UKBR) or NZ beetroot juice (NZBR). Baseline represents 0 h on day 7 of supplementation and 2 h represents the 2 h post-meal timepoint on day 7. Values are mean (n = 13) with error bars representing 95% confidence intervals. * indicates a significant difference between the relevant data sets ($p < 0.05$). ** indicates a significant difference between the relevant data sets ($p < 0.001$).

6.3.4 Blood Glucose and Lipid Levels

Comparisons of levels of blood glucose and lipid parameters are presented in Table 6.4. Twelve participants completed fasting blood glucose measurements at familiarisation and in all three treatment conditions. There was no difference in participants' fasting glucose concentrations during familiarisation and after 6 days of supplementation with any of the intervention drinks.

Eleven participants completed a full lipid panel at familiarisation and in all three treatment conditions; however, one participant returned a low-density lipoprotein result outside the device's testing range, and therefore no result was returned. There were no differences between participants' triglycerides, total cholesterol and high-density lipoprotein cholesterol at familiarisation vs after 6 days of supplementation with any intervention drink. There was a difference in participants' high-density lipoprotein cholesterol between familiarisation (FAM) and PL at 6 days of intervention (FAM = 1.709 ± 0.087 mmol/L, PL = 1.581 ± 0.099 mmol/L, p = 0.047) and between FAM and the UKBR intervention (FAM = 1.709 ± 0.087 mmol/L, UKBR = 1.579 ± 0.082 mmol/L, p = 0.011), whilst HDL-C in the NZBR intervention trended lower than FAM (FAM = 1.709 ± 0.087 mmol/L, NZBR = 1.583 ± 0.096 mmol/L, p = 0.082).

No adverse events related to consumption of the intervention or placebo beverages were reported during the trial.

Table 6.4. Fasting blood glucose and lipid profiles at familiarisation and at baseline on day 7, after 6 days of supplementation with either placebo, UK beetroot juice or NZ beetroot juice.

	Mean ± SD (mmol/L)				Difference vs familiarisation Mean (95% CI)		
Parameter	**Familiarisation**	**UKBR**	**NZBR**	**Placebo**	**UKBR – FAM**	**NZBR – FAM**	**PL – FAM**
Fasting blood glucose *(mmol/L)* n = 11	5.14 ± 0.46 *(4.83, 5.45)*	5.13 ± 0.51 *(4.78, 5.47)*	5.15 ± 0.74 *(4.66, 5.65)*	5.34 ± 0.76 *(4.83, 5.85)*	-0.01 *(-0.46, 0.44)*	0.02 *(-0.47, 0.51)*	0.2 *(-0.37, 0.77)*
Total cholesterol *(mmol/L)* n = 11	4.36 ± 0.76 *(3.86, 4.87)*	4.12 ± 0.58 *(3.73, 4.51)*	4.27 ± 0.81 *(3.73, 4.81)*	4.14 ± 0.76 *(3.63, 4.65)*	-0.24 *(-0.64, 0.16)*	-0.09 *(-0.59, 0.4)*	-0.22 *(-0.45, 0.01)*
Triglycerides *(mmol/L)* n = 11	1.57 ± 0.8 *(1.04, 2.11)*	1.28 ± 1.23 *(0.45, 2.11)*	1.31 ± 0.43 *(1.02, 1.6)*	1.73 ± 0.7 *(1.26, 2.2)*	-0.3 *(-1.27, 0.67)*	-0.26 *(-0.78, 0.26)*	0.16 *(-0.55, 0.86)*
HDL-C *(mmol/L)* n = 11	1.71 ± 0.29 *(1.51, 1.9)*	1.58 ± 0.27 *(1.4, 1.76)*	1.58 ± 0.32 *(1.37, 1.8)*	1.58 ± 0.33 *(1.36, 1.8)*	-0.13 *(-0.26, 0)*	-0.13 *(-0.27, 0.02)*	-0.13 *(-0.22, -0.04)*
LDL-C *(mmol/L)* n = 10	2 ± 1.03 *(1.26, 2.74)*	2.14 ± 0.53 *(1.75, 2.52)*	1.98 ± 0.66 *(1.51, 2.45)*	1.89 ± 0.66 *(1.42, 2.36)*	0.14 *(-0.52, 0.8)*	-0.02 *(-0.52, 0.48)*	-0.11 *(-0.6, 0.38)*

Values are mean ± SD with 95% confidence intervals in parentheses. n varies per parameter as indicated (n = 11 for fasting blood glucose; n = 10-11 for lipid parameters). Differences are unadjusted paired means vs familiarisation. Positive values indicate condition exceeded familiarisation. UKBR, UK beetroot juice; NZBR, NZ beetroot juice; PL, placebo; FAM, familiarisation (pre-intervention baseline); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

6.4 Discussion

The aim of this study was to assess and compare the effects of consuming two different BR juice beverages on metabolic syndrome risk factors after 6 days of daily juice consumption. We also aimed to compare the impact of acute ingestion of two different BR juice beverages on measures of vascular function, blood pressure, and plasma nitrate and nitrite levels. By investigating BR juices containing different concentrations of nitrates and betalains, we aimed to gain a better understanding of the contributions of these bioactive compounds to any observed changes in the parameters measured. The results from this study were consistent with previous findings that acute ingestion of BR juice reduces blood pressure compared to placebo (16, 20, 21, 42) and that BR juice reduces SVR in healthy participants.

6.4.1 Plasma Nitrate and Nitrite

As expected, plasma nitrate concentrations were elevated following 6 days of ingestion of UKBR and NZBR compared to PL. Simultaneously, plasma nitrite concentrations were elevated in the UKBR compared to PL after 6 days of ingestion. Previous studies have also shown an accumulation of nitrite in the blood following short-term dietary nitrate supplementation (4-6 days with 372 mg nitrate/day) (43, 44). McDonagh and colleagues used a similar protocol, providing 2 x 70-mL shots of BR juice containing ~400 mg of nitrate and saw increases in plasma nitrate and nitrite acutely at 2 h and 5 h, as well as at baseline on day 7 after 6 days of supplementation (45). The present study showed that plasma nitrite levels increased following 6 consecutive days of nitrate supplementation above 1100 mg/day, as well as acutely following ingestion. The UKBR condition, which contained ~160 mg more nitrate than the NZBR condition resulted in greater changes in plasma nitrate and nitrite, suggesting relatively small changes in nitrate doses can have significant effects on plasma nitrate and nitrite levels, and subsequently on NO availability.

Even after 6 days of daily consumption, an acute dose increased both plasma nitrate and nitrite concentrations significantly following both UKBR and NZBR consumption compared to the placebo. The UKBR resulted in higher plasma nitrate levels than NZBR at both the 2 h and 5 h timepoint, as well as higher nitrite levels at the 2 h timepoint. Given the UKBR treatment had a higher nitrate dose than the NZBR, this suggests a dose-response relationship between the amount of ingested dietary nitrate and subsequent circulating plasma nitrate levels, a precursor for NO production which can subsequently be utilised by the vascular endothelial cells and vascular smooth muscle cells to maintain vascular homeostasis (46).

6.4.2 Blood pressure

In this study, while SBP did not decrease between baseline and 2 h after ingestion of any of the intervention beverages, in the UK intervention, DPB was significantly reduced 2 h post-ingestion. Beetroot juice supplementation has been shown to acutely decrease both SBP and DBP (16, 20, 21), and a comparison of meta-analyses shows that those with only a hypertensive adult cohort show greater reductions in SBP (-4.96 mmHg) (47) than meta-analyses that included both hypertensive and healthy participants (-3.55 mmHg) (48). The present study used participants who had a normotensive SBP, but they still showed reductions in systolic blood pressure of ~5 mmHg between groups consuming NZBR juice and placebo at baseline on day 7 (after 6 days of supplementation) and 2 h post-supplementation.

Supplementation on day 7 did not cause an acute reduction in SBP compared to baseline in the UKBR or NZBR interventions, and this may suggest that a 'saturation point' of nitrite utilisation is reached after the 6-day loading period, meaning further reductions in SBP are not possible. Whilst this may be the case in our study, previous research in older adults has shown continued improvements after 28 days of supplementation (16). Further, plasma nitrite levels were significantly higher following ingestion of UKBR than both PL and NZBR after 6 days, yet SBP was significantly lower in the NZBR juice intervention group compared to PL, whilst UKBR was not. It appears the reduction in SBP following short-term BR juice ingestion may not be solely due to the blood pressure-lowering effects of dietary nitrate, but that other compounds, such as betalains, which were present at higher concentrations in the NZBR, may be contributing to the observed reductions in SBP following the NZBR intervention. Shepherd and colleagues (42) showed a reduction in BP following ingestion of nitrate-depleted BR juice (NDBR), highlighting that the lowering of blood pressure observed in previous studies may not be caused entirely by nitrate alone. In Shepherd and colleagues' study, plasma concentrations of the anti-inflammatory cytokine interleukin-10 (IL-10), which reduces pro-inflammatory cytokine expression (49), increases endothelial nitric oxide expression (50), and inhibits angiotensin-II-induced vascular dysfunction (51), increased following ingestion of both BR and NDBR. Previous studies have shown betalain-rich dyes augment IL-10 production in rat models (52, 53), and this pathway may explain why the betalain-rich NZBR was more effective at reducing SBP than UKBR. The nitrate-independent potential for betalains to reduce blood pressure is corroborated by Rahimi and colleagues' study which supplemented individuals with atherosclerotic cardiovascular disease with either *Opuntia stricta* (prickly pear cactus; known to be comparable to beetroot in betalain content (54) but ~20x lower in dietary nitrate (55)) or red beetroot, for two weeks, and showed

improvements in SBP in both treatments compared to placebo, but greater reductions were observed in the red beetroot juice condition (56).

6.4.3 Systemic Vascular Resistance and Stroke Volume

Significant reductions in SVR were observed after 6 days of supplementation with NZBR, but not with UKBR, compared to PL. The effect observed with the NZBR is notable because SVR is primarily controlled by the microvasculature, where endothelial dysfunction tends to precede the development of hypertension (29). The NZBR contained lower levels of nitrate but higher levels of betalains than UKBR, and betalains have been shown to have strong antioxidant and anti-inflammatory effects (57), which may improve microvascular endothelial function. Previous studies have shown that tumour necrosis factor-alpha (TNF-α) and reactive oxygen species (ROS) are major contributors to endothelial dysfunction (58, 59), however betalains have been shown to reduce TNF-α and ROS levels in rat models (52, 60), and this mechanism may, in part, explain why consumption of NZBR resulted in reduced SVR compared to PL, but UKBR did not.

Systemic vascular resistance was also reduced in all conditions 2 h postprandially. Previous studies have shown splanchnic blood flow is increased following meal consumption to secure essential nutrients and oxygen to the intestines for digestion and absorption of digested substances to the liver (61, 62). This is caused by vasodilation of blood vessels supplying the stomach, intestines, pancreas and liver, which is primarily mediated by increases in NO signalling (63). Due to this vasodilation of microvascular blood flow, SVR is reduced throughout the whole body, meaning any acute changes to SVR caused by ingesting the BR juice may have been masked by the effect of concurrent meal consumption.

6.4.4 Lipid Profile and Blood Glucose Levels

Despite prior studies suggesting that acute consumption of beetroot juice can improve lipid profiles (64-67) and glucose metabolism (66, 68-70), in this study no significant changes were observed in fasting blood glucose, triglycerides, or cholesterol levels after 6 days of supplementation. There are several possible explanations for these findings. Firstly, the relatively short duration of the intervention (6 days) may have been insufficient to produce measurable changes in lipid metabolism or glucose homeostasis, which typically require more prolonged dietary interventions ranging from 4-12 weeks (71-74). Secondly, the participants in this study were generally healthy and normolipidemic, so their baseline lipid and glucose levels were already within a normal range, potentially limiting the capacity for further improvement. The only significant changes observed in lipid parameters were in HDL-C; both UKBR and PL showed lower HDL than FAM after 6 days of supplementation, however they were not significantly

different to NZBR, and NZBR trended lower than at familiarisation. It is possible that introducing a beverage to the diet may have caused nutrient displacement, particularly at breakfast when fibre intake is generally highest (75). Despite this, in a study by Moftah and colleagues (76), hypercholesterolemic rats fed 200, 400, or 600 mg/kg/day of BR juice for 4 weeks showed elevated HDL-C levels compared to placebo. A clear dose-response relationship was observed, with the 600 mg/kg/day group having the highest HDL-C levels after 4 weeks of supplementation. Moftah and colleagues' study notably took place over a longer duration than the present study (4 weeks vs 6 days), as well as in a population of hypercholesterolemic rats, whereas our study took place in healthy humans. Future research should explore supplementation in populations at risk of hypercholesterolaemia or MetS over periods of 4 weeks or more to investigate the potential of BR juice consumption to improve glucose and lipid metabolism.

6.4.5 Potential Combined Effects of Consumption of Nitrates and Betalains

This study provides new insights into how the different bioactive components in beetroot juice, nitrates and betalains, produce beneficial health effects. While dietary nitrate clearly plays a role in acute vasodilation and blood pressure reduction, our findings that SVR was reduced in the NZBR juice group suggest that betalains may contribute to more sustained improvements in vascular function, particularly in the microvasculature. The greater reduction in SVR observed with NZBR consumption, despite its lower nitrate content, underscores the potential of betalains to have bioactivity that complements that of dietary nitrate, possibly by enhancing NO bioavailability (50).

Polyphenols have been shown to improve oral health and reduce the proliferation of *Streptococcus mutans* (77), which may compete with other nitrate-reducing bacteria in the oral cavity. Whilst betalains are not polyphenols, they may induce similar effects on the oral microbiome due to their similar antioxidant activity (78). As hypertensive individuals have reduced abundance of the nitrate-reducing bacteria taxa *Veillonella* compared with normotensive individuals (79), and oral mouthwash prior to dietary nitrate supplementation reduces the effectiveness of interventions which reduce blood pressure (80), it is possible that the greater reductions in BP seen after 6 days of supplementation with NZBR were due to an increase in nitrate-reducing bacteria compared to the groups consuming the placebo and UKBR.

Further, betalains have an anti-inflammatory effect by scavenging ROS (57). Nitric oxide, once formed, is highly susceptible to degradation by superoxide anions (a form of ROS), leading to the formation of peroxynitrite (81); by preventing this reaction, betalains may increase the bioavailability of NO produced from nitrate and nitrite reduction. Finally, it has been shown that

antioxidants and polyphenols in vegetables and fruits increase the rate of NO production in the gut (82), meaning concurrent ingestion of polyphenols and dietary nitrate results in greater NO bioavailability postprandially (83).

6.4.6 Limitations and Future Directions

Whilst this study provided insight into the effects of BR juice on plasma nitrate concentrations and haemodynamics, several limitations in the study design may have limited our understanding of the effects of BR supplementation on glucose control and lipid parameters. Firstly, the duration of the intervention (6 days) may have been insufficient for detecting more substantial changes in glucose metabolism and lipid profiles. Previous studies often involved longer intervention periods from 4-12 weeks (71-74) or used a population already at risk of MetS, such as those with hypercholesterolaemia or T2DM (84, 85). Our study used a healthy, normotensive population capable of 2 h of continuous physical activity. The use of healthy, moderately trained participants likely limited the ability of the study to observe significant changes in blood pressure, lipid profiles and glucose metabolism. Future studies should include a longer supplementation period as well as focusing on populations at risk of MetS development or who already present with MetS risk factors.

A further limitation is that the two active beverages were not nitrate-matched (UKBR: 1280 mg vs NZBR: 1120 mg per daily dose). This modest difference clearly influenced circulating nitrate/nitrite kinetics as reflected in the higher plasma nitrate/nitrite in the UKBR condition. This complicates attribution of vascular effects to betalains versus nitrate. Consequently, the possibility that between-arm differences in SBP and SVR reflect interactions between dose-dependent NO bioavailability and betalain content rather than betalains alone cannot be dismissed. Future studies should equate nitrate dose and manipulate betalain content independently.

To establish in greater depth the contribution of betalains improvements in vascular function seen in this study and other studies of its type, studies using nitrate-depleted BR juice, dietary nitrate without betalains and BR juice with both bioactives should be employed. Establishing the relative contributions of betalains and nitrates to mitigation of different MetS risk factors may help inform intervention strategies in the future.

6.5 Conclusion

This study confirms that beetroot juice containing dietary nitrate and betalains improves vascular function by reducing systemic vascular resistance and systolic blood pressure after 6 days of

supplementation. Plasma nitrate and nitrite levels increased more at higher nitrate doses, but this did not result in a linear improvement in vascular function. Betalains may have a significant role in the observed beneficial effects that BR juice had on blood pressure. This should be considered in the formulation of beetroot juice products to optimise health benefits. Future research should be carried out to confirm these findings and establish mechanisms of action to explain the observed effects, as well as investigate whether the acute effects translate to longer term health benefits.

6.6 References

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Student name:	Cameron Haswell
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In which chapter is the manuscript/published work?	Chapter 7

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Cameron Haswell (C.H.), Kay Rutherfurd Markwick (K.R.M.), Ajmol Ali (A.A.), Rachel Page (R.P.) and Roger Hurst (R.H.) contributed to the conceptualization of the manuscript; CH., K.R.M., A.A., R.P., and RH contributed to the methodology; C.H., performed formal analysis; C.H., conducted data collection and sample analysis; C.H., performed writing — original draft preparation; C.H., K.R.M., A.A., R.P., and R.H., contributed to writing — review and editing.

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Chapter 7 – Insights from a 12-Week Nutritional Intervention Trial in People with Prediabetes: Screening, Recruitment and Retention

This chapter was originally intended to address research Question 5 (see Section 1.3.4) and aimed to evaluate whether chronic supplementation with beetroot and blackcurrant juice could improve fasting glucose, HbA1c, blood pressure, and lipid profiles in individuals with prediabetes. However, due to significant recruitment challenges and the high resource demands of delivering a long-term intervention in this at-risk population, the manuscript was adapted. Instead, this chapter presents the challenges discovered in recruitment and retention of participants with prediabetes into a 12-week daily nutritional intervention study. This chapter has been presented in manuscript format.

Abstract

Introduction

Prediabetes, an intermediate state of hyperglycaemia, significantly increases the risk of developing type 2 diabetes mellitus (T2DM). Despite the potential for dietary interventions to prevent progression to T2DM, recruitment and retention of participants in clinical trials investigating prediabetes and other chronic diseases remains challenging due to low awareness of health status and large sample size requirements. This study aimed to investigate and learn from challenges in recruitment and retention during a 12-week daily nutritional intervention using beetroot and blackcurrant juices to improve glycaemic control in individuals with prediabetes.

Methods

A single-blind, placebo-controlled pilot study was conducted in Auckland, New Zealand. Participants aged 30-75 years with prediabetes (HbA1c 41 - 49 mmol/mol, fasting glucose >5.6 mmol/L) were recruited via healthcare providers, social media, university mailing lists, and community outreach. Eligible participants were randomised into 4 intervention arms: beetroot juice (600 mg nitrate/person/day), blackcurrant juice (300 mg anthocyanins/person/day), a beetroot and blackcurrant mix (600 mg nitrate and 300 mg anthocyanins/person/day), or placebo. Daily supplementation was provided for 12 weeks, with primary outcome measures including glycated haemoglobin (HbA1c), fasting blood glucose, lipid profile, and blood pressure. Recruitment and retention rates were analysed to assess study challenges and participants also completed a feasibility questionnaire to evaluate acceptability of the intervention beverages.

Results

Recruitment of participants occurred over the period of August-November 2023. Of the 45 individuals screened, 12 (26.7% of screened) participants were randomised, with 8 completing the full intervention (67% of randomised participants). Healthcare provider advertisement and social media advertising each accounted for 33% of total recruitment, whilst word of mouth and participant mailing lists accounted for 16% of total recruitment, each. Reasons for participant attrition (33%) included time constraints and perceived lack of immediate benefit of the juice. The intervention was well-tolerated, with compliance exceeding 90% in participants who completed the study. Taste, texture, and portion size were key factors affecting compliance, with the mix and blackcurrant drinks receiving lower palatability ratings than the beetroot and placebo drinks. Blood glucose and metabolic outcomes varied among individuals and further investigation with significantly larger sample sizes is required to view any significant effects.

Conclusion

This study highlights the challenges of recruiting and retaining participants in long-term dietary intervention trials targeting glycaemic control in prediabetes. Cost and time effective recruitment strategies, including healthcare provider outreach and targeted advertising, are essential to achieve adequate sample sizes. These insights are also relevant for trials in other underdiagnosed populations, such as hypertension, osteoarthritis, and chronic kidney disease.

Keywords: Participant recruitment; participant retention; participant screening; dietary intervention; beetroot juice; blackcurrant juice.

7.1 Introduction

People with prediabetes, an intermediate state of hyperglycaemia (1), are at high-risk of developing type 2 diabetes mellitus (T2DM)(2). In New Zealand there are over 300,000 people with T2DM (~6% of the population) and the prevalence of prediabetes in adults aged >15 years is 25.5% (3). 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 intervention may lead to preventing development of T2DM (1, 5, 6). The benefits of preventing T2DM are substantial, however intervention studies investigating those at risk of T2DM face challenges in recruiting and retaining participants. Prediabetes is often undiagnosed, as people living with prediabetes are often asymptomatic (5) and prevalence rates of undiagnosed prediabetes in US adults may be as high as 45.8% (7). In contrast, the rate of undiagnosed T2DM is only 6.8% in US adults (7), suggesting higher rates of diagnoses in T2DM than in prediabetes. Low awareness of prediabetes status may be a considerable barrier to effective recruitment into research trials investigating lifestyle or dietary intervention during prediabetes as many potential participants are unaware they are eligible to participate. Previous studies investigating interventions in people at risk of T2DM have employed large-scale screening and recruitment strategies to capture potential participants at great cost, both financially and time (8-10); however literature regarding the efficacy of recruitment strategies in prediabetes is limited.

Polyphenol-rich foods have been investigated for their ability to modulate blood glucose levels (11-19) and reduce postprandial glucose responses in both healthy subjects and in those with T2DM. Beetroot 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 pressure (22) and also mediates glucose uptake from the intestines and skeletal muscle (23, 24). These bioactive compounds may be capable of improving blood glucose control (13, 20, 25-27) as well as other modifiable markers associated with diabetes such as blood pressure (28-32) and lipid profile (25, 27). Longer-term supplementation with dietary nitrate through sodium nitrate in drinking water has been shown to improve insulin sensitivity and glucose control in a rat model (33), however, a recent randomised control trial in people with T2DM supplemented with beetroot juice (containing 180 mg of nitrate) daily for 12 weeks, showed no differences in glycated haemoglobin A1c (HbA1c), homeostatic model assessment for insulin resistance (HOMA-IR), total cholesterol (TC), low-density lipoprotein (LDL-C), triglycerides (TG) and blood pressure (BP) between the intervention and control group (25). The authors did note that due to large participant withdrawal (17.5%) during

the study period, the sample size was reduced from 46 to 38 participants and the study lacked the statistical power to elucidate any changes resulting from the intervention drinks (25). Furthermore, the nitrate dose of 180 mg daily is below that used in previous studies investigating dietary nitrate consumption that have shown significant changes in insulin sensitivity and blood pressure(34, 35). These findings highlight the importance of selecting the correct dose and ensuring adequate sample sizes in studies investigating the impact of beetroot juice on T2DM risk factors.

Blackcurrants, rich in polyphenols in the form of anthocyanins, are another food reported to improve blood glucose control (12, 36, 37), blood pressure (38) and lipid profile (39). Previous studies in rats have shown an antidiabetic effect of blackcurrant anthocyanins through increased 5' adenosine monophosphate-activated protein kinase (AMPK) activation (40-42), whilst studies in humans have shown that blackcurrant anthocyanins are able to reduce the postprandial glucose response following acute carbohydrate ingestion in the early response phase (0-30 min) (12, 36). This may be due to an inhibition of salivary α-amylase and α-glucosidase by blackcurrant anthocyanins (43).

Beetroot and blackcurrant juice may have the potential to be natural, dietary interventions that could have a significant impact on glycaemic control in people with prediabetes by attenuating postprandial hyperinsulinaemia and preserving β-cell function, which can delay the onset of T2DM (44, 45). Previous studies investigating interventions in people living with prediabetes have required significant recruitment budget requirements (8-10) and faced large participant withdrawal rates (25, 46).

This PhD study initially aimed to investigate the impact of beetroot and blackcurrant juice consumption on HbA1c and other metabolic outcomes in people with prediabetes; however challenges in recruitment and retention meant the study was underpowered. Therefore, the present paper aims to provide an analysis and review of the difficulties encountered in recruitment and retention of participants in a long-term (12 weeks) supplementation study in people living with prediabetes. This pilot study will inform methods of recruitment and drink dosages for a larger study investigating the efficacy of longer-term supplementation with beetroot and blackcurrant juice in people with prediabetes. The results will also inform recruitment and retention strategies for other studies investigating long-term dietary supplementation in populations with undiagnosed chronic disease.

7.2 Methods

7.2.1 Study Population

The study was approved in June 2022 by the Health and Disability Ethics Committee (HDEC; Ref: 2022 EXP 11682). The clinical trial was registered prospectively at anzctr.org.au (Australia and New Zealand Clinical Trials Registry ID: ACTRN12622001108707). All participants were required to give written informed consent prior to participation in the study.

Participants were recruited from Auckland, New Zealand using a combination of poster advertising, liaison with health practitioners (general practitioners and dietitians), shopping centre visits and social media targeting. Inclusion criteria for the study were: (i) 30 - 75 years, (ii) body mass index (BMI) 18.5 - 40 kg/m^2, (iii) HbA1c 41 - 49 mmol/mol, (iv) fasting glucose > 5.6 mmol/L, (v) not pregnant or breastfeeding, (vi) not allergic to beetroot or blackcurrant, (vii) non-smoker, (viii) able to communicate well in English, (ix) not started taking glucose-lowering prescriptions within the previous 3 months. Participants were enrolled into the study from August 2023 until November 2023.

7.2.2 Recruitment Methods

This pilot study had an initial recruitment target of 48 participants (12 per group) with an expected drop-out rate of ~20%, resulting in 10 participants per group at final analysis. As this was a pilot study, sample size calculation were not performed, however this recruitment target aligned with the final analysis numbers reported by Babateen and colleagues (46) who achieved n = 10 per group after ~20% dropout in a similar 13-week intervention study investigating daily beetroot juice supplementation with a similar nitrate dose to our intervention.

Academic Institution

Recruitment strategy involved email communication through Massey University email lists and recruitment databases as well as poster advertising in shared communal and study spaces such as the University library, lecture halls, student lounges and eating areas.

Administrators for each department were contacted with a request to email department staff with study details and posters for recruitment. Posters were displayed around the university campus with relevant study details for potential participants, as well as contact details for the principal investigator.

The recruitment database included people who had previously expressed interest in participating in research and participants from previous trials in the School of Sport, Exercise and

Nutrition. People were contacted via emails containing the quarterly participant newsletter to inform them of the trial and invite them to consider participating.

The School of Sport, Exercise and Nutrition also runs a dietetics clinic, including participants referred from local GP clinics. The dietetics department agreed to distribute study information to participants who were eligible through previous prediabetes diagnoses.

Healthcare Professionals

Trial information including an introductory email outlining the study design, list of inclusion criteria and participant information sheet were sent to 6 local healthcare clinics along with an invitation for face-to-face discussion of further details regarding the study and the potential benefits for participants. Of the 6 healthcare clinics contacted, 2 agreed to face-to-face meetings and both agreed to distribute the study information to participants with HbA1c levels within the prediabetic range (41 - 49 mmol/mol). The other 4 healthcare clinics contacted did not respond, and it was assumed the study information was not disseminated. There was no reward or payment for healthcare centres for distributing this information.

Diabetes Foundation Aotearoa were also contacted to discuss options regarding dissemination of study information but did not respond.

Events

Two members of the study team attended The Asian Network Inc.'s (TANI) local event to network with health professionals and promote the study. A study poster was also included in the subsequent TANI quarterly newsletter. The research team also conducted random screening at a local shopping centre, offering free HbA1c tests to the public. Volunteers were then informed of the study and given the opportunity to participate in the research trial, or to take the information away for friends/family that may be interested.

Social Media

Online advertisements were posted to community Facebook groups in the local area without paid promotion. These advertisements included study information and contact information for potential interest and included links to the study webpage on the Massey University website.

Word of Mouth

Some people reported hearing about the trial through friends, family and/or colleagues. Participants who had already started the trial often spoke to friends and family about their participation which garnered interest.

Further detail on the yield and resource cost of each channel is given in Appendix 4.2.

7.2.3 Study Design

The study was a 12-week, placebo-controlled, single-blind pilot study with 4 testing arms: beetroot juice (BR), blackcurrant juice (BC), beetroot and blackcurrant juice mix (MIX) or placebo (PL). Participants were required to either attend the laboratory at Massey University's Auckland campus or receive an in-home visit by the principal investigator on 2 occasions, separated by a 12-week intervention period (Figure 7.1).

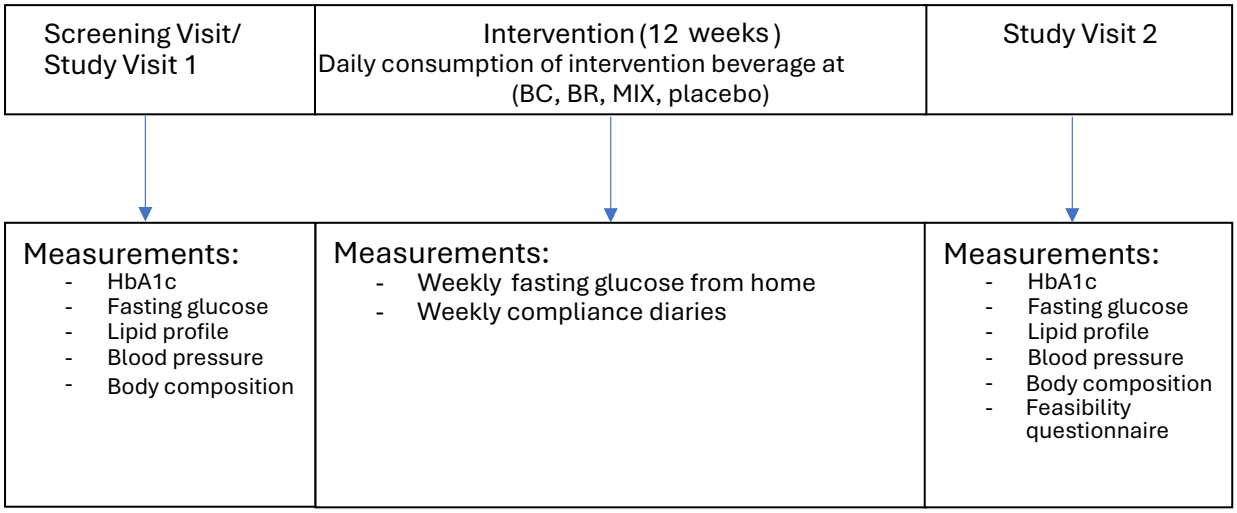

Figure 7.1. Prediabetes study design overview. BC, blackcurrant beverage (300 mg anthocyanins/person/day); BR, beetroot beverage (600 mg nitrate/person/day); MIX, mixed beverage (300 mg anthocyanins and 600 mg nitrate/person/day); HbA1c, glycated haemoglobin A1c.

A food frequency questionnaire (FFQ) was also administered at both study visits as part of the original protocol. However, dietary intake data are not reported here, as they did not become relevant to the feasibility objectives that this chapter ultimately focused on. Additionally, the small sample size meant that meaningful paired baseline–endpoint comparisons were not feasible had the analysis been pursued

Pre-screening: Participants were pre-screened via a phone call to establish likely eligibility for the study. Participants were screened out if they had recently started taking metformin or

another glucose-lowering pharmaceutical or had undergone a significant amount of body weight change in the previous 6 months (Δ > 10% of body mass).

Study Visit 1/ Screening visit: Upon establishing likely eligibility, participants either attended the laboratory at Massey University or received an at-home visit during which measures were taken via finger prick blood sampling for fasting blood glucose (HemoCue Glucose 201+ System, Hemocue, Ängelholm, Sweden), lipid profile and HbA1c (Cobas® b 101 system, Roche Diagnostics, Basel, Switzerland). The volunteer was then invited to participate in the study visits if screening indicated they were eligible. If eligible, stature, mass, body mass index (BMI), and body composition (using a bioelectrical impedance scale (BIA), InBody 270, Inbody, Seoul, South Korea) were then measured. Blood pressure (Omron HEM-7121, Omron, Kyoto, Japan) and resting heart rate (Omron HEM-7121, Omron, Kyoto, Japan) were also measured. The participants were instructed on how to use the handheld glucometer (Caresens Dual, i-SENS, Seoul, South Korea) by the principal investigator and were issued with their study drinks and instructions/compliance record (Appendix 4.6) and a glucometer with a document for recording weekly fasting blood glucose levels (Appendix 4.8).

Intervention period: Participants were assigned to an intervention group and asked to consume 1 study drink daily for 12 weeks (84 days). During this time, they completed weekly fasting glucose measurements using the handheld glucometer issued during their first visit, and ticked a compliance record each time a drink was consumed. Participants were included in data analysis if compliance was >80%, in accordance with studies for T2DM, hypertension and dyslipidaemia (47). Participants were advised to avoid using antibacterial mouthwash but otherwise were instructed to maintain their habitual diet and physical activity levels throughout the 12-week intervention period. No formal dietary monitoring was conducted; however, participants were advised not to substantially alter their diet, begin new exercise programmes, or start any new medications that might affect glycaemic control during the study. Participants who required further intervention drinks during the intervention period were contacted by the principal investigator and an in-person drop-off was arranged at a convenient time. During study visit 1, participants were invited to contact the principal investigator at any time should they required assistance with the at-home glucose monitoring or any other aspect of the study.

Study Visit 2: Study visit 2 was conducted after 12 weeks (84 days) of intervention, and measures for HbA1c, lipid profile, fasting glucose, stature, BMI, body composition, blood pressure and

heart rate were repeated. Participants also completed a feasibility questionnaire to establish the palatability, ease of consumption and practicality of their allocated intervention beverage. Participants responded to statements with ratings between 1 and 5 where 1 represented strong disagreement, 2 represented disagreement, 3 represented neutral feelings, 4 represented agreement and 5 represented strong agreement with the statement (Appendix 4.7).

7.2.4 Treatment Conditions

Beetroot and blackcurrant juices were purchased as concentrates from Juice Products New Zealand (JP-NZ, Timaru, Aotearoa New Zealand) and The New Zealand Blackcurrant Cooperative (The New Zealand Blackcurrant Co-operative Ltd, Nelson, Aotearoa New Zealand), respectively. Beetroot concentrates were analysed for nitrate using HPLC methods modified from Chou and colleagues (48) whilst blackcurrant juice concentrates were analysed for anthocyanin content using spectrophotometric methods established by Özgen and colleagues (49).

The test drinks were aliquoted using aseptic procedures in a food-grade laminar flow hood to ensure sterility and minimise contamination. Equipment that came into direct contact with the drinks (such as measuring jugs, mixing spoons and pails) was sanitised in Mikro-chlor solution (200 ppm) for a minimum of 15 minutes, and work surfaces were cleaned with 70% ethanol. Sterile sample containers were used for all aliquots, and the laminar flow hood and balance were re-sanitised at 30-min intervals or sooner if required.

All concentrates were stored at –20°C before aliquoting. For preparation, beetroot and blackcurrant concentrates were transferred into sterile measuring jugs and weighed into pre-labelled sterile containers within the laminar flow hood. Beetroot and blackcurrant drinks were aliquoted into sterile 120-mL containers containing 4 days' supply, and the mixed drink was prepared in a sterilised 10-L pail before being aliquoted into 250-mL sterile containers in 2 - 3-day quantities. Placebo drinks were aliquoted into sterile 120-mL containers containing 10 days' supply. After aliquoting, the intervention drinks were immediately placed on ice and subsequently stored at –20°C until distribution.

Quality control testing was performed on 2 samples from each batch to assess both pH and microbial safety. pH was measured immediately after aliquoting and after 1, 4, 8, and 12 weeks of frozen storage using calibrated pH meters. Microbial testing, including assessments for yeast, mould, and total plate count (TPC), were conducted at Massey University's Auckland campus (Massey University Food Technology Laboratory) immediately after preparation, following

refrigerated storage (4 days at 5°C), and after frozen storage (up to 12 weeks at –20°C), including subsequent thawing periods to simulate participant handling (Appendix 4.5).

Participants received a compliance diary with instructions to store drinks in their freezers (-20°C) and thaw them in a refrigerator (5°C) 24 hours prior to consumption. Once thawed, drinks were kept refrigerated when not in active use. Participants were instructed to weigh out one dose daily and dilute with cold water to taste. Participants were asked to consume the prepared beverage within a 15-min period. (Appendix 4.6). Beverages could be consumed at any time of day but were recommended to be taken at breakfast for consistency. Doses were not divided across the day. Participants who found the volume difficult to tolerate were advised to increase dilution but not to reduce the dose weight.

In the BR intervention arm, participants consumed beetroot concentrate containing 600 mg of nitrate (NO_3^-) daily (approximately 53 g or 67 g depending on batch, see Appendix 4.5). In the BC intervention arm, participants consumed blackcurrant concentrate containing 300 mg anthocyanins daily (24 g concentrate). In the MIX intervention arm, participants consumed a combination of the BR and BC intervention group doses (either 77 g total or 91 g total depending on BR batch). Participants in the PL intervention arm consumed 10 g daily of a commercially available berry-flavoured sugar free syrup. The doses chosen were based on the doses used in the previous intervention studies within this thesis (Chapters 5 and 6).

7.3 Results

7.3.1 Participants

Recruitment and data collection took place between August 2023 and February 2024. A total of 45 participants responded and were screened for this study with 14 participants meeting the inclusion criteria. All the participants who failed to meet the inclusion criteria had HbA1c values outside the prediabetic range in NZ (41 – 49 mmol/mol). Two participants declined to participate after screening, whilst 4 participants were randomised but did not complete the full intervention period (see Figure 7.2). Of the 12 participants who started the intervention period, 8 participants completed the full 12 weeks, all of whom met the compliance target of > 80% of beverages consumed (47).

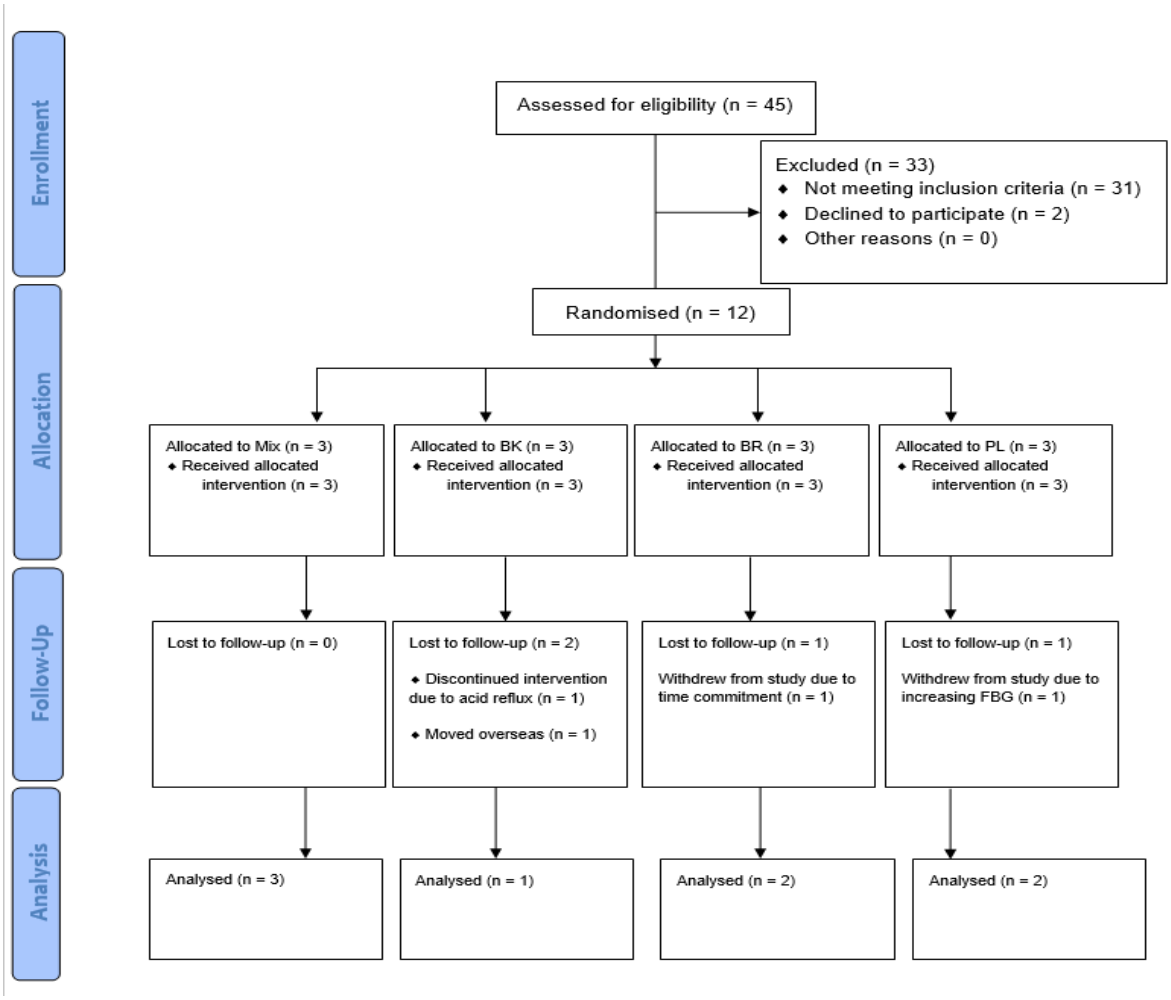

Figure 7.2. Study CONSORT flow diagram. A total of 45 participants were screened with 8 participants completing data collection. Mix; beetroot and blackcurrant juice mix intervention. BC; blackcurrant juice intervention; BR, beetroot juice intervention; PL, placebo intervention; FBG, fasting blood glucose.

Of the 12 participants randomised into the study, 4 were recruited from local healthcare provider referral, 4 from social media advertising, 2 from university participant mailing lists and 2 from word-of-mouth (Figure 7.3). Random finger prick screening for HbA1c at local shopping

centres resulted in 2 eligible participants out of 8 who consented to screening. However, both eligible participants declined to participate (Figure 7.3).

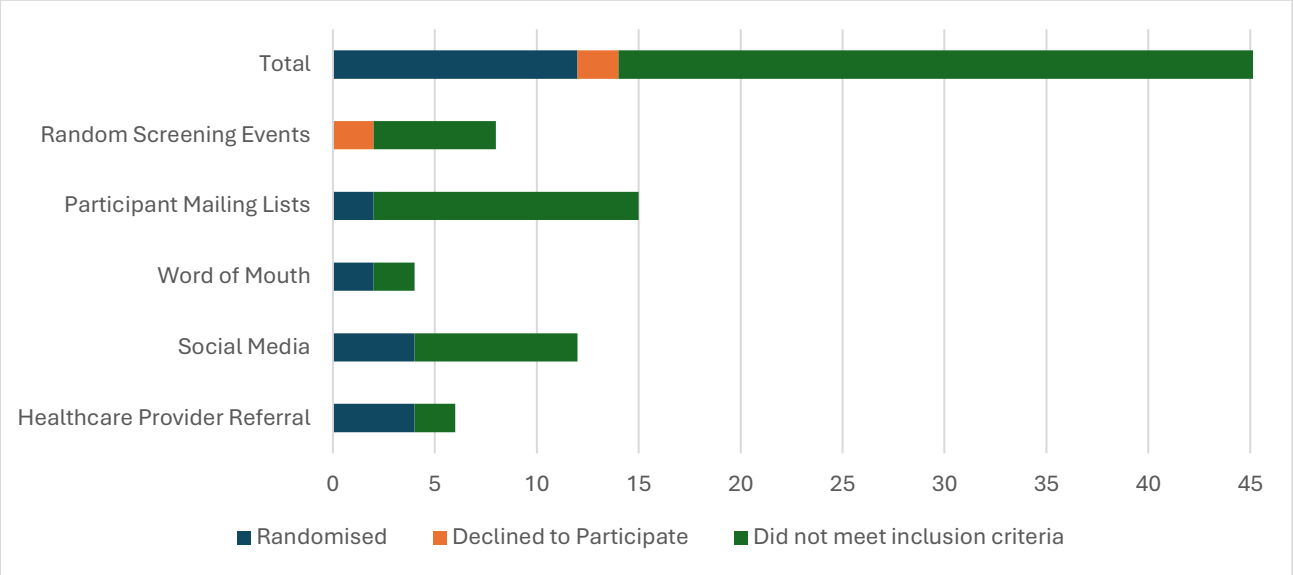

Figure 7.3. Recruitment efficacy by strategy. Numbers of participants who were randomised, declined to participate following confirmed inclusion eligibility and those not meeting inclusion criteria from each recruitment strategy.

Baseline characteristics of the participants who completed the intervention and study visit 2 are shown in Table 7.1.

Table 7.1 Baseline characteristics stratified by sex.

Characteristic	Male	Female
n	4	4
Age (y)	61.0 ± 8.8	62.3 ± 5.5
Stature (cm)	171.9 ± 0.6	160.4 ± 7.5
Weight (kg)	80.5 ± 16.8	76.1 ± 17.4
BMI (kg/m^2)	27.3 ± 5.6	29.3 ± 4.7
Body fat (%)	29.8 ± 4.7	40.2 ± 5.2
FBG (mmol/L)	7.4 ± 1.8	6.7 ± 2.0
HbA1c (mmol/mol)	43.8 ± 2.4	44.0 ± 1.5
Clinical blood pressure		
SBP (mmHg)	155.0 ± 37.7	135.3 ± 9.5
DBP (mmHg)	94.3 ± 14.8	71.8 ± 2.6

Data are presented as mean ± standard deviation. BMI, Body Mass Index; FBG, Fasting Blood Glucose; HbA1c, Glycated Haemoglobin A1c; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure.

7.3.2 Fasting Blood Glucose

Due to the small sample size, participant weekly fasted blood glucose results are presented individually in Figure 7.4.

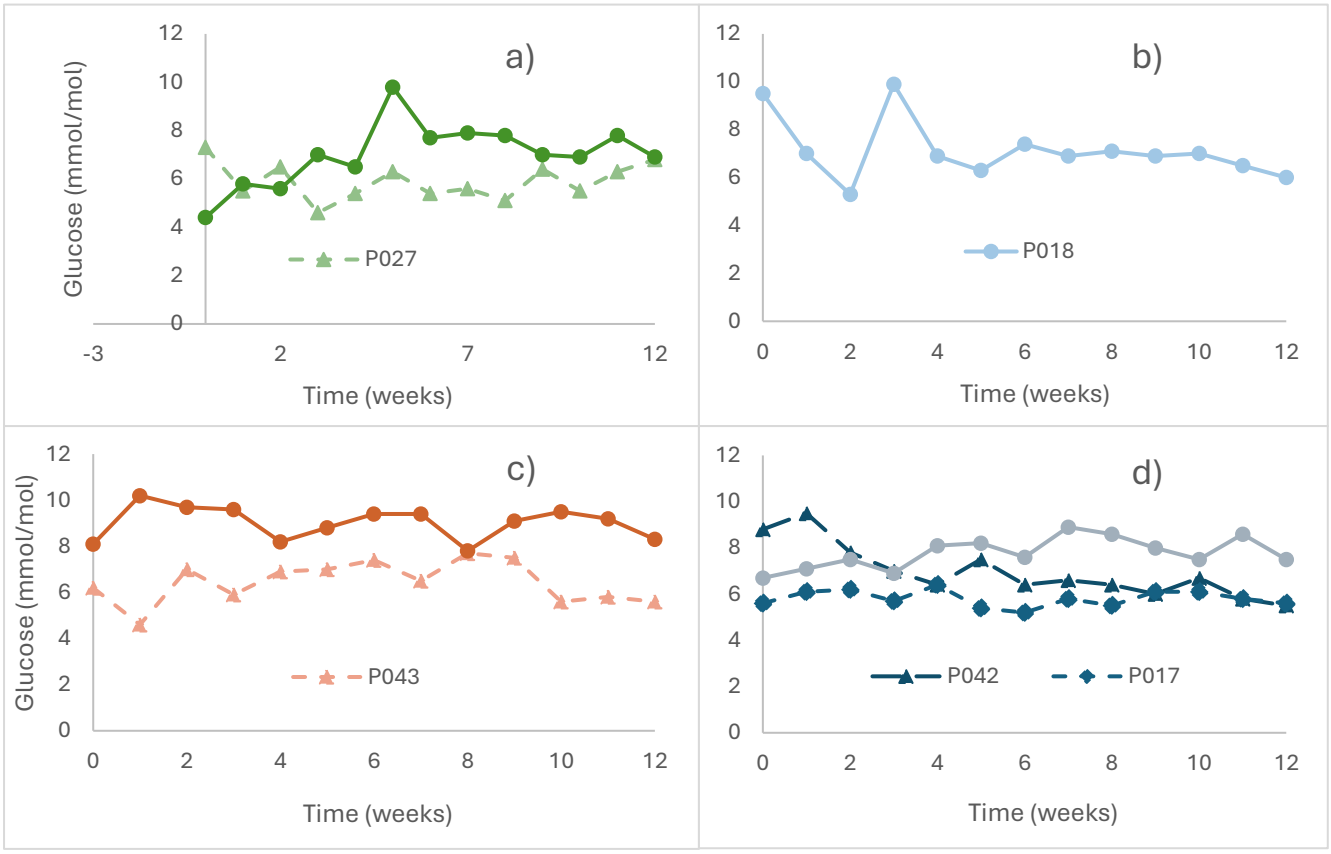

Figure 7.4. Weekly fasting blood glucose levels of individual participants plotted by intervention group from baseline to 12 weeks (84 days) of supplementation with either Placebo (a), Blackcurrant (b), Beetroot (c) or Mix (d). Participant results are identified by individual anonymised study identification number (P007 – P044).

Changes in the outcome measures: HbA1c, fasting blood glucose, blood pressure and lipid profiles from baseline to 12 weeks for the participants in each intervention are presented in Table 7.2.

Due to small sample sizes for each intervention, no significant differences were detected between groups for any parameter. It was noted that systolic blood pressure was reduced in all three participants in the MIX intervention group and reduced in one of the two participants in the BR intervention. Fasting blood glucose levels in the participant in the BC intervention decreased from 9.5 mmol/L to 7.0 mmol/L, however their HbA1c increased from 44 mmol/mol to 47 mmol/mol. Two participants saw reduced HbA1c from the prediabetic range (41 – 49 mmol/mol) to the healthy range, one participant in the PL intervention group, and the other in the MIX intervention group.

Table 7.2. Participant variables per individual participant pre- and post- intervention.

RCT Variables	Placebo (n = 2)				Beetroot juice (n = 2)				Blackcurrant juice (n = 1)		Mix (n = 3)					
	P027		P044		P007		P043		P004		P042		P017		P018	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
HbA1c (mmol/mol)	45	44	43	40	47	54	42	42	44	47	47	38	42	43	41	45
Fasting blood glucose (mmol/L)	7.3	6.8	4.0	5.1	8.1	8.3	6.2	5.6	9.5	7.0	8.8	5.5	5.6	5.6	6.7	7.5
Systolic blood pressure (mmHg)	135	136	133	130	200	181	121	132	127	134	125	106	172	155	148	134
Diastolic blood pressure (mmHg)	70	69	74	73	116	97	84	91	86	92	69	60	91	86	74	79
TC (mmol/L)	6.44	5.81	5.92	6.76	5.94	6.81	3.65	5.18	3.31	3.24	4.00	3.21	5.57	5.10	7.63	6.66
TG (mmol/L)	1.55	1.44	0.87	0.80	0.79	0.94	1.31	2.17	1.34	1.32	2.33	4.87	1.16	1.01	2.07	1.81
HDL-C (mmol/L)	2.16	1.70	1.72	2.10	2.37	2.09	1.38	1.59	1.17	1.08	1.10	1.07	1.76	1.80	2.25	1.81
LDL-C (mmol/L)	3.58	3.46	3.80	4.29	3.21	4.30	1.67	2.60	1.53	1.55	1.83	N/A	3.28	3.10	4.44	4.01
CHOL/HDL	3.0	3.4	3.4	3.2	3.6	4.7	2.6	3.3	2.8	3.0	3.6	3.0	3.2	2.8	3.4	3.7

Abbreviations: HbA1c, glycated haemoglobin A1c; TC, Total Cholesterol; HDL-C, High-Density Lipoprotein; LDL-C, Low-Density Lipoprotein; CHOL/HDL; Cholesterol to High-Density Lipoprotein Ratio; N/A, Value outside reference range of device.

7.3.3 Beverage Consumption Questionnaires

Beverage consumption questionnaire results are presented in Table 7.3. Overall, beverage acceptability was moderate, with all participants in the BR, PL and BC groups agreeing or strongly agreeing that the drinks were easy to consume (ratings 4 or 5). The MIX beverage however, received either neutral or disagree ratings (ratings 2-3).

Taste perceptions were variable, with BR and PL receiving agreement or strong agreement (ratings 4-5), while BC and the MIX had more variable responses (ratings 2-4). Reports of a bad aftertaste were minimal, with most participants disagreeing or strongly disagreeing, other than the BC participant (rating 4). Texture followed a similar trend to taste, with the MIX receiving the least favourable responses (ratings 2-4) and BR and PL the most favourable responses (ratings 1-2).

Concerns about portion size was raised, with all participants in the MIX group agreeing the volume was too great (ratings 4-5); other beverages ranged from neutral to strongly disagree (ratings 1-3).

Responses regarding whether the supplement would be easier to consume in another form (e.g. capsule) generally favoured an alternative, with 6/8 participants agreeing or strongly agreeing (ratings 4-5), 1 neutral (rating 3), and 1 PL participant strongly disagreeing (rating 1). Supplement frequency was largely acceptable, with most participants strongly disagreeing or just disagreeing (ratings 1-2) that it was too frequent, except for the 1 MIX participant who was neutral (rating 3).

Five participants provided qualitative comments, highlighting issues such as difficulty freezing the beverage (BR), staining of surfaces and clothes, tarry stool (BC), and reflux (MIX). One participant commented that the daily supplementation became burdensome after 6 weeks (BC). Difficulty mixing the supplement was reported (MIX) as well as coughing whilst drinking (PL).

Overall, the MIX and BC supplements appeared to have the most neutral and negative feedback whereas PL and BR were generally well-accepted with higher agreement ratings.

Table 7.3. Beverage Consumption Questionnaire Results

Question	Placebo (n = 2)		Beetroot juice (n = 2)		Blackcurrant juice (n = 1)	Mix (n = 3)		
	P027	P044	P007	P043	P004	P042	P017	P018
The beverage was easy to drink	4	5	4	4	4	3	3	2
The beverage tasted nice	4	5	5	4	2	3	4	3
The beverage left a bad aftertaste	1	1	1	1	4	1	2	3
The texture of the beverage made it easy to eat or drink	4	5	4	4	3	4	2	2
The amount of liquid to consume was too much	1	1	3	1	1	3	4	4
The supplement would be easier to consume if it was in a different form (e.g., capsule)	1	4	5	4	3	4	5	4
The frequency of supplementation was too high	1	2	1	1	1	1	2	3
Additional Comments	The beverage caused me to cough while drinking it	N/A	Didn't freeze well	N/A	Tarry stool 6 weeks was fine but thereafter a chore Taste not clear Stained benchtop and clothes	Didn't mix great with water – left some sediment	N/A	Thick & syrupy Gave reflux issues Half amount would be enough

Questions were marked on a scale of 1-5 by participants during study visit 2. 1, Strongly Disagree. 2, Disagree. 3, Neutral. 4, Agree. 5, Strongly Agree. Additional comments are abbreviated for ease of reading.

7.4 Discussion

Whilst the study initially aimed to evaluate differences in metabolic outcomes over 12 weeks, including changes in HbA1c, between intervention groups, the study was underpowered due to limited participant numbers. As a result, formal assessment of intervention efficacy was not possible. Although preliminary data were collected from the participants who completed the 12-week intervention, the high degree of inter-individual variability, particularly in glucose-related biomarkers, indicates a substantially larger sample size would be required to detect meaningful changes. Despite this variability, the direction of change in certain outcomes (fasting glucose and triglycerides) appeared promising. The intervention should be conducted in a larger cohort to evaluate its efficacy robustly.

The two most significant challenges encountered in this study were effective recruitment of participants meeting the inclusion criteria and participant retention through the 12-week nutritional intervention. Of the 45 individuals screened, 31 (69%) failed to meet the eligibility criteria based on HbA1c values. Among those who were randomised, attrition was also high, with 4 (33%) failing to complete the full intervention period. These recruitment and retention difficulties limit the interpretability of outcomes and highlight key areas for improvement in future studies. Specifically, broader inclusion criteria, enhanced community outreach, and alternative delivery formats may be needed to optimise both enrolment and adherence in prediabetic populations.

7.4.1 Screening and Recruitment

Participant attrition was observed primarily during two phases of the study: at screening and midway through the intervention phase. During screening visits, many participants were excluded due to the stringent inclusion criteria, particularly the requirements for HbA1c (41 – 49 mmol/mol) and fasting blood glucose (> 5.6 mmol/L) levels within the prediabetic range. These criteria, while necessary for study validity, narrowed the number of eligible candidates, leading to a high proportion of ineligible vs eligible participants for this study. Whilst we attempted to recruit participants through healthcare providers, dietitian referrals and through social media advertising, the low conversion of screenings to randomised participants underscores the need for refining recruitment strategies to reach a larger group of eligible candidates. Alternatively, including more targeted recruitment strategies to increase the likelihood of each screened person being eligible to participate may have helped us reach our recruitment target. All participants were informed of the expectations of their time and commitment for the study prior to the screening visit via the screening phone call, and only one randomised

participant cited the time commitment as the reason for withdrawal from the study, indicating early communication of time commitments was effective in reducing participant withdrawal.

Whilst the proportion of screened participants who met the eligibility criteria was low (14/45 (31%)), this is not unusual for chronic studies investigating specific population groups, such as those with prediabetes. The Diabetes Prevention Program (DPP) (9) screened 158,177 people, 30,985 of which reached screening step 2 (oral glucose tolerance test; OGTT), and only 3,819 of which were randomised (2.4% of those screened) into the study. Compared with the present study, the DPP had more stringent inclusion criteria (BMI ≥ 24 kg/m^2, 2-h plasma glucose 7.8 – 11 mmol/L based on 75 g OGTT, elevated fasting plasma glucose (5.3 – 6.9 mmol/L) (50), was a longer intervention and had a longer follow-up period (24 week lifestyle intervention, 3 ⅓ – 5 year follow-up). The DPP also had a larger burden for participants, which included 16 sessions involving lifestyle coaching, supervised exercise sessions, dietary modification and clearly defined weight loss and physical activity goals, intravenous blood sampling at start and end, a longer intervention period and a longer follow-up period (51). Although the protocol for the DPP likely made participation more challenging, the low-screening-to-randomisation rate highlights the challenges faced in recruiting large numbers of participants for cohort studies.

The Diabetes Community Lifestyle Improvement Program (D-CLIP) used a 2-step screening process involving a random capillary blood glucose test (step 1) to identify those at high risk for prediabetes, followed by a clinic-based OGTT (step 2) to identify prediabetes. In step 1, 19,377 individuals were screened at residential locations, offices, educational institutes, places of worship, parks and beaches. Of the 19,377 individuals screened via finger-prick sampling, 3,535 (18.2%) were eligible for OGTT screening (step 2). Only 21.5% of those eligible via finger-prick screening participated in the OGTT screening, and 521 additional participants entered directly at step 2 after referral from clinics or study databases. By the end of the screening step 2, 602 participants were randomised into the trial (3.1% of those screened) (8).

The DPP and D-CLIP studies highlight the difficulty of targeted screening for large cohorts in studies requiring diagnostic inclusion criteria. Our study used more targeted methods, with 4 of the 12 randomised participants coming from healthcare provider advertising. Another 4 participants were recruited through social media advertising, 2 from university participant mailing lists and 2 from word-of-mouth. Random finger-prick screening sessions at local shopping centres resulted in 2 eligible participants who declined to participate out of 8 who consented to screening. This low conversion rate from random finger-prick screening reflected the conversion rates seen in the D-CLIP screening step 1 (8). Social media advertising and healthcare provider clinic advertising were the two most successful

methods of participant recruitment, and were significantly more cost and time effective, than random finger-prick sampling at public venues. Our screening-to-randomisation rate of 26.7% exceeded that of the DPP and D-CLIP studies, despite each of those studies having dedicated recruitment teams targeting participants through direct mail, print, screening events, radio/TV, medical referral, phone calls and posters (8, 9). Future studies recruiting large cohorts of people living with prediabetes should be aware of the large time and monetary cost of advertising and screening associated with successful recruitment.

One recruitment method not utilised in the present study was radio/television promotion/advertising. A 2024 study by Bessell and colleagues (10) investigating the cost-effectiveness of different recruitment strategies for a diabetes prevention trial in Sydney, Australia, found that radio advertising was the most cost-effective strategy for recruitment, at AUD 286 per randomisation vs an average of AUD 545 across all strategies (including radio, newspapers, healthcare, online news and television). The DPP study (9) also found that radio/TV was the most effective recruitment strategy in terms of time spent to recruit each randomised participant at 26 h per participant compared with 106.8 h for direct mail, 125 h for screening events, 33.1 h for medical referral and 36.3 h for phone calls.

7.4.2 Participant Withdrawal

In the current study, 4 participants failed to complete the full 12-week intervention, despite committing at the first study visit. The reasons for mid-intervention withdrawal varied, with self-reported barriers including time constraints, perceived lack of immediate benefit, and challenges with intervention adherence (e.g., consistent daily consumption of study beverages). The feasibility questionnaire indicated that taste, texture, and portion size of the study drinks impacted participant experience, with lower taste and aftertaste ratings reported for the blackcurrant and lower taste, texture and volume ratings for the mixed drinks compared with beetroot and placebo, and this may have affected participant retention in the blackcurrant intervention arm.

A systematic review of weight loss interventions also identified dissatisfaction with diet or treatment as a predictor of participant withdrawal (52). A 13-week feasibility study using beetroot juice by Babateen et al. (46) randomised participants into 4 intervention arms; high-nitrate (HN; 2 x 70-mL BR daily), medium-nitrate (MN; 70 mL of beetroot juice daily), low-nitrate (LN; 70 mL of beetroot juice every other day) and placebo (PL; 70 mL of nitrate-depleted beetroot juice every other day). This study saw attrition rates of 38% in the HN group, 24% in the MN and 20% in the PL group, with no withdrawal from the LN group. The highest attrition rates were observed in the

HN and MN groups, suggesting that higher doses of beetroot juice were less acceptable or feasible for prolonged consumption (46). Reasons given for withdrawal from the study included gastrointestinal symptoms, uncomfortable bowel movements (25% of withdrawals), and taste/smell causing nausea and/or inability to consume the whole dose (25% of withdrawals), highlighting how unfavourable opinions of the intervention can contribute to non-compliance. Gastrointestinal issues (acid reflux) were also reported by two participants in our study, with one participant withdrawing from the study as a result. We were unable to find similar findings for studies investigating short-medium term daily supplementation of blackcurrant juice

The current study experienced withdrawal rates of 33%, which is similar to those seen in the study by Babeteen and colleagues (46). Despite this, given the MIX intervention in this study contained the highest overall dose and volume (600 mg of nitrate and 300 mg of anthocyanins, 77g or 91g dependent upon batch), and Babateen and colleagues saw a dose-response relationship to participant withdrawal (46), it seems unusual we did not observe a larger withdrawal rate in this intervention arm compared with other arms, with the MIX arm having no participant withdrawals. Although participant numbers were limited, the MIX intervention generally had less favourable ratings than the BR and PL interventions, but more favourable than the BC, and this may have resulted in less drop out compared with BC, despite the higher volume/dosage.

A study which involved daily consumption of a multi-strain probiotic capsule over a 12-week period saw withdrawal rates of 21% (53), which may indicate that capsules may be more easily consumed and/or convenient than a drink intervention. Future studies may look to utilise blackcurrant and/or beetroot extracts in the form of capsules/powders to administer bioactive ingredients such as anthocyanins and nitrate and betalains, respectively.

The use of capsules for delivery of blackcurrant anthocyanins seems feasible, as several methods of anthocyanin extraction are possible including microwave-assisted extraction (54), ultrasonic-assisted extraction (55-57) and enzyme-assisted extraction (58, 59). Previous studies have shown improvements of glucose control parameters following blackcurrant extract supplementation both acutely (36, 60) and following 8 days of supplementation (37). Other medium-to-long-term supplementation studies using blackcurrant capsules have resulted in lower withdrawal rates than observed in this and other juice intervention studies. Hurst and colleagues supplemented 34 healthy individuals with 240 mg of blackcurrant anthocyanins in capsules or a placebo capsule for 5 weeks and observed no withdrawals from either intervention group (61). In a 6-month study, Nosal and colleagues (62) supplemented 48 peri and early

postmenopausal women with either one (Low-BC) or two (High-BC) capsules daily (392 mg anthocyanins per capsule). This study saw withdrawal rates of 5.9% and 31.3% in the low-BC and high-BC groups respectively. However, failure to take the supplement was not cited as a reason for withdrawal in either intervention group (62). These findings suggest that capsule-based delivery of blackcurrant anthocyanins is not only feasible but may also improve participant adherence in future longer-term interventions.

Beetroot supplementation is currently less feasible in powder or capsule form than BC, as beetroot extracts tend to be low in dietary nitrate, a key bioactive contributing to beetroots' effects on glucose metabolism (33). Typically, sliced beetroot is crushed then dried either through sun drying, tray drying or freeze drying to make a powder (63) before either ultrasound, Soxhlet, cold or superficial extraction techniques (64-66). An analysis of commercially available beetroot capsules and powders showed mean nitrate quantities of 6.1 mg and 43 mg per recommended daily dose, respectively (67); far lower than the 600 mg administered in this study and shown to be effective in previous research (46).

In addition to nitrate, betalains, which may contribute to the improvements in vascular (Chapter 6) and glycaemic outcomes observed following beetroot supplementation, are known to be sensitive to light, oxygen and heat, and may degrade significantly during drying and processing steps, particularly under high temperatures (68), however recent advances have been made to preserve betalains during extraction (69). Finding methods to incorporate both bioactives into a palatable supplement seems likely to result in the best outcomes for consumers and is an important area for future research.

Furthermore, although blackcurrant appears more amenable to capsule-based delivery, there is limited information on the palatability, acceptability and adherence to blackcurrant-based beverage consumption over short to medium-term time periods. More evidence is needed to compare different methods of blackcurrant anthocyanin delivery to determine how best to increase adherence to long-term supplementation.

7.4.3 Insights for Future Research

Evidence from previous studies (8-10) involving large cohorts with specific diagnostic inclusion criteria show that mass screening appears to be the most effective form of recruitment. Future studies need to budget for large recruitment drives including allowance for advertising and participant screening. Across published studies, radio advertising (9, 10), along with healthcare provider referral (9, 10) appear to be the most time- and cost-effective methods of recruitment. Bessell and colleagues' study (2015-2018) recruited 65% of their randomised prediabetic participants through radio advertising (46%) and healthcare provider referral (19%) alone (10). No other method (including online articles, academic/institutional adverts, word of mouth, newspapers, events, pharmacies and social media) contributed more than 6% of total randomised participants in this study (10). Therefore, radio advertisements and healthcare provider referrals should be prioritised to ensure recruitment in large cohort studies is effective enabling subsequent studies to be more likely to be sufficiently powered.

While participant information sheets (Appendix 4.2) and initial discussions with the potential participants aimed to highlight our study's objectives and potential benefits, they may have underemphasised the logistical demands of study participation. Detailed, clear communication about time commitments, study procedures, and the importance of compliance may help set realistic expectations and enhance commitment. Tailored approaches could include emphasising potential health benefits to participants and addressing common concerns about time and effort early on, making participants better prepared and more invested in participating.

The current study design required weekly fasting blood glucose measurements and daily consumption of intervention beverages. These tasks, though necessary for data collection, may have been perceived as burdensome, or participants may not have felt they had the necessary knowledge to perform fasting blood glucose measurements from home. Efficacy of intervention has been shown as an important predictor of attrition in weight-loss interventions (52), and participants not seeing an immediate lowering of FBG levels in the present study may have contributed to withdrawal, with one participant citing rising FBG values in the first 3 weeks as the reason for withdrawal. Managing expectations around the rate of improvement in at-home blood glucose readings may have reduced this attrition.

Improving the palatability of the study interventions may also improve participant retention. Utilising capsules/powders rather than beverages may reduce gastrointestinal symptoms as well as reduce the burden on participants when consuming the dietary intervention. This may lead to a subsequent reduction in withdrawal from studies. Administering the bioactive compounds

extracted from blackcurrant and/or beetroot juice through capsules should be considered, or in the case of blackcurrant, more widely used. However, further investigation into the production of beetroot capsules/powders with functionally meaningful quantities of both betalains and dietary nitrate is required to enable this method of supplementation to be utilised.

Most importantly, dietary interventions for metabolic risk reduction must be evaluated not only for efficacy but also for accessibility and cultural relevance. Evidence shows that people with prediabetes, particularly Māori and Pasifika, face barriers to preventive nutrition and health programs due to cost, acceptability, and limited culturally tailored options (70, 71). Evidence from New Zealand shows that nutraceutical intervention use is most common among higher-income, European, and tertiary educated populations (72), suggesting that intervention based on juice supplements may be preferentially adopted by groups already advantaged in terms of health resources and access. Unless reformulated into more affordable formats such as powders or extracts and delivered through trusted Kaupapa Māori and community-based channels, nutraceutical interventions risk reinforcing health inequalities. Embedding cultural tailoring and equitable distribution models is therefore critical to ensuring that nutraceutical intervention contribute to reducing, rather than widening, health disparities in Aotearoa New Zealand.

7.5 Conclusion

This study highlights the challenges of recruiting and retaining participants in a longer-term nutritional intervention trial in individuals with prediabetes as well other underdiagnosed populations, such as hypertension, osteoarthritis, and chronic kidney disease, to ensure meaningful outcomes. Specific key insights include addressing the perceived burdens of participation, such as time commitment and adherence to intervention protocols, refining recruitment strategies to better communicate expectations and engage a larger cohort of eligible candidates and providing a dietary supplement which is both easy to ingest and effective at improving health outcomes. These insights highlight areas for future improvement to ensure the feasibility and success of similar intervention studies.

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Chapter 8 – Discussion and Conclusion

This chapter provides an overview of findings from this PhD study, assessing the strengths and limitations of the research, and outlining recommendations for future research.

8.1 Summary of Key Findings

This PhD research investigated the hypoglycaemic potential of beetroot and blackcurrant juice supplementation across several research studies, addressing specific questions under the overarching hypothesis that these functional foods may improve glycaemic control and other metabolic syndrome biomarkers. A summary of these findings aligned with the research questions formulated for this PhD (Chapter 1) are outlined below:

1. **Does beetroot and/or blackcurrant juice supplementation have the potential to improve metabolic syndrome risk factors and glucose control?**

Animal studies and human clinical trials examined in the narrative review (Chapter 3) (1) indicated that both beetroot bioactives (dietary nitrate and betalains) and blackcurrant bioactives (anthocyanins) have the potential to improve metabolic syndrome risk factors such as hyperglycaemia, hypertension and dyslipidaemia. The evidence suggests that beetroot juice, rich in dietary nitrate, can improve glucose metabolism through enhanced nitric oxide availability, potentially improving insulin sensitivity and reducing postprandial glucose peaks. Similarly, blackcurrant juice/extract, abundant in anthocyanins, may beneficially influence blood glucose control by slowing carbohydrate digestion through inhibition of the enzymes alpha-amylase and alpha-glucosidase. Both beetroot and blackcurrant juice intake also demonstrate hypotensive effects: beetroot through nitric oxide-dependent vasodilation and blackcurrant through endothelial nitric oxide synthase activation and antioxidant action. Additionally, there is evidence suggesting that compounds from both beetroot and blackcurrant juice/extract may positively influence lipid profiles: beetroot may inhibit lipid peroxidation and thus reduce oxidative stress, while blackcurrant juice/extract may attenuate the postprandial elevation of circulating free fatty acids compared with control meals, however more investigation is needed as studies investigating the impact of beetroot and blackcurrant compounds on dyslipidaemia are limited.

2. **Are we using the correct site and method of blood collection to observe differences in acute blood glucose control following interventions targeting glucose uptake at the skeletal muscle?**

Analysis of the results from Chapter 4 examined whether the chosen blood sampling site and method of blood collection was able to accurately detect acute difference in blood glucose levels following dietary interventions. Chapter 4 summarised the key differences in blood glucose results obtained between 3 different blood glucose sampling methods: plasma glucose (via glucose oxidase-peroxidase (GOD-PAP) assay), venous whole blood glucose (via HemoCue

201+ glucose analyser) levels and capillary glucose (via HemoCue 201+ glucose analyser). Blood glucose levels were compared during oral glucose tolerance tests (OGTTs) after acute ingestion of either placebo or beetroot juice and toast (totalling 75 g of carbohydrate, with matched carbohydrate content in the juices and the remaining carbohydrate provided by the toast to complete the OGTT meal). The results indicated significant differences between the blood glucose results using the different collection and testing methods. Plasma glucose, commonly used as a gold standard measure of systemic glucose levels, and venous whole blood were unable to capture localised, early-phase differences in blood glucose levels between the placebo and beetroot juice interventions. Capillary blood glucose sampling, however, effectively detected early-phase glucose reductions following the beetroot juice intervention, highlighting its usefulness and sensitivity to localised skeletal muscle glucose uptake. Thus, the study concluded that while plasma sampling remains standard for systemic assessments, capillary glucose sampling may be preferable when evaluating acute interventions targeting peripheral glucose metabolism.

3. **Does acute beetroot juice or blackcurrant juice consumption improve postprandial blood glucose responses in healthy individuals?**

Analysis of the results from Chapter 5 indicated that beetroot juice (BR) intake prior to a carbohydrate load attenuated the early phase (0-60 min) rise in blood glucose levels compared with placebo (PL). Specifically, the peak blood glucose concentration was reduced compared with PL (BR = 2.51, PL = 3.40, $p = 0.002$) and the early phase incremental area under the curve (iAUC) was lower in BR than PL between 0-30 min (BR = 52.24, PL = 66.50; $p = 0.017$) and 0-60 min (BR = 95.27, PL = 122.88; $p = 0.010$). No significant differences were detected between blackcurrant juice (BC) and PL for the mean capillary blood glucose peak or iAUC in the early-phase response. There was a significant within-subjects effect of intervention on early-phase insulin response (0-30 mins iAUC) ($p = 0.045$, partial $\eta^2 = 0.291$) with BC having the lowest iAUC (BC = 772 μU·min/mL, BR = 836 μU·min/mL, PL = 848 μU·min/mL), however, pairwise comparisons were not statistically significant. These results indicate that a single dose of beetroot juice can blunt postprandial glucose peaks, which has positive implications for reducing oxidative stress and vascular damage associated with hyperglycaemia, whilst a single dose of blackcurrant juice may improve insulin sensitivity. Further investigation is needed to elucidate the long-term effects of beetroot and blackcurrant supplementation on blood glucose control.

4. **Do the betalains in beetroot juice contribute to previously observed improvements in blood pressure, lipid profile and blood glucose control following supplementation in healthy individuals?**

Chapter 6 presents the results of an investigation of the effects of consumption of two different beetroot juices, sourced from the UK and New Zealand (UKBR; 1280 mg nitrate, 147 mg betalains, daily; and NZBR; 1120 mg nitrate, 506 mg betalains, daily) with different nitrate and betalain compositions, with a placebo beverage (PL) on metabolic syndrome biomarkers after 6 days of daily ingestion. Consumption of both UKBR and NZBR significantly elevated plasma nitrate and nitrite levels compared with PL. Systolic blood pressure (SBP) was reduced after 6 days of supplementation with NZBR compared with placebo (-5.3 mmHg for NZBR vs PL, $p = 0.038$) but not for UKBR. Systolic blood pressure was reduced 2 h post ingestion in both the UKBR intervention condition (-5.7 mmHg, $p = 0.003$) and the NZBR intervention condition (-5.6 mmHg, $p = 0.029$) compared with the PL condition. Systemic vascular resistance (SVR) was reduced after 6 days in the NZBR condition (-219 dyne-sec-cm^{-5} vs. PL, $p = 0.024$) but not for the UKBR condition. No significant differences were observed for fasting blood glucose and lipid indices following either intervention. These results indicate that BR supplementation improves vascular function, with NZBR causing greater reductions in SBP and SVR despite having a lower nitrate content than the UKBR, suggesting an important role for betalains in the observed differences. As no differences were seen for blood glucose levels and lipid profile in either intervention group, more investigation into the chronic effects of supplementation may be needed to discern the effects of BR consumption on these biomarkers.

5. **Can chronic consumption of beetroot and blackcurrant juices improve blood glucose control, blood pressure and lipid profiles in people living with prediabetes?**

This research question was initially intended to be investigated through a chronic intervention trial supplementing with either beetroot juice, blackcurrant juice, a beetroot/blackcurrant juice mix or placebo. However, time limitations to recruitment meant a full trial with sufficient power to investigate this question was unable to be conducted. Instead, we used the study to investigate challenges in recruitment and retention of participants during a 12-week daily nutritional intervention to improve glycaemic control in individuals with prediabetes and investigate strengths and limitations of the research design to improve future research investigating long-term supplementation in specialist population groups. Recruitment of study participants was through a combination of social media advertising, advertising through healthcare providers, university mailing lists and community outreach. Of the 45 participants

screened, 12 (26.7%) were randomised, with 8 completing the 12-week intervention (67%). Recruitment was most successful through healthcare providers (33% of randomised participants) and social media advertising (33% of randomised participants). Of the participants who met the inclusion criteria at screening and started the intervention, reasons for withdrawal included time constraints and lack of immediate benefit for improving their health. The palatability of the intervention beverages was also assessed, with the blackcurrant juice and the mix drink receiving the lowest ratings for taste, and texture, possibly affecting the withdrawal rate in the blackcurrant intervention. Most participants (75%) either agreed or strongly agreed that an alternative form of intervention delivery such as encapsulation would be preferable to juice. Overall, financially cost effective and time efficient recruitment strategies, including healthcare provider outreach and targeted advertising, are essential to achieve adequate sample sizes for chronic intervention studies in underdiagnosed or asymptomatic populations. The selection of interventions which are easy to administer and palatable may enhance participants' perceived tolerability and overall experience of the intervention, which may in turn lead to increased acceptability and compliance during intervention studies.

8.2 Main Findings and Implications

The narrative review (1) set the stage in understanding the potential impact of consumption of beetroot and blackcurrant juices on three key components of metabolic syndrome; hyperglycaemia, hypertension and dyslipidaemia. Both beetroot and blackcurrant juice supplementation have the potential to improve metabolic syndrome biomarkers, however, several key gaps were identified within the literature. Firstly, most of the studies investigating glucose control in humans were conducted in an acute setting, with both beetroot (2-4) and blackcurrant (5-7) juice supplementation resulting in improvements in early-phase glucose responses. Only a single medium or long-term intervention study (6 weeks of supplementation) was identified (8) investigating the effect of beetroot or blackcurrant juices on glucose metabolism. This study did not use a control group, nor did it report betalain or nitrate doses in its findings, meaning comparison to other studies is challenging. The investigation of long-term supplementation of beetroot and blackcurrant juices was therefore identified as a major gap in the literature. The review also highlighted that studies investigating beetroot juice tend to focus on achieving sufficient nitrate quantity to elicit effects on glucose control. The review of the literature offered us insight into the potential importance of betalains in affecting blood glucose metabolism and highlighted the importance of investigating the effects of betalains and their contribution to improvements in glycaemic control and other metabolic syndrome risk factors.

8.2.1 Glucose Kinetics and the Role of Blood Sampling Method

The methodological findings from Chapter 4 have important implications for the design and interpretation of dietary intervention studies targeting acute glucose metabolism. The finding that only capillary sampling detected significant reductions in early-phase postprandial glucose responses to OGTTs provides context for previous research, particularly studies reporting equivocal results, and suggests that some null findings may be attributable to sampling from sites that were less sensitive to acute postprandial changes. For example, Wootton-Beard and colleagues (2) saw significant decreases in early-phase blood glucose levels following beetroot ingestion using capillary blood sampling (2), whereas neither Fuchs and colleagues (9) or Shepherd and colleagues (10) observed differences in acute glucose responses following beetroot supplementation when measuring plasma glucose from venous samples. These discrepancies are likely due to differences in the blood glucose sampling site. Importantly, this result may also be applicable to other analytes influenced by peripheral uptake dynamics, such as ketones (11) and lactate (12), particularly in interventions involving exercise, polyphenols or vasodilatory compounds such as nitrate, where metabolism at the skeletal muscle is likely to be affected.

From a practical perspective, capillary sampling is less invasive and uncomfortable for the participant than venepuncture. Finger-prick tests are associated with lower ethical burden compared with intravenous sampling as they involve minimal discomfort and carry a lower risk of adverse events such as haematoma or infection. From the participant's perspective, finger pricks are less invasive which may lower barriers to study participation. This is particularly pertinent for studies involving individuals with T2DM, who are often familiar with capillary sampling through self-monitoring. A study assessing patient preferences (13) for chronic care patients (T2DM, cardiovascular disease, chronic kidney disease and thyroid disease) for blood sampling methods found that 35.1% of participants preferred finger prick sampling, compared with 21.7% for venous draws; among patients with T2DM, 45% expressed preference for finger pricks vs 15% who preferred venous. Moreover, 46% of participants reported physical discomfort following venous sampling, and 12% reported anticipatory anxiety before phlebotomy appointments (13). The current study supports the use of finger-prick sampling as a means to reduce procedural burden and improve participant experience in blood glucose sampling. Additionally, finger-prick testing requires less tissue sampling and avoids the need for formal sample storage registration or long-term sample handling, associated with intravenous sampling. Furthermore, analysis via point-of-care devices requires less technical expertise and is cheaper than laboratory-based analysis via enzymatic assays. The use of finger-prick blood

sampling may improve recruitment rates and facilitate greater compliance in repeated measures or home-based study protocols. It is important to note however, that without testing both capillary and venous glucose simultaneously, it is not possible to discern whether an intervention is changing peripheral glucose kinetics or systemic glucose (such as through suppressed hepatic glucose output or slowed intestinal glucose absorption), making a combination of both sampling techniques ideal from a study robustness perspective.

8.2.2 Acute Supplementation Study Results

The acute findings from Chapter 5 suggest that consumption of beetroot juice has the potential to offer meaningful reductions in early-phase postprandial glucose peaks when consumed as part of a carbohydrate meal, an outcome relevant for both healthy individuals and those at increased risk of type 2 diabetes and other metabolic disorders associated with hyperglycaemia. Further, by measuring both venous and capillary blood glucose levels and establishing a change in blood glucose only at the capillary, we can infer that the changes in glucose result from either increased GLUT 4 translocation at the skeletal muscle or enhancement of nitric-oxide mediated perfusion, or both. While consumption of blackcurrant juice at the dose tested (120 mg anthocyanins) did not significantly affect blood glucose levels, a trend towards reduced early-phase insulin response was noted, implying possible improvements in insulin sensitivity which merit further exploration at higher anthocyanin dosages. Castro-Acosta and colleagues (5) showed a dose-response relationship between blackcurrant anthocyanin ingestion and improvements in postprandial glucose, implying 120 mg may be too low a dose to elicit significant changes in glucose control.

An important key barrier to the translation of research into practical dietary recommendations, is that commercially available products lack standardisation in anthocyanin, betalain and nitrate dosage. Many beetroot and blackcurrant beverages do not report the content of these compounds, making it difficult for consumers to select effective products or for researchers to compare results across studies. All future studies should report the anthocyanin or nitrate/betalain dosage for blackcurrant and beetroot interventions, respectively. Overall, the BOP study underscores the need for dosage optimisation of both blackcurrant and beetroot juice beverages before widespread consumer use can be advised, and the BOP study also gives insights into potential mechanisms through which beetroot juice may alter blood glucose dynamics.

8.2.3 Emerging Role of Betalains

The UKBR vs NZBR juice trial (Chapter 6), is one of the first studies to directly investigate the role of betalains, alongside nitrate, in mediating the metabolic benefits of beetroot juice consumption. The study provides novel and compelling evidence that betalains, which have been underexplored in human nutrition trials, may independently contribute to improvements in vascular function. As highlighted in the narrative review (1), betalains are known for their antioxidant and anti-inflammatory properties (14) and are proposed to protect nitric oxide from oxidative degradation (41, 43). Whilst most beetroot research to date has focussed on nitrate as the principal bioactive compound, our results showed that consumption of the higher-betalain, lower-nitrate NZBR beverage resulted in greater reductions in SVR after 6 days of daily consumption than the lower-betalain, higher-nitrate UKBR beverage. This is a novel and significant finding, because SVR reflects microvascular function, an early and sensitive predictor of future hypertension risk (15). These results challenge the prevailing nitrate-centric paradigm that has dominated beetroot juice research, and supports the idea that optimisation of betalain content is also important to fully realise the therapeutic potential of beetroot-based products and interventions. Whilst this result is very promising, further investigation is required to establish optimal doses and establish the pharmacokinetics of betalains and their metabolites following ingestion. Longer-term studies with at-risk populations, including those with hypertension and metabolic syndrome are needed to establish whether betalain metabolism is similar in metabolically compromised populations as well as healthy populations. If betalains are established as key bioactive compounds contributing to the previously observed health benefits associated with beetroot consumption, future interventions may benefit from selectively enhancing betalain content or combining betalain-rich extracts with other bioactives.

8.2.4 Implications for Recruitment and Feasibility in Long-Term Nutritional Trials

The 12-week intervention feasibility study (Chapter 7) highlighted a series of recruitment and retention challenges with important implications relevant for research in asymptomatic or underdiagnosed populations, such as individuals with prediabetes or metabolic syndrome. Only 27% of screened individuals were eligible and enrolled and the study included a preliminary screening phone call prior to an invitation for a screening visit. This finding reflects the broader issue identified in the narrative review: prediabetes is often undiagnosed, and recruitment strategies must therefore go beyond passive advertising, such as posters, flyers or general online advertisement. Previous studies have shown that asymptomatic conditions such as prediabetes create a limited perception of urgency for care due to the long-term nature of the health risks,

leading to less compulsion to change or participate in interventions (16). Similarly, participants who are unaware of, or in denial about their condition also pose recruitment challenges (17) as their limited awareness or reluctance to acknowledge risk can make them less likely to view participation as beneficial or necessary. Therefore, targeted recruitment strategies including healthcare provider outreach should be used as a cost and time effective strategy to recruit participants in underdiagnosed and difficult to engage populations. Healthcare providers are particularly valuable in this context, with 70% of Americans identifying them as their most trusted source of health information and 59% stating they would consult their healthcare provider first when considering clinical trial enrolment (18). Whilst NZ-specific surveys asking these exact questions are not readily available, existing data shows that New Zealanders demonstrate similar high levels of trust in their healthcare providers and systems, comparable to trends observed in other countries (19). In addition, healthcare providers have access to diagnostic information relating to inclusion criteria that researchers may not, which puts them in a unique position to both identify eligible participants and encourage participation through trusted communication that can reduce hesitancy and enhance legitimacy, as observed in recruitment strategies used in other studies (20, 21).

Results from the feasibility study (Chapter 7) also highlighted the importance of palatability, portion size and delivery format for long term supplementation intervention trials, with participants indicating a preference for an alternative delivery form (encapsulation or powder). Previous studies have noted that dislike of the taste of the intervention is among the core reasons for low adherence to healthy diet regimens (22, 23). Furthermore, in single food intervention trials, taste fatigue can cause significant dropout even if participants initially like the taste at the start of the intervention trial. Results from other studies (24) suggest that use of smaller-volume, nutrient-dense formulations may improve adherence, an approach potentially preferable for beetroot and blackcurrant juice supplementation. Some participants in this PhD prediabetes study reported gastrointestinal issues when consuming the juice. Previous research has shown reduced prevalence of gastrointestinal side effects following cranberry encapsulation (2% reported issues) compared with cranberry juice (23% reported issues) (25, 26). These findings, along with the feedback from participants in the current prediabetes study, suggest that encapsulation may be more appropriate for future long-term intervention trials. This approach could also enhance public health impact by improving adherence at scale by reducing barriers relating to taste, storage and preparation and, while offering practical recommendations for commercial producers looking to harness the health benefits of different polyphenol or nitrate-rich functional foods. However, cost-effectiveness for consumers must also be considered,

particularly if such interventions are to be integrated into public health messaging; formulations must remain affordable and accessible to ensure equitable access across diverse and at-risk populations.

8.3 Limitations

This thesis offers novel insights into the potential of beetroot and blackcurrant juices to modulate metabolic biomarkers, however there are limitations that must be acknowledged. Constraints across study design, sample size, recruitment timelines, biomarker selection and intervention composition reflect the challenges of early-phase nutritional intervention trials and are important to consider in the interpretation and generalisation of findings presented in this thesis.

8.3.1 Study Design

This thesis presents powerful insights into acute and short-term (hours or days) ingestion of both beetroot and blackcurrant juices and their effects on measures of metabolic syndrome. The narrative review (1) highlighted that longer-term trials were needed to capture longer-term adaptations. Chapter 7 was initially conceived as a 12-week intervention in people with prediabetes, but due to recruitment shortfalls and time constraints, it could not be completed with adequate power (only 8 participants finished, out of 12 randomised), curtailing our ability to evaluate the chronic efficacy of the interventions. Therefore, sufficiently powered long-term intervention trials investigating the effects of beetroot and blackcurrant juices on metabolic syndrome biomarkers remains a critical gap in the literature.

8.3.2 Study Population

The use of healthy volunteers, while being convenient and controlling for comorbidities, does limit the generalisability of the findings to a healthy population The lack of older and high-risk participants means we could not directly assess efficacy in the very populations that stand to benefit most (e.g. those with baseline hypertension or impaired glucose tolerance). Further, whilst efforts were made to reach wider demographics in New Zealand (Māori, Pacific and Asian populations), our studies contained a narrow demographic (predominantly New Zealand European adults in an urban setting) meaning cultural and genetic factors could not be explored.

8.3.3 Mechanistic Understanding

While certain measures such as plasma nitrate/nitrite levels, capillary vs venous glucose were useful to infer peripheral glucose uptake effects, these were relatively narrow in scope. The metabolic responses to beetroot and blackcurrant involve complex pathways, including vascular perfusion (27-36), insulin secretion and signalling (2, 5-7, 32, 37) and inflammation and oxidative stress (28, 38-41), many of which were not directly measured.

Another mechanistic limitation is the breadth of biomarkers assessed. Our focus was on glucose, insulin (Chapter 5), blood pressure, and standard lipids, with only limited exploration of other pathways. Inflammatory markers (e.g. IL-6, CRP) and oxidative stress indicators were not measured, and mechanistic tools such as endothelial function testing (e.g. flow-mediated dilation) (42) or gold-standard insulin sensitivity assays (e.g. hyperinsulinaemic clamp) (43) were beyond the scope of this PhD. The absence of these measurements limits our mechanistic understanding meaning we must speculate whether the observed outcomes were driven by improved insulin action, enhanced blood flow, or slower carbohydrate absorption.

8.3.4 Intervention Composition

Finally, there are important limitations related to the interventions themselves, particularly regarding their composition, dosing, and mode of delivery. The variability of dosages used in previous studies meant it was challenging to standardise betalain, nitrate and anthocyanin doses across interventions. Further, the blackcurrant dose used in our acute study (120 mg anthocyanins) may be suboptimal compared with previous studies observing clear metabolic benefits at 600 mg anthocyanins (5, 37). Another practical limitation concerns the formulation and delivery of the interventions. Using a food-based rather than capsule-based approach introduces issues surrounding sugar content. Each juice dose contained a substantial sugar load (42.9 g of sugar in Chapter 5 beverages), which may counteract some of the metabolic benefits received from their ingestion. Having a sugar-free or encapsulated alternative may have mitigated this issue.

8.4 Future Directions

Building on the key findings and limitations outlined in this thesis, several clear directions emerge for future research. Key priorities include the need for greater standardisation of intervention formulations and dosing, enhanced mechanistic investigation through more comprehensive biomarker panels and sampling strategies, and the completion of longer-term intervention studies in metabolically at-risk populations. Given the recruitment and retention challenges encountered in the current study and previous longer-term intervention studies, future trials should also consider more evidence-based and innovative approaches to participant engagement. Addressing these future directions will be critical to advancing the translational impact of research into beetroot and blackcurrant bioactives in the context of metabolic disease prevention.

8.4.1 Dosing Optimisation

Firstly, future studies should ensure that the doses of active compounds (nitrate, betalains, anthocyanins) are precisely quantified, consistent and sufficient to be effective. This should include:

- Dose-response trials testing low, medium and high quantities of each of the active ingredients individually, and in combination to determine the minimum and maximal effective doses and safe doses.
- Trials exploring whether a loading period or daily administration is necessary for certain bioactives to elicit benefits, as opposed to acute usage.
- Trials investigating how the timing of doses relative to meals or time of day of consumption affects the efficacy of the intervention.

Determining these characteristics will allow for consistent and effective interventions across trials, allowing more direct comparisons of intervention studies and accurate determination of the efficacy of these interventions.

8.4.2 Longer-term Interventions in At-Risk Populations

Longer-term studies (in the order of 8–12 weeks or more) involving individuals with prediabetes, insulin resistance, or other features of metabolic syndrome should be carried out to address the research gap caused by the short duration and healthy volunteer focus of current studies:

- Given blood pressure and glycaemic control may improve incrementally as biochemical pathways adapt or as small daily improvements compound, study durations above 12 weeks would be ideal to allow this adaptation to occur, as well as be reflected in clinical biomarkers such as HbA1c.
- Studies must consider real-world feasibility of chronic use by ensuring the intervention is easy to integrate into daily life. This should also recognise that a “one-size-fits-all” approach is unlikely to be effective, and cultural context and individual preferences need to be incorporated into intervention design.
- Studies should consider more palatable or easy-to-consume delivery forms such as capsules and powders for long-term intake.
- Targeting metabolically at-risk populations such as individuals with prediabetes will increase the clinical relevance of the results and may yield greater observable benefits than studies carried out in healthy individuals.

These adaptations to study designs are crucial given betalain metabolism changes in response to chronic supplementation (44) and anthocyanin ingestion leads to improved subsequent anthocyanin metabolism (45) and increased short-chain fatty acid production through gut microbiota (46-48). Further, long-term intervention provides the ability to measure real-world changes in metabolic status of at-risk individuals such as those with prediabetes.8.4.3 Mechanistic Studies

Future work should include mechanistic studies to elucidate *how* beetroot and blackcurrant bioactives exert their metabolic effects. This should include studies to isolate the contributions of individual compounds.

- Comparative studies should assess nitrate-rich versus nitrate-depleted preparations (e.g. beetroot juice vs betalain-matched prickly pear cactus juice) to determine the relative contributions of nitric oxide and betalains to vascular and glycaemic outcomes.
- Measuring plasma and urine levels of nitrate, nitrite, and key anthocyanin and betalain metabolites over time may help correlate peak bioavailability and metabolite concentrations with physiological effects.
- Future trials should include more comprehensive biomarker panels, such as insulin, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), C-reactive protein (CRP), interleukin-6 (IL-6) and lipoprotein subfractions to better elucidate the metabolic, inflammatory, and hormonal pathways affected by these dietary interventions.
- Multi-sample techniques (e.g. capillary and venous blood sampling, blood pressure monitoring, and flow-mediated dilation) should be incorporated to assess dynamic glucose responses and endothelial function.

These approaches would help progress from observational associations to mechanistic validation by linking levels of specific bioactive metabolites with physiological outcomes. Expanded biomarker panels could also identify ancillary effects on metabolic inflammation as shown in previous studies where anthocyanin reduced CRP levels (37) and betalains improved biomarkers of oxidative stress and inflammation (14, 38-40, 49). Measuring insulin, GLP-1 and GIP concentrations would determine whether observed improvements in glucose control are caused by enhanced insulin secretion/sensitivity or altered glucose absorption.

8.4.3 Improving Recruitment and Delivery for Future Long-Term Trials

Recruitment and retention are critical challenges for long-term studies targeting at-risk populations. While digital methods offer cost-efficiency and reach, our findings suggest that recruitment is most effective when combined with trusted healthcare provider referrals and integrated into existing health services. Future studies should:

- Use radio and digital recruitment methods (e.g. Facebook, Instagram) to reach at-risk individuals to reduce recruitment cost per participant. Leveraging digital tools (e.g. intake logging apps, virtual visits, online support groups) to increase convenience and engagement for participants.
- Partner with primary care providers to identify people living with/at risk of prediabetes through routine screening and offer immediate enrolment.
- Continue established practice of co-designing studies with Māori and Pacific communities, embedding tikanga, whānau-centred messaging, and visible Māori/Pacific leadership across research roles. Engagement with iwi and hapū should build on long-term relationships and community advisory processes, with alignment to Kaupapa Māori research principles and *Te Tiriti o Waitangi*. Successful precedents such as OL@-OR@ (50) and a culturally adapted pregnancy trial (51) provide models for culturally grounded communication and leadership.
- Improve retention by providing interim health feedback (e.g. blood pressure or fitness improvements) to reinforce perceived benefits.

Social media advertising often lowers the cost of recruitment per participant (median ~12USD via Facebook/Instagram vs. 214USD via traditional methods) (52), and leveraging digital recruitment through social media and online forums could help find individuals with prediabetes who might not be actively aware of their condition. However, platform selection may have significant bearing when targeting age groups: younger adults and middle-aged adults tend to use Instagram and TikTok more frequently, while Facebook remains the most effective platform for reaching adults aged 40 years and older (53). For older adults, those living rurally, or those with limited internet access or digital literacy, traditional media channels such as radio can still serve as effective recruitment strategies (54, 55). A mixed-method recruitment approach would be necessary to achieve representative, age-inclusive, and equitable sampling across socioeconomic and geographic groups, and reduce selection bias towards only those with high technological literacy (56).

Partnering with healthcare providers may improve the integrity of study recruitment and allow direct access to participants living with prediabetes. However, many healthcare providers are difficult to engage, highlighting the importance of incentivisation or clear communication of the benefits to their patients when seeking collaboration. Recruitment and delivery approaches should also be *Te Tiriti*-led to ensure equitable research outcomes for all population groups, particularly Māori and Pacific communities. In terms of retention, previous studies have found success in providing participants with interim results (57) and leveraging digital tools such as gamification as incentivisation (58, 59). These approaches not only enhance engagement and retention but are vital for ensuring equity and ethical rigour in metabolic health research.

8.5 Conclusions

This thesis provides novel evidence that beetroot and blackcurrant juice supplementation can modulate key biomarkers of metabolic health, including improving glycaemic control and reducing blood pressure. Our findings contribute to the growing body of evidence supporting the potential of nitrate, anthocyanin and betalain-rich foods in the prevention of metabolic disease. Importantly, our results highlight the likely independent role of betalains in supporting vascular function. This underscores the need for future studies to include betalain quantification and dosage consideration in their designs, particularly in beetroot-based interventions where betalains are often present but unquantified. This work also demonstrated that capillary blood sampling more sensitively reflects changes in peripheral glucose uptake than venous sampling alone, which has important methodological implications for future trials seeking to assess postprandial glycaemic responses.

Overall, this research not only confirms the acute efficacy of polyphenol and nitrate-rich juice supplementation in healthy adults to improve glycaemic and vascular markers following single-dose intake, but provides a foundation for larger, longer-term interventions in at-risk populations, with the potential to inform dietary strategies targeting type 2 diabetes and metabolic syndrome prevention.

8.6 References

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Appendices

Appendix 1 – Research Outputs

Peer-Reviewed Journal Publication
Haswell, Cameron & Ali, Ajmol & Page, Rachel & Hurst, Roger & Rutherfurd-Markwick, Kay. (2021). Potential of Beetroot and Blackcurrant Compounds to Improve Metabolic Syndrome Risk Factors. Metabolites. 11. 338. 10.3390/metabo11060338.
Conference Proceedings
Haswell, Cameron & Ali, Ajmol & Page, Rachel & Hurst, Roger & Rutherfurd-Markwick, K.. (2022). Acute supplementation of beetroot but not blackcurrant juice manages postprandial blood glucose responses in healthy adults. Proceedings of the Nutrition Society. 81. 10.1017/S0029665122002610.

Appendix 2 – BOP study documents (Chapter 5)

The following section of the Appendices includes participant recruitment and documents, and data collection forms pertaining to the BOP study:

Appendix 2.1 *– BOP study recruitment poster* _____ *250*

Appendix 2.2 *– BOP study participant information sheet* _____ *250*

Appendix 2.3 *– BOP study participant Screening Questionnaire* _____ *257*

Appendix 2.4 *– BOP study case record form* _____ *259*

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Appendix 2.7 *– BOP study results sheet for participants to take home* _____ *269*

Appendix 2.1 – BOP study recruitment poster

Interested in Scientific Research?
Love Beetroot and/or Blackcurrant?

The School of Health Sciences at Massey University is conducting a study on blood glucose responses to antioxidant-rich beetroot and blackcurrant juices in people aged between **18-60** years, **living in Auckland.**

All participants will receive

- **FREE body composition, blood glucose, and blood pressure assessments**
- **FREE breakfast for three visits**
- **$20 when screened for eligibility***
- **$90 on completion of three trial visits***
- *In the form of vouchers

Can you help?

If you participate in this study, you will be required to:

- Make four* visits to the Massey University Nutrition Laboratory in Albany, Auckland. Blood samples will be taken.
- Eat a breakfast meal with one of three different beverages. We will see if these beverages help control blood sugar levels.

*screening visit and 3 trial visits

For further information or **to express interest,** email c.haswell@massey.ac.nz or phone [redacted]

Please pass this information on to anyone you know who could be interested.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

This study has been approved by the Human Ethics Southern A SOA 20/34

Appendix 2.2 – BOP study participant information sheet

Participant Information Sheet

Study title:	**A study to assess the effects of beetroot and blackcurrant juices on glycaemic (blood sugar) responses in healthy participants**		
Locality:	**Massey University Albany Campus, Auckland**	Ethics committee ref.:	**Southern A, Application 20/34**

Researcher Introduction and invitation to take part

My name is Cameron Haswell, and I am a PhD student at Massey University, School of Health Sciences. My supervisors are A/Prof Kay Rutherfurd-Markwick, A/Prof Ajmol Ali, A/Prof Rachel Page and Dr Roger Hurst.

You are invited to take part in a study to assess the effects of beetroot and blackcurrant juices on glycaemic (blood sugar) responses in healthy participants.

VOLUNTARY PARTICIPATION AND WITHDRAWAL FROM THIS STUDY

Participating in this study is completely voluntary and you are free to decline to participate, decline to answer any particular question, or to withdraw from the research at any practicable time. Should you withdraw, you will still receive a koha for your participation up until the point of withdrawal.

WHAT IS THE PURPOSE OF THE STUDY?

Obesity and diabetes are epidemic in New Zealand (and in other Western countries), and many individuals now have problems maintaining their blood sugar levels within normal levels.

Certain foods and drinks, including berries, teas and vegetables have been shown to reduce blood sugar levels. This may be due to the high levels of antioxidants that are present in these foods, though other substances such as inorganic nitrate may also be involved. The effects of New Zealand beetroot and blackcurrant juices on blood glucose control have not been studied, thus, this study aims to determine whether blood sugar levels and regulation in prediabetic participants can be improved when consuming a beetroot or blackcurrant beverage, or mix, for 12 weeks daily.

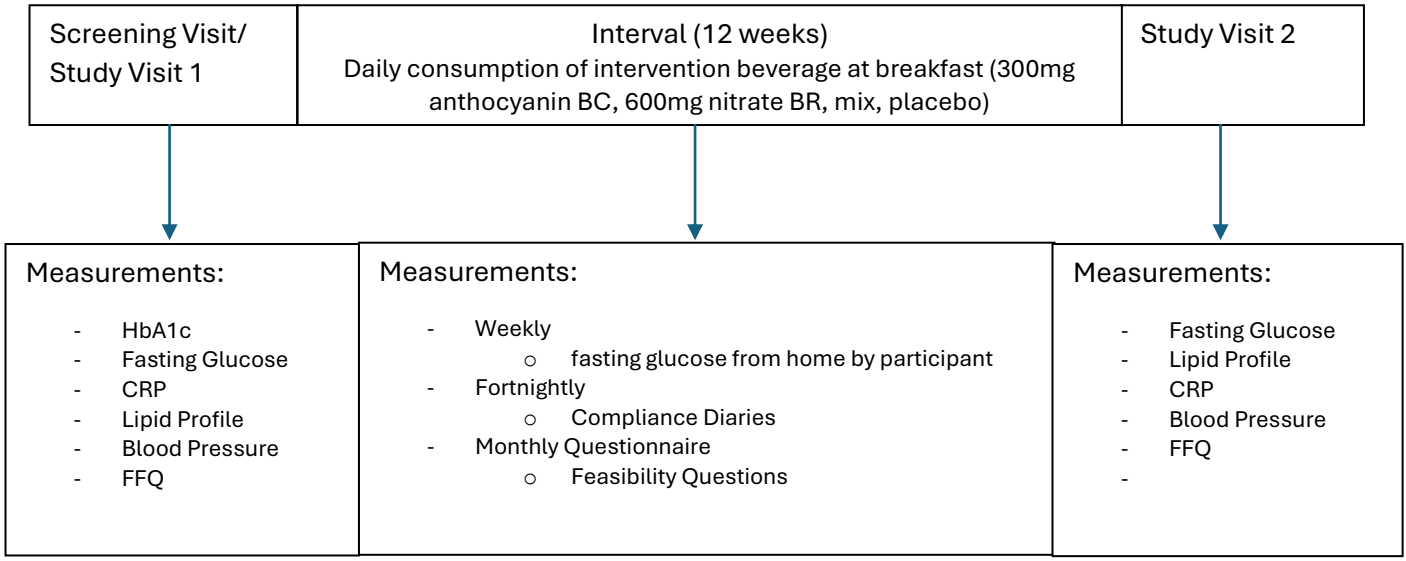
WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

You have been chosen to participate in the study because you have met the criteria to be included in the study. The criteria include:

- 30-65 years of age
- BMI 18.5 - 35.0 kg/ m^2
- 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

The study will involve you coming to the Massey University Sports Lab, located at the Albany campus. If you are eligible after an initial screening questionnaire, you will need to be available for TWO in-person morning visits separated by a 12-week intervention period. Each of these visits will take place in the morning and will last approximately 1 hour. Each session, including the screening visit, will require you to fast overnight (i.e. no food or drink except water) for at least 10 hours before coming to the research facility.

Whole Study at a Glance



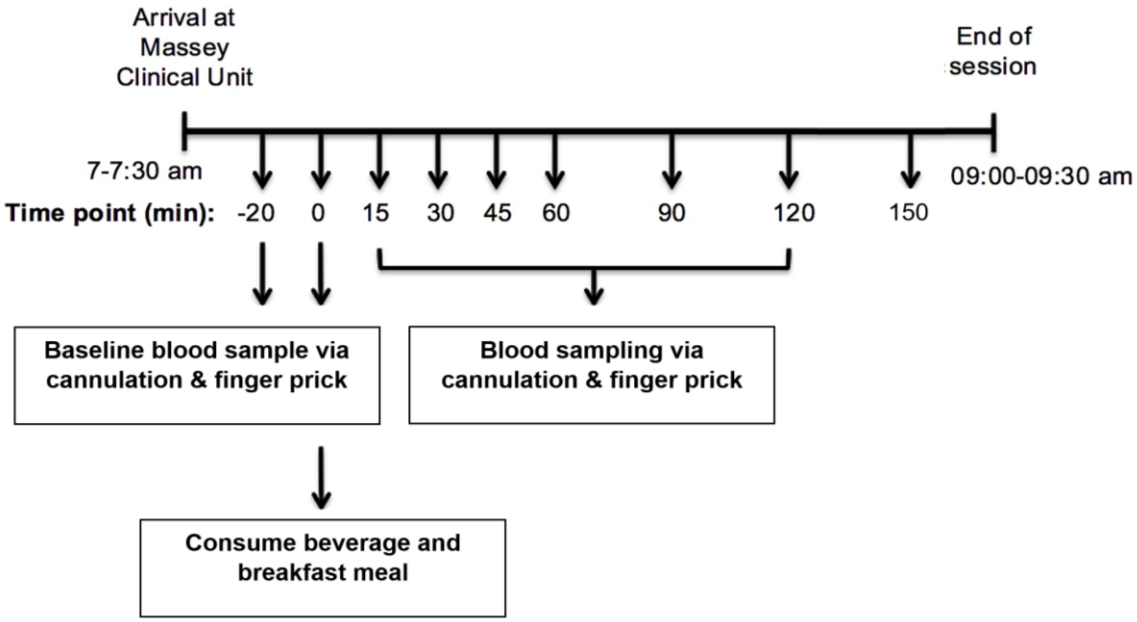
Screening visit (Visit 1)

During an initial screening session, we will assess whether you fit the study inclusion criteria. You will be required to arrive at the Massey University Human Nutrition Research Unit at 7:30am ONE morning to have a screening fasted blood sample collected. The session should take less than one hour. A qualified phlebotomist will measure your fasting blood sugar, lipid profile and HbA1c levels through a finger prick blood sample. You will be invited to participate

in the study visits if screening indicates you do not have any metabolic issues which may affect the intervention.

In addition, trained researchers will determine your body mass index (BMI) by measuring your mass and height. They will also be measuring your waist and hip circumference, and your percentage of body fat and lean body mass (using a bioelectrical impedance scale). This can be done privately, and you do not need to undress, however you will need to remove socks and shoes. Blood pressure and heart rate will also be measured. You will also be asked about your relevant medical history, and any medication use.

Visits 2 to 4



During the test visits (visits 2-4), one or two finger-prick blood samples will be taken to measure your baseline blood sugar level, and a cannula will be inserted into the upper forearm/inside of the elbow at the beginning of testing. You will then consume a meal (toast) and a beverage (either placebo, beetroot juice or blackcurrant juice) containing 75g of carbohydrate in total. Further blood samples will be taken over the next 150 minutes (see diagram) after consuming the meal, to measure your blood sugar levels. Approximately 5ml of blood will be taken through cannulation at each timepoint. Following completion, you may eat and drink as normal (this will be provided). We will have DVDs available for viewing to help pass the time.

WHAT ARE THE POSSIBLE BENEFITS AND RISKS OF THIS STUDY?

By participating in the study, you will obtain information about your general health status, HbA1c value, body composition and blood pressure measurement entirely at no cost to you.

These results are not diagnostic, and should you be concerned about any of the results obtained, we would ask that you refer to your normal healthcare provider to seek further information.

In addition, there will be a koha for involvement in the study.

It is highly unlikely that you will be injured during this study. Finger prick blood sampling and intravenous cannulation are safe and routinely used practices. A trained phlebotomist and first aider will be available during each study session and will assess for adverse events (i.e. feeling nauseous, dehydrated or faint) during each blood sample collection.

WHO PAYS FOR THE STUDY?

In recognition of your participation in this study, you will receive a koha of $20 for screening and $30 for completion of each subsequent session. You will receive a total of $110 for completing the whole study (1 screening visit and 3 study visits) in the form of vouchers. If for any reason you are unable to complete the study, you will only receive a koha for each of the visits you completed.

COVID-19

If you are ill, please contact the researchers and postpone your trial. If symptomatic of COVID -19 (see Ministry of Health website – www.health.govt.nz) please contact your health care provider immediately and seek advice. If you have been tested for COVID-19 please wait to receive your test result before coming to campus. You will also be asked to download the Ministry of Health contact tracing application on your smart phone for contact tracing purposes. As per Massey University guidelines you will be asked to sign into the Ministry of Health app each time you are on campus. The researchers can assist you with this.

The researchers will be wearing appropriate PPE and whenever possible will maintain a physical distance of 1m to reduce the likelihood of transmission, and you will not be brought into direct contact with any other participants during your visits to the research centre.

WHAT ARE MY RIGHTS?

Participating in this study is completely voluntary and you are free to decline to participate, decline to answer any particular question, or to withdraw from the research at any practicable time. Should you withdraw, you will still receive a koha for your participation up until the point of withdrawal.

You, the participant, have the right to access information about you, collected as part of this study. You will be told of any new information about adverse or beneficial effects related to this study which may impact upon your health.

It is important to us that we maintain your privacy throughout this study. Your name and contact information will be held electronically and stored on the Principal Investigators computer only

for data recording purposes. Each participant in the study will be allocated a number. Staff involved in blood sampling and analysis will have access to participant numbers only. All data from study sessions will be recorded against your participant ID number and your name will never be used in any report, correspondence or publication. Your involvement in this study is confidential.

WHAT HAPPENS AFTER THE STUDY OR IF I CHANGE MY MIND?

You can withdraw from the study at any time and will be compensated according to the number of visits made. Furthermore, you are welcome to discuss any concerns you have with the research team at any time, and you have free access to your data. If you withdraw from the study all of the data that was related to you will be shredded.

The treatment intervention (beetroot and blackcurrant juices) will not be available to any participant after the study.

The study data will be stored at a secure location at Massey University Albany Campus. Electronic data and records will be the responsibility of the Principal investigator. All data will be kept for 5 years, at which point it will be destroyed using University Security methods for removal of confidential material.

Participants are welcome to discuss the findings of this study with the researchers at any time. You will also be provided with a full copy of the final study report, if requested.

It is very likely that the results of this study will be written up for publication in a peer-reviewed journal and/or presentation and a Nutrition conference within 12 months of completing the study. If this happens no participant identification information will be included.

WHO DO I CONTACT FOR MORE INFORMATION OR IF I HAVE CONCERNS?

If you have any questions, concerns or complaints about the study at any stage, you can contact the following researchers involved in the study:

Cameron Haswell: PhD student, School of Health Sciences, Massey University, Albany

Phone: +[redacted]

Email: c.haswell@massey.ac.nz

A/Prof Kay Rutherfurd-Markwick: Associate Professor, School of Health Sciences, Massey University, Albany

Ph: 09 414 0800 ext. 43646

Email: k.j.rutherfurd@massey.ac.nz

A/Prof Rachel Page: Head of School of Health Sciences, Massey University, Wellington

Phone: 04 801 5799 ext. 63462

Email: R.A.Page@massey.ac.nz

A/Prof Ajmol Ali: Associate Professor, School of Sport, Exercise and Nutrition, Massey University, Albany

Phone: 09 213 6414

Email: A.Ali@massey.ac.nz

Dr Roger Hurst: Principal Scientist, Food Innovation Portfolio, Plant & Food Research Ltd

Phone: 06 953 7677 Mobile - [redacted]

Email: Roger.Hurst@plantandfood.co.nz

COMMITTEE APPROVAL STATEMENT

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 20/34. If you have any concerns about the conduct of this research, please contact Dr Negar Partow, Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63363, email humanethicsoutha@massey.ac.nz).

WHAT IF SOMETHING GOES WRONG?

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic, and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted, you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Appendix 2.3 – BOP study participant Screening Questionnaire

Participant Screening Questionnaire

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UNIVERSITY OF NEW ZEALAND

A study to assess the effects of beetroot and blackcurrant juices on glycaemic (blood sugar) responses in healthy participants

School of Health Sciences, College of Health, Albany, Auckland

PRINCIPAL INVESTIGATORS:
Cameron Haswell
Assoc Prof Kay Rutherfurd-Markwick
Assoc Prof Ajmol Ali
Assoc Prof Rachel Page
Dr Roger Hurst

Inclusion Criteria:

1. Are you aged 18-60? YES / NO / UNSURE
2. Do you have a BMI between 18.5-30.0? YES / NO / UNSURE
3. Do you have an HbA1c of ≤ 40 mmol/ mol YES / NO / UNSURE
4. Do you have a fasting glucose < 5.7 mmol/ L YES / NO / UNSURE
5. Do you smoke? YES / NO / UNSURE

Exclusion Criteria:

1. Do you have any known glucose control issues / diabetes? YES / NO
2. Do you have any known clinically significant disease? YES / NO
3. Are you taking any medications that include blood glucose/sugar lowering prescriptions YES / NO
4. Do you have any allergies to beetroot or blackcurrant? YES / NO
5. Are you pregnant or breastfeeding? YES / NO

If you answered YES to Questions 1, 2 or 3 in the Exclusion Criteria section please provide more details:

_____

_____

_____

_____

_____

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

CASE RECORD FORM

A study to assess the effects of beetroot and blackcurrant juices on glycaemic (blood sugar) responses in healthy participants

School of Health Sciences, College of Health, Albany, Auckland

PRINCIPAL INVESTIGATORS:
Cameron Haswell
Assoc Prof Kay Rutherfurd-Markwick
Assoc Prof Ajmol Ali
Assoc Prof Rachel Page
Dr Roger Hurst

Participant Initials:			
Participant ID Number:			

Date of Birth: _____

DD **MM** **YYYY**

Participant ID:				Participant Initials:			

Inclusion Criteria:

1. Is the participant aged 18-60? YES / NO
2. Does the participant have a BMI between 18.5-35.0? YES / NO
3. Does the participant have an HbA1c of ≤ 40 mmol/ mol YES / NO
4. Does the participant have a fasting glucose < 5.7 mmol/ L YES / NO
5. Is the participant non-smoking? YES / NO
6. Is the participant able to communicate with the investigators? YES / NO

Exclusion Criteria:

1. Does the participant have any known glucose control issues / diabetes? YES / NO
2. Does the participant have any known clinically significant disease? YES / NO
3. Not taking any medications that include blood glucose/ sugar lowering prescriptions YES / NO
4. Does the participant have any allergies to beetroot/blackcurrant? YES / NO
5. Is the participant pregnant or breastfeeding? YES / NO

Participant ID:				Participant Initials:			

VISIT ONE: HISTORY AND MEASUREMENTS

INFORMED CONSENT:

Written consent must be given before any study procedures can be carried out.

Has the participant given written informed consent? **YES / NO**

DEMOGRAPHIC DATA

Gender: **MALE / FEMALE**

Date of Birth _____

AGE _____

ETHNICITY

- NZ European
- NZ Māori
- Pacific Islander
- European
- North American
- Middle Eastern/African
- Indian
- South Asian
- Other Asian

  _____
- Other

  _____

MEASUREMENTS

Weight: _____ (kg)

_____ (kg)

Height: _____ (cm)

_____ (cm)

BMI: _____ (kg/m^2)

Waist: _____ (cm)

_____ (cm)

Hip: _____ (cm)

_____ (cm)

Waist/ Hip circumference ratio:

_____

Body fat: _____ (%)

Lean body mass: _____ (%)

Participant ID: | | | | Participant Initials: | | | |

MEDICATIONS TAKEN

Is the participant currently taking any concomitant medications/ significant non-drug therapy?

Yes ☐ No ☐

DETAILS:

_____

_____

_____

_____

_____

KNOWN MEDICAL HISTORY

Is there any known medical history in the following systems:

	System	Yes	No			System	Yes	No
1	Cardiovascular				9	Neoplasia		
2	Respiratory				10	Neurological		
3	Hepato-billary				11	Psychological		
4	Gastro-intestinal				12	Immunological		
5	Genito-urinary				13	Dermatological		
6	Endocrine				14	Allergies		
7	Haematological				15	Eyes, ears, nose, throat		
8	Musculo-skeletal				16	Other		

If YES, please note details below:
(include any known family history of disease below)

_____

_____

_____

_____

_____

Participant ID:				Participant Initials:			

SCREENING BLOOD TEST

Is participant fasting? YES / NO Time of last FOOD/DRINK _____

Site of Collection: _____

Time of Collection: _____

Date of Collection: _____

FASTING GLUCOSE _____

HbA1c _____

Meet Inclusion? YES / NO

Blood pressure: _____ (mmHg) Heart rate: _____ (bpm)

Blood pressure: _____ (mmHg) Heart rate: _____ (bpm)

Blood pressure: _____ (mmHg) Heart rate: _____ (bpm)

Average blood pressure: _____ (mmHg) Average heart rate: _____ (bpm)

Lipid values (mmol/ L):

CHO		**HDL**		Non-HDL	
TG		**LDL**		**CHOL/HDL**	

NOTES:

_____

_____

_____

Adverse Events Schedule

The occurrence of adverse events will be sought by non-directive questioning of the participant at each visit during the study.
Adverse events also may be detected when they are volunteered by the participant during or between visits or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event will be evaluated as per the below schedule.

Participant ID..... Date of adverse event.....

Description of Adverse Event:	Comments
1. The severity (mild/ moderate/ severe)	
2. Relationship to the study intervention product (suspected/not suspected)?	
3. Duration (start and end dates or if continuing at end of study)	
4. Action taken (no action taken; intervention product permanently discontinued due to this adverse event; concomitant medication taken/ non-drug therapy given)?	
5. Whether serious/not serious, where a serious adverse event (SAE) is defined as one which is: ☐ Is fatal or life-threatening ☐ Results in persistent or significant disability/incapacity ☐ Constitutes a congenital anomaly/birth defect ☐ Requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for: ☐ Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition ☐ Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent ☐ Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission ☐ Social reasons and respite care in the absence of any deterioration in the participant's general condition ☐ Is medically significant, i.e., defined as an event that jeopardizes the participant or may require medical or surgical intervention to prevent one of the outcomes listed above	
Additional Comments:	

Research Team member's name.....

Participant ID:					Participant Initials:		

DATA COLLECTION

Blood Glucose

Finger Prick	**Blood glucose levels (mmol/ L) at each time point (min)**									
Intervention	**Date**	**-20**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**
V1										
V2										
V3										
Serum	**Blood glucose levels (mmol/ L) at each time point (min)**									
Intervention	**Date**	**-20**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**
V1										
V2										
V3										

Blood Pressure

Intervention	Date	-20	60	120
V1				
V2				
V3				

Remarks:

_____
_____
_____
_____

Appendix 2.5 – BOP study participant consent form

Consent for Participation

A study to assess the effects of beetroot and blackcurrant juices on glycaemic (blood sugar) responses in healthy participants

Would you like any unused blood samples to be returned to you on completion of the study?
YES / NO

☐ I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

☐ I agree/ do not agree to participate as per the outlined requirements and am aware that I may withdraw up to two weeks following the completion of data collection.

☐ In signing this consent form I declare that I am in no way being subjected to coercion or duress and am consenting of free will.

☐ I agree to participate in this study under the conditions set out in the Participant Information Sheet.

☐ I would be willing to be added to the volunteer database for future nutrition research notifications

Signed_____ Date_____

Full Name – printed _____

This consent form will be kept securely according to Massey University Human Ethics Committee (MUHEC) requirements for a period of five years following the study and remain confidential throughout.

Appendix 2.6 – BOP study sample handling procedure

BOP – Blood sample measurements

Tube	-20	0	15	30	45	60	90	120	150
Grey	X	X	X	X	X	X	X	X	X
Red	X	X	X	X	X	X	X	X	X
Purple	X	X	0	X	0	X	0	X	0

Marker	**Tubes / Volume**	**Sample type**	**Storage**
Glucose	3 Eppendorfs 500ul ml	Plasma EDTA	Sports Lab -80

Red Vacutainer (Gel)

Insulin	3 Eppendorfs 500ul	Plasma	Sports Lab -80

Purple Vacutainer (EDTA)

GLP-1	3 Eppendorfs 120ul	Plasma	Sports Lab -80

Grey Vacutainer (Fluoride/EDTA)

Glucose	2 x cuvette	Plasma and Capillary	Disposed

Per draw
Grey: 1 x 4ml
Red: 1 x 6ml
Purple 1 x 500ul
Eppendorfs – 9
Glucose MicroCuvettes – 1

Following blood collection:

Add 8.06ul of aprotinin and 10ul of DPP-IV inhibitor to purple EDTA vacutainers

Take capillary glucose sample and analyse immediately with haemocue 201+

Take 20ul of blood from Grey vacutainer and place in glucose cuvette for immediate analysis with haemocue 201+

Allow red vacutainers 20-30mins to clot

Spin bloods at 10000rpm for 10mins

Aliquot according to guidelines shown

Appendix 2.7 – BOP study results sheet for participants to take home

The Beetroot and Blackcurrant Juice Study

Date:

Thank you for attending the Beetroot and Blackcurrant Juice Study Screening. Your results from the visit are reported below.

Height (cm)		
Weight (kg)		
BMI (kg/ m2)* ** Based on NZ Heart Foundation*		< 18.5 = Underweight 18.5 - 24.9 = Normal weight 25.0 - 29.9 = Overweight > 30.0 = Obese
Waist circumference (cm)* **Based on International Diabetes Federation (IDF)*		Increased disease risk if: Men ≥ 90, while for European men ≥ 94 Women ≥ 80
Hip circumference (cm)		
Blood pressure (mm/ Hg)* ** Based on American Heart Foundation (AHA) recommendations*		Systolic Diastolic < 120 and < 80 - Normal 120 - 129 and < 80 - Elevated 130 - 139 or 80 - 89 - S1 Hypertension ≥ 140 or ≥ 90 - S2 Hypertension > 180 and/ or > 120 - Hypertension Crisis
Heart rate (bpm)* ** Based on AHA*		60 - 100 = Normal
HbA1c (mmol/ mol)* **Based on New Zealand Medical Association (NZMA)*		≤ 40 = Normal 41-49 = Prediabetes ≥ 50 = Type 2 Diabetes
Fasting glucose (mmol/ L)* ** Based on Diabetes New Zealand*		<5.7 = Normal 5.7 - 6.9 = Prediabetes ≥ 7.0 = Diabetes

Lipid Profile*: ** Based on Southern Cross Medical Society*		Recommended to be:
Total cholesterol (TC) (mmol/ L), **which is HDL + LDL +20% TG**		< 4.0
Triglycerides (TG) (mmol/ L)		< 1.7
High-density lipoprotein (HDL) (mmol/ L)		> 1.0
Low-density lipoprotein (LDL) (mmol/ L)		< 2.0
Non-HDL (total bad cholesterol) (mmol/ L) ** Based on Heart UK*		< 4.0
TC/HDL ratio		< 4.0

Appendix 3 – UK vs NZ study documents (Chapter 6)

The following section of the Appendices includes participant recruitment and documents, and

data collection forms pertaining to the UK vs NZ study:

Appendix 3.1 *– UK vs NZ study recruitment poster* _____ *272*

Appendix 3.2 *– UK vs NZ study participant information sheet* _____ *273*

Appendix 3.3 *– UK vs NZ study consent form* _____ *277*

Appendix 3.1 – UK vs NZ study recruitment poster

MASSEY UNIVERSITY

NZ VS. UK BEETROOT JUICE

Which is best for exercise performance, cognition and cardiovascular function?

CONSIDER TAKING PART IN THIS STUDY?

REQUIREMENTS:

- Healthy males and females
- Aged 18-45 years
- undertake some regular training.
- Fully vaccinated

COMPENSATION

4 visits = $200 vouchers

Contact:
d.holten@massey.ac.nz
a.ali@massey.ac.nz

Appendix 3.2 – UK vs NZ study participant information sheet

Comparison of NZ beetroot juice with UK beetroot juice supplementation on exercise performance, cognition and cardiovascular function and blood glucose levels in recreationally trained exercisers

Researcher Introduction
We are researchers at Massey University and Loughborough University (UK) and are interested in comparing the benefits of beetroot juice grown in NZ and UK on exercise and cognitive performance, cardiovascular function, fasting blood glucose levels and lipid profile.

Invitation to Participate in Research Study
Beetroot juice has been shown to improve exercise performance, cognition, and cardiovascular function, and may reduce blood glucose levels and alter lipid profiles. NZ soil is conducive to growing high-nutrient-containing fruits and vegetables e.g., blackcurrants, blueberries, kiwifruit – all of which have been shown to enhance exercise performance and/or aid in recovery from exercise. Beet-It, the most popular commercially available beetroot juice drink, is grown in the UK; however, no research has compared NZ vs. UK beetroot for exercise performance, cognition, and cardiovascular function and blood glucose levels. The aim of this study is to compare exercise and cognitive performance, as well as cardiovascular function, blood glucose levels and lipid profile following 7 days' supplementation with NZ beetroot juice relative to supplementation with beetroot juice from the UK. We would like to invite 15 well-trained male and female exercisers to take part in this study.

Participant Recruitment
If you are aged 18-45 years and undertake regular training, we'd like to invite you to participate in this study. If you are interested in participating, we would like to check whether you are suitable and have a set of questions to ask and discuss with you. If you are unsure about any of the questions and/or how they relate to you, then please consult with the researchers. You will receive a $50 Westfield/MTA voucher per visit for travel expenses for participation in this study on a pro-rata basis upon completion of the study (4 visits = $200).

Project Procedures and Participant Involvement
We ask you to come in for one familiarisation trial and 3 main trials. For all trials you will be asked to wear comfortable exercising apparel such as trainers, shorts and a shirt or cycling gear if you have it. Please bring a towel as showering facilities will be available.

Familiarisation trial (single trial)
During the familiarisation trial you will be asked to provide a fasting finger prick blood sample (blood glucose and lipid profile). You will then be provided with breakfast (cereal or toast) and will be acquainted with the study protocols and shown how to complete the food record diary which you will be required to keep prior to the main trial. You will then complete the maximal oxygen uptake (VO_2max) test. After a brief (15 min) rest, we will ask you to complete a familiarisation of the 4-km cycling time trial test.

Main trials (3 trials)
For the main trials, we want you to undertake 6 days' supplementation (with one of the three drinks: placebo, UK beetroot juice drink or NZ beetroot juice drink); on the 7th day, you will be asked to come into the lab fasted and after providing a finger prick blood sample you will be provided with breakfast and then undergo baseline tests, before consuming the beverage and then, 2.5 h later, completing the 120-minute cycling exercise followed by the 4-km time trial test. The test beverage will also be provided 60 min into the 120-minute exercise test. Outcome measures will be taken before, during and after the time trial. There will be a 7 days' washout period after each trial.

Female participants taking contraceptive pills will be able to come in for the main trial during days 3-7 of their placebo; those not taking any contraceptives will be required to come during the early follicular phase (days 3-7); therefore, there will be longer duration between study visits for female participants.

Blood samples (6 ml) will be taken via venepuncture before the drink is ingested (-2.5 h), immediately pre-exercise and immediately post-exercise to assess plasma nitrate and plasma nitrite.

Cognitive function tests (rapid visual information processing (RVIP); Stroop test; and Corsi blocks) will be assessed before supplementation, 2.5 h after supplementation and immediately post-exercise.

Hydration status will be measured using urine specific gravity to ensure you are sufficiently hydrated for exercise when you arrive at each trial day.

Perceptual measures (ratings of perceived exertion, feeling scale, felt arousal scale) and other physiological/anthropometric measures (blood pressure, body mass, heart rate, blood flow) will be taken before, during and after the exercise test.

We will also monitor diet (food records) during the 3-day period leading to the main trial. Please refrain from exercise for the 48 h before the cycling time trial and avoid consumption of caffeine-containing food and beverages 24 h before the exercise trial. Please follow the same procedures prior to each main trial.

Qualified first aiders, trained in resuscitation (NZ Red Cross First Aid, Level 2) and the use of a defibrillator will be present for all exercise sessions. In addition, the researchers will be constantly monitoring physiological and perceptual variables that will aid in identifying any issues. All researchers are double vaccinated and boosted for Covid-19; as per Massey University and Government protocols, all participants must be double vaccinated for Covid-19 as well. Researchers will be wearing protective face masks and barrier gloves, and we ask you to always wear a face mask while on campus (apart from when exercising).

Participant's Rights
You are under no obligation to accept this invitation. Should you choose to participate, you have the right to:
• decline to answer any particular question
• withdraw from the study at any time, even after signing a consent form (if you choose to withdraw you cannot withdraw your data from the analysis after the data collection has been completed)
• ask any questions about the study at any time during participation

• provide information on the understanding that your name will not be used unless you give permission to the researcher
• be given access to a summary of the project findings when it is concluded

Good Practice and Cultural Safety for Massey University Research

The study was discussed with the Student Recruitment Adviser - Māori Academic Support. We have considered the inclusion of Māori and indigenous values and concepts, allowing for the use of whānau support and appropriate Māori protocols. We acknowledge the concept of manaakitanga, respecting the participant`s inherent dignity and acting in a caring manner towards them by way of:

- Taking full responsibility to perform research in a safe and ethical manner (aroha)
- Providing the participant with all of the critical information regarding the study in a clear way, so they can make informed decisions (tūmanako and whakapono)
- An awareness of the cultural significance and sensitivity for a culturally safe implementation of the study (māhaki)
- Respect for the privacy and confidentiality of Māori participants
- Acknowledging the tapu (sacred) nature of blood/human tissue by offering remaining blood samples (if appropriate) back to the donor and keeping human samples secured and separated from other biological material, to ensure that the tapu māheuheu is not mixed with or contaminated by other tapu or noa (profane) substances.

Confidentiality

All data collected will be used solely for research purposes and has the possibility of being presented in a professional journal. All personal information will be kept confidential by assigning numbers to each participant. No names will be visible on any papers on which you provide information. All data/information will be dealt with confidentiality and will be stored in a secure location for five years on the Massey University Albany Campus. After this time, it will be disposed of by an appropriate staff member from the School of Sport, Exercise and Nutrition.

Project Contacts

If you have any questions regarding this study, please do not hesitate to contact either of the following people for assistance:

Principal researchers

Prof Ajmol Ali (School of Sport, Exercise and Nutrition, Massey University)
a.ali@massey.ac.nz (09) 213 6414

A/Prof Kay Rutherfurd-Markwick (School of Health Sciences, Massey University)
k.j.rutherfurd@massey.ac.nz (09) 213 6646

Prof Marie Wong (School of Food and Advanced Technology, Massey University)
M.Wong@massey.ac.nz (09) 213 6656

Dr Stephen Bailey (School of Sport, Exercise and Health Sciences, Loughborough University, UK)
S.Bailey2@lboro.ac.uk

Research assistants:

Cameron Haswell (PhD student) c.haswell@massey.ac.nz	Luke Stanaway (PhD student) l.stanaway@massey.ac.nz	Max Dowson (Research assistant) m.dowson@massey.ac.nz
Krutika Nanavati (PhD student) k.nanavati@massey.ac.nz	Owen Mugridge (Clinical Trials Manager) o.mugridge@massey.ac.nz	Brianna Harrison (Research assistant) b.harrison@massey.ac.nz

Committee Approval Statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 21/36. If you have any concerns about the conduct of this research, please contact Dr Negar Partow, Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63363, email humanethicsoutha@massey.ac.nz.

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic, and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted, you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Appendix 3.3 – UK vs NZ study consent form

Comparison of NZ beetroot juice with UK beetroot juice supplementation on exercise performance, cognition and cardiovascular and blood glucose levels in recreationally trained exercisers

CONSENT FORM FOR STUDY VOLUNTEERS

This consent form will be held for a minimum period of five (5) years

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.
I understand that I have the right to withdraw from the study at any time and to decline to answer any particular questions (if I choose to withdraw I cannot withdraw my data from the analysis after the data collection has been completed).
I agree to provide information to the researcher on the understanding that my name will not be used without my permission. (The information will be used only for this research and publications arising from this research project.)
I agree to participate in this study under the conditions set out in the Information Sheet.

Signature: _____ Date_____

Full Name (printed) _____

Phone Number_____ Age _____ Date of Birth _____

Are you willing to be contacted regarding future research projects within the School of Sport, Exercise and Nutrition? Your name and email address will be saved in a secure location. You will be sent periodic newsletters regarding research studies within the School. You can opt out of this newsletter at any time.

☐ Tick here if you accept.

Appendix 4 – Prediabetes study documents (Chapter 7)

The following section of the Appendices includes participant recruitment and documents, and data collection forms pertaining to the Prediabetes study:

Appendix 4.1 *– Prediabetes study recruitment poster* ____ *279*

Appendix 4.2 *– Prediabetes study participant information sheet* ____ *280*

Appendix 4.3 *– Prediabetes study participant information sheet* ____ *290*

Appendix 4.4 *– Prediabetes study consent form* ____ *292*

Appendix 4.5 *– Prediabetes study Protocol Document* ____ *299*

Appendix 4.6 *- Prediabetes study drink preparation procedure* ____ *301*

Appendix 4.7 *– Prediabetes study drink compliance diaries* ____ *312*

Appendix 4.8 *– Prediabetes study supplementation questionnaire* ____ *315*

Appendix 4.9 *– Prediabetes study at home glucose monitoring sheet* ____ *317*

Appendix 4.1 – Prediabetes study recruitment poster

Do you have high blood sugar? Are you pre-diabetic?

The School of Sport, Exercise & Nutrition at Massey University is conducting a study on the blood glucose response to antioxidant-rich plant extracts in people aged between **40-60** years, **living in Auckland.**

If you have high blood sugar levels, antioxidant rich plant extracts may help improve your blood glucose levels.

All participants will receive

- **$20 when screened for eligibility***
- **$200 on completion of four trial visits***
- **FREE breakfast for four visits**
- **FREE body composition, blood glucose, and blood pressure assessments.**

*In the form of a prezzy card.

Can you help?

If you participate in this study, you will be required to:

- Make five* visits to the Massey University Nutrition Laboratory in Albany, Auckland between August-November 2017. Blood samples will be taken.
- Drink a sugary drink along with three different antioxidant-rich foods. We will see if these help control blood sugar levels.

*screening visit and 4 trial visits

For further information or **to express interest,** please visit **www.massey.ac.nz/glarestudy** , email glarestudy@massey.ac.nz, scan the QR code above, or phone (09) 213 6650

Please pass this information on to anyone you know who could be interested.

This study has been approved by the Southern Health & Disabilities Ethics Committee (HDEC. HDEC reference number is 17/STH/82
You can contact HDEC via email on hdecs@moh.govt.nz

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UNIVERSITY OF NEW ZEALAND

Appendix 4.2 Prediabetes study recruitment methodology

This appendix expands on the recruitment and screening activities summarised in Section 7.2.2, providing channel-level detail on each strategy used, the yield each produced, the time and resources invested, and a reflective summary of what worked and what did not. As participant recruitment and retention emerged as the central feasibility challenge of this trial, the level of detail provided here is intended to inform the design of future long-term dietary intervention trials in underdiagnosed populations such as those with prediabetes, hypertension or chronic kidney disease.

A4.2.1 Overview of recruitment activity

Recruitment ran as a hard-stop window between August and November 2023. Across this period, 77 self-initiated pre-screening enquiries were received through a combination of email correspondence, telephone calls and direct messages via Facebook (comprising 32 via Facebook, 29 via the participant mailing list newsletter, 12 via university posters, and 4 by word of mouth). A further 14 individuals entered the pre-screening pathway through study-initiated channels: 6 referrals from engaged GP clinics, and 8 walk-up HbA1c tests at the shopping centre screening event. Of all 91 pre-screen contacts, 45 progressed to an in-person HbA1c finger-prick screening visit, as illustrated in the CONSORT flow diagram presented in Figure 7.2 of the main text.

The 46 pre-screen enquiries that did not progress to in-person screening were excluded predominantly for two reasons. The first was residence outside the Auckland region, most commonly from respondents reached through the participant mailing list newsletter, which had national rather than regional distribution, including those who had previously participated in research trials but moved out of the local area. The second was falling outside the eligible age range of 30–75 years, most commonly from respondents to university posters; many of these enquiries came from undergraduate students who appeared to be motivated more by the $50 NZD koha than by an established likelihood of eligibility.

Of the 45 individuals who progressed to in-person screening, 12 were randomised, giving an overall screen-to-randomisation rate of 27%. Of the remaining 33, 31 did not meet the inclusion criteria; 27 because their HbA1c fell within the normoglycaemic

range (< 41 mmol/mol), with 4 individuals presenting HbA1c values above the upper limit of the prediabetic range (> 49 mmol/mol), consistent with previously undetected type 2 diabetes. No exclusions occurred on the basis of BMI. Two further individuals declined to participate after screening, both of whom had been screened at the random shopping-centre screening event and were unwilling to subsequently travel to the North Shore campus for the formal study visits.

A4.2.2 Recruitment channels

Academic institution

Recruitment through Massey University used two parallel channels: emails distributed via departmental administrators to staff and student mailing lists, and printed posters (*Appendix 4.1*) displayed in shared communal and study spaces including the University library, lecture halls, student lounges and eating areas. Posters were also displayed in local areas immediately surrounding the Albany campus.

Posters generated 12 pre-screen enquiries but, on reflection, were poorly targeted for a study of prediabetes. The majority of poster-driven enquiries came from undergraduate students who fell outside the age range and, in many cases, appeared to be motivated by the koha rather than by an established likelihood of prediabetes. None of the poster-driven enquiries progressed to in-person screening. While posters were inexpensive to produce and display, this channel effectively yielded a return of zero for the time spent responding to the resulting enquiries.

The participant recruitment database, administered as a quarterly participant newsletter by the School of Sport, Exercise and Nutrition, generated 29 pre-screen enquiries, the second-largest volume of any channel. However, its national distribution meant that a substantial proportion of respondents lived outside Auckland and were excluded at pre-screen on geographic grounds. Of the 15 individuals from this channel who progressed to in-person screening, 13 did not meet the inclusion criteria and 2 were randomised, giving a screen-to-randomisation conversion of 13% and an enquiry-to-randomisation rate of just 7%, the lowest yield of any channel that produced a randomised participant.

The School of Sport, Exercise and Nutrition's dietetics clinic, which sees patients referred from local GP clinics including some with previous prediabetes diagnoses, agreed to distribute study information to potentially eligible patients. This channel produced 2 in-person screens, neither of whom met the inclusion criteria at screening.

Healthcare professionals

Eight general practice (GP) clinics across the North Shore of Auckland were contacted by email with an introductory letter outlining the study aims, inclusion criteria and potential benefits to participants. The full text of the introductory email used is reproduced at the end of this appendix (A4.2.5). Each email included an invitation to meet face-to-face to discuss the study and the option of distributing the study poster to potentially eligible patients. No incentive or payment was offered to clinics for participating in dissemination.

Two of the eight clinics responded and engaged with the research team: North Shore Medical and Health Centre (Sunset Road) and Albany Family Medical Centre. Both face-to-face meetings were short (5–10 minutes), held directly with a GP rather than with a practice manager or nurse, and consisted of an overview of the study aims and likely participant burden. Following each meeting, the study poster was emailed to the clinic for internal distribution to potentially eligible patients.

The remaining six clinics did not respond to the initial email, and a follow-up email also went unanswered. On reflection, attempting an in-person introductory visit at non-responding clinics, rather than relying solely on email follow-up, would likely have been a more effective second contact strategy.

Despite engaging only two clinics, healthcare provider referral was by some margin the most successful recruitment channel of the study in terms of time and cost efficiency. Of the 6 individuals referred and screened in-person, 4 (67%) met the inclusion criteria and were randomised, representing both the highest screen-to-randomisation conversion rate and the highest enquiry-to-randomisation rate of any channel. Critically, all 4 of these randomised participants came from a single clinic, meaning that one engaged GP practice accounted for one-third of the entire study's enrolment. This finding directly supports the recommendation made in Section 7.4.1 that targeted

healthcare provider engagement should be the cornerstone of recruitment strategies for studies in asymptomatic and underdiagnosed populations.

Diabetes Foundation Aotearoa was also contacted by email to discuss dissemination of study information through their networks. No response was received.

Random screening events

A single random screening event was conducted at a North Shore shopping centre, at which two members of the study team approached members of the public and offered free HbA1c testing. Several hundred people were approached over the course of the day, of whom eight agreed to undergo a finger-prick HbA1c test on-site. Of those eight, six fell outside the eligible HbA1c range; the remaining two, while eligible, subsequently declined to participate, citing unwillingness to travel to the North Shore campus for the formal study visits, an outcome that, given the screening event itself took place on the North Shore, points to a more general aversion to additional study-related travel rather than to distance per se.

Although random screening returned zero randomisations. The exercise demonstrated that even where eligibility is confirmed at the point of screening, perceived travel burden can be a decisive barrier to enrolment, a finding consistent with the broader literature on recruitment friction in chronic-disease trials.

Social media

Online advertising consisted of unpaid posts in approximately 10–15 community Facebook groups specific to suburbs on the North Shore of Auckland. Posts contained brief study information, eligibility criteria, contact details for the principal investigator, and a link to the study webpage hosted on the Massey University website.

Facebook generated 32 pre-screen enquiries. Of the 32 enquiries, 12 progressed to in-person screening and 4 were randomised, giving a screen-to-randomisation conversion of 33% and an enquiry-to-randomisation rate of 13%. This channel was inexpensive (unpaid) and required only modest management, primarily responding to comments and direct messages, but performed well relative to most other channels, particularly where local geographic targeting matched the catchment area.

While unpaid posts proved effective and are cost efficient, paid targeted advertising through social media may have produced higher yield with less researcher time. Targeting by age, geographic radius and inferred interested categories drawn from users' browsing and platform behaviours would allow advertisements to reach an audience pre-filtered for both eligibility and likely interest. This would directly cut researcher time spend phone-screening eligible enquiries generated by untargeted adverts. Subject to budget availability, paid social media advertising should be considered in future research, with the important caveat that it may systemically under-represent older adults, those from lower socioeconomic backgrounds, and those who restrict cookie-based platform tracking.

Word of mouth

A small number of pre-screen enquiries (n = 4) and 4 in-person screens were generated by word of mouth, primarily through participants already enrolled in the trial speaking to friends, family or colleagues. Two of these four (50%) were randomised. While the absolute numbers are small, the conversion rate is consistent with the broader principle that referred participants, whether by a healthcare provider or a known peer, tend to arrive with a stronger prior likelihood of eligibility and motivation than self-referred respondents to broadcast advertising.

Community events

Two members of the study team attended a local event hosted by The Asian Network Inc. (TANI) to network with health professionals and promote the study, and a study advertisement was subsequently included in the TANI quarterly newsletter. No enquiries were traced to this channel. While the absence of yield does not necessarily reflect the value of the community relationship itself, community engagement having intrinsic value beyond direct recruitment, it does suggest that single-event attendance and a one-off newsletter inclusion are insufficient to drive enrolment in a short-window trial. Sustained engagement with community networks over a longer recruitment period would likely be needed for this channel to deliver measurable recruitment yield.

A4.2.3 Recruitment yield by channel

Table A4.2.1 summarises the yield of each recruitment channel across the recruitment window. Pre-screen enquiry figures, in-person screening figures, ineligibility figures and randomisation figures are presented as recorded; the screen-to-randomisation column reports the proportion of in-person screens that resulted in randomisation, while the enquiry-to-randomisation column reports the proportion of all pre-screen enquiries (including those filtered out before in-person screening) that resulted in randomisation. The latter is the more useful metric for planning purposes, as it captures the true yield of each channel relative to the recruitment effort it generated.

Table A4.2.1. Recruitment yield by channel across the August–November 2023 recruitment window.

Recruitment channel	Pre-screen enquiries	In-person screens	Ineligible	Randomised	Screen → randomisation	Enquiry → randomisation
Healthcare provider referral	6	6	2	**4**	**67%**	**67%**
Social media (Facebook)	32	12	8	4	33%	13%
Word of mouth	4	4	2	2	50%	50%
Participant mailing list	29	15	13	2	13%	7%
University posters	12	0	—	0	—	0%
Shopping centre screening	8 (on-site)	8	6	0 (2 declined)	0%	0%
TANI event + newsletter	0	0	—	0	—	—
Total	**91**	**45**	**31**	**12**	**27%**	**13%**

TANI, The Asian Network Inc.

A4.2.4 Resource investment

Table A4.2.2 summarises the researcher time invested across recruitment activities. Total recruitment-related researcher time was approximately 97.5 hours over the four-

month recruitment window, exclusive of intervention delivery itself and Study Visit 2. Two activities accounted for the substantial majority of this time: telephone pre-screening (~27 hours for ~80 enquiries at approximately 20 minutes each) and in-person screening visits (~45 hours for 45 screens). Together, these two activities represent roughly 74% of the total recruitment time investment.

This distribution carries an important methodological implication. Because in-person screening visits incur approximately one hour of researcher time per visit, and because telephone pre-screening, while shorter per contact, scales linearly with enquiry volume, recruitment channels that produce high volumes of ineligible enquiries impose a disproportionate hidden cost on the research team. The participant mailing list channel, for example, generated 15 in-person screens but only 2 randomisations, meaning approximately 13 hours of in-person screening time were effectively spent on candidates who would not be randomised. In contrast, healthcare provider referral generated 6 in-person screens for 4 randomisations, with only approximately 2 hours of screening time spent on ineligible candidates. Across the study as a whole, of the 45 hours spent on in-person screening, approximately 33 hours (73%) were spent on individuals who were ultimately not randomised. This finding strongly reinforces the value of targeted, pre-filtered recruitment channels, particularly healthcare provider referral, for studies in underdiagnosed populations, since each ineligible in-person screen represents a fixed and non-recoverable researcher time cost.

Direct cash costs were modest. Poster printing was on the order of cents per poster. HbA1c cartridges for the Cobas b 101 system cost $12 NZD each, with eight cartridges used at the shopping centre event. Travel and parking costs for the shopping centre and GP clinic visits were negligible. Koha payments totalled approximately $1,300 NZD ($20 NZD voucher provided at the in-person screening visit, regardless of eligibility outcome, for the 45 individuals screened, plus a further $50 NZD voucher at study completion for the 8 participants who completed the 12-week intervention). The Facebook recruitment used organic (unpaid) posts only, incurring no advertising cost.

Table A4.2.2. Researcher time investment across recruitment activities.

Activity	Researcher hours	Notes
Poster design and distribution (campus and local areas)	4	Printing costs minimal (cents per poster)
Recruitment mailing list emails (drafting, liaison with department administrators)	1	National distribution; many out-of-Auckland enquiries
Social media posts (setup, monitoring, responding to messages)	4.5	No paid promotion; ~10–15 community Facebook groups
TANI event attendance	4	Two staff attended; newsletter follow-up included
Shopping centre screening event (one day)	8	HbA1c cartridges $12 NZD each; 8 tests performed
GP clinic outreach (email drafting, research, follow-up)	2	8 clinics contacted
GP clinic face-to-face meetings and travel	2	2 clinics engaged; meetings 5–10 min each
Telephone pre-screening (~80 enquiries × 20 min)	~27	Largest single time use bar in-person screening visits
In-person screening visits (45 × ~1 hr)	45	HbA1c, fasting glucose, lipid profile, BP, body composition
Approximate total researcher time	**~97.5 hrs**	**Recruitment activities only; excludes intervention delivery and Study Visit 2**
Approximate koha paid (NZD)	~$1,300	45 in-person screens × $20 voucher + 8 completers × $50 voucher
HbA1c cartridges used for screening (NZD)	~$540	45 cartridges × $12
Approximate total costs for recruitment	~$1840	

TANI; The Asian Network Inc. NZD; New Zealand Dollars.

A4.2.5 Reflective summary

Three observations are worth highlighting from the recruitment experience of this trial, each of which informs the recommendations made in Section 7.4 of the main text.

First, channel conversion rates varied substantially across the channels that produced randomised participants. Enquiry-to-randomisation rates ranged from 7% (participant mailing list) to 67% (healthcare provider referral), almost a ten-fold difference. The conversion rate of a recruitment channel is a more useful planning metric than its absolute reach, because the cost to the research team of an ineligible in-person screen is approximately equal to that of an eligible one. Channels that pre-filter for likely eligibility such as healthcare provider referral, where the referrer has access to diagnostic information unavailable to the research team, are therefore disproportionately valuable, even when their absolute reach is small.

Second, broadcast advertising channels (posters, national mailing lists, broad-reach social media) produced large volumes of pre-screen enquiries but tended to misalign with the study's eligibility criteria. University posters in particular generated 12 enquiries dominated by undergraduate students drawn by the *koha* rather than by any likelihood of prediabetes and yielded zero in-person screens despite the time invested in responding to these enquiries. Locally targeted Facebook posts, by contrast, performed substantially better, suggesting that broadcast channels can be made viable through tight geographic and demographic targeting but lose value when targeting is loose.

Third, the single most productive recruitment event of the entire trial was a 5–10 minute face-to-face meeting with a single GP at one North Shore practice, which ultimately accounted for one-third of the study's total enrolment. The contrast between this outcome and the zero yield from email-only outreach to six non-responding clinics suggests that in-person engagement with primary care providers, even briefly, is a substantially more effective recruitment investment than equivalent time spent on broadcast advertising. For future trials in similar populations, an early and persistent in-person GP engagement strategy is therefore recommended as the primary

recruitment channel, with locally targeted social media used as a secondary channel and broadcast methods de-prioritised.

A4.2.6 Healthcare provider introductory email (text)

The following text was used as the introductory email sent to the eight North Shore GP clinics contacted during recruitment. Minor formatting and contact details have been retained as sent.

> *Kia ora,*
>
> *My name is Cameron Haswell, I am PhD student and health science tutor at Massey University, Albany. As part of my PhD research, I am conducting a 12-week intervention study to investigate the effects of vegetable and/or berry concentrates on glycaemic control in prediabetics. I am reaching out to see if it would be possible for me to come and have a brief chat to give the doctors and nurses an overview of what the study involves, and perhaps hand out some flyers to give to patients who are found to be living with prediabetes?*
>
> *The intervention involves daily consumption of a beverage concentrate for 84 days, which will be provided to participants, and a 1hr study visit at the start and end of the intervention period. The study visit can take place either at Massey's Albany Campus, or at a location convenient for the participant, as all the testing equipment is portable. Participants will be provided with a glucometer to utilise for the 12 weeks of the study and will be asked to take a weekly fasting glucose sample on a Caresens Dual glucometer following training from a qualified phlebotomist.*
>
> *Participants will receive information about their HbA1c, fasting glucose, lipid profiles and blood pressure during each study visit, as well as body composition and anthropometric measures. They will also receive a $50 petrol voucher as a koha for their time. As there are 4 intervention arms, participants have a 75% chance of being in an active intervention group.*
>
> *Please let me know if this would be of interest to you or your patients,*
>
> *Kind regards,*
>
> *Cameron*

Appendix 4.3 – Prediabetes study participant information sheet

Participant Information Sheet

Beating Prediabetes Study

Formal Study title: **A study to assess the effects of beetroot and blackcurrant juices on glycaemic (blood sugar) responses in prediabetes**

Lead Researcher: Cameron Haswell

Study Site: Massey University Sports Lab

Ethics committee ref: 2022 EXP 11682

Researcher Introduction and invitation to take part

My name is Cameron Haswell, and I am a PhD student at Massey University, School of Health Sciences. My supervisors are A/Prof Kay Rutherfurd-Markwick, Prof Ajmol Ali, A/Prof Rachel Page and Dr Roger Hurst.

You are invited to take part in a study to assess the effects of beetroot and blackcurrant compounds on glucose (blood sugar) control in people with prediabetes.

This Participant Information Sheet will help you decide if you'd like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign the Consent Form on the last page of this document. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep.

This document is 11 pages long, including the Consent Form. Please make sure you have read and understood all the pages.

VOLUNTARY PARTICIPATION AND WITHDRAWAL FROM THIS STUDY

Participating in this study is completely voluntary and you are free to decline to participate, decline to answer any particular question, or to withdraw from the research at any practicable time. Should you withdraw, you will still receive a koha for your participation up until the point of withdrawal.

WHAT IS THE PURPOSE OF THE STUDY?

Obesity and diabetes are epidemic in New Zealand (and in other Western countries), and many individuals now have problems maintaining their blood sugar levels within normal levels.

Certain foods and drinks, including berries, teas and some vegetables have been shown to reduce blood sugar levels. This may be due to the high levels of antioxidants that are present in these foods, though other substances such as inorganic nitrate may also be involved. The effects of New Zealand beetroot and blackcurrant juices on blood sugar control have not been studied, thus, this study aims to determine whether blood sugar levels in prediabetes can be improved following daily consumption of a beetroot or blackcurrant beverage.

HOW IS THE STUDY DESIGNED?

The study will take place in NZ and will include a total of 160 participants. You will be enrolled in the study for a total of 12 weeks and will be required to come to the lab on 2 separate occasions at the start and end of the study period.

There are 4 groups in the study, and you will be randomly assigned to either beetroot, blackcurrant, a beetroot/blackcurrant mix or placebo and will consume a beverage each morning for 12 weeks. There is a 25% chance that you will be in any given group.

You will be asked to provide a finger prick blood samples during both study visits where we will measure HbA1c, lipid profile and fasting glucose. These visits will also involve measures of body composition and a food frequency questionnaire (FFQ).

During the 12-week study intervention, you will be asked to measure your fasting glucose weekly and complete weekly compliance diaries. These diaries and questionnaires will be used to check the appropriateness of the intervention and to ensure there are no adverse effects of the intervention drink.

WHO CAN TAKE PART IN THE STUDY?

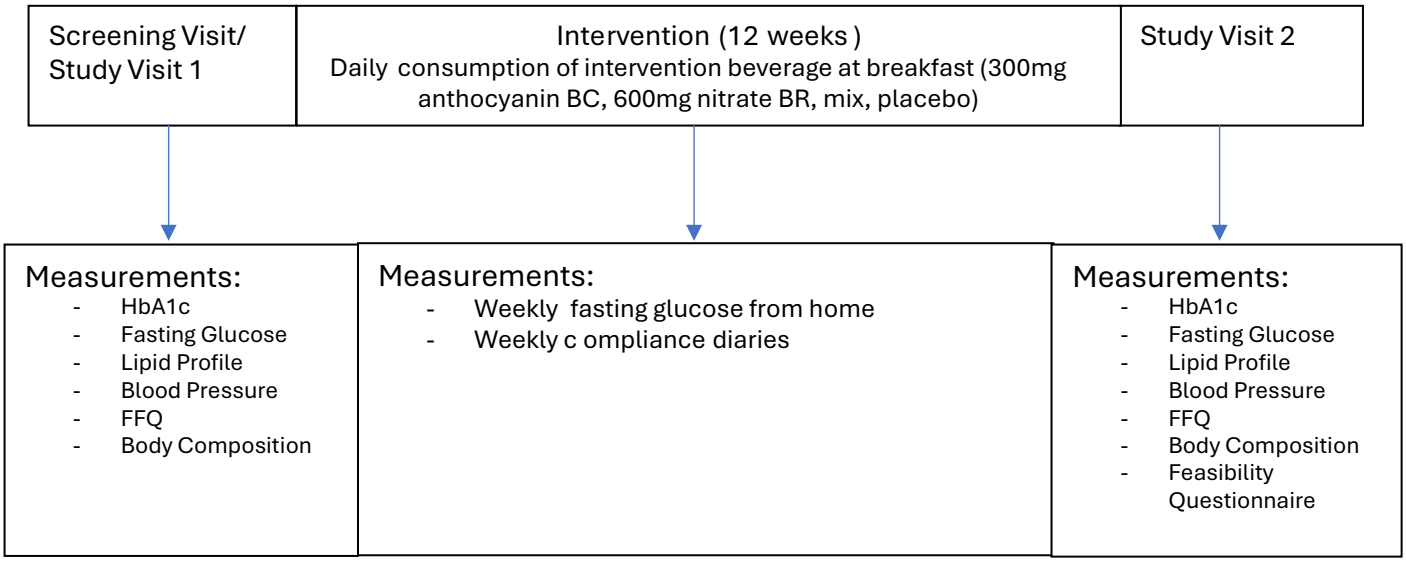
You have been chosen to participate in the study because you have met the criteria to be included in the study. The criteria include:

- 30-65 years of age
- BMI 18.5 - 40.0 kg/ m^2
- 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

WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

The study will involve you coming to the Massey University Sports Lab, located at the Albany campus. If you are eligible after an initial screening questionnaire, you will need to be available for TWO in-person morning visits separated by a 12-week intervention period. Each of these visits will take place in the morning and will last approximately 1 hour. Each session, including the screening visit, will require you to fast overnight (i.e. no food or drink except water) for at least 10 hours before coming to the research facility.

Whole Study at a Glance



Screening visit (Visit 1)

During your screening and first study visit, we will assess whether you meet the study inclusion criteria. You will be required to 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. A qualified phlebotomist will measure your fasting blood sugar, lipid profile and HbA1c levels through a finger prick blood sample. You will be invited to participate in the study visits if screening indicates you are eligible for the study.

In addition, trained researchers will determine your body mass index (BMI) by measuring your weight and height. They will also be measuring your waist and hip circumference, and your body composition (using a bioelectrical impedance scale). This can be done privately, and you do not need to undress, however you will need to remove socks and shoes. Blood pressure and heart rate will also be measured. You will also be asked about your relevant medical history, and any medication use. Finally, you will complete a food frequency questionnaire online which will provide us information on your eating habits over the previous 30 days.

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

Study Interval (12 weeks)

Between study visits 1 and 2 there will be a 12-week interval during which time you will be asked to consume a beverage daily. This beverage will be either placebo, blackcurrant, beetroot or a blackcurrant/beetroot mix. During this time, you will be asked to complete a weekly fasting glucose measurement using the handheld glucometer issued to you during your first visit (or your own).

You will be asked to complete a compliance diary each week including questions about how you are feeling and how you are finding the intervention drink. This should take no longer than 5 minutes to complete.

Study Visit 2

Study visit 2 will be identical to study visit 1. You are required to come to the lab fasted 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. You will also complete a feasibility questionnaire to assess the long-term viability of the intervention drink. You will then be thanked for your time and receive a *koha* for you participation.

WHAT ARE THE POSSIBLE RISKS OF THIS STUDY?

It is highly unlikely that you will be injured during this study. Finger prick blood sampling is a safe and routinely used practice. A trained phlebotomist and first aider will be available during each study session and will assess for adverse events (i.e. feeling nauseous, dehydrated or faint) during each finger prick sample collection.

You may also find that the study intervention drink is not to your taste but there is no risk to you in consuming the beverages, apart from allergic reactions to either blackcurrant or beetroot, which is extremely rare.

WHAT ARE THE POSSIBLE BENEFITS OF THIS STUDY?

By participating in the study, you will obtain information about your general health status, HbA1c value, body composition and blood pressure measurement entirely at no cost to you. These results are not diagnostic, and should you be concerned about any of the results obtained, we would ask that you refer to your normal healthcare provider to seek further information.

In addition, there will be a $50 voucher as a *koha* for involvement in the study.

WHAT ARE THE ALTERNATIVES TO TAKING PART?

Other treatments for prediabetes are available and you should consult with your general practitioner for more information.

WILL ANY COSTS BE REIMBURSED?

There will be a $50 *koha* to thank you and reimburse your travel costs for the study.

WHAT IF SOMETHING GOES WRONG?

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic, and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted, you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

WHAT WILL HAPPEN TO MY INFORMATION?

During this study researchers, and other staff will record information about you and your study participation. This includes the results of measurements mentioned in the protocol. You cannot take part in this study if you do not consent to the collection of this information.

De-identified (Coded) Information

To make sure your personal information is kept confidential, information that identifies you will not be included in any report generated by the researchers. Instead, you will be identified by a code. The researchers will keep a list linking your code with your name, so that you can be identified by your coded data if needed. Only the research group will have access to your coded information.

The results of the study may be published or presented, but not in a form that would reasonably be expected to identify you.

Security and Storage of Your Information.

Your identifiable information is held at Massey University during the study. After the study, it will be transferred to a secure archiving site and stored for at least 10 years as per standard regulations, then destroyed. Your coded information will be entered into electronic case report forms. All storage will comply with local and/or international data security guidelines.

Risks.

Although efforts will be made to protect your privacy, absolute confidentiality of your information cannot be guaranteed. Even with coded and anonymised information, there is no guarantee that you cannot be identified. The risk of people accessing and misusing your information (e.g. making it harder for you to get or keep a job or health insurance) is currently very small, but may increase in the future as people find new ways of tracing information.

This research includes basic information such as your ethnic group, geographic region, age range, and sex. It is possible that this research could one day help people in the same groups as you. However, it is also possible that research findings could be used inappropriately to support negative stereotypes, stigmatize, or discriminate against members of the same groups as you.

Rights to Access Your Information.

You have the right to request access to your information held by the research team. You also have the right to request that any information you disagree with is corrected.

Please ask if you would like to access the results of your screening and safety tests during the study.

If you have any questions about the collection and use of information about you, you should ask the primary researcher, Cameron Haswell.

Rights to Withdraw Your Information.

You may withdraw your consent for the collection and use of your information at any time, by informing the primary researcher.

If you withdraw your consent, your study participation will end, and the study team will stop collecting information from you.

If you agree, information collected up until your withdrawal from the study will continue to be used and included in the study. You may ask for it to be deleted when you withdraw, unless you withdraw after the study analyses have been undertaken.

Information from this study may lead to discoveries and inventions or the development of a commercial product. The rights to these will belong to The Beverage Lab. You and your family will not receive any financial benefits or compensation, nor have any rights in any developments, inventions, or other discoveries that might come from this information.

WHAT HAPPENS AFTER THE STUDY OR IF I CHANGE MY MIND?

You can withdraw from the study at any time and will be compensated according to the number of visits made. Furthermore, you are welcome to discuss any concerns you have with the research team at any time, and you have free access to your data. If you withdraw from the study all of the data that was related to you will be shredded.

The treatment intervention (beetroot and blackcurrant juices) will not be available to any participant after the study.

The study data will be stored at a secure location at Massey University Albany Campus. Electronic data and records will be the responsibility of the Principal investigator. All data will be kept for 10 years, at which point it will be destroyed using University Security methods for removal of confidential material.

It is very likely that the results of this study will be written up for publication in a peer-reviewed journal and/or presentation and a scientific conference within 12 months of completing the study. If this happens no participant identification information will be included.

CAN I FIND OUT THE RESULTS OF THE STUDY?

Participants are welcome to discuss the findings of this study with the researchers at any time. You will also be provided with a full copy of the final study report, if requested, as well as a lay summary of the results at the end of the study duration.

The study details are available on the ANZCTR under number ACTRN12622001108707.

WHO IS FUNDING THE STUDY?

The study is being funded by Massey University, School of Health Sciences. The blackcurrant juice used in the study has been provided by The Blackcurrant Cooperative.

WHO HAS APPROVED THE STUDY?

This study has been approved by an independent group of people called a Health and Disability Ethics Committee (HDEC), who check that studies meet established ethical standards. The HDEC committee has approved this study.

WHO DO I CONTACT FOR MORE INFORMATION OR IF I HAVE CONCERNS?

If you have any questions, concerns or complaints about the study at any stage, you can contact the following researchers involved in the study:

Cameron Haswell: PhD student, School of Health Sciences, Massey University, Albany

Phone: +[redacted]

Email: c.haswell@massey.ac.nz

A/Prof Kay Rutherfurd-Markwick: Associate Professor, School of Health Sciences, Massey University, Albany

Ph: 09 414 0800 ext. 43646

Email: k.j.rutherfurd@massey.ac.nz

A/Prof Rachel Page: Head of School of Health Sciences, Massey University, Wellington

Phone: 04 801 5799 ext. 63462

Email: R.A.Page@massey.ac.nz

Prof Ajmol Ali: Associate Professor, School of Sport, Exercise and Nutrition, Massey University, Albany

Phone: 09 213 6414

Email: A.Ali@massey.ac.nz

Dr Roger Hurst: Principal Scientist, Food Innovation Portfolio, Plant & Food Research Ltd

Phone: 06 953 7677 Mobile -[redacted]

Email: Roger.Hurst@plantandfood.co.nz

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

Phone: 0800 555 050

Fax: 0800 2 SUPPORT (085 00 2787 7678)

Email: advocacy@advocacy.org.nz

Website: https://www.advocacy.org.nz/

For Māori health support, please contact:

Dr Bevan Erueti: Senior Lecturer - Health Promotion/Associate Dean – Māori, School of Health Sciences, Massey University, Wellington

Phone: 04 695 16087

Email: B.Erueti@massey.ac.nz

You can also contact the health and disability ethics committee (HDEC) that approved this study on:

Phone: 0800 4 ETHIC

Email: hdecs@health.govt.nz

Appendix 4.4 – Prediabetes study consent form

Consent Form

Please note, an interpreter may be available if required

Please tick to indicate you consent to the following

I have read or have had read to me in my first language, and I understand the Participant Information Sheet.		
I have been given sufficient time to consider whether or not to participate in this study.		
I have had the opportunity to use a legal representative, whānau/ family support or a friend to help me ask questions and understand the study.		
I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and information sheet.		
I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care.		
I consent to the research staff collecting and processing my information, including information about my health.		
If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed.	Yes ☐	No ☐
I agree to an approved auditor appointed by the New Zealand Health and Disability Ethics Committees, or any relevant regulatory authority or their approved representative reviewing my relevant medical records for the sole purpose of checking the accuracy of the information recorded for the study.		
I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.		
I understand the compensation provisions in case of injury during the study.		
I know who to contact if I have any questions about the study in general.		

I understand my responsibilities as a study participant.

I wish to receive a summary of the results from the study. Yes ☐ No ☐

Declaration by participant:
I hereby consent to take part in this study.

Participant's name:

Signature: Date:

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name:

Signature: Date:

Appendix 4.5 – Prediabetes study Protocol Document

Prediabetes study protocol

1. Background

Background: People with prediabetes, an intermediate state of hyperglycaemia[1], 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 T2DM[1,5,6.]

Polyphenol-rich foods have been investigated for their ability to modulate blood glucose levels[7-14] 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 pressure[15,16] and postprandial blood glucose response[7, 17,18] in healthy subjects and in people with prediabetes[19.]

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 pressure[22] and also mediates glucose uptake from the intestines and skeletal muscle[23,24]. Bk is high in polyphenols including anthocyanins which are proposed to modify postprandial glycaemia[25]. 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 HbA1c, fasting blood sugar, CRP and lipid profiles in people with prediabetes.

2. Hypothesis

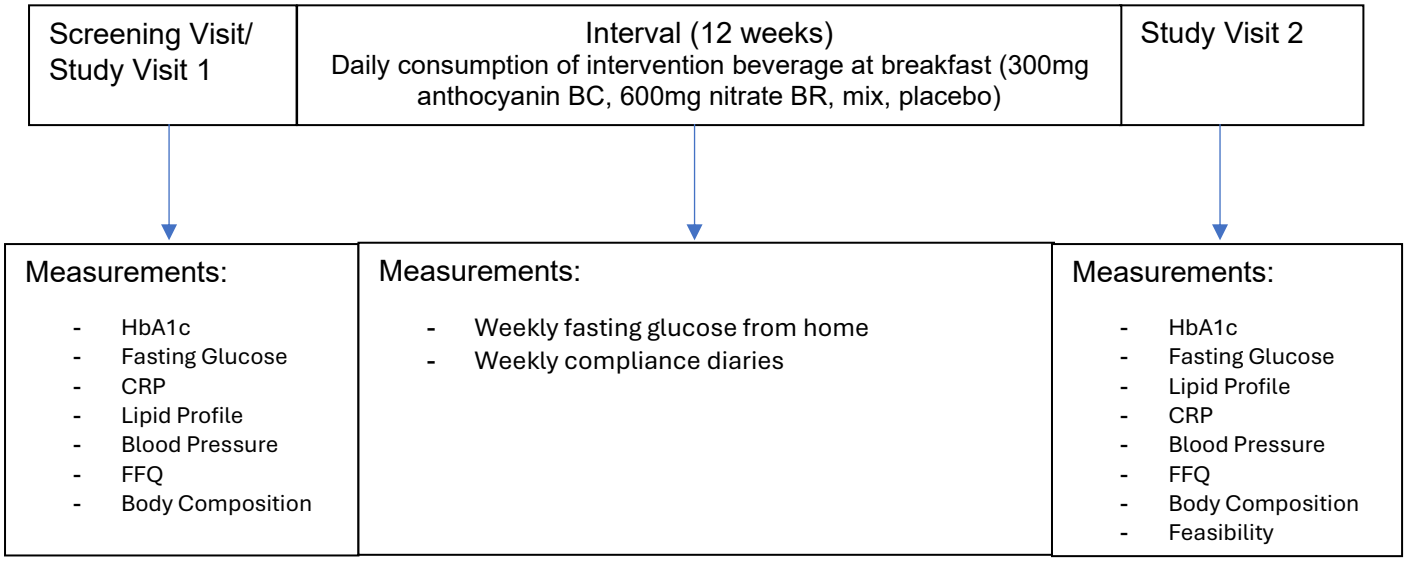
12 weeks of supplementation of blackcurrant anthocyanins or beetroot compounds, or both, will improve HbA1c and measures of metabolic syndrome compared with placebo.

3. Experimental design

The study will be a double-blind, randomized, placebo-controlled, parallel study with four testing arms (placebo, blackcurrant juice, beetroot juice, beet 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. Measures will be taken for fasting blood

glucose, HbA1c, lipid profile, CRP, 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.

Whole Study at a Glance



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, HbA1c levels and CRP 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. 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 to 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. This should take no longer than 5 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 and the same measurements that were taken at visit 1, will be taken by the researcher, to determine if the intervention has had an effect on any of the measures stated above. The participant will also complete a feasibility questionnaire to assess the long-term viability of the intervention drink.

4. Methods and procedures

4.1 Sample size

The study will recruit 40 people into each group (160 participants total). As there is no robust previous literature using chronic supplementation of either BR or Bk in prediabetes, the study is a polit 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.

4.2 Inclusion criteria

- 30-65 years of age
- BMI 18.5 - 40.0 kg/ m^2
- 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
- Double vaccinated with booster shot against COVID-19

4.3 Exclusion criteria

- Use insulin
- Chronic kidney disease
- Significant weight loss in previous 12 months
- Regularly participating in >5hrs exercise weekly
- Other medical factors that may affect study

4.4 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, CRP, 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.

4.5 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.

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Appendix 4.6 - Prediabetes study drink preparation procedure

Prediabetes Study: Drinks Aliquoting and Handling Procedures

Scope of SOP

The purpose of this SOP is to outline the procedures for aliquoting, and handling of the Prediabetes drink samples in a consistent and controlled manner. This SOP outlines the steps for the preparation of the trial sample drinks, and the proper storage and handling of the samples to minimize contamination and deterioration.

Drinks:

Placebo (commercial product)		
Beetroot concentrate	Brix 70.00	pH 3.99
Blackcurrant concentrate	Brix 65.30	pH 3.25
Mix of Beetroot and blackcurrant concentrates mixed in approximate proportions (based on anthocyanin and nitrate values)		pH 3.69

Starting Materials: Beetroot and Blackcurrant Concentrates, Placebo

a) Beetroot and blackcurrant concentrates were purchased or provided by JP-NZ and The New Zealand Blackcurrant Cooperative, respectively.
b) Concentrates were stored at -20°C before aliquoting.
c) Placebo drinks are commercially available Sodastream pre-packaged Sugar Free Cranberry Raspberry concentrate. The drinks will be stored at room temperature, provided to participants chilled and refrigerated by participants once opened.

Equipment

Food grade lab coat
Safety glasses
Hair net, beard cover
Food grade barrier gloves

120-150ml Sterile Sample Containers (purchased as sterile)
250ml Sterile Sample Container (purchased as sterile)

Tablespoons/Dessert Spoons
Spatula
Ladle Spoon
Measuring Jug
10L Pails

Balance
pH meter
Ice/ice packs
Microchlor solution
70% Ethanol in spray bottle

Sterilising and Sanitising equipment:

Prior to starting:

a) All personnel will wear appropriate laboratory lab coat, glasses, gloves, hair nets, beard coverings as required.
b) All equipment (measuring jugs, pails, spoons etc) coming in direct contact with the drink samples as part of the aliquoting process will be sterilised by immersion in Mikro-chlor sanitising solution (70g in 5L of water; 200ppm available chlorine), with minimum 15minutes contact time. Wherever possible items will be removed from the Mikro-chlor solution in the laminar flow hood and placed in a sterile container in the hood to drain prior to use.
c) Sterile sample containers will be used to store the drinks.
d) Workbenches will be sanitized using 70% ethanol solution.
e) Laminar flow hood will be sanitized using 70% ethanol solution.
f) Balance will be positioned in the laminar flow hood and sanitized with 70% ethanol.

During Aliquoting

- The laminar flow hood and the balance will be re sanitised with 70% ethanol at a minimum of 30min intervals or immediately following any spills or if any possible contamination is deemed to have occurred.
- Freshly sanitised equipment (jugs, spoons etc) will be employed between each set of samples (sufficient for 1 participant) or if any possible contamination is deemed to have occurred.
- Personnel will change gloves at least hourly or if any possible contamination is deemed to have occurred. Gloves will be changed following the sterilisation procedures prior to starting of aliquoting.

Aliquoting Beetroot and Blackcurrant Drinks to be carried out aseptically:

d) Beetroot and Blackcurrant juice concentrates will be transferred into sterilized measuring jugs in the laminar flow hood.
e) A sterile sample container with lid removed (in hood) will be placed on the balance, tared and the required weight (see below) of concentrate or mix will be poured from the measuring jug into the sample container.
f) The lid will be reapplied, and the container placed into a Tupperware container which is sitting on ice.
g) Once one participant's worth of drinks and spares for testing have been aliquoted the Tupperware container and spares will be removed from the hood and refrigerated unless it can be immediately frozen.

h) For beetroot drinks, a maximum 4 days' worth of juice will be weighed into each 250ml sterile sample container from a measuring jug (quantity dependent upon analysis of each pail of concentrate).
i) For blackcurrant drinks, 4 days' worth will be weighed into 120ml sterile sample containers from a measuring jug.
j) For the beetroot/blackcurrant mix, beetroot and blackcurrant concentrate will be mixed in a 10L pail sanitised with michro-chlor and mixed with a ladle spoon (quantities dependent upon beetroot batch). 2-3 days' worth of mixed concentrates will then be aliquoted into sterile sample containers via a sterilised measuring jug.
k) Once aliquoted, drinks will be stored at -20°C before testing days and distribution to participants.

Testing of aliquoted drinks

Following aliquoting procedures, 2 drinks from each batch will be used for pH and micro-testing at various timepoints. Details of these procedures are listed below.

Immediate

One drink will be measured immediately following the aliquoting procedure. This will confirm aseptic techniques were followed and that the concentrates have not spoiled during storage.

During storage etc

At 1, 4, 8 and 12 weeks following aliquoting, the stored sample drink will be thawed and tested for changes in pH, as well as micro testing. 12 weeks is the maximum duration of time a participant may have aliquoted drinks stored in their freezer.

Testing methods

pH Measurement

a) pH of drinks is measured using one of the following, and all drinks will be tested prior to and after aliquoting, and 4 spare bottles of each drink type will be retained to measure pH after 1, 4, 8 and 12 weeks:
   i) Hanna Instruments, H1 2221 Calibration Check pH/ORP Meter
   ii) 2. Sartorius-101, BIOLAB

   Method:

   a) Perform a two-point pH electrode calibration using pH 4.0 and 7.0 buffers.
   b) Gently invert the drink sample two to three times.
   c) Transfer the sample from the bottle into 20ml Universal Bottles.
   d) Add the pH probe into the universal bottle containing the sample.
   e) Wait for the reading on the pH meter to stabilize and record the reading.
   f) Repeat steps d) and e) 3 times for replicates, washing probe between measures with distilled water, and record average pH.

Micro Testing to be carried out by Niki Wang

Extra sample bottles will be kept for Micro Testing. Drinks will be tested at multiple timepoints and under multiple conditions for testing for Yeast and Mould and Total Plate Count (TPC):

1. Immediately after bottling
2. After 4 days of refrigeration at 5°C.
3. After 1, 4, 8 and 12 weeks frozen at -20°C.
4. After 1, 4, 8 and 12 weeks frozen at -20°C, then thawed for 4 days at 5°C.

These times and durations will reflect the conditions of the drinks during the intervention period at the participant's residence. 5°C simulates a home fridge whilst -20°C simulates a home freezer.

Media will be prepared and autoclaved as required. Pour plating method will be used for Y&M (Yeasts and Mould) and TPC (Total Plate Count) tests. Milli-Q water will be used to prepare media. Peptone bottles and tips will be prepared and autoclaved as required. YGC Agar (Yeast Extract Glucose Chloramphenicol Agar) will be used for Y&M test. PCA (Plate Count Agar) will be used for TPC test. Plates will be incubated at 25°C for 5 days for Y&M and at 30°C for 2 days for TPC.

Food Technology: Food Microbiology and Safety Laboratory Manual (141.311) will be followed for test procedures. Niki Wang will carry out the micro testing procedures for this study.

Drinks Storage

a)Drinks will be stored at -20°C in chest freezers available in the IC building sports lab until required by participants.

b)The sample containers will also be stored in Tupperware by batch, with 6 sample containers per Tupperware. This is to ease transport as well as keep the juice separate from potential contaminants during at-home freezer storage once given to participants.

c) Participants will be instructed to keep the sample containers in the freezer unless in active use, as which point containers should be stored in the fridge between at-home aliquoting.

Appendix 4.7 – Prediabetes study drink compliance diaries

Prediabetes Study – Drinks Instructions and diary – Drink C

Each bottle contains enough product for **2 days** of supplementation.

Consume 1 dose (67g) within a 15-minute period, **once each day** (you may consume the drink at any time of the day).

To prepare the drink, please measure 50mls using the graduated sample container provided, or if you own a digital scale, please weigh 67g. Dilute to taste with cold water (hot or boiling water will alter the active ingredients in the intervention).

Store any bottles not in-use in the freezer, in the Tupperware container provided, and keep the lids on the drinks tightly closed until use.

Please remember to keep any open bottles refrigerated to reduce the likelihood of spoiling. Any unopened bottles should be kept in the freezer unless thawing for use. To thaw, place in the fridge 24hrs before consumption.

Please tick the table below after consuming your drink. If you forget to consume a drink one day, please put a cross in the table below.

Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Mon													
Tues													
Wed													
Thurs													
Fri													
Sat													
Sun													

Lost drinks or missing drinks? Please get in contact ASAP!

Contact details:

Cameron Haswell: c.haswell@massey.ac.nz or [redacted]

Thanks for participating the Prediabetes Study!

Prediabetes Study – Drinks Instructions and diary – Drink A

Each bottle contains enough product for **4 days** of supplementation.

Consume 1 whole dose (24g) within a 15-minute period, **once each day** (you may consume the drink at any time of the day).

To prepare the drink, please measure 18mls using the graduated sample container provided, or if you own a digital scale, please weigh 24g. Dilute to taste with cold water (hot or boiling water will alter the active ingredients in the intervention).

Store any bottles not in-use in the freezer, in the Tupperware container provided, and keep the lids on the drinks tightly closed until use.

Please remember to keep any open bottles refrigerated to reduce the likelihood of spoiling. Any unopened bottles should be kept in the freezer unless thawing for use. To thaw, place in the fridge 24hrs before consumption.

Please tick the table below after consuming your drink. If you forget to consume a drink one day, please put a cross in the table below.

Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Mon													
Tues													
Wed													
Thurs													
Fri													
Sat													
Sun													

Please remember to keep any open bottle refrigerated to reduce the likelihood of spoiling. Any unopened bottles should be kept in the freezer unless thawing for use.

Lost drinks or missing drinks? Please get in contact ASAP!

Contact details:

Cameron Haswell: c.haswell@massey.ac.nz or [redacted]

Thanks for participating the Prediabetes Study!

Prediabetes Study – Drinks Instructions and diary – Drink B

Each bottle contains enough product for **50 days** of supplementation.

Consume 1 whole dose (10g) within a 15-minute period, **once each day** (you may consume the drink at any time of the day).

To measure out the drink, please measure 10mls using the graduated shot glass provided, then dilute in water to taste. If you own digital scales, please measure 8g instead. Dilute to taste with cold water (hot or boiling water will alter the active ingredients in the intervention).

Please remember to keep any open bottle refrigerated to reduce the likelihood of spoiling. Any unopened bottles may be stored in cupboards in a cool, dark place.

Please tick the table below after consuming your drink. If you forget to consume a drink one day, please put a cross in the table below.

Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Mon													
Tues													
Wed													
Thurs													
Fri													
Sat													
Sun													

Lost drinks or missing drinks? Please get in contact ASAP!

Contact details:

Cameron Haswell: c.haswell@massey.ac.nz or [redacted]

Thanks for participating the Prediabetes Study!

Prediabetes Study – Drinks Instructions and diary – Drink D

Each bottle contains enough product for **3 days** of supplementation.

Consume 1 dose (91g) within a 15-minute period, **once each day** (you may consume the drink at any time of the day).

To prepare the drink, please measure 70mls using the graduated sample container provided, or if you own a digital scale, please weigh 91g. Dilute to taste with cold water (hot or boiling water will alter the active ingredients in the intervention).

Store any bottles not in-use in the freezer, in the Tupperware container provided, and keep the lids on the drinks tightly closed until use.

Please remember to keep any open bottles refrigerated to reduce the likelihood of spoiling. Any unopened bottles should be kept in the freezer unless thawing for use. To thaw, place in the fridge 24hrs before consumption.

Please tick the table below after consuming your drink. If you forget to consume a drink one day, please put a cross in the table below.

Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Mon													
Tues													
Wed													
Thurs													
Fri													
Sat													
Sun													

Lost drinks or missing drinks? Please get in contact ASAP!

Contact details:

Cameron Haswell: c.haswell@massey.ac.nz or [redacted]

Thanks for participating the Prediabetes Study!

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Effects of beetroot compounds and blackcurrants anthocynanins on glucose control in prediabetes: A pilot study

Questionnaire on Beverage Consumed

School of Health Sciences, College of Health, Albany, Auckland

PRINCIPAL INVESTIGATORS:

Cameron Haswell
A/Prof Rachel Page
A/Prof Kay Rutherfurd
Prof Ajmol Ali
Dr Roger Hurst

Please answer the following questions. These questions are about the beverage which you have been taking in the past 8 weeks.

Circle the number that best links with how you feel: 1 is strongly disagree and 5 is strongly agree. For example:

Question	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
Do you like the colour of the supplement?	1	2	3	(4)	5

If you have any problems with the questions, then please ask the investigator for help. Your answers will be kept strictly confidential.

Participant Identifier Code:

Please circle the number that provides your feelings with respect to the supplement you consumed.

Question	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
The beverage was easy to drink	1	2	3	4	5
The beverage tasted nice	1	2	3	4	5
The beverage left a bad aftertaste	1	2	3	4	5
The texture of the beverage made it easy to eat or drink	1	2	3	4	5
The amount of liquid to consume was too much	1	2	3	4	5
The supplement would be easier to consume if it was in a different form e.g. capsule	1	2	3	4	5
The frequency of supplementation was too high	1	2	3	4	5
Any other comments on use of the supplement?					

Appendix 4.9 – Prediabetes study at home glucose monitoring sheet

Participant ID _____ Date of Visit 1_____

Date of Visit 2_____

Please use this sheet to record fasting blood glucose measurements throughout the 12-week intervention period.

Please try your best to take your sample at as similar a time and day of each week as possible, (e.g. Wednesdays at 7am). Please remember that the sample needs to be taken before you first eat or drink anything other than water for the day. If for any reason you forget to take your sample, or there is anything of note which you think might be relevant, please note this in the comment section of the table.

The back of this sheet contains reminder instructions on how to take the fasting glucose sample.

Please write the date of your first drink: _____

Week Number	Date	Time	Fasting Glucose Value (mmol/L)	Comments
0				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				

Thank you for your help in completing this log,

If you have any problems, please contact Cameron Haswell on [redacted] or c.haswell@massey.ac.nz

Preparation

Prior to sample collection please make sure you have fasted for at least 12 hours from the previous evening. This is important to get accurate results.

Please also wash your hands and any surfaces you may use during the sample collection.

Step 1

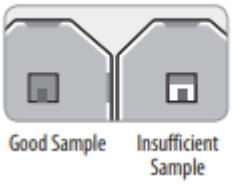
Insert a test strip with the contact bars facing upwards into the meter's test strip port. Push the strip in gently until the meter beeps. Be careful not to bend the test strip.

Step 2

Obtain a blood sample using the lancing device. To do this place the device against the pad of the finger as shown in the diagram. Press the release button. Remove the device from the finger. Wait a few seconds for a blood drop to form and wipe away the first drop with a tissue. You will need a drop no larger than a pinhead for the sample.

Step 3

After the drop symbol appears on the screen, apply the blood sample to the narrow end of the test strip until the meter beeps. The diagram below shows the best way to collect the blood sample with the test strip, which is by holding the test strip vertically to the blood sample on the finger.



Step 4

Once the sample has been successfully applied, the display segments will rotate clockwise on the meter while the measure is being performed. You can now take your finger away from the meter.

The test result will appear after the meter counts down from 5 to 1. The result will be automatically stored in the meter's memory.

If the test strip is removed after the test result is displayed, the meter will automatically switch off after 3 seconds. Discard used test strips safely in appropriate containers.

If for some reason the blood collection is not successful, then the Er4 message may appear. If this happens remove and discard the test strip and repeat the process. You may need to do another finger prick.