# Effect of beetroot pomace consumption on acute blood pressure and postprandial blood glucose responses on healthy adults; a pilot study

A thesis presented in the partial fulfilment of the requirements for the degree of

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## Abstract

**Background:** Nitrate-rich beetroot juice (BRJ) supplementation has been shown to improve blood pressure (BP) levels in younger and older adults via increased nitric oxide (NO) production leading to dilation of blood vessels. Similarly, to some extent a reduction of blood glucose (BG) levels through several different mechanisms. However, limited research exists on the by-product of beetroot juice production, beetroot pomace (BRPOM), and its role in controlling BP and BG levels.

**Objective:** To investigate and compare the changes in BP and BG levels in a 3 h period following the ingestion of BRPOM, BRJ and placebo as part of an oral glucose tolerance test (OGTT).

**Methods:** Following an overnight fast, twelve healthy adults consumed either BRPOM (600 mg nitrate, 10 g fibre, 75 g carbohydrate), BRJ (600 mg nitrate, <1 g fibre, 75 g carbohydrate), or placebo (0 mg nitrate, <1 g fibre, 75 g carbohydrate) 15 minutes prior to the OGTT, separated by 1-week washout periods, in a single-blind, crossover design, pilot study. Blood pressure and BG levels were measured at baseline, then every 15 minutes for BG and every hour for BP during a 3 h OGTT.

**Results:** No significant changes in SBP for BRJ (-2.3 ± 5.56 mmHg, $p = 0.174$), BRPOM (-3 ± 13.4 mmHg, $p = 0.456$), or PLA (5.2 ± 11.3 mmHg, $p = 0.142$) treatment group after 3 h. However, there was a trend towards significance in the differences between the three groups ($p = 0.075$, $\eta^2_p = 0.404$). Clinically meaningful reductions in SBP were observed for BRJ (-2.3 ± 5.56 mmHg) and BRPOM (-3 ± 13.4 mmHg). There were no differences ($p = 0.739$, $\eta^2_p = 0.059$) in mean glucose incremental area under the curve (iAUC) between BRJ, BRPOM and PLA (232.8 ± 61.9, 242 ± 54.7 and 252.4 ± 60.6 mmol/L/min, respectively). There were no differences between BRJ, BRPOM and PLA in time to peak ($p = 0.269$, $\eta^2_p = 0.313$), peak glucose levels ($p = 0.241$, $\eta^2_p = 0.247$), 2 h glucose levels ($p = 0.565$, $\eta^2_p = 0.150$), 3 h glucose levels ($p = 0.395$, $\eta^2_p = 0.233$) and percentage increment of postprandial BG ($p = 0.783$, $\eta^2_p = 0.048$).

**Conclusion:** This pilot study with a small sample size showed a large effect size and clinically meaningful attenuations for acute SBP changes following the consumption of beetroot pomace. Further investigations would be needed to explore the applicability of these findings to hypertensive individuals. However, its impact on acute BG levels remains non-significant, possibly influenced by the small sample size and participants' baseline normoglycaemic levels. Further research is needed to explore potential effects in a hyperglycaemic population.

**Key words:** Beetroot juice; Beetroot pomace; Dietary fibre; Nitric oxide; Blood pressure; Blood glucose.

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## List of abbreviations

| Abbreviation | Term |
|---|---|
| ACEI | Angiotensin-converting enzyme inhibitor |
| ARB | Angiotensin receptor blocker |
| BIA | Bioelectrical impedance analyser |
| BG | Blood glucose |
| BMI | Body mass index |
| BP | Blood pressure |
| BRJ | Beetroot juice |
| BRPOM | Beetroot pomace |
| CVD | Cardiovascular disease |
| DASH | Dietary Approaches to Stop Hypertension |
| DBP | Diastolic blood pressure |
| FBG | Fasting blood glucose |
| GAE | Gallic acid equivalent |
| HbA1c | Glycosylated haemoglobin |
| HDLC-C | High-density lipoprotein cholesterol |
| HPLC | High-performance liquid chromatography |
| iAUC | Glucose incremental area under the curve |
| IDF | Insoluble dietary fibre |
| $KNO_3$- | Potassium nitrate |
| MAP | Mean arterial pressure |
| NO | Nitrite oxide |
| $NO_2^-$ | Nitrite |
| $NO_3^-$ | Nitrate |
| NOS | Nitric oxide synthase |
| NZ | New Zealand |
| OGTT | Oral glucose tolerance test |
| PLA | Placebo |
| SBP | Systolic blood pressure |
| SD | Standard deviation |
| SDF | Soluble dietary fibre |
| SVR | Systemic vascular resistance |
| T2DM | Type 2 diabetes mellitus |
| TDF | Total dietary fibre |
| USA | United States of America |

# CHAPTER 1: INTRODUCTION, AIM AND OBJECTIVES

## 1.1 Background

Hypertension is a common comorbidity of cardiovascular disease (CVD) and is also associated with type 2 diabetes mellitus (T2DM) (Petrie et al., 2018). Hypertension can lead to other conditions, such as heart attack, stroke, kidney disease/failure, eyesight problems, peripheral artery disease and heart failure (Mills et al., 2020; Oparil et al., 2018). Worldwide rates for hypertension have increased exponentially from 648 million in 1990 to 1.38 billion in 2019 (Zhou et al., 2021). In New Zealand (NZ), hypertension rates were relatively consistent throughout 2015-2019, averaging 32.7% of the population; however, in 2021, the highest rates of hypertension were observed with 33.4% of people in NZ with hypertension (Ministry of Health, 2021a).

Approximately 451 million people worldwide were diagnosed with diabetes mellitus in 2017, and this number is expected to increase to over 650 million by 2045 (Cho et al., 2018).
In NZ, approximately over 220,000 people currently have T2DM (Holder-Pearson & Chase, 2022). By 2040, the number of New Zealanders with T2DM alone is expected to increase by 70–90% to over 400,000 persons (Holder-Pearson & Chase, 2022).

Research indicates that diet can play a key role in the development of CVD and T2DM. Increased consumption of energy-dense foods with high levels of added sugars and saturated fat has played a key role in developing the current obesogenic environment (D'Innocenzo et al., 2019). Obesity is a risk factor for a multitude of comorbid conditions, such as gastrointestinal disorders, muscular and joint disorders, and psychological issues (Fruh, 2017). Obesity is also a leading risk factor for both CVD and T2DM (Hruby & Hu, 2015). People who consume diets rich in fruit and vegetables, such as the Mediterranean diet, have demonstrated the greatest improvement in their glycaemic control and reducing CVD by 29% over 4.8 years compared to other diets such as paelolithic, low carbohydrate, high-protein, vegetarian and nut-enriched diets (Joseph et al., 2022).

Additionally, other nutritional interventions including the Dietary Approaches to Stop Hypertension (DASH) diet are popular strategies to lower CVD and T2DM risk. The foundation of this diet is high fruit and vegetable intake, low sodium and high dietary fibre intake (Mohanlal et al., 2012). Many fruits and vegetables contain nutrients such as phytochemicals, antioxidants, vitamins, minerals and fibre, which can work independently or synergistically to reduce CVD and T2DM risk.

There has also been growing interest in the utilisation of functional foods as a means of managing blood glucose levels (BG) and blood pressure (BP) to reduce harmful effects associated with elevated BG and BP levels (Bumrungpert et al., 2020; Lim et al., 2020). Beetroot juice, chia seed flour, watermelon extract, and pine bark extract are a few examples of the functional foods that have been shown to help reduce postprandial BG in healthy adults (Lim et al., 2020; Wootton-Beard et al., 2014) and BP levels in both healthy younger and older adults as well as adults with prehypertension and hypertension (Massa et al., 2016; Stanaway et al., 2019; Toscano et al., 2014). In addition, since functional foods are whole foods, they are likely to be cost-effective and may be associated with fewer side effects compared to current medications associated with helping BG and BP control (Sica, 2004; Tahrani et al., 2016).

Nitrate ($NO_3^-$), which is naturally found in vegetables such as spinach, rocket, and red beetroot (Hord et al., 2009), has been investigated for its potential cardioprotective properties (Kapil et al., 2015; Liu et al., 2013). Nitrate is a precursor to the bioactive nitric oxide (NO), which acts as a vasodilator by relaxing the muscles in the inner wall of blood vessels, thus causing blood vessels to dilate, causing a decrease in systemic vascular resistance (SVR) thereby increasing blood flow which consequently reduces BP (Ma et al., 2018). Given the vasodilating properties of NO, consuming foods naturally high in $NO_3^-$ may aid in improving endothelial function in people with T2DM or hypertension. Hyperglycaemia in T2DM is a key causal factor of endothelial dysfunction, causing the constriction of the blood vessels in the absence of blockages (Hadi & Suwaidi, 2007). Therefore, through vasodilation brought about by $NO_3^-$ consumption, movement of insulin and glucose to skeletal muscle may be enhanced, leading to improvements in insulin signalling and postprandial glucose uptake into skeletal muscle cells (Kim et al., 2006).

Additionally, fibre consumption has been shown to help reduce the risk of developing T2DM and CVD risk (Barber et al., 2020). While some studies have shown beneficial effects to BP after fibre consumption, some have failed to find significant findings (Keenan et al., 2002; Sarriá et al., 2012; Swain et al., 1990). These inconsistent findings may be attributed to different fibre types (insoluble or soluble), and baseline BP levels (Dong et al., 2019; Shahoud et al., 2021). Many studies have shown that increased ingestion of total dietary fibre (TDF) attenuates postprandial BG and lowers the glucose area under the curve (iAUC)(Cassidy et al., 2018; Fuller et al., 2016; Goff et al., 2018; Yu et al., 2014). Delaying gastric emptying and glucose absorption are plausible mechanisms by which fibre may reduce postprandial BG levels (Lattimer & Haub, 2010; Meng et al., 2017).

Studies in overweight or obese men have shown that orange pomace consumption (average 5.4 g TDF) significantly delays the time to reaching peak glucose levels, but no significant effect on iAUC (Chen et al., 2017; Dong et al., 2016). A study in healthy individuals found similar results with the consumption of orange juice fortified with orange pomace (5g TDF) reducing peak postprandial BG levels compared to orange juice alone, but had comparable results to whole orange (Guzman et al., 2021). However, there was no significant effect of pomace on postprandial iAUC compared to orange juice.

The total amount of fibre required to attenuate BG and BP levels varies between studies. In studies using orange pomace, an average of 5 g TDF was required to delay time reaching peak glucose in an acute setting (Chen et al., 2017; Dong et al., 2016; Guzman et al., 2021). It is suggested when TDF consumption is above 25 g or more per day, this was associated with lower T2DM risk (Yao et al., 2014). Similarly, when consumption of insoluble dietary fibre (IDF) was over 14 g per day, this was associated with better BG levels; however, effects plateaued when IDF intake was above 25 g per day (Kabisch et al., 2021). Therefore, the ratio of IDF and soluble dietary fibre (SDF) may require different amounts of fibre required to reduce BG and BP levels.

Many studies have investigated the health potential of beetroot juice (BRJ) supplementation due to its high dietary $NO_3^-$ content (Mirmiran et al., 2020). Some studies have shown BRJ supplementation significantly decreases both systolic and diastolic BP in hypertensive adults as well as younger and older healthy adults (Kapil et al., 2015; Stanaway et al., 2019), and other studies have shown it to significantly decrease only the systolic blood pressure (SBP) in hypertensive and healthy adults (Bondonno et al., 2015; Webb et al., 2008).

There is limited research exploring the role of $NO_3^-$ on glycaemic control. Some studies have shown that BRJ supplementation significantly lowers postprandial BG levels (Chang et al., 2018; Wootton-Beard et al., 2014). However, when $NO_3^-$ was given via sodium-nitrate supplementation, it failed to show significant attenuation of postprandial BG response levels (Cermak et al., 2015). Therefore, $NO_3^-$ may not be the sole ingredient leading to attenuation of postprandial BG responses, but it may work in combination with other components that are present in beetroot juice such as betalains and polyphenols.

Juicing beetroot produces the by-product, pomace, which is generally considered a waste product, used either for animal feed or discarded in landfills (Vulić et al., 2014). Based on published nutritional analyses, beetroot pomace (BRPOM) contains high amounts of dietary fibre, polyphenols, and betalains (Costa et al., 2017; Mirmiran et al., 2020; Shyamala & Jamuna, 2010). Evidence suggests that fibre, polyphenols and betalains are anti-inflammatory and can lower BP and attenuate postprandial BG levels (Godos et al., 2019; Madadi et al., 2020; Rahimi et al., 2019).

Despite the amount of research undertaken on BRJ and other fruit pomaces, to date no studies have investigated the potential of BRPOM consumption, with its natural $NO_3^-$ and fibre content to improve postprandial BP and BG levels in humans.

### 1.2 Aim, objectives and hypothesis

#### 1.2.1 Aim:

The aim of the current study was to evaluate and compare the acute effects of beetroot pomace, beetroot juice and placebo on postprandial BP and BG in healthy adults in an acute randomised cross-over trial.

#### 1.2.2 Objectives:

Specific objectives for this study include investigating and comparing;

1. Changes in BP in the 3-h period following ingestion of beetroot pomace (600 mg $NO_3^-$, 10 g TDF), beetroot juice (600 mg $NO_3^-$, <1 g TDF), and placebo (0 mg $NO_3^-$, <1 g TDF) when consumed as part of an oral glucose tolerance test (75 g carbohydrates).
2. Changes in BG (iAUC, peak time, peak value, 2-h and 3-h values, and percentage increment) in the 3 h period following ingestion of beetroot pomace (600 mg $NO_3^-$, 10 g TDF), beetroot juice (600 mg $NO_3^-$, <1 g TDF), and placebo (0 mg $NO_3^-$, <1 g TDF) when consumed as part of an oral glucose tolerance test (75 g carbohydrates).

#### 1.2.3 Hypotheses:

It was hypothesised that acute intake of:

1. Beetroot pomace will lead to a similar decrease in BP (SBP and DBP) compared to BRJ due to both containing the same $NO_3^-$ levels but a greater BP decrease compared to placebo, which contains no $NO_3^-$ and is not expected to induce any reductions in BP
2. Beetroot pomace will lead to greater decreases in BG levels (iAUC, peak time, peak value, 2 h and 3h values, and percentage increment) due to its additional fibre content compared to BRJ and placebo, which both lack fibre.

To examine these hypotheses, we conducted a randomised, single-blind cross-over pilot trial to investigate and compare acute changes in postprandial BP and BG levels following the consumption of beetroot pomace (600 mg $NO_3^-$, 10 g TDF), beetroot juice drink (600 mg $NO_3^-$, <1 g TDF) and placebo (0 mg $NO_3^-$, <1 g TDF; all treatments contained 75 g carbohydrate)

### 1.3 Thesis Structure

This thesis is divided into four chapters. Chapter one is an introduction of the background, purpose, aim, objectives, and hypotheses of this research study. Chapter two is a narrative review of the current literature on beetroot juice/pomace and its acute effect on BP and BG levels. Chapter three is a manuscript of the research study for publication which includes an abstract, introduction, methods, results, discussion, and references to the relevant appendices. The final chapter (Chapter four), contains the conclusion to the thesis, outlining strengths, limitations, and recommendations for future research. The appendices include advertisement material for recruitment, participant information sheet, ethics consent form, health screening questionnaire, nutritional analysis of the beetroot pomace, and beetroot pomace development methods.

### 1.4 Researcher Contributions

Table 1. 1 Researchers Contributions to thesis study

| **Contributors** | **Contributions to Research** |
|---|---|
| Elnaz Gorbani | Primary researcher and author. Completed ethics application, recruited participants, data collection, literature review, analysed and completed statistical analysis, interpretation of results and wrote thesis manuscript. |
| Associate Professor Kay Rutherfurd-Markwick | Primary academic supervisor. Assisted with completion of ethics application, planned research design, analysed beetroot pomace nitrate and nitrite content, assisted in reading and editing of thesis. |
| Professor Ajmol Ali | Co-academic supervisor. Assisted with completion of ethics application, planned research design, assisted with interpretation of data, assisted in reading and editing of thesis. |
| Dr Cheryl Gammon | Co-academic supervisor. Planned research design, assisted in reading and editing of thesis. |
| Associate Professor Rachel Page | Co-academic supervisor. Planned research design, assisted in reading and editing of thesis. |
| Professor Marie Wong | Co-academic supervisor. Assisted with creating the beetroot pomace product, assisted in the |

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|---|---|
| | microbiology testing for beetroot pomace, assisted in reading and editing of thesis. |
| Niki Wang | Performed heat treatment and microbiology testing for beetroot pomace. |

## CHAPTER 2: REVIEW OF THE LITERATURE

### 2.1 Introduction

Cardiovascular disease (CVD) is the leading cause of disease burden in the world, where CVD deaths increased from 12.1 million in 1990 to 18.6 million in 2019 (Roth et al., 2020). Similarly, more than 450 million people worldwide (approximately 6.20% of world's population) were diagnosed with Type 2 diabetes mellitus (T2DM) and more than 1 million deaths occurred due to this condition in 2017 alone (Khan et al., 2020). In New Zealand (NZ), approximately over 220,000 people currently have T2DM and by 2040, this is expected to increase by 70–90% to over 400,000 persons (Holder-Pearson & Chase, 2022). In NZ, CVD was the leading cause of death for both the indigenous population, Māori, and non-Māori between 2010-2012 (Ministry of Health, 2018). However, diabetes was ranked in the top 5 causes of death for Māori but was not for the non-Māori population (Ministry of Health, 2018). High blood pressure (BP; hypertension) is a common comorbidity of CVD which is also associated with T2DM. Type 2 diabetes, hypertension and CVD are closely interlinked due to their similar risk factors and shared complex physiological pathways. Atherosclerosis, endothelial dysfunction, insulin resistance and chronic inflammation are common factors that contribute to this interconnection. Additionally, the risk of CVD is increased by TD2M and hypertension (Petrie et al., 2018).

Research indicates that diet can play a significant role in preventing CVD and T2DM. In particular, diets high in fruits and vegetables have been found to reduce the risk of T2DM by 7% and are inversely associated with hypertension risk (Halvorsen et al., 2021; Li et al., 2016; Wang et al., 2016).

Many fruits and vegetables contain nutrients such as phytochemicals, antioxidants, vitamins, minerals and fibre, consumption of which may aid in reducing/preventing the development of CVD and T2DM (Bazzano et al., 2003; Wallace et al., 2020; Zheng et al., 2020). The biological mechanisms by which fruits and vegetables exert their effects may involve multiple pathways. Nutrients present in fruits and vegetables may independently or work together to reduce the risk of CVD. For example, the high water and fibre, and low-energy content of whole fruits and vegetables increase satiety, and therefore may displace high-energy foods leading to overall reduction in total energy intake (Bazzano et al., 2003). In addition, certain vegetables contain dietary nitrate ($NO_3^-$), which has been shown to have beneficial effects on BP and blood glucose (BG) levels (Bondonno et al., 2015; Chang et al., 2018; Kapil et al., 2015; Stanaway et al., 2019; Wootton-Beard et al., 2014). Nitrate is a precursor to bioactive nitric oxide (NO), which dilates blood vessels and enables the blood to flow more easily due to decreased SVR. Decreased SVR lowers BP and enhances the movement of insulin to tissue such as skeletal muscle, and has been shown that glucose uptake in the skeletal muscles has increased by 25-40% (Gheibi & Ghasemi, 2020; Kim et al., 2006; Ma et al., 2018).

Most supplementation research investigating the effects of $NO_3^-$ has used beetroot juice (BRJ) which is naturally high in $NO_3^-$. This research has shown potential hypotensive and hypoglycaemic effects following BRJ supplementation(Chang et al., 2018; Jonvik et al., 2016; Mirmiran et al., 2020; Stanaway et al., 2019; Wootton-Beard et al., 2014). Juicing beetroot results in the production of pomace, which is generally considered a waste product and used either for animal feed or discarded in landfills (Vulić et al., 2014). Despite over 6000 research studies undertaken on BRJ's effects on BP and BG levels, there are currently no scientific studies exploring the potential of the waste product, beetroot pomace (BRPOM), for improving BP and BG control.

The following sections of the literature review provides an overview of CVD and hypertension (Section 2.2), overview of T2DM (Section 2.3), and current medications for treating high BP and BG (Section 2.4). Functional foods and the mechanisms of dietary fibre and their role in BP and BG management are then examined (Sections 2.5 – Section 2.7). Current research on pomace sources including BRPOM (Section 2.8 and 2.9), is then reviewed. The role of $NO_3^-$ in beetroot on BP and BG control (Section 2.10 and 2.11) is addressed, followed by a short review of the potential for BRPOM as a health promoting ingredient and the evidence required to support this (Section 2.12)

**2.2 Cardiovascular disease and hypertension**

Cardiovascular disease is a general term used to describe the thickening of arterial walls resulting in disorders of the heart and blood vessels (Lopez et al., 2021; Stewart et al., 2017). These include coronary heart disease, where there is a build-up of plaque in the arteries resulting in insufficient blood flow and oxygen to the heart; cerebrovascular disease (including ischemic or haemorrhagic stroke), where there is insufficient blood flow and oxygen to the brain; peripheral artery disease, where there is insufficient blood flow to the heart, brain and limbs; and hypertension, where BP is increased over defined limits (Lopez et al., 2021).

Although medical advances have resulted in a nearly 50% reduction in CVD-related deaths over the last several decades (Okwuosa et al., 2016), CVD remains the number one cause of death both globally and in NZ (Ministry of Health, 2018; World  Health Organization, 2019). About 1 in 3 deaths in NZ are attributed to CVD (Heart Foundation NZ, 2017). Cardiovascular disease mortality and morbidity accounted for 12.5% of total public health expenditure ($4.8 billion) in NZ between 2007 and 2014, and as the aging population increases in NZ, health costs associated with CVD are expected to increase in parallel (Blakely et al., 2019).

Risk factors for CVD encompass non-modifiable factors such as genetics/familial hypercholesterolemia, older age, family history and ethnicity (for example Māori and Pacific Islanders are at higher risk in NZ (Selak et al., 2020)) and modifiable behavioural risk factors which include tobacco smoking, alcohol usage, low physical activity, insufficient intake of fruits and vegetables, and high intakes of saturated fats and sodium (Cogswell et al., 2016; Ruan et al., 2018; Siri-Tarino et al., 2010). The behavioural risk factors can eventually lead to modifiable metabolic risk factors comprising of overweight/obesity, dyslipidaemia, increased BG levels and hypertension (Benjamin et al., 2019).

Blood pressure is usually measured while at rest and in a seated position, with two or measures taken at least two minutes apart. Diagnosis of hypertension (high BP) is not made in a single office visit. Consistent high BP measurements across 2-3 office visits over a 1-4 week interval (depending on BP level) is required to confirm a diagnosis of hypertension (Unger et al., 2020). Hypertension is classified as having a systolic blood pressure (SBP) greater than and equal to 140 mmHg and a diastolic blood pressure (DBP) greater than or equal to 90 mmHg (McLean et al., 2013). Consistent hypertension can damage blood vessels which can lead to angina, heart attack, stroke, kidney disease/failure, eyesight problems, peripheral artery disease and/or heart failure (Mills et al., 2020; Oparil et al., 2018).

From 2017-2020 there was a decreasing trend in high BP diagnoses with 30.8% of New Zealanders living with high BP in 2020. However, in 2021, the highest percentage of hypertension of 33.4% was recorded in NZ adults (Ministry of Health, 2021a). Data collection for the high BP cases in 2021 may have been impacted by COVID-19, resulting in a lower sample size which may mean this estimate is less accurate. However, the higher percentage of people with hypertension in 2021 may be due to the increased stress associated with the COVID-19 pandemic, including factors such as unemployment (Nghiem et al., 2020).

A number of studies have shown increased fruit and vegetable intake is associated with lower body weight (Zheng et al., 2020), cholesterol levels, BP (Bazzano et al., 2003; Wallace et al., 2020), and better BG control (Halvorsen et al., 2021; Zheng et al., 2020), all of which help reduce the risk of CVD and T2DM. Fruits and vegetables contain important nutrients, including phytochemicals, antioxidants, vitamins, minerals, fibre and some contain compounds such as $NO_3^-$. Nitrates in BRJ have been shown to facilitate attenuation of BP in both younger and older adults (Stanaway et al., 2019). Similarly, consumption of dietary fibre has also been shown to reduce BP as well as reducing BG levels, increasing satiety, and alleviating constipation (Anderson et al., 2009; Ozen et al., 2020; Threapleton Diane et al., 2013). The beetroot juice by product, pomace, would contain nearly all of the fibre found in fresh beetroot, making it a high fibre food ingredient (Mirmiran et al., 2020). However, to date there have been no studies investigating the impact of the fibre and $NO_3^-$ content in BRPOM, on improving health biomarkers such as BP and BG levels.

**2.3 Diabetes and blood glucose control**

Diabetes mellitus (DM) is defined by high levels of BG (hyperglycaemia) as a result of impaired insulin action. Type 1 diabetes mellitus is an autoimmune disease where the pancreatic insulin-producing cells are attacked by the immune system rendering them incapable of producing insulin. In comparison, T2DM occurs when the tissue (such as liver, muscle and adipose tissue) responses to insulin is diminished, leading to insulin resistance. The development of T2DM can be attributed to a variety of factors such as genetic predisposition, ageing, ethnicity, and lifestyle factors including a sedentary lifestyle, being overweight or obese, low physical activity, and/or consumption of diets high in energy-dense foods (Goyal & Jialal, 2020).

In 2021, about 245,680 people in NZ had been diagnosed with diabetes, with 90% of these being T2DM. The estimated prevalence of diabetes increased from 35.7 per 1000 population in 2012 to 41.5 per 1000 population in 2021 (Diabetes New Zealand, 2018; Te Whatu Ora - Health New Zealand, 2022). Type 2 diabetes tends to occur more often in persons over 45 years of age. However, in recent years in NZ, T2DM has been increasingly diagnosed at younger ages of 25-44 years (Goyal & Jialal, 2020; Ministry of Health, 2015), with about 16% of under 45-year-olds being identified as having prediabetes (Best Practice Advocacy Centre New Zealand, 2018).

Glycosylated haemoglobin (HbA1c) is an important tool used to measure glycaemic control over the previous 2-3 months (Sherwani et al., 2016). It is used in NZ and around the world to diagnose both T2DM and prediabetes. Prediabetes is the time when medical, nutritional and exercise interventions can have a vital role in slowing progression to T2DM. However, the threshold values for diagnosis of prediabetes differs internationally. In NZ, values of 41-49 mmol/mol are used, whereas in the United States of America (USA) the range is 39-46 mmol/mol and in the UK it is 42-47 mmol/mol. One reason for these differences is the different ethnic populations in each country. African Americans in the USA and Māori and Pacific people in NZ have higher HbA1c levels compared to the rest of the population (Cohen et al., 2016; Metcalf et al., 2018), leading to implementation of lower prediabetes thresholds in these countries. In addition, ethnic populations within each country may have variations in red blood cell turnover (Sequeira & Poppitt, 2017). Red blood cell lifespan averages 120 days, but can vary between 70-140 days, leading to equivalent changes in HbA1c at a constant BG (Cohen et al., 2016; Franco, 2012). For example, if HbA1c levels fall by 8% when the average red blood cell age falls by 8%, a HbA1c level of 48 mmol/mol will instead be 42 mmol/mol, resulting in a missed diabetes diagnosis. High HbA1c levels in certain ethnic groups may also be due to inequitable access to care and socioeconomic factors for these groups, therefore leading to the observed differences in HbA1c levels between certain ethnicities (Cohen et al., 2016; Metcalf et al., 2018). This disparity in HbA1c between ethnic populations is important as it may result in incorrect diagnosis and over- or under-treatment and reporting of diabetes and prediabetes.

Without appropriate interventions, about 5-10% of people with prediabetes will develop T2DM annually, with individuals who are overweight or obese being at greater risk (Bennasar-Veny et al., 2020; Tabák et al., 2012). Many risk factors are modifiable including high BP, low HDL-C, poor intake of nutrient-dense foods, obesity, and low physical activity, and therefore can be targeted to help prevent individuals from developing T2DM. There is a 40-70% relative risk reduction if lifestyle modifications are implemented for individuals with prediabetes (Tabák et al., 2012).

Persistant hyperglycaemia is a high risk for people with T2DM and can lead to complications and risks such as chronic kidney disease, blindness, limb amputation, CVD and early death (Ministry of Health, 2015; Zheng et al., 2018). Deterioration in physical health can also lead to a higher prevalence of anxiety and depression (Trikkalinou et al., 2017). Therefore, the physical and mental demands of T2DM can negatively impact both the individual's and their family's quality of life.

The rising number of T2DM cases in NZ comes at a great cost to the economy with the annual cost for T2DM being an estimated $2.1 billion – 0.67% of NZ's total Gross Domestic Product (GDP). This is estimated to increase by 63% to $3.5 billion by 2040. Public funded costs for treating T2DM are estimated at around $1.0 billion per annum. Additionally, lost wages are increasing due to activity-impairing complications due to T2DM. This was estimated to cost the economy $562 million in 2020, and by 2040 this figure is estimated to increase by 47%, to $755 million (Holder-Pearson & Chase, 2022). The additive costs of general practice, medication, transport, clinic visits, and taking time off work unpaid can cause a financial burden for people with T2DM and prediabetes living in lower socioeconomic environments, thereby rendering individuals incapable of meeting costs to prevent/treat diabetes-related complications (Holder-Pearson & Chase, 2022; Simmons et al., 1999).

As the cost of most diabetes medications is funded for people living in NZ, it is part of the $1.0 billion public funded health costs. This provides a compelling case for the Government to look at investing in the prevention and possible treatment of T2DM with dietary solutions, such as beetroot pomace/juice, which may be cost effective in the long term.

### 2.4 Medications for treating high blood glucose and blood pressure

Although current medications for T2DM and hypertension have helped improve BG control and BP control, respectively, and thus improved life expectancy and quality of life, medications can also lead to adverse side effects (Sica, 2004; Wang et al., 2017). Metformin is the first line medication used in patients with T2DM who are not able to control their BG levels through diet and exercise and is used in combination with lifestyle management (Ministry of Health, 2021b). Although metformin has a good long-term safety record, it has been associated with gastrointestinal discomfort and may have contraindications to renal impairment and hypoxaemia (Tahrani et al., 2016). Another T2DM medication, pioglitazone has been associated with fractures, weight gain, oedema and heart failure (Tahrani et al., 2016), whereas diuretics for hypertension have been associated with hyperlipidaemia, hyperglycaemia, and hypokalaemia (Sica, 2004).

Due in part to adverse effects, low compliance or non-compliance in taking metformin and antihypertensive drugs has been documented (Abbas et al., 2020; Horsburgh et al., 2019). Functional foods or natural food compounds have shown promise as alternatives or adjuncts to current medicines for managing BG and BP levels (Alkhatib et al., 2017; Kurtz et al., 2018; Lim et al., 2020; Stanaway et al., 2019). There have been trends toward using nutrition to manage health conditions as an alternative or alongside current medication (Downer et al., 2020). Therefore, individuals might find alternative treatments, such as using functional foods, more acceptable if it means they can either reduce the dosage of their current medication or cease to take it.

### 2.5 Effects of functional foods on high blood pressure and high blood glucose levels

Functional foods are foods or parts of food containing specific components that promote the consumer's health and well-being (Tur & Bibiloni, 2016). As mentioned previously, there has been growing interest in using nutrition to help prevent and alleviate symptoms of many common diseases, with several studies investigating the potential of functional foods for the prevention or treatment of health conditions such as hypertension and hyperglycaemia (Alkhatib et al., 2017; Kurtz et al., 2018). In addition, since functional foods are whole foods rather than supplements provided in a pill/tablet form, they are likely to be more cost-effective and may be associated with fewer side effects compared to current medications.

Tables 2.1 and 2.2 provides examples of studies investigating the effects of some functional foods and food extracts on postprandial BP and BG levels, respectively. The studies shown were selected because they were either single-blind or double-blinded which helps reduce conscious and unconscious bias from researchers and participants, therefore, maximising the validity of any observed differences in effectiveness between the functional foods compared to a placebo. Additionally, these studies were selected featuring diverse participant characteristics (hypertensives, people with diabetes, healthy etc), which provides a range of perspectives and results to consider when reviewing which functional foods were effective.

Chia seed flour (Toscano et al., 2014), watermelon extract (Massa et al., 2016) , black tea (Grassi et al., 2015) and beetroot juice (Jajja et al., 2014; Kapil et al., 2015; Stanaway et al., 2019; Webb et al., 2008) are a few of the foods which have been shown to help lower BP in younger and/or older adults. Bioactive components in each food including $NO_3^-$ (BRJ), α-linolenic acid (chia seed flour), L-citrulline (watermelon), and high flavonoid content (black tea) (Table 2.1) may be responsible for eliciting health benefits. However, results from different studies using the same food ingredient are sometimes equivocal. For example, one study demonstrated that acute consumption of beetroot juice was effective in lowering SBP in both older and younger adults, and DBP in older adults (Stanaway et al., 2019). In contrast, a study in hypertensive treated adults found no change in participants' home or ambulatory BP after consumption of beetroot juice for one week (Bondonno et al., 2015). One reason for these differences could be due to the hypertensive medication participants were taking in the Bondonno et al. (2015) study influencing nitric oxide (NO) production, thereby influencing responses to the active component, dietary $NO_3^-$ (Louis et al., 2002). Another reason may have been that the amount of $NO_3^-$ in the BRJ was insufficient to achieve significant changes in BP, as the study by Stanaway et al. (2019) used 651 mg nitrate compared to 434 mg in the Bondonno et al. (2015) study.

Functional foods are also able to improve glycaemic control (Table 2.2), with consumption of BRJ reducing postprandial BG levels in healthy adults (Wootton-Beard et al., 2014), and bitter melon juice having a similar effect in obese adults (Lee & Ma, 2019). In contrast, consumption of blueberries led to an extended postprandial BG response compared to the control group but no significant reductions in mean glucose incremental area under the curve (iAUC) (Bell et al., 2017). Consumption of pine bark extract, also showed promising results in reducing postprandial BG in healthy adults (Lim et al., 2020).

It is important to note that although these whole foods and extracts have active constituents which play an important role in eliciting BP and BG attenuation, they also contain a myriad of other ingredients, which may work independently or synergistically to help control BP and BG levels. For example, all the foods mentioned in Table 2.1 and Table 2.2 also contain polyphenols such as flavonoids, anthocyanins and in the case of beetroot, betalains. Black tea also contains catechins and caffeine which may contribute to its BG attenuating effects (Meng et al., 2019). Polyphenols have also been found to have a role in positively influencing BP and BG levels in both human and animal studies (Aryaeian et al., 2017; Godos et al., 2019; Meng et al., 2019; Zhang et al., 2020).

Table 2. 1 Selected studies investigating the acute and chronic effects of functional foods and extracts on blood pressure

| **Functional foods** | **Participants*** | **Study design** | **Blood pressure outcomes** | **Reference** |
|---|---|---|---|---|
| Beetroot juice | 13 young adults:<br>9M, 4F (age 25 ± 3 years)<br>11 older adults:<br>3M, 8F (age 56 ± 6 years) | Randomised double-blind, placebo controlled, crossover design<br><br>Dose: 150 ml BRJ or placebo<br>Duration: Acute supplementation during an overnight fasted state on two separate occasions (2.25 h period each) separated by a one-week washout period | Significant ↓ in SBP in both groups<br>Significant ↓ DBP in older adults but not in younger adults | Stanaway et al. (2019) |
| Beetroot juice | 27 hypertensive treated adults<br>10M, 17F<br>(age 63.2 ± 4.4 years) | Randomised, double-blind, placebo-controlled, crossover design<br><br>Dose: 2 x 70 ml/day of either nitrate-rich BRJ or nitrate depleted BRJ<br>Duration: Chronic daily supplementation during two 1-week intervention period separated by a 2-week washout period | No difference in home BP and ambulatory BP between beetroot juice and placebo | Bondonno et al. (2015) |
| Chia seed flour | 10 hypertensive drug treated adults<br>9 hypertensive non-drug treated adults | Randomised, double-blind, parallel, placebo controlled | Significant ↓ in ambulatory and clinical BP in hypertensive drug | Toscano et al. (2014) |

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| | 7 placebo<br><br>(age 35-65 years) | Dose: 35 g/day of chia flour or 35 g placebo (roasted wheat bran)<br>Duration: Chronic daily supplementation for 12 weeks | + chia seed treated group<br><br>Nonsignificant ↓ in ambulatory and clinical BP in group consuming chia seed without hypertensive drugs, however had significant ↓ in SBP | |
| Watermelon extract | 40 prehypertensive and hypertensive adults<br>Watermelon group:<br>10M, 10F (age: 48.7 ± 1.9 years)<br>Placebo group:<br>11M, 9F (age 47.4 ± 1.2 years) | Chronic, randomised, double-blind, experimental, placebo controlled<br><br>Dose: 6 g/day of watermelon extract or placebo<br>Duration: Chronic daily supplementation for 6 weeks | Significant ↓ in SBP and DBP in the watermelon group but no significant differences to final BP compared to the placebo | Massa et al. (2016) |
| Black tea | 19 untreated hypertensive patients<br>5M, 14F<br>(age 51.5 ± 8.4 years) | Randomised, double-blind, placebo-controlled,<br>Crossover design<br><br>Dose: 150 mg tea polyphenols or placebo<br>Duration: Chronic twice daily intake for 8 days separated by a 13-day washout period | Significant ↓ in SBP and DBP compared to placebo | Grassi et al. (2015) |

SBP; systolic blood pressure, DBP; diastolic blood pressure, BP; blood pressure, BRJ; beetroot juice, F; female, M; male, ↓ decrease

* Age written as mean and standard deviation or as a range

Table 2. 2 Selected studies investigating the acute effects of functional foods and extracts on blood glucose levels

| **Functional foods** | **Participants*** | **Study design** | **blood glucose outcomes** | **Reference** |
|---|---|---|---|---|
| NZ Pine bark extract | 25 healthy adults<br>10M, 15F<br>(age 24.8 ± 0.8 years) | Single-blind, cross-over, placebo-controlled, dose response<br><br>Dose: 50 mg or 400 mg pine bark extract, or placebo<br>Duration: Acute supplementation following an overnight fast. Different dosages of pine bark extract, and placebo consumed on three separate occasions before an OGTT. Treatments were separated by at least 48 h washout period between treatments | Significant ↓ in mean glucose iAUC compared to placebo | Lim et al. (2020) |
| Beetroot juice | 16 healthy adults<br>6M, 10F<br>(age 27 ± 5 years) | Randomised, single-blind, placebo controlled, cross-over design<br><br>Dose: 225 ml BRJ, control matched or placebo<br>Duration: Acute supplementation following an overnight fast. Three separate treatments (2.5 h period | Significant ↓ in postprandial BG levels in 0–30-min phase compared to glucose drink<br><br>No significant difference in mean | Wootton-Beard et al. (2014) |

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| | | each) separated by at least 48 h washout period | glucose iAUC between beetroot juice and matched macronutrient control | |
| Bitter melon juice | 16 obese adults<br>1M, 15F<br>(age 29.5 years) | Acute, randomised double-blind, placebo controlled<br><br>Dose: 100 ml bitter melon juice or placebo<br>Duration: Acute supplementation following an overnight fast, 30 min prior to an OGTT (2 h period) | Significant ↓ in postprandial BG lower compared to control group after 120 min | Lee and Ma (2019) |
| Blueberry | 17 healthy adults<br>4M, 13F<br>(age 24.1 ± 4.9 years) | Double-blind, five-condition, counterbalanced, crossover design<br><br>Dose: Anthocyanin rich blueberry powder containing 0 mg, 310 mg, and 745 mg anthocyanins in both sugar matched and no added sugar conditions<br>Duration: acute supplementation of treatments following a 2 h morning fast. Five separate treatments separated by a minimum of four days washout period | Significant extended postprandial BG response compared to sugar-matched control<br><br>Nonsignificant ↓ in peak postprandial BG levels in blueberries<br><br>No significant difference in mean glucose iAUC between different treatments | Bell et al. (2017) |
| Black tea | 13 healthy adults 7M, 6F<br>11 pre-diabetic adults 5M, 6F | Acute, randomised, double-blind, placebo-controlled crossover design | Significant ↓ in iAUC compared to placebo | Butacnum et al. (2017) |

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| | (age 20-60 years) | | | |

BG; blood glucose, F; female, M; male, OGTT; oral glucose tolerance test, iAUC; incremental area under the curve, BRJ; beetroot juice, ↓ decrease

* Age written as mean and standard deviation.

The dietary fibre present in many plant foods has been shown to reduce the absorption rate of postprandial glucose in the intestines (Goff et al., 2018). A meta-analysis of the effects of fibre on BP has also found that increased fibre intake helped in lowering BP in hypertensive patients, with a smaller, non-significant reduction observed in normotensive individuals (Whelton et al., 2005). Higher fibre intake is associated with enhanced insulin sensitivity, meaning the cells respond better to the effects of insulin and remove glucose from the blood stream and into the bodies' cells at a faster rate (Perry & Ying, 2016). Insulin also stimulates NO production in the endothelium. Nitric oxide's vasodilatory properties increase blood flow and therefore facilitates a quicker transfer of BG to the skeletal muscle. In addition, due to the vasodilatory effects of NO, BP will also be lowered due to improved blood flow (Kim et al., 2006; Zhou et al., 2014).

Preliminary research has shown that BRJ consumption has hypoglycaemic and hypotensive effects (Brown & Briggs, 2017; Mirmiran et al., 2020; Olumese & Oboh, 2016; Stanaway et al., 2019). The main active ingredient of BRJ thought to be responsible for these effects is $NO_3^-$. However, BRJ also contains other bioactive ingredients, such as polyphenols, betalains and vitamin C (Mirmiran et al., 2020) which may act individually or synergistically to help lower BP. Beetroot pomace however, differs in composition from beetroot juice with pomace also containing dietary fibre in addition to dietary $NO_3^-$ and other potential bioactive ingredients such as betalains. Therefore, it is possible that the presence of fibre in beetroot pomace could have additive/synergistic effects in regulating BP and BG.

**2.6 Current research on pomace**

Pomace is the by-product that remains after juicing fruit and vegetables. Studies have shown pomace from fruit and vegetables are usually high in TDF (Table 2.3) with the pomace fibre content depending on the variety of the fruit or vegetable. Therefore, a range of TDF values have been reported for the same fruit and vegetable pomace due to the different varieties analysed. Certain pomaces have been utilised as flours to enrich food products such as muffins, cakes, and cereal to increase the fibre content (Costa et al., 2017; Quiles et al., 2018). Additionally, pomace can be used as a substitute for sugar and fat, thus reducing the caloric load while enhancing the nutritional value of the food product, which can also be very useful for aiding in weight loss (Quiles et al., 2018).

Table 2. 3 Total dietary fibre levels of different pomace sources

| Pomace source | Amount of TDF / 100 g | Reference |
|---|---|---|
| Apple | 26.8 – 82.0 | Antonic et al. (2020) |
| Orange | 37.8 – 64.3 | (Figuerola et al., 2005); Grigelmo-Miguel and Martín-Belloso (1999) |
| Grape | 50 – 75 | Quiles et al. (2018) |
| Beetroot | 11.2 – 60.8 | (Sahni & Shere, 2017); Shyamala and Jamuna (2010) |
| Carrot | 45.4 | Shyamala and Jamuna (2010) |

TDF, total dietary fibre

Studies investigating uses for pomace from fruit and vegetable sources such as orange (Chen et al., 2017), apple (Ravn-Haren et al., 2013), sour cherry (Bajerska et al., 2016) and grape (Urquiaga et al., 2015) have also found potential for reducing postprandial BG levels and providing cardioprotective properties (Table 2.4).

In a randomised cross-over study, 23 healthy participants consumed either 22 g apple pomace, 550 g whole apples, 550 ml apple juice or no supplement, every day for four weeks, before switching to the next supplement (Ravn-Haren et al., 2013). In this study, consumption of apple pomace was found to help lower serum low-density lipoprotein (LDL) cholesterol concentrations the most compared to whole apples, apple juice and no supplement. This potential effect of the apple pomace was attributed to its bioactive constituents: fibre, and polyphenols. Consumption of orange pomace also helped attenuate postprandial glycaemic responses, possibly due to its fibre and flavonoid content (Chen et al., 2017). When sour cherry pomace was used as a substitute for wheat flour in a muffin formula, participants who consumed the enriched muffin had significantly lower BG responses 30, 45 and 60-minutes post consumption than when they consumed a plain wheat flour muffin. Consumption of the sour cherry enriched muffins also led to lower iAUC for BG than wheat flour muffins (Bajerska et al., 2016). Similarly, daily consumption of 20 g of grape pomace (10 g fibre) with lunch for 16 weeks resulted in significant decreases in SBP and DBP and fasting glucose levels in individuals with at least one component of metabolic syndrome (Urquiaga et al., 2015). These effects were attributed to dietary fibre and polyphenols in the grape pomace.

Table 2. 4 Results of selected studies investigating different pomace types and their effect on postprandial blood glucose and blood pressure in humans

| Pomace source | Total dietary fibre and fibre type (soluble:insoluble)* | Participants | Study design | Results | Reference |
|---|---|---|---|---|---|
| Orange | 5.48 g (40:60) | 34 overweight men (age 52.1 ± 7.7 years) | Randomised, Placebo-controlled, double-Blind, crossover trial<br><br>Acute supplementation with 240 ml beverage of either placebo, low pomace dose or high pomace dose with a breakfast meal. A washout period of 2-weeks or more between the three trials was followed | Did not impact the overall iAUC or BG.<br>Significant↓ glucose levels at most time points.<br>Delayed time to reach maximal glucose concentrations. | Chen et al. (2017) |
| Orange | 5.4 g (40:60) | 36 men with increased cardiometabolic risk (age 48 ± 1 years) | Randomised, placebo-controlled, double-blind, crossover trial<br><br>Acute supplementation with 240 ml beverage of plain orange juice, orange juice with pomace or control with a breakfast meal. A washout period of 2-weeks or more between the three treatments was followed | No significant effect on postprandial iAUC between different treatments.<br>Delayed time to reach maximal glucose concentrations.<br>Significant ↓ maximal change in maximal glucose concentration. | Dong et al. (2016) |
| Apple | 5 g | 58 healthy subjects (age 25 ± 3 years) | Randomised,<br>Placebo-controlled,<br>crossover trial<br>Acute supplementation with 235 ml beverage of plain apple juice, | No attenuation of maximal BG concentration | Guzman et al. (2020) |

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| | | | apple juice with apple pomace fibre or whole apple after an overnight fast over a 2-h period. A washout period of 2-weeks or more between the three treatments. Carbohydrates were matched in all three treatments, 24 g | Delayed time to reach maximal glucose and insulin concentrations | |
| Enzyme-treated orange | 5 g (40:60) | Study 1: 17 healthy subjects (age 39.3 ± 3.1 years)<br>Study 2: 45 healthy subjects (age 25.1 ± 4.3 years) | Randomised, placebo-controlled, crossover trial<br><br>Acute supplementation with 250 ml beverage of plain orange juice, orange juice with enzyme-treated orange pomace fibre or whole orange (fibre matched) after an overnight fast over a 2-h period. A washout period of 2-weeks or more between the three treatments. Sugars were matched in all three treatments, 19.3 g | No significant effect of pomace on postprandial iAUC compared to orange juice<br><br>Pomace and whole orange attenuated maximal glucose concentration. | Guzman et al. (2021) |
| Orange | 3.5 g (41:59) | 10 healthy subjects (age 29.6 ± 5.8 years) | Randomised, placebo-controlled, double-Blind, crossover trial<br><br>Acute supplementation with 250 ml beverage of plain orange juice, or orange juice with orange pomace fibre after an overnight fast over a 2-h period. A washout period of 1-2-weeks or more | No significant difference between pomace and placebo in iAUC. Significant differences between pomace and placebo at 15 min post ingestion. | Bosch-Sierra et al. (2019) |

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| | | | between the two treatments was followed. | | |
| Enzyme treated Orange | 5 g | 10 healthy subjects (age 27.9 ± 7.7 years) | Randomised crossover trial<br><br>Acute supplementation with 250 ml beverage of plain orange juice, or orange juice with enzyme-treated orange pomace fibre after an overnight fast over a 2-h period. A washout period of 3-7 days between the two treatments was followed. Carbohydrates were matched in both treatments, 24.2 g | Significant ↓ iAUC for pomace compared to control.<br>Significant ↓ peak glucose concentration for pomace. | Huang et al. (2019) |
| Wine grape | 10 (7:93) | 38 men with at least one metabolic syndrome (age. Experimental: 44.5 ± 9.3 years; control: 43.1 ± 8.4 years) | Prospective, randomised, controlled parallel-group trial<br><br>Chronic supplementation with 20 g wine grape pomace (10 g fibre) every day at lunch for 16-weeks compared to control | Intake of pomace significantly ↓ SBP and DBP, and fasting BG compared to control group | Urquiaga et al. (2015) |
| Wine grape | 2 g/day for 6 weeks | 17 high-risk cardiovascular subjects<br><br>12 healthy subjects | Prospective, randomised,intervention trial<br><br>Chronic supplementation with 2 g wine grape pomace (as seasoning in food) every day for 6-weeks compared to control | BP and fasting BG significantly decreased compared to control group. | Taladrid et al. (2022) |

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iAUC; incremental area under the curve, BP; blood pressure, SBP; systolic blood pressure, DBP; diastolic blood pressure, BG; blood glucose, ↓ decrease

* Soluble fibre and insoluble fibre ratio are in percentage %

At the time of writing, the potential effects of BRPOM on human health remains largely unexplored despite extensive research demonstrating the effects of BRJ effects, particularly on BP. This lack of research has prompted the current study investigating whether BRPOM may confer similar or equivalent effects to BRJ.

**2.7 Beetroot pomace**

Red beetroot *(Beta vulgaris;* BR*)* is a rich source of biologically active substances such as inorganic $NO_3^-$, betalains, polyphenols, carotenoids, B-vitamins, and fibre (Ceclu & Nistor, 2020; Mirmiran et al., 2020). The dietary $NO_3^-$ content of beetroot varies considerably between varieties and is also dependent on a range of external factors, including the level of nitrogen fertiliser used, temperature and processing conditions (Hobbs et al., 2013). Therefore, the $NO_3^-$ levels present in different batches of beetroot can vary considerably. Nitrate concentrations in commercial BRJ have been reported to range between 388 ±19.9 to 3968 ±252 mg/L (Mirmiran et al., 2020). Red BRJ has been used as the source of $NO_3^-$ in many supplementation studies (see Tables 2.2 and 2.5). As previously discussed, BRJ intake has shown the potential to decrease BP (Jajja et al., 2014; Kapil et al., 2015; Stanaway et al., 2019; Webb et al., 2008) and may have a role in attenuating postprandial BG responses (Chang et al., 2018; Holy et al., 2017; Wootton-Beard et al., 2014).

The juicing process involves inserting whole pieces of beetroot through a fast-spinning metal blade that separates the juice from the pulpy flesh by centrifugal force (Khaksar et al., 2019). The resulting juice contains $NO_3^-$, as well as 98% of the sugars but 0% of the fibre present in the whole raw beetroot it was juiced from. Hence the by-product of the beetroot juicing process is the pomace which contains 100% of the fibre from the beetroot vegetable (Mirmiran et al., 2020) as well as other potential bioactive compounds such as $NO_3^-$, betalains and polyphenols (Sotirios, 2020).

Processing beetroot juice results in high amounts of waste material (approximately 15-30% of the whole beetroot) which is not limited to the pomace by-product but may also include the crown and peel (removed before processing). This by-product is often used for animal feed and biofuel but it can also be discarded in landfills, creating environmental challenges (Patel & Shukla, 2017; Vulić et al., 2014).

The reported levels of fibre in beetroot pomace vary considerably, one study found 11.2 g / 100 g TDF (Sahni & Shere, 2017) while another study found 60.8 g / 100 g TDF (Shyamala & Jamuna, 2010). This range may be due to the processing methods the studies utilised and/or the BR variety. In addition to fibre, BRPOM also contains polyphenols, betalains and natural sugars. The phenolic content of raw beetroot is 255 mg GAE (gallic acid equivalent)/100 g (Mirmiran et al., 2020), and BRPOM ranges from 100-5000 mg GAE/100 g (Sotirios, 2020). Again, the differences in the phenolic range could be because of different beetroot varieties and/or methods of obtaining the beetroot pomace from its raw form. The sugar content of beetroot pomace is 8.79 g / 100g, compared to beetroot juice 4.8-6.6 g / 100g, and raw beetroot 6.76 g / 100g (Mirmiran et al., 2020; Sobhy et al., 2020).

To date no studies have reported the $NO_3^-$ content of BRPOM. As previously discussed, BRJ contains high levels of nitrate; therefore, it is likely that some $NO_3^-$ will also be present in the BRPOM. The combination of TDF, polyphenols and $NO_3^-$ present in BRPOM may make it an attractive ingredient in food products designed to elicit reductions in BP and attenuation of BG responses.

**2.8 Effect of fibre consumption on blood glucose levels**

The beneficial effects of dietary fibre consumption on overall human health were recently documented in a narrative review indicating the inverse relationship between dietary fibre consumption and the development of T2DM and CVD (Barber et al., 2020). Dietary fibre is a type of plant carbohydrate which is undigested and may be fermented in the colon. There are two types of fibre: soluble dietary fibre (SDF) and insoluble dietary fibre (IDF). Soluble fibre dissolves in water in the gastrointestinal tract and forms a viscous gel substance, which bypasses digestion in the small intestine and travels to the large intestines, where it is fermented by the microflora (Lattimer & Haub, 2010). In contrast, IDF does not dissolve in water; instead, it remains mostly intact and helps promote the absorption of water, thereby improving stool volume, allowing for faster transit time through the intestine (Goff et al., 2018; Lattimer & Haub, 2010; Tan et al., 2020).

Many studies have shown that increased ingestion of total dietary fibre (TDF) attenuates postprandial BG and lowers the glucose area under the curve to some extent (Cassidy et al., 2018; Fuller et al., 2016; Goff et al., 2018; Yu et al., 2014). The mechanisms by which fibre helps control glycaemic responses are still not fully understood. There are several plausible mechanisms, including delay in gastric emptying rate; delay in glucose absorption and rate of starch digestion; increases in insulin secretion and sensitivity; inhibition of amylase activity; and/or modification of release of digestion and fermentation-related hormones (Lattimer & Haub, 2010; Meng et al., 2017; Nyambe-Silavwe & Williamson, 2016). The degree to which these mechanisms are involved in glycaemic control depends on the physiochemical characteristics of the different fibres such as their viscosity, fermentability and water solubility, but also the amount of fibre ingested (Müller et al., 2018).

The amount of TDF required to result in attenuation of BG levels varies between studies. In a dose-response meta-analysis (Yao et al., 2014), higher consumption of TDF, IDF, fruit fibre and cereal fibre was found to be inversely associated with T2DM risk. There was however, a non-linear association between TDF intake and T2DM risk, suggesting that there is a threshold TDF intake amount beyond which TDF can lower T2DM risk. When TDF consumption was 25 g or more per day, this was associated with lower T2DM risk (Yao et al., 2014). Similarly, consumption of >14 g IDF per day was assciated with better BG levels, and those effects plateaued when IDF intake was above 25 g per day (Kabisch et al., 2021). The ratio of SDF and IDF may lead to differing amounts of fibre being required to elicit reductions in postprandial BG.

Soluble dietary fibre's ability to form a viscous solution inside the gastrointestinal tract is one of the main reasons that consumption slows the gastric emptying rate, reducing postprandial glucose levels. An early study (Jenkins et al., 1978) investigating the effects of SDF consumption within a test meal showed that 12 g of TDF (mainly SDF) helped decrease postprandial BG levels in six healthy participants compared to a control meal. However, a review of the literature (Tosh, 2013) on the effects of oat and barley SDF β-glucan consumption reported only 4 g was required to elicit a reduction in glycaemic responses. In contrast, another study (Meng et al., 2017) where participants consumed 4.8 g or 9.6 g of oat cereal showed no significant effect on postprandial glucose iAUC. However, this study used Cheerios oat cereal as the source of fibre, and it is possible that the β-glucan may have been degraded during the food processing steps either by exposure to heat or physical disruption, thus resulting in no effect on glycaemic control.

The role of IDF on glycaemic control is unclear. Studies investigating the effects of consumption of foods with a higher proportion of IDF compared to SDF have shown mixed results in decreasing postprandial BG levels (Bosch-Sierra et al., 2019; Chen et al., 2017; Dong et al., 2016). One study (Bosch-Sierra et al., 2019) in healthy adults who consumed 3.5 g of citrus fibre consisting of 58.9% IDF and 41.1% SDF showed negligible effects in attenuating postprandial BG levels. However, other studies (Chen et al., 2017; Dong et al., 2016) demonstrated consumption of orange pomace (5.4 g TDF) consisting of 60% IDF and 40% SDF fibre was able to delay the time reached to maximum postprandial BG levels. Therefore, it is possible that the 3.5 g of citrus fibre in the Bossch-Sierra et al. (2019) study was insufficient to make significant changes in the postprandial BG due to its overall lower IDF and SDF content.

Consumption of insoluble fibre may attenuate BG responses by reducing digestive enzyme activity due to non-specific binding with insoluble fibre and expanding faecal volume, which aids in decreasing transit time in the colon (Goff et al., 2018). These findings indicate IDF may also contribute to the effects of SDF in reducing postprandial BG levels and the potential of IDF should not be disregarded. In summary, dietary fibre type and intake amount may have varying effects on postprandial BG levels and therefore T2DM risk

**2.9 Effect of dietary fibre consumption on blood pressure**

High BP is associated with increased risk of ischaemic and haemorrhagic stroke, chronic kidney disease and end-stage renal disease. Additionally, the coexistence of hypertension and diabetes can worsen microvascular and macrovascular complications (Mills et al., 2020). Small decreases in BP can lead to clinically significant reductions in diabetic complications and CVD risk. For example, in a recent 29ypothesiz controlled trial, a reduction of 5.6/2.2 (SBP/DBP) mmHg in the treatment group resulted in a 9% reduction in major macrovascular or microvascular complications in people with T2DM (Rahman et al., 2019). Similarly, a systematic review found a reduction in SBP by 10 mmHg in hypertensive participants reduced CVD risk by 29% (Bundy et al., 2017). Studies have shown SBP and DBP attenuation of as little as 2 mmHg can be considered clinically significant to reduce CVD in both normotensive and hypertensive individuals (Hess et al., 2016).

Nutritional interventions have been popular strategies for achieving a healthy BP range and reducing CVD risk. One such nutritional intervention has been the DASH diet, a diet high in fruits and vegetables, low-fat dairy products, and other food products low in saturated fats, low in sodium and high in fibre, potassium, and magnesium (Mohanlal et al., 2012). The DASH diet has been shown to significantly decrease SBP by 6 to 11 mm Hg in both normotensive and hypertensive patients (Challa et al., 2021). Many clinical trials have demonstrated that the DASH diet is inversely associated with CVD, stroke, T2DM and obesity (Challa et al., 2021). The diet recommends five daily servings of both fruits and vegetables. It has been proposed that the natural presence of $NO_3^-$ in leafy greens and root vegetables like beetroot are important factors contributing to the antihypertensive effects in the DASH diet (Morris et al., 2019).

However, compliance to this diet outside of clinical settings is low due to challenges in adhering to specific dietary advice when guidance from a health professional is not present (Challa et al., 2021). Therefore, if specific fruits and vegetables with the greatest hypotensive effects are utilised, then consumers of these foods may still experience BP lowering benefits even if the DASH diet is not strictly followed.

Consumption of high fibre foods has been shown to have beneficial effects on BP. For example, beta-glucan is a fibre that is well researched for its effects on lowering BP, with consumption of oat fibre β-glucan shown to decrease SBP or mean arterial pressure (MAP) in normotensive, prehypertensive and hypertensive humans (Keenan et al., 2002; Pins et al., 2002; Saltzman et al., 2001). Administration of 5.5 g β-glucan to 18 untreated hypertensive patients for 6 weeks resulted in reductions in both SBP and DBP by 7.5 mmHg and 5.5 mmHg, respectively (Keenan et al., 2002).

Other fibre types have also been shown to exhibit BP-lowering actions. For example, consuming cocoa husks rich in fibre and polyphenols significantly decreased SBP by 8 mmHg and DBP by 8.3 mmHg and significantly decreased serum BG by 0.3 mmol/l after eight weeks of supplementation (Sarriá et al., 2012). Consumption of 7.5 g per day (5.25 g TDF) of grape antioxidant dietary fibre for 16-weeks was sufficient to significantly lower both SBP and DBP (8.5 mmHg and 3.8 mmHg reduction, respectively) in healthy and hypercholesterolemic subjects (Jiménez et al., 2008). One study, however, showed that consumption of a high-fibre oat bran supplement (11.7g TDF) did not significantly lower BP in participants any more than the low-fibre supplement alternative (Swain et al., 1990). These inconsistent findings may be due to BP responses to different fibre types (IDF or SDF) or more likely because the participants had a lower healthy baseline BP of 112/68 mm Hg, and the body has regulatory mechanisms to inhibit BP from going any lower (Shahoud et al., 2021). Additionally, 16 out of the 20 participants were dietitians, consuming a baseline of 23.3 g TDF per day in their usual diet. Therefore, adding a high or low fibre supplement may not have affected their overall BP due to the effects of fibre potentially plateauing when above 25 g per day (Kabisch et al., 2021).
One source of dietary fibre that is frequently overlooked is the utilisation of fruit and vegetable waste by-products formed as a result of processing, also referred to as pomace. Pomace can potentially serve as a valuable source of fibre (Nawirska & Uklańska, 2008).

### 2.10 Nitric oxide and its effects on blood pressure

Nitrite oxide (NO) is produced via two known pathways: endogenous and exogenous. The endogenous pathway begins by converting L-arginine to NO, a reaction catalysed by the enzyme nitric oxide synthase (NOS). In contrast, the exogenous pathway is dependent on the consumption of dietary $NO_3^-$ and nitrite ($NO_2^-$) (Clifford et al., 2015). Following $NO_3^-$ consumption, it is almost entirely absorbed by the small intestine and stomach. About 75% of $NO_3^-$ is excreted in the urine and 25% is absorbed across the upper gastrointestinal tract from which it is then extracted from the blood circulation and secreted into the oral cavity. At this site, $NO_3^-$ becomes concentrated and is rapidly reduced to $NO_2^-$ by anaerobic bacteria in the mouth. The $NO_2^-$ is then swallowed to re-enter the stomach, some of which is absorbed into the systemic circulation where it is metabolised to NO. Nitrite reductase converts the remaining $NO_2^-$ in the stomach to NO where it helps kills pathogenic bacteria, stimulates mucosal blood flow and mucus production (Hobbs et al., 2013; Kapil et al., 2015). Nitric oxide acts as a vasodilator by relaxing the muscles in the inner wall of the blood vessels, thereby causing blood vessels to dilate, allowing greater blood flow and consequently lowering BP (d'El-Rei et al., 2016).

The highest dietary sources of $NO_3^-$ and $NO_2^-$ are found in certain green leafy vegetables such as spinach and rocket and root vegetables such as red beetroot, which all contain >250 mg $NO_3^-$ (4 mmol)/100 g (Hord et al., 2009; Ma et al., 2018). A study (Stanaway et al., 2019) in which 150 ml BRJ (651 mg $NO_3^-$) was administered to 24 healthy adults resulted in significant decreases in acute SBP in both healthy younger and older adults and reduced DBP in older adults. The researchers hypothesised that the more significant decrease in DBP in the older cohort may be attributed to their lower initial NO levels. Similarly, the administration of BRJ to hypertensive and/or overweight adults resulted in a significant decrease in SBP (Jajja et al., 2014; Kapil et al., 2015) and DBP (Kapil et al., 2015). Table 2.5 summarises the results from several studies investigating the effects of $NO_3^-$ consumption on BP. The studies highlighted in Table 2.5 utilised different quantities of dietary $NO_3^-$ given to various cohorts, including individuals who were hypertensive, overweight, or healthy. The use of BRJ as the main source of $NO_3^-$ in these studies is a particular emphasis; however, additional studies using alternate $NO_3^-$ sources were also included to examine any potential differences on BP.

Table 2. 5 Results of selected acute and chronic studies investigating the effects of nitrate consumption on blood pressure

| Nitrate source | Nitrate amount (mg) | Participants* | Study design | Blood pressure outcomes | Reference |
|---|---|---|---|---|---|
| Beetroot juice concentrate | 300-400 mg | 21 older overweight adults<br>10M, 11F<br><br>(age 62.0 ± 1.4 years) | Randomised, 2-arm, parallel trial<br><br>Chronic supplementation with either 70 ml BRJ concentrate or 200 ml black current juice a day for 21 days | Significant ↓ SBP in week 3<br>No significant changes in week 1 and week 2 | Jajja et al. (2014) |
| Beetroot juice concentrate | ~384 mg | Study 1: 13 hypertensive adults on medication<br>10M, 3 F<br>(age 53 ± 12 years)<br><br>Study 2: 14 hypertensive adults without medication<br>11M, 3F<br>(age 49 ± 13 years) | Two separate double-blind, counterbalanced measures design<br><br>Acute supplementation with either 70 ml nitrate-rich BRJ concentrate or 70 ml nitrate-depleted BRJ concentrate a day for 3 days in two treatments separated by a minimum of 1-week washout period | No difference on SBP and DBP in study 1 participants<br><br>In study 2, participants had Significant ↓ SBP and ↓DBP | Broxterman et al. (2019) |
| Beetroot juice | 397 mg | 64 hypertensive adults<br>26M, 38F<br>(age 56.3 ± 16.4 years) | Randomised, placebo-controlled, double-blind trial<br><br>Chronic daily supplementation with 250 ml BRJ or placebo for 4 weeks | Significant ↓ SBP and ↓DBP | Kapil et al. (2015) |

| | | | | | |
|---|---|---|---|---|---|
| Beetroot juice concentrate | ~397 mg | 17 young healthy adults (24.2 ± 3.5 years)<br>7 older healthy adults (68.7 ± 10.3 years) | Randomised, placebo-controlled, crossover trial<br><br>Acute supplementation with 70 ml BRJ concentrate or placebo following an overnight fast. Two separate treatments (3 h period each) separated by a 1-week washout period | No effect on SBP and DBP in both older and younger adults | Rogerson et al. (2022) |
| Beetroot juice | 434 mg | 27 hypertensive adults<br>10M, 17F<br>(age 53-70 years) | Randomised, placebo-controlled, double-blind, crossover trial<br><br>Chronic supplementation with 2 x 70 ml/day of either nitrate-rich BRJ or nitrate depleted BRJ for 1-week separated by a 2-week washout period | No significant changes to both SBP and DBP | Bondonno et al. (2015) |
| Beetroot juice | 651 mg | 13 young adults (age 18-30 years)<br>11 older adults (age 50-70 years) | Randomised, double-blind, crossover trial<br><br>Acute supplementation with 150 ml BRJ or placebo following an overnight fast. Two separate tretments (2.25 h period each) separated by a 1-week washout period | Significant ↓ SBP in both groups<br>Significant ↓ DBP in older adults | Stanaway et al. (2019) |
| Spinach | 854 mg | 27 healthy adults<br>11M, 16F<br>(24.5 ± 11 years) | Randomised, placebo-controlled, crossover trial<br><br>Chronic daily supplementation with high-nitrate or low-nitrate soup for 1-week. Two separate occasions separated by a 1-week washout period | Significant ↓ central SBP, DBP and brachial SBP at 180 min following 1-week of supplementation | Jovanovski et al. (2015) |

| | | | | | |
|---|---|---|---|---|---|
| Beetroot juice | 1395 mg | 14 healthy adults (no age or gender details included) | Open-label, randomised, crossover trial<br><br>Acute supplementation with 150 ml BRJ or placebo (water) following an overnight fast. Two separate treatments separated by a 1-week washout period | Significant ↓ SBP at 24 hours after ingestion compared to 1-hour post ingestion<br>No significant decrease for DBP | Webb et al. (2008) |
| Potassium nitrate supplement | 496 or 1488 mg | 33 healthy adults<br>15M, 18F<br>(age 18-45 years) | Acute, randomised placebo-controlled, double-blind, crossover trial<br><br>Acute supplementation with 'high dose', 'low dose' or placebo following an overnight fast. Three separate treatments separated by a 1-week washout period | No significant changes to both SBP and DBP | Floyd et al. (2019) |

SBP; systolic blood pressure, DBP; diastolic blood pressure, BP; blood pressure, BRJ; beetroot juice, F; female, M; male, ↓ decrease

* Age written as mean and standard deviation or a range

Not all studies (Table 2.5) have shown that consumption of $NO_3^-$ results in a lowering of BP. For example, one study where healthy participants consumed either a high (1488 mg) or a low (496 mg) $NO_3^-$ dose via potassium nitrate supplementation ($KNO_3^-$), or placebo 1 hour prior to ingesting 75 g glucose, did not find any significant changes in SBP or DBP (Floyd et al., 2019). The researchers hypothesised that their participants' acute high BG and insulin may have rendered the $NO_3^-$ incapable of reducing BP. However, the participants in the study (Floyd et al., 2019) had a low baseline SBP (113 ± 10 mmHg), which may have prevented the effects of $NO_3^-$ from further decreasing BP. Furthermore, research suggests that a higher baseline SBP will result in a larger decrease in SBP following $NO_3^-$ intake from beetroot (Bahadoran et al., 2017). This finding suggests a hypertensive population may see more reductions in their BP compared to a healthy population with lower SBP baseline.

Additionally, using a single ingredient $NO_3^-$ supplement as opposed to a $NO_3^-$ rich whole food, may not achieve the same result, as whole foods may contain other bioactive ingredients such as polyphenols, betalains and fibre which may also individually or synergistically confer hypotensive effects (Jiménez et al., 2008; Miranda et al., 2016; Moreno-Luna et al., 2012; Sarriá et al., 2012). For example, a study investigating the effects of supplementing 50 mg betalain extracted from red beetroot found consumption lowered both SBP and DBP in participants (Rahimi et al., 2019). This suggests that betalain and $NO_3^-$ together may have synergistic or additive effects.

Bondonno et al. (2015) also failed to show changes in SBP and DBP after administering $NO_3^-$ rich BRJ containing 434 mg $NO_3^-$ daily for 1 week to individuals on hypertensive medication. The authors (Bondonno et al., 2015) reasoned the lack of effect was due to the participants' loss of endothelial function due to hypertension, suggesting the endothelium was not able to effectively vasodilate in response to $NO_3^-$. Furthermore, the hypertensive medications the participants were taking may have prevented additional reductions in BP if their hypertension was already well controlled.

In summary, the ability of dietary $NO_3^-$ to lower BP may be dependent on the absence of low baseline SBP, high BG/insulin levels, endothelial function, and whether $NO_3$- is taken through whole foods where other bioactive ingredients may also be present or in a single ingredient supplement.

**2.11 Nitric oxide and its effects on blood glucose responses**

There is limited research exploring the role of dietary $NO_3$- on glycaemic control. Given the ability of $NO_3^-$ to lead to dilation of blood vessels, it has been theorised that consumption of $NO_3^-$ may enhance the movement of insulin to skeletal muscle, increasing glucose uptake by 25-40%, thereby improving postprandial BG uptake and insulin signaling (Kim et al., 2006).

However, the results from previous research in this area have been conflicting. Chang et al. (2018) administered 270 ml BRJ ($NO_3^-$ dose unknown) to 10 healthy participants aged 20-24 years and showed that postprandial BG levels were significantly lower compared to the sugar-matched control. Similarly, Wootton-Beard et al. (2014) showed that consumption of 225 ml BRJ (992 mg $NO_3^-$) elicited significantly lower postprandial glucose and insulin responses in 16 healthy participants. Both sets of authors attributed their positive results to the presence of dietary $NO_3^-$, betalains, and polyphenols, suggesting possible inhibition of digestive enzymes and/or increased BG uptake as the mechanism of action for altering postprandial glycaemia (Chang et al., 2018; Wootton-Beard et al., 2014). In contrast, another study (Cermak et al., 2015) failed to demonstrate attenuation of postprandial BG responses despite an increase in plasma $NO_3^-$ and $NO_2^-$ following administration of 9.3 mg/kg sodium $NO_3^-$ (equivalent to 651 for a 70 kg individual; 930 mg for 100 kg individual) to 17 men with T2DM. Sodium nitrate was used to avoid confounding effects from other components such as carbohydrates on BG. This result suggests that attenuation of postprandial BG responses may not be solely based on $NO_3^-$ content alone but also reliant on the presence of other bioactive components such as the betalains and polyphenols found in BRJ.

**2.12 Current research and summary**

Several dietary supplementation studies have investigated the health effects of consuming pomace from sources such as orange, apple, sour cherry and grape to help reduce postprandial BG levels and provide cardioprotective properties. Additionally, pomace can be used as a substitute for sugar and fat, thus reducing caloric load while enhancing the nutritional value of the food product due to the added fibre. This is particularly important to help reduce obesity which is a risk factor for hypertension and type 2 diabetes.

Beetroot juice, which is high in nitrates, has been shown to help reduce BP and postprandial BG levels. Its by-product, beetroot pomace, contains high amounts of fibre, polyphenols and betalains and evidence suggests that these three compounds can also lower BP and attenuate postprandial BG. To date, no studies have reported the nitrate content of beetroot pomace, however, after juicing, there will likely be some nitrate present in the pomace. Hence beetroot pomace with its higher fibre content may have a similar if not greater health-promoting effect as beetroot juice.

A limited number of studies have utilised beetroot pomace for the enrichment of pasta, cakes and cookies to increase its fibre content. However, at the time of writing, no studies have investigated the health-promoting potential of beetroot pomace consumption. It is therefore important to investigate this further, particularly as it may offer a cost-effective and sustainable waste stream option to promote cardiovascular health. Research is needed to analyse the nutritional properties of beetroot pomace and explore its potential as a functional food for reducing BP and improving BG control.

## CHAPTER 3: RESEARCH STUDY MANUSCRIPT


### 3.1 Abstract

**Background:** Nitrate-rich beetroot juice (BRJ) supplementation has been shown to improve blood pressure (BP) levels in younger and older adults via increased nitric oxide (NO) production leading to dilation of blood vessels. Similarly, to some extent a reduction of blood glucose (BG) levels through several different mechanisms. However, limited research exists on the by-product of beetroot juice production, beetroot pomace (BRPOM), and its role in controlling BP and BG levels.

**Objective:** To investigate and compare the changes in BP and BG levels in a 3 h period following the ingestion of BRPOM, BRJ and placebo as part of an oral glucose tolerance test (OGTT).

**Methods:** Following an overnight fast, twelve healthy adults consumed either BRPOM (600 mg nitrate, 10 g fibre, 75 g carbohydrate), BRJ (600 mg nitrate, <1 g fibre, 75 g carbohydrate), or placebo (0 mg nitrate, <1 g fibre, 75 g carbohydrate) 15 minutes prior to the OGTT, separated by 1-week washout periods, in a single-blind, crossover design, pilot study. Blood pressure and BG levels were measured at baseline, then every 15 minutes for BG and every hour for BP during a 3 h OGTT.

**Results:** No significant changes in SBP for BRJ (-2.3 ± 5.56 mmHg, $p = 0.174$), BRPOM (-3 ± 13.4 mmHg, $p = 0.456$), or PLA (5.2 ± 11.3 mmHg, $p = 0.142$) treatment group after 3 h. However, there was a trend towards significance in the differences between the three groups ($p = 0.075$, $\eta^2_p = 0.404$). Clinically meaningful reductions in SBP were observed for BRJ (-2.3 ± 5.56 mmHg) and BRPOM (-3 ± 13.4 mmHg). There were no differences ($p = 0.739$, $\eta^2_p = 0.059$) in mean glucose incremental area under the curve (iAUC) between BRJ, BRPOM and PLA (232.8 ± 61.9, 242 ± 54.7 and 252.4 ± 60.6 mmol/L/min, respectively). There were no differences between BRJ, BRPOM and PLA in time to peak ($p = 0.269$, $\eta^2_p = 0.313$), peak glucose levels ($p = 0.241$, $\eta^2_p = 0.247$), 2 h glucose levels ($p = 0.565$, $\eta^2_p = 0.150$), 3 h glucose levels ($p = 0.395$, $\eta^2_p = 0.233$) and percentage increment of postprandial BG ($p = 0.783$, $\eta^2_p = 0.048$).

**Conclusion:** This pilot study with a small sample size showed large effect size and clinically meaningful attenuations for acute SBP changes following the consumption of beetroot pomace. Further investigations would be needed to explore the applicability of these findings to hypertensive individuals. However, its impact on acute BG levels remains non-significant, possibly influenced by the small sample size and participants' baseline normoglycaemic levels. Further research is needed to explore potential effects in a hyperglycaemic population.

**Key words:** Beetroot juice; Beetroot pomace; Dietary fibre; Nitric oxide; Blood pressure; Blood glucose.

### 3.2 Introduction

Cardiovascular disease (CVD) is often linked to high blood pressure (hypertension) and diabetes (Petrie et al., 2018), which can also lead to other conditions, such as heart attack, stroke, kidney disease/failure, eyesight problems, peripheral artery disease and heart failure (Mills et al., 2020; Oparil et al., 2018). In New Zealand (NZ), hypertension rates were relatively consistent throughout 2015-2019, averaging 32.7% of the population; however in 2021 the highest rates of hypertension were observed with 33.4% of people in NZ with hypertension (Ministry of Health, 2021a). Additionally, NZ has approximately over 220,000 people who currently have type 2 diabetes mellitus (T2DM) (Holder-Pearson & Chase, 2022). By 2040, the number of New Zealanders with T2DM alone is expected to increase by 70–90% to over 400,000 persons (Holder-Pearson & Chase, 2022). Current research indicates that diet can play a key role in influencing the onset of CVD and T2DM. Increased consumption of energy-dense foods with high levels of added sugars and saturated fat have emerged as central contributors to developing the current obesogenic environment (D'Innocenzo et al., 2019). Obesity is a risk factor for a multitude of comorbid conditions, such as gastrointestinal disorders, muscular and joint disorders, and psychological issues (Fruh, 2017). People who consume diets rich in fruit and vegetables, such as the Mediterranean diet, have demonstrated the greatest improvement in their glycaemic control and reduced CVD by 29% over 4.8 years compared to other diets such as paleolithic, low carbohydrate, high-protein, vegetarian and nut-enriched diets (Joseph et al., 2022).

There has also been growing interest in the utilisation of functional foods as a viable strategy for managing elevated blood glucose levels (BG) and blood pressure (BP), aiming at adverse effects associated with elevated BG and BP levels (Bumrungpert et al., 2020; Lim et al., 2020). Nitrate ($NO_3^-$), which is naturally found in vegetables such as spinach, rocket, and red beetroot (Hord et al., 2009), has been studied for its potential cardioprotective properties (Kapil et al., 2015; Liu et al., 2013). Nitrate is a precursor to bioactive nitric oxide (NO), which acts as a vasodilator by relaxing the muscles in the inner wall of blood vessels. This dilation leads to an increase in blood flow, which, in turn, aids with the reduction of BP (Ma et al., 2018) and potentially BG levels as there is an increased glucose uptake by the skeletal muscles (Kim et al., 2006).

Some studies have shown that beetroot juice (BRJ) contains high levels of $NO_3^-$ (Mirmiran et al., 2020) and the consumption is associated with significant attenuations to both systolic and diastolic BP (Kapil et al., 2015; Stanaway et al., 2019) and other studies have shown it to significantly decrease only the systolic blood pressure (SBP) (Bondonno et al., 2015; Webb et al., 2008). There is, however, limited research impact of $NO_3^-$ on glycaemic control. Some studies have shown that BRJ supplementation significantly lowers postprandial BG levels (Chang et al., 2018; Wootton-Beard et al., 2014). However, when $NO_3^-$ was given via sodium-nitrate capsule supplementation, it failed to show significant attenuation of postprandial BG levels (Cermak et al., 2015). This implies $NO_3^-$ may work in conjunction with other components that are present in BRJ such as betalains and polyphenols to attenuate postprandial BG levels.

Juicing beetroot produces the by-product, pomace, which is generally considered a waste product, used either for animal feed or discarded in landfills (Vulić et al., 2014). Based on published nutritional analyses, beetroot pomace (BRPOM) contains high amounts of dietary fibre, polyphenols, and betalains (Costa et al., 2017; Mirmiran et al., 2020; Shyamala & Jamuna, 2010).

Fibre consumption has been shown to help reduce the risk of developing T2DM and CVD risk (Barber et al., 2020). While some studies have shown beneficial effects on BP after fibre consumption, some have failed to find significant findings (Keenan et al., 2002; Sarriá et al., 2012; Swain et al., 1990). These inconsistent findings may be attributed to different fibre types (insoluble or soluble), and baseline BP levels (Dong et al., 2019; Shahoud et al., 2021). Many studies have shown that increased ingestion of total dietary fibre (TDF) attenuates postprandial BG and lowers the glucose incremental area under the curve (iAUC)(Cassidy et al., 2018; Fuller et al., 2016; Goff et al., 2018; Yu et al., 2014). Delaying gastric emptying and glucose absorption are plausible mechanisms by which fibre may reduce postprandial BG levels (Lattimer & Haub, 2010; Meng et al., 2017).

Studies in overweight or obese men have shown that orange pomace consumption (average 5.4 g TDF) significantly delays the time to reach peak glucose levels, but has no significant effect on iAUC (Chen et al., 2017; Dong et al., 2016). A study in healthy individuals found similar results with the consumption of orange juice fortified with orange pomace (5g TDF) reducing peak postprandial BG levels compared to orange juice alone but had comparable results to whole orange (Guzman et al., 2021). However, no significant effect of orange pomace on postprandial iAUC compared to orange juice was observed.

Despite the amount of research undertaken on BRJ and other fruit pomaces, to date, no studies have investigated the potential health benefits of BRPOM consumption. Here, we evaluate and compare the acute effects of BRPOM, BRJ and placebo on postprandial BP and BG in healthy adults.

## 3.3 Materials and Methods

### 3.3.1 Study and participants

This pilot study was conducted between June 2021 and April 2022. Participants were recruited from Auckland, New Zealand using poster and social media advertisements (Appendix A) within Massey University and the Auckland, North Shore area. All interested individuals were provided with a participant information sheet (Appendix B). All participants provided written informed consent before taking part in the study (Appendix C). Ethical approval was obtained from the Massey University Human Ethics Committee (MUHEC; SOA 21/10). The study was conducted according to the guidelines laid down in the Declaration of Helsinki.

A sample size of 12 participants (7M; 5F) was recruited based on the average sample size of previous studies with similar designs (Stanaway et al., 2019; Webb et al., 2008). To confirm eligibility interested participants were required to complete a health-screening questionnaire (Appendix D) and visit the research laboratory. At this visit, anthropometric measures including height (m) using a stadiometer and weight (kg) and percentage body fat using a bioelectrical impedance analyser (BIA; Inbody 230) occurred (Appendix H and I). Metabolic parameters including fasting BG (FBG) via finger pricking (Appendix G) and glucose monitor (MediSense, Optium, Abbott, Auckland, New Zealand), HbA1C (Cobas b 101, Roche Diagnostics) and BP (mean value of three measurements; Riester ri-champion®N) were also measured while seated, allowing 2 minutes between each measurement.

Inclusion criteria for the study included: (i) body mass index (BMI) of 18.5-30.0 kg/ $m^2$, (ii) aged 20-60 years, (iii) fasting BG <5.7 mmol/L, (iv) glycated haemoglobin A1c (HbA1c) <40 mmol/mol, (v) BP > 120/80 mmHg, (vi) not taking any medications that included BG and/or BP-lowering prescriptions, (vii) not taking nitrate-based dietary supplements, (viii) free from cardiovascular disease or diabetes. Participants were not eligible if they were pregnant, breastfeeding, had any form of cardiovascular and metabolic disease, if they smoked, or if they had any known allergies to beetroot.

### 3.3.2 Development of beetroot pomace

Beetroot pomace (BRPOM) was collected following the juicing of fresh beetroot (*Beta vulgaris, Pablo*). This pomace was frozen and freeze dried on plastic sheets before being powdered, heat treated and packaged (more information on product development can be found in (Appendix F).

Before use, the BRPOM powder was tested for microbial safety by carrying out aerobic plate count and the presence of yeast and mould, lactic acid bacteria, *Staphylococcus aureus*, and spores' tests. Yeast, mould and lactic acid bacteria detection were carried out by plating serial-diluted samples on appropriate selective media-yeast extract glucose chloramphenicol agar, standard plate count, deMan Rogosa and Sharpe media (Barbu et al., 2020; Nisa et al., 2021). Detection of *S. aureus* was conducted using Baired-Parker agar plates (Thaker et al., 2013) and the presence of spores was analysed using the method of McHugh et al. (2017). All the experiments were carried out in triplicate in the School of Food and Advanced Technology, microbiology laboratories at Massey University Auckland campus.

### 3.3.3 Intervention products

All three dietary intervention products contained the same amount of maltodextrin and apple juice (Just Juice; 11.2°Brix), with varying amounts of sucrose to make up a total of 75 g of carbohydrates for each intervention product. BRPOM powder (29g; 600 mg $NO_3$-) or BRJ freeze-dried powder (35g; Nature's Way; 600 mg $NO_3$-) was added to the apple juice, maltodextrin and sucrose mixture to either create a thick pudding or a drink, respectively (Table 3.1). Beetroot pomace was stored in the freezer until the morning of the intervention visit. The rest of the intervention ingredients except for maltodextrin and sucrose were stored in the fridge prior to each visit. Intervention products were prepared 20 min before consumption.

Table 3. 1 Composition of intervention products

| **Ingredients** | **BRJ*** | **BRPOM pudding**** | **PLA*** |
|---|---|---|---|
| **Apple juice (ml)** | 250 | 250 | 250 |
| **Sucrose (g)** | 7.3 | 15.7 | 37.7 |
| **Maltodextrin (g)** | 10 | 10 | 10 |
| **Beetroot (g)** | 35 [1] | 29 [2] | 0 |

BRPOM, beetroot pomace; BRJ, beetroot juice; PLA, placebo.

* Formulated as drinks, ** Formulated as food

[1] Beetroot juice powder

[2] Beetroot pomace powder

The final concentrations for $NO_3^-$ within the BRJ and BRPOM drink/food were determined using high-performance liquid chromatography (HPLC; Section 3.3.5). The nutritional composition of the freeze-dried BRPOM (g/100 g) and the three dietary intervention products are shown in Table 3.2.

Table 3. 2 Nutritional composition of freeze-dried pomace, and the three dietary intervention products

| **Variable** | **Treatment group** | | | |
|---|---|---|---|---|
| | POM$ | BRJ | BRPOM pudding | PLA |
| Nitrate (mg) | 2069 | 600 | 600 | 0 |
| Carbohydrate (g) | 40.2 | 75 | 75 | 75 |
| Fat (g) | 4.4 | < 1 | 1.3 | < 1 |
| Protein (g) | 12 | < 1 | 3.5 | < 1 |
| Energy (kJ) | 1326 | 1092.7 | 1022.4 | 685.8 |
| TDF (g) | 34.5 | < 1 | 10 | < 1 |
| IDF (g) | 25.9 | < 1 | 7.5 | < 1 |
| SDF (g) | 8.6 | < 1 | 2.5 | < 1 |

BRPOM; beetroot pomace, BRJ; beetroot juice, PLA; placebo, TDF; total dietary fibre, IDF; Insoluble dietary fibre, SDF; soluble dietary fibre

[$]Based on 100 g of beetroot pomace powder

### 3.3.4 Study design and procedures

This acute, single-blind, randomised, crossover study involved participants consuming one of three intervention products: 250 ml beetroot juice (BRJ), 270g beetroot pomace pudding (BRPOM), and 250 ml placebo (PLA) given in a randomised order (using a random number generator) on three separate visits. Researchers who performed the sample and data analyses were completely blinded and absent when the participants were consuming the treatments, which were given by a separate volunteer. A minimum 7-day washout period was applied between visits to allow the normalisation of $NO_3^-$ levels in the body (Lansley et al., 2011).

At every visit, participants were instructed to arrive at the laboratory after observing at least a 10-h overnight fast (water was permitted) and refrain from excessive exercise and alcohol consumption 24 h and caffeine 6 h prior to each study visit. Participants were encouraged to eat the same meal the night prior to each visit, however no dietary recall was conducted and $NO_3^-$ intake was not monitored or restricted to investigate the effects of beetroot pomace and beetroot juice in conjunction with a normal daily oral intake). All trials were conducted in the morning at approximately the same time of the day (±2 h).

A schematic of the study protocol for the three intervention visits is illustrated in Figure 3.1. For each of the intervention visits a BG measurement was taken 5 min before (baseline) the participants consumed (within a 5 min period), one of the three intervention products. This was followed by a 3 h oral glucose tolerance test (OGTT) with BG measurements taken at 15, 30, 45, 60, 90, 120, 150 and 180 min. Blood pressure measurements were taken 5 min prior to consumption of each of the intervention products (baseline) and again at 60, 120 and 180 min. Participants remained in the laboratory for the duration of each intervention trial.

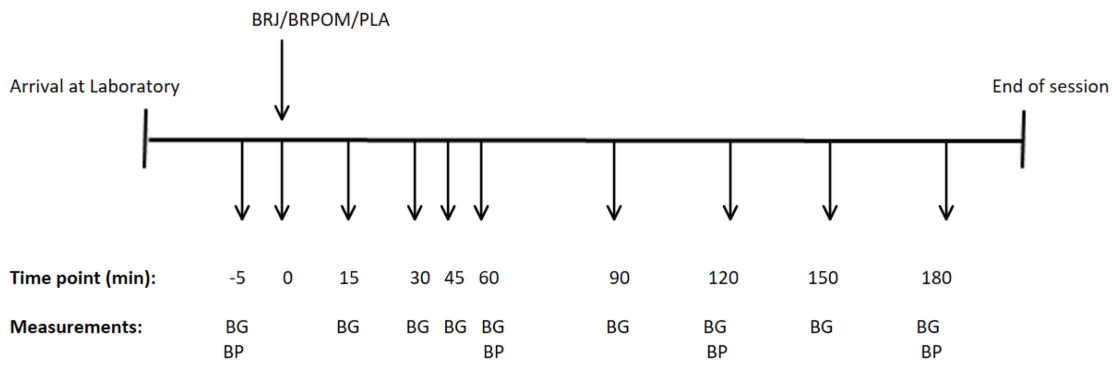



Figure 3.1. Schematic of the study protocol. Abbreviations: BRJ; beetroot juice, BRPOM; beetroot pomace, PLA; placebo, BG; blood glucose, BP; blood pressure.

### 3.3.5 HPLC analysis of nitrate

To determine the quantity of $NO_3^-$, beetroot pomace was first weighed and ground with water in a glass Potter-Elvehjem grinder. The supernatant was then removed, further water was added, and grinding was repeated two more times. All supernatants and any remaining pomace material were quantitively transferred to a tube, and the final weight of pomace/material was determined by weighing. Next, 1 ml of the extracted pomace mixture was centrifuged at 14560 *g* for 10 min and filtered using a 0.45-µm syringe filter (Thermofisher Scientific, New Zealand). For BRJ powder, it was first weighed, resuspended in a known volume of water, and then vortexed to mix well and 1 ml taken for centrifuging and filtering. All samples for both BRPOM and BRJ powder were diluted with water as required and placed in 2 ml glass HPLC vials for analysis.

Nitrate was then measured using a Gracesmart RP 18 HPLC column (Thermofisher Scientific, USA) using a mobile phase of 0.01 M Octylammonium orthophosphate solution (pH 3-3.5) at a flow rate of 0.8 mL/min at 20 °C. Nitrate was quantified at 213 nm using $NO_3^-$ external standards. Samples were analysed in triplicate.

### 3.3.6 Data and statistical analysis

**Data analysis**

Different parameters were used to analyse the response of the treatments during the OGTT. The iAUC of postprandial BG was calculated from 0 to 180 min during the OGTT using the trapezoidal rule (Wolever & Jenkins, 1986). The percentage increment of postprandial BG was calculated from 3 h postprandial BG (3hPG) in reference to the fasting blood glucose (FBG), using the formula [(3hPG-FBG)/FBG] x 100 (Carnevale Schianca et al., 2010). The time to peak BG concentration was determined by the time point during the OGTT when glucose levels were the highest. Peak glucose levels were identified as the highest BG levels during the OGTT.

For secondary analysis investigation, participants were stratified based on their baseline BG levels at the starting of each intervention treatment. Participants were stratified at cut-off values of ≤4.9 mmol/L or ≥5 mmol/L to determine whether treatment effects differed between lower and higher BG ranges.

To analyse BP responses, mean changes in SBP and DBP were analysed from baseline pre-supplementation to 120 min and 180 min post-supplementation.

Participants were also stratified according to their baseline SBP for each intervention treatment. Due to the wide range of starting BP on each intervention treatment day, participants were stratified using cut-off values of ≥124 mmHg or ≤123 mmHg to represent elevated and normal SBP groupings (respectively). These values were chosen to ensure there was a similar number of participants in each grouping within each intervention treatment for analysis.

**Statistical analysis**

All statistical analyses were completed using IBM SPSS (Version 22.0). All analyses were carried out on raw data and delta-changed data to counteract the impact of baseline variations. Data are presented as mean and standard deviation. Statistical significance was considered at $P < 0.05$. All data were examined using repeated-measures ANOVA (95% confidence interval), apart from physical features and baseline measurements. Time and treatment were under within-subject factors when looking at main effect of time. Analysis of each treatment considered pre-treatment baseline measures in comparison with 2h and 3h post-treatment BP and BG results. Relative effect sizes were calculated using eta squared and defined as small ($\eta^2_p$ = 0.01), medium ($\eta^2_p$ = 0.06), or large ($\eta^2_p$ = 0.14).

## 3.4 Results

### 3.4.1 Participants

The physical characteristics, and HbA1c concentrations of the 12 participants (7 men and 5 women, mean age 34.4 ± 8.9 years) who completed this study are reported in Table 3.3**.** Baseline BP for each participant were different before each treatment. Average baseline SBP was 122 ± 9.4, 124.5 ± 8.3 and 122.1 ± 11.4 mmHg for BRJ, BRPOM and placebo PLA, respectively. Average baseline DBP was 80.9 ± 8.8, 81.8 ± 7.6, and 80.3 ± 10.5 mmHg for BRJ, BRPOM and PLA, respectively.

Table 3. 3 Physical characteristics and HbA1c measurements of participants (n = 12)

| Characteristics | Mean ± SD |
|---|---|
| **Gender (M/F%)** | 58/42 |
| **Age (years)** | 34.4 ± 8.9 |
| **BMI (kg / m$^2$)** | 24.7 ± 3.4 |
| **HbA1c (mmol/mol)** | 34.5 ± 4.7 |
| **Body fat (%)** | 24.5 ± 9.8 |

Values are means (± SD). Abbreviations: BMI; body mass index, HbA1c; glycated haemoglobin A1c.

### 3.4.2 Glucose incremental area under the curve

The glucose iAUC for the three dietary treatments were 232.8 ± 61.9, 242 ± 54.7 and 252.4 ± 60.6 mmol/L/min for BRJ, BRPOM and PLA, respectively. There was no significant difference in mean iAUC between the three dietary treatments (p = 0.739, $\eta^2_p$ = 0.059). Additionally, there was no interaction between treatment effect and order of treatments (p = 0.667, $\eta^2_p$ = 0.204).

### 3.4.3 Time to peak glucose concentration

There was no significant difference in the mean time to achieve peak glucose concentrations between the treatments ($p = 0.269$, $\eta^2_p = 0.313$). The mean time for BG levels to peak was 26.6 ± 11.54, 33.75 ± 6.78 and 28.75 ± 13.5 min for BRJ, BRPOM and PLA, respectively (Figure 3.2).

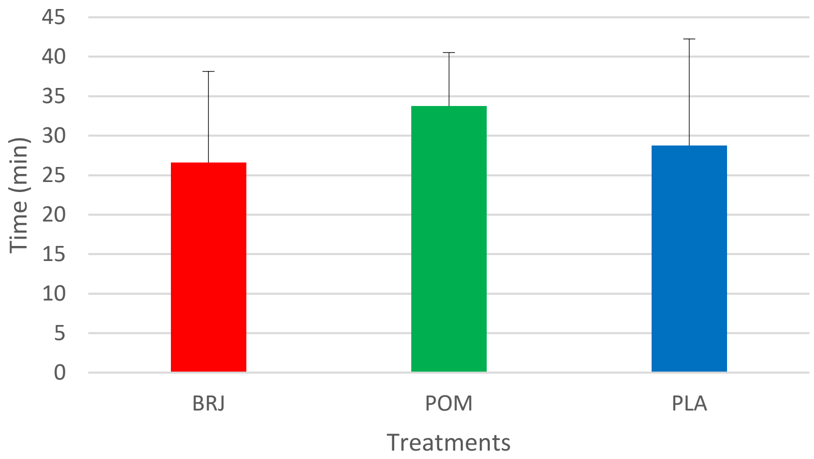



Figure 3.2 Mean peak time to peak glucose concentrations following the ingestion of beetroot juice (BRJ), beetroot pomace (POM) and placebo (PLA) treatments.

### 3.4.4 Peak glucose concentration levels

The mean peak glucose concentrations for the three dietary treatments were 8.7 ± 1.68, 9.1 ± 1.77 and 9.3 ± 1.90 mmol/L for BRJ, BRPOM and PLA, respectively. There were no significant differences in the peak glucose levels between the three treatments ($p = 0.241$, $\eta^2_p = 0.247$).

### 3.4.5 2-h and 3-h postprandial blood glucose concentration levels

At the 2-h time point during the OGTT, mean BG concentration levels were 5.1 ± 0.51, 4.8 ± 0.36 and 4.7 ± 0.48 mmol/L for BRJ, BRPOM and PLA, respectively. There were no significant differences between the three treatments ($p = 0.565$, $\eta^2_p = 0.150$).

At the 3-h time point, the mean BG concentration levels were 3.9 ± 0.26, 4.3 ± 0.35, and 4.2 ± 0.26 mmol/L for BJ, POM and PLA, respectively. There were also no significant differences in the BG concentration levels between the three dietary treatments ($p = 0.395$, $\eta^2_p = 0.233$; Figure 3.3).

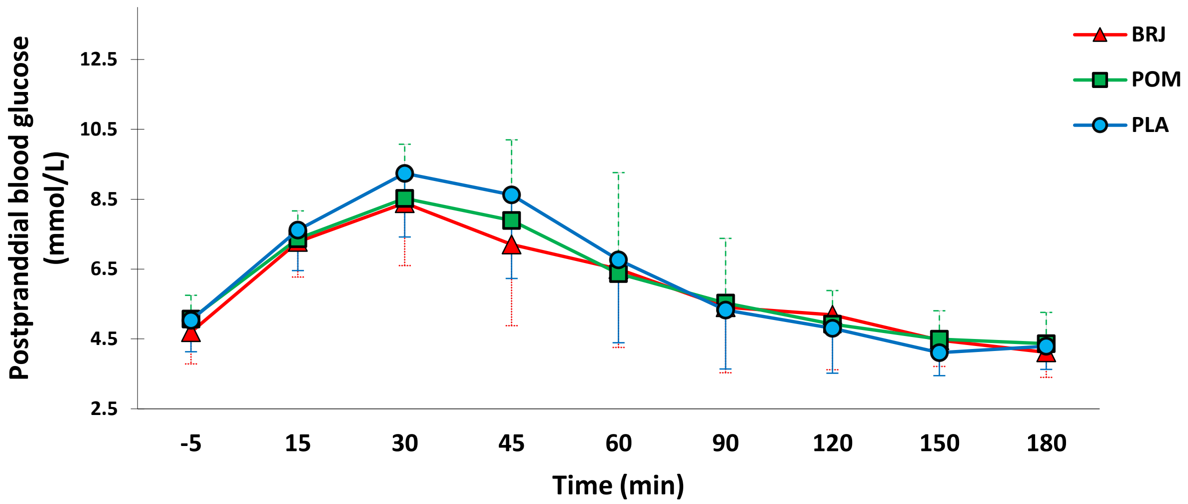



Figure 3.3 Mean postprandial blood glucose levels (±SD) before and after supplementation with beetroot juice (BRJ), beetroot pomace (POM) and placebo (PLA) treatments.

### 3.4.6 Percentage increment of postprandial blood glucose

The percent reduction in plasma glucose for the three treatments pre-supplementation to post-supplementation (180 min) were 12.7% ± 6.3%, 12.1% ± 4.8% and 10.8% ± 6.2% for BRJ, BRPOM and PLA, respectively. No significant differences in mean percent postprandial BG were observed between the three treatments ($p = 0.783$, $\eta^2_p = 0.048$).

### 3.4.7 Blood glucose secondary analysis

Participants who had a starting baseline BG level ≥ 5 mmol/L (n = 4) exhibited significant decreases in their BG levels 180 min after consumption of the BRJ treatment (-1.38 ± 0.9 mmol/L) compared to participants who had a starting BG level of ≤ 4.9 mmol/L (n = 8; -0.18 ± 0.69 mmol/L; $p = 0.025$, $\eta^2_p$ = 0.390). In the BRPOM treatment, 180 min after consumption, participants with a starting baseline BG level of ≤ 4.9 mmol/L (n = 6) showed non-significant decreases in their BG levels of 0.5 ± 0.8 mmol/L, and those with a baseline BG of ≥ 5mmol/L (n = 6) showed non-significant decreases of 0.8 ± 0.72 mmol/L ($p = 0.456$, $\eta^2_p = 0.049$). The differences in BG responses for the PLA treatment were not significantly different between participants having baseline BG levels of ≤ 4.9 mmol/L (n = 5; -0.5 ± 0.81 mmol/L) and ≥ 5mmol/L (n = 7; -0.8 ± 0.72 mmol/L; $p = 0.213$, $\eta^2_p = 0.128$; Figure 3.4).

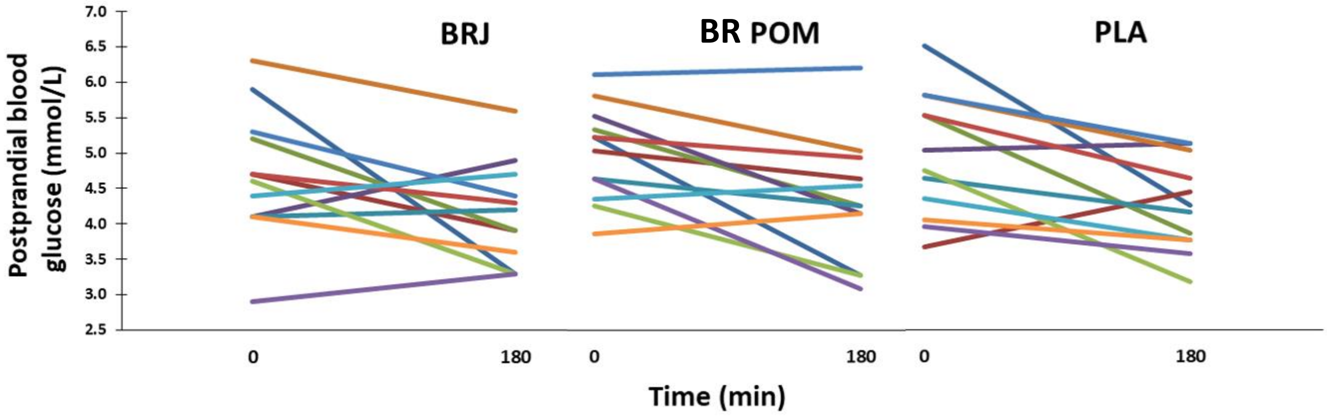



Figure 3.4 Postprandial blood glucose levels before and 180 min after supplementation for each treatment group for all participants (each colour representing a different participant). Abbreviations: BRJ: beetroot juice; BRPOM: beetroot pomace; PLA: placebo

**3.4.8 Blood pressure**

The mean SBP during the 180-min trial was 121.5 ± 2.8, 121.2 ± 2.4 and 123.5 ± 3.2 mmHg for BRJ, BRPOM and PLA, respectively. The mean DBP during the 180-min trial was 80.7 ± 2.4, 81.2 ± 2.4 and 80.7 ± 3.1 mmHg for BRJ, BRPOM and PLA, respectively.

There was no significant interaction between treatment * time for either SBP ($p = 0.297$,) or DBP ($p = 0.537$) but the effect sizes for SBP and DBP were $\eta^2_p = 0.612$ and $\eta^2_p = 0.480$; Figure 3.5.

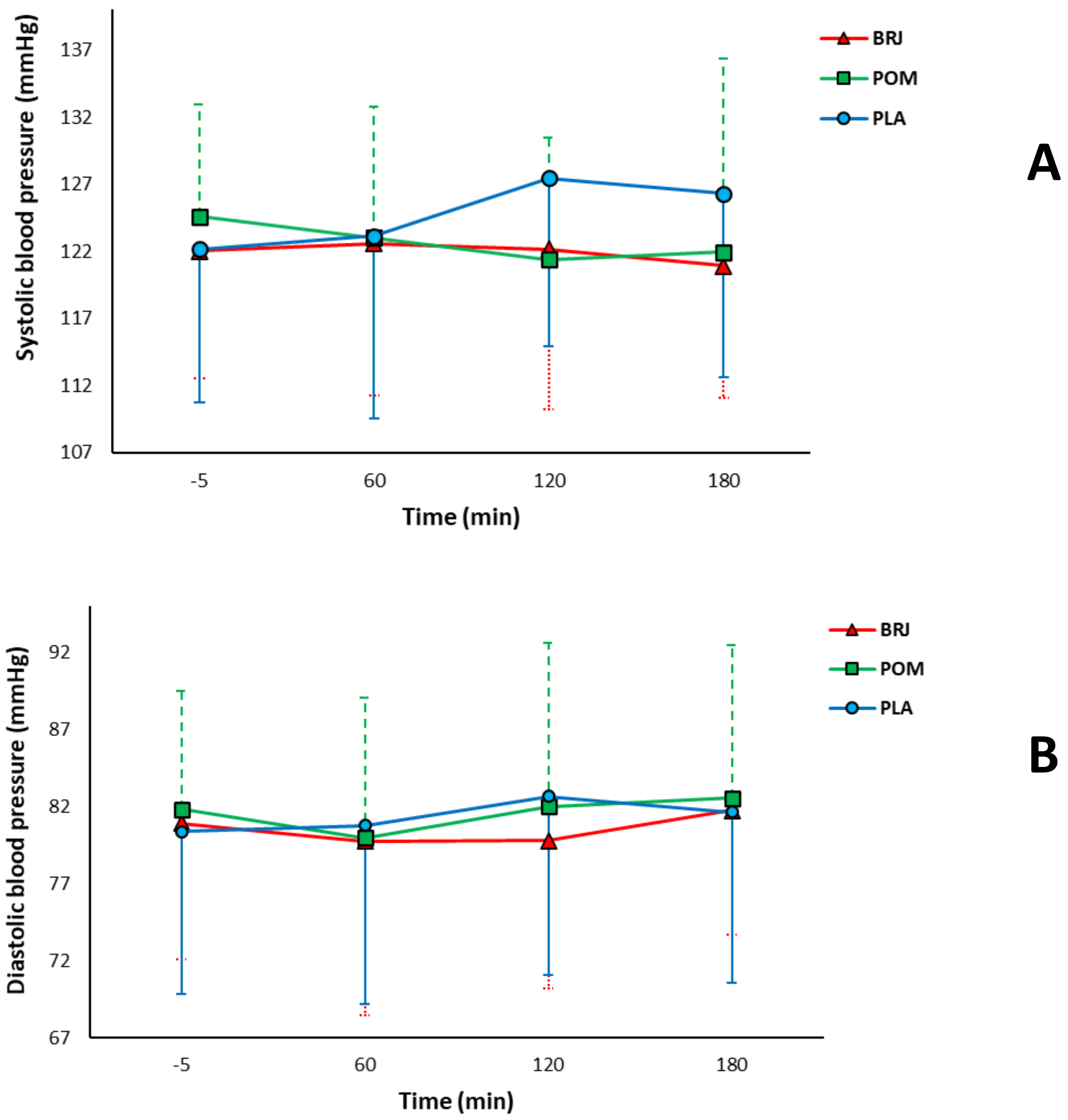
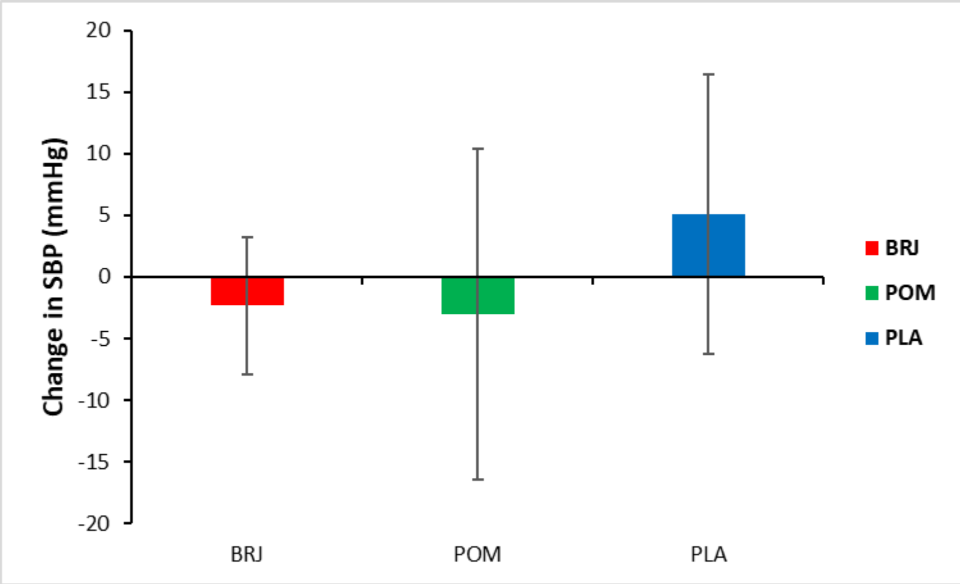


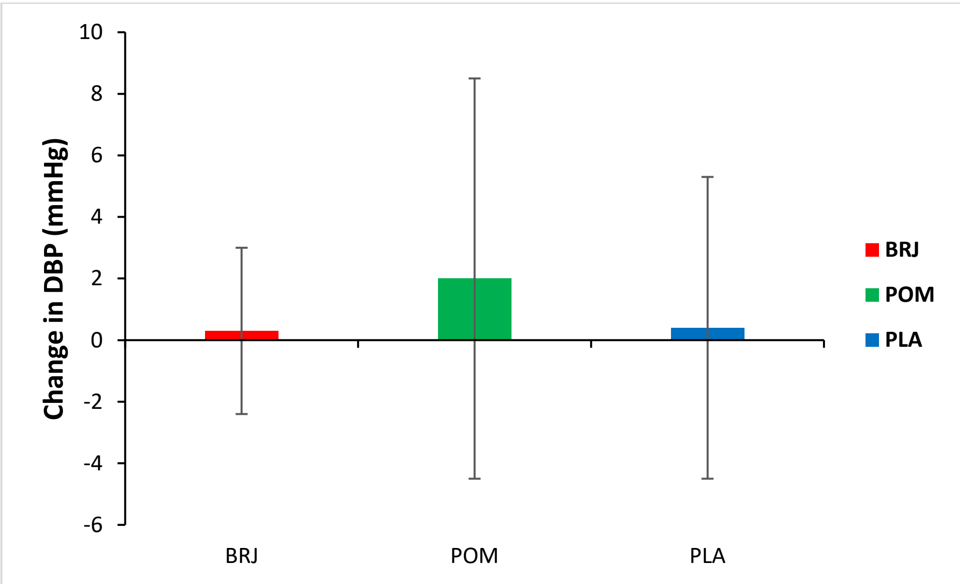

Figure 3.5 Mean (A) systolic and (B) diastolic blood pressure (±SD) pre-supplementation and over the 180 min after supplementation for each treatment group. Abbreviations: beetroot juice (BRJ), beetroot pomace (POM) and placebo (PLA).

There was a non-significant reduction in the change in SBP for both BRJ (-2.3 ± 5.56 mmHg, $p$ = 0.174) and BRPOM (-3 ± 13.4 mmHg, $p$ = 0.456) 180 min post-supplementation compared to pre-supplementation. In the PLA group the SBP increased (5.2 ± 11.3 mmHg, $p$ = 0.142) 180 min post-supplementation compared to pre-supplementation. There was a trend for interaction in the differences between the three treatments ($p$ = 0.075, $\eta^2_p$ = 0.404; Figure 3.6A).

There was no interaction effect for DBP of treatment*time for all treatments ($p$ = 0.774, $\eta^2_p$ = 0.050; Figure 3.6B).




A




B

Figure 3.6. (A) Change in systolic blood pressure (SBP) (mmHg) pre- and post-supplementation (180 min) with beetroot juice (BRJ), beetroot pomace (POM) and placebo (PLA). (B) Change in diastolic blood pressure (DBP) (mmHg) pre- and post-supplementation (180 min) with beetroot juice (BRJ), beetroot pomace (POM) and placebo (PLA).

The mean delta change in SBP from pre-supplementation to 120 min post-supplementation was significantly different between the three treatments ($p = 0.029$, $\eta^2_p = 0.507$). Although the following results were not statistically significant it is interesting to note that the consumption of BRPOM reduced SBP the most (-1.8 ± 10.9 mmHg, $p = 0.572$), followed by BRJ (-1.6 ± 7.2 mmHg, $p = 0.462$), whereas consumption of the PLA increased SBP (5 ± 8.9 mmHg, p = 0.080) at 120 min post-supplementation. There were no significant differences in change in DBP between the three dietary treatments from pre-supplementation to 120 min post-supplementation (p = 0.130, $\eta^2_p$ = 0.335).

**3.4.9 Blood pressure secondary analysis**

Following consumption of BRJ, participants with a starting baseline SBP of ≥ 124 mmHg (n = 5) exhibited a non-significant reduction (-4.6 ± 6.1 mmHg, p = 0.172, $\eta^2_p$ = 0.408) in their SBP from pre-supplementation to 180 min post-supplementation. During the BRJ treatment, participants with an initial baseline SBP of ≤ 123 mmHg (n=7) showed non-significant reductions of -0.7 ± 4.8 mmHg, p = 0.712, $\eta^2_p$ = 0.024 (180 min post-supplementation). There was no difference in the change in SBP from baseline to post-supplementation between the SBP ≤ 123 mmHg vs the SBP ≥ 124 mmHg groups in the BRJ treatment (p = 0.251, $\eta^2_p$ = 0.879)

Similar findings were observed in the BRPOM treatment, where participants with an initial baseline SBP of ≥ 124 mmHg (n=6) showed a non-significant reduction in their SBP of -4 ± 9.6 mmHg (p = 0.359, $\eta^2_p$ = 0.051) whereas participants that had a baseline SBP of ≤ 123 mmHg (n=6) exhibited a trend for lower decrease in SBP of -2 ± 17.3 mmHg (p 0.052, $\eta^2_p$ = 0.702). The change in SBP from baseline to post-supplementation between the SBP ≤123 mmHg vs the SBP ≥124 mmHg groups during the BRPOM treatment were not significant (p = 0.810, $\eta^2_p$ = 0.980).

In the PLA treatment, all participants exhibited a non-significant increase in their SBP post-supplementation (180 min) regardless of their starting baseline SBP (SBP ≤ 123 mmHg = 4 ± 12.3 mmHg, p = 0.245, $\eta^2_p$ = 0.216 vs. SBP ≥ 124 mmHg = 6.8 ± 11 mmHg, p = 0.460, $\eta^2_p$ = 0.143). When comparing the two groups together, there was no significant difference (p = 0.693, $\eta^2_p$ = 0.972) between the SBP ≤ 123 mmHg vs the SBP ≥ 124 mmHg groups post PLA supplementation.

**3.5 Discussion**

This pilot is the first study to examine the acute effects of beetroot pomace (BRPOM), beetroot juice (BRJ), and placebo (PLA) on postprandial blood pressure (BP) and blood glucose (BG) in normotensive adults. Most studies investigating the effects of food on BP and BG levels are on whole foods or juices (Bondonno et al., 2015; Lim et al., 2020; Stanaway et al., 2019; Wootton-Beard et al., 2014). One food popularly used to measure its effects on BP has been BRJ (Bondonno et al., 2015; Jajja et al., 2014; Kapil et al., 2015; Stanaway et al., 2019; Webb et al., 2008). The main active component in BRJ, nitrate ($NO_3^-$) is known to be responsible for eliciting vasodilating effects to allow greater blood flow. In this study, investigation of the potential benefits of BRPOM, the waste product after beetroot juice production, was undertaken. Beetroot pomace contains $NO_3^-$ and 100% of the fibre of whole beetroot which BRJ does not contain. Therefore, the study objectives were to measure and compare the changes during a 3-h oral glucose tolerance test (OGTT) to the BG levels after consuming either of three treatments (BRPOM, BRJ, and PLA) and to examine changes in BP over the 3-hour period. A crossover trial design was performed, and the cohort were 12 healthy adults, age range 23-48 years (mean 34.4 ± 8.9 years).

The main findings of this study demonstrate that; 1) there were no statistically significant differences between the three treatments for both BP and BG; 2) there was a trend towards differences in changes in systolic blood pressure (SBP) between the three treatments from pre-supplementation to 3 h post-supplementation ($p = 0.075$); 3) although reductions in SBP were not statistically significant in BRJ and BRPOM treatments, they appeared to be of clinical importance showing a 2-3 mmHg drop (Hess et al., 2016); 4) there were no significant differences for incremental area under the curve (iAUC), peak glucose levels, time to peak glucose level and percent increment of postprandial BG between the three treatments.

### 3.5.1 Systolic and diastolic blood pressure

This is the first study to investigate the effects of BRPOM on SBP and DBP. No significant differences were observed between the three treatments from pre-supplementation to 3 h post-supplementation. There was a trend ($p = 0.075$) in differences in changes in SBP between the three treatments from pre- to post-supplementation. This was a pilot study, and as such, a sample size calculation was not a requirement, and not conducted. However, even with a small sample size (n= 12), we observed a large effect size ($\eta^2_p > 0.14$) between the treatments.

A large effect size observes the strength of the differences between treatments, therefore, a large effect size suggests there is a substantial and practical significant difference observed between the different treatments (Sullivan & Feinn, 2012). Additionally, the amount which the BRPOM treatment decreased SBP can be considered clinically meaningful. The BRPOM treatment decreased SBP by 3 mmHg, BRJ by 2.3 mmHg whereas SBP increased by 5.2 mmHg in the PLA trial. It is important to note that even attenuations as little as 2 mmHg have been shown to have significant impact on reducing cardiovascular disease (CVD) risk in both normotensive and hypertensive individuals (Hess et al., 2016). Therefore, despite not achieving statistical significance, our findings suggest that BRPOM has promising effects in reducing SBP.

Stanaway et al. (2019) found by administering an acute dose of 651 mg dietary $NO_3^-$ to 13 younger and 11 older adults, they were able to significantly decrease SBP in both age groups and the older adults experienced greater reductions in diastolic blood pressure (DBP) compared to the younger group. In our study, there were no statistically significant changes in the DBP of the participants despite administering 600 mg $NO_3^-$ from BRJ or BRPOM. One explanation for these results may be attributed to having younger participants included in our study with an average age of 34.4 years old. The availability of nitric oxide synthase (NOS) substrate L-arginine and cofactor tetrahydrobiopterin declines naturally with ageing. The older population also have lower plasma nitrite ($NO_2^-$) (Capper et al., 2021), which is a biomarker of nitric oxide (NO) production and availability (Lansley et al., 2011). Therefore, lower levels of NOS and $NO_2^-$ indicate a potential impairment in the synthesis of NO via the NOS-NO pathway during the ageing process. Populations aged 60-70 years may experience greater advantages from dietary $NO_3^-$ supplementation as it might enhance NO bioavailability. Consequently, this could lead to more discernible effects or benefits after supplementation (Kelly et al., 2013), which was exemplified in the Stanaway et al. (2019) study in the older group. We also did not measure plasma $NO_3^-$ and $NO_2^-$, therefore it was not known whether the treatments increased plasma $NO_3^-$ and $NO_2^-$ levels in the participants. Many studies have measured plasma $NO_3^-$ and $NO_2^-$ levels (Bondonno et al., 2015; Jajja et al., 2014; Kapil et al., 2015; Stanaway et al., 2019; Webb et al., 2008), which gave them a better insight to whether $NO_3^-$ supplementation is working based on their participants' $NO_3^-$ plasma levels. However, responses to dietary supplementation may be variable amongst individuals. For instance, individuals with higher baseline plasma $NO_3^-$ levels may not experience increase in plasma $NO_3^-$ levels after $NO_3^-$ supplementation, thus may not experience the same effects compared to someone starting with a lower baseline $NO_3^-$ level. This was observed by (Coggan et al., 2018), where the women in the study were higher responders to $NO_3^-$ supplementation because they had lower baseline plasma $NO_3^-$.

A minimum amount of dietary $NO_3^-$ is required to make changes to BP, and based on current research, the threshold could be 500 mg (Ashworth & Bescos, 2017). Rogerson et al. (2022) examined the effect of BRJ concentrate (~397 mg $NO_3^-$) on healthy older and younger adults and found no effect on SBP and DBP in both older and younger groups. In contrast, (Stanaway et al., 2019) administered 651 mg $NO_3^-$ to a similar participant demographic as Rogerson et al. (2022) but their results showed reductions to SBP and DBP in the both older and younger group. In our study, we administered 600 mg $NO_3^-$ and found that BRPOM reduced SBP by 3 mmHg which is clinically relevant but had no significant effects on DBP. Therefore, the dosage of dietary $NO_3^-$ may have varying effects based on the initial baseline BP levels.

In our study, we had on average younger participants (34.4 ± 8.9 years) who were normotensive with an average baseline SBP of 121 mm Hg before each treatment. Research suggests the higher the baseline SBP, the greater attenuation of SBP following $NO_3^-$ intake from beetroot (Bahadoran et al., 2017). This could suggest why Rogerson et al. (2022) saw no effect as the average SBP baseline for the young and older participants were 106.8 and 115.1 mmHg, respectively, and baseline DBP was 66.8 and 81.3 mmHg respectively. Individuals with low baseline BP levels may have regulatory mechanisms in the body to inhibit BP from going any lower towards hypotension (Shahoud et al., 2021). This was similar to what we observed in our study where participants' regulatory mechanisms prevented them from becoming hypotensive, which is the other end of the BP spectrum we wanted to avoid. Therefore, a hypertensive population may exhibit a more pronounced response to dietary $NO_3^-$ supplementation. For example, Broxterman et al. (2019) provided a dosage 385 mg dietary $NO_3^-$ (for 3 days) from BRJ concentrate to a hypertensive adult cohort. This dosage was similar to the Rogerson et al. (2022) study. However, (Broxterman et al., 2019) found their supplement and 385 mg $NO_3^-$ dosage led to significant drop in SBP and DBP in hypertensive patients not taking antihypertensive medications. In the antihypertensive medication group, 77% of participants were taking one type of antihypertensive medication (ACEI; angiotensin-converting enzyme inhibitor, or ARB; angiotensin receptor blocker) and 23% were taking two types of different antihypertensive medications (beta receptor blocker, calcium channel blockers, and/or diuretics). Those taking antihypertensive medication did not see an effect to their BP because their starting baseline BP was already well controlled by the medication. However, participants in the Broxterman et al. (2019) study consumed the supplemente for three days, which could have led to increased and sustained blood $NO_3^-$ levels compared to the acute dose administered in Rogerson et al. (2022), thus resulting in the observed BP lowering effects.

We conducted secondary analysis to examine the different responses to the three treatments based on their baseline BP. The 12 participants were stratified into two groups: SBP ≥ 124 mmHg and ≤ 123 mmHg. This specific classification was chosen mainly for statistical purposes to ensure similar number of participants were in each group. Although the results were not statistically significant, we discovered that participants with SBP ≥ 124 mmHg had SBP reductions of ≥4 mmHg in the BRJ and BRPOM treatments. In contrast, participants with SBP ≤ 123 mmHg in the BRJ group had a non-significant 0.7 mmHg reduction, but 2 mmHg drop in the BRPOM, which is considered clinically relevant. These results indicate that BRJ and BRPOM both containing $NO_3^-$ may have potential to influence SBP when it is ≥ 124 mmHg. However, even for BRPOM when SBP was ≤ 123 mmHg, it still managed to provide a clinically relevant reduction in SBP. This suggests that BRPOM has additional factors to support attenuation of SBP, which could be attributed to its fibre content. Possible mechanisms may include TDF's role in reducing the uptake of LDL cholesterol and triglycerides, thereby enhancing the elasticity of blood vessel walls. This, in turn, decreases vascular resistance, ensuring sufficient tissue perfusion without necessitating a subsequent increase in heart rate to sustain stroke volume. Additionally, TDF intake improves insulin sensitivity which is a contributing factor to healthy endothelial function and improving hypertension (Reynolds et al., 2022).

For this study, an oral glucose tolerance test (OGTT) was conducted. Each participant consumed 75 g total carbohydrate at each treatment day. Floyd et al. (2019) hypothesised that the lack of BP-lowering action after administering a dietary $NO_3^-$ supplement was due to the acute elevations of BG and insulin. Elevations of BG and insulin may have blunted the actions of $NO_3^-$. As BG levels rise, there is a potential disruption to the insulin-mediated vasodilation process in skeletal muscle, impacting the synergistic relationship with the vasodilatory effects of NO derived from the dietary $NO_3^-$ supplement. Consequently, this disturbance may play a role to the observed inability of $NO_3^-$ to effectively lower BP in this context.

This may have occurred in our study as we measured BG levels rising after the OGTT, therefore rendering $NO_3^-$ incapable of attenuating BP levels. Future studies could investigate the administration of BRPOM as a single ingredient that is not part of an OGTT to get a better understanding of the effects on BP in an acute setting.

In conclusion, $NO_3^-$ supplementation from BRJ and BRPOM may show statistically significant results in populations with higher baseline BP and older age. This study did not show statistically significant attenuation; however, it is noteworthy that we observed clinically relevant results with large effect size comparing SBP from pre- to post-supplementation for both BRJ and BRPOM. Further research will be necessary to confirm these observations. Additionally, these results suggest further research on a larger population size with higher baseline BP and older age may see more significant results.

### 3.5.2 Blood glucose

This was the first study to investigate the effects of BRPOM on postprandial BG. Our results showed that BRPOM did not significantly alter iAUC, peak glucose concentration levels, time to reach peak glucose concentration, 2-h and 3-h postprandial BG levels, and percentage increment of postprandial BG.

In this pilot study, we only recruited 12 participants, and although we did not achieve statistical significance, there were large effect sizes for peak glucose concentrations, time to peak BG, and 2-h and 3-h BG concentration levels between the three different treatments. Large effect sizes may be attributed to large intra-individual variability, where each participant's results were vastly different compared to other participants. This variability might be influenced by gender, ethnicity, age, and dietary habits. Furthermore, inter-individual variability including differences in baseline BG at each treatment and other factors such as recent unreported strenuous physical activity within 24 hours, could have contributed to different glycaemic responses. Other studies observing reduced BG responses following intake of fruit pomaces had more than 30 participants in their study (Chen et al., 2017; Dong et al., 2016; Guzman et al., 2020; Guzman et al., 2021; Urquiaga et al., 2015). Two studies (Chen et al., 2017; Dong et al., 2016) found that the orange pomace delayed time to reach maximal glucose concentrations. Chen et al. (2017) found there was a significant attenuation to BG in most time points, whereas Dong et al. (2016) found significant attenuations in changes to pre-supplementation and peak glucose concentration. However, both studies' cohorts were overweight men with increased metabolic disease risk which may explain why there were different physiological effects compared to our study which consisted of healthy individuals. Additionally, both studies (Chen et al., 2017; Dong et al., 2016) supplemented orange pomace with a standardised breakfast meal as opposed to an OGTT. This may have contributed to the different results in BG changes compared to our study. Nevertheless, in our secondary analysis where we found participants during the BRJ treatment who started with a baseline BG of ≥ 5 mmol/L (n =4) had a significant (-1.38 ± 0.9 mmol/L; p = 0.025) reduction to their BG levels compared to others with baseline BG of ≤ 4.9 mmol/L (n = 8; -0.18 ± 0.69 mmol/L). However, only 4 people in our study had BG levels ≥ 5 mmol/L, therefore the results will not be representative of a wider population.

In our study, the BRPOM had a percentage of 75% insoluble dietary fibre (IDF) which is higher than other studies that investigated orange pomace consisting of an average of 60% IDF (Bosch-Sierra et al., 2019; Chen et al., 2017; Dong et al., 2016; Guzman et al., 2021). Soluble dietary fibre (SDF) forms a viscous gel-like substance that has been shown to help slow gastric emptying and has a greater potential of attenuating postprandial BG than IDF (Dong et al., 2019; Tabatabai & Li, 2000). It's plausible that the elevated IDF content in this present study is responsible for not yielding significant effects on BG. Studies have shown that IDF may contribute to the effects of SDF of attenuating BG by reducing digestive enzyme activity due to non-specific binding with insoluble fibre and expanding faecal volume, which aids in decreasing transit time in the colon (Goff et al., 2018). In our study, we also used a greater Total dietary fibre (TDF) dosage of 10 g, in contrast to previous studies mentioned wherein an average TDF administration of 5 g was observed. Therefore, the lack of significant effect seen in this study could be attributed to our small, healthy participant sample size.

Additionally, our study looked at the acute effects of BRPOM and not chronic intake effects. A study looking at wine grape pomace (Taladrid et al., 2022) administered 2 g per day to high-risk cardiovascular and healthy participants found it helped significantly lower fasting BG after 6 weeks. Further long-term studies will need to be performed to get a better understanding of the range of health effects of BRPOM.

Lastly, a study looking at the impact of BRJ on BG levels found that BRJ helped lower postprandial BG levels during 0-30 minutes post-supplementation (Wootton-Beard et al., 2014). This effect was attributed to the polyphenols present in BRJ. For this study, there were no statistically significant results observed for any of the glucose parameters measured. In this study, we did not measure the amount of polyphenols in the BRPOM or BRJ to compare. It might be possible that after juicing, there will be differing amounts of polyphenols in the juice compared to the pomace.  Future studies are needed to investigate impact of BRPOM polyphenol content on BG.

**3.5.3 Conclusion**

In conclusion, our study revealed no significant differences between the three treatment groups from pre-supplementation to 3h post-supplementation for both BP and BG measures. However, we did notice a trend towards statistical significance in the differences in changes in SBP following supplementation between the three treatments. We had a healthy cohort of 12 participants, which may have contributed to the lack of statistical significance. Despite this limitation, we observed large effect sizes across different measures such as differences in BP and BG measures between the three treatments. Additionally, we observed clinically relevant reductions to SBP in the BRPOM and BRJ group suggesting promising potential for BRPOM. The participants had on average a low baseline BP and BG values that may have influenced the overall outcomes. Those who had higher baseline SBP values (≥124 mmHg) saw greater clinically relevant reductions (-4.6 ± 6.1 mmHg vs -4 ± 9.6 mmHg for BRJ and BRPOM, respectively) than those who had lower baseline SBP values (≤123 mmHg; -0.7 ± 4.8 mmHg vs -2 ± 17.3 mmHg for BRJ and BRPOM, respectively). This was similar to higher baseline BG values (≥ 5 mmol/L), where the BRJ group saw a significant reduction (-1.38 ± 0.9 mmol/L) in the BG values after supplementation compared to those who had a lower BG baseline value (≤4.9 mmol/L; -0.18 ± 0.69 mmol/L).

This study has highlighted the potential of BRPOM, a by-product of juicing beetroot, in the role of helping to reduce BP and attenuate BG levels. Future work is required to investigate long term effects and impact in different populations.

## CHAPTER 4: CONCLUSION AND RECOMMENDATIONS

### 4.1 Summary

Previous research has shown that acute beetroot juice (BRJ) supplementation can help reduce blood pressure (BP), which on a population level will result in meaningful reductions in the incidence of CVD (Strachan & Rose, 1991). Some research has shown BRJ also has the potential to attenuate blood glucose levels (BG) in healthy adults. These results were linked to the high dietary nitrate ($NO_3^-$) and polyphenol content found in BRJ. Dietary $NO_3^-$, via an exogenous pathway, creating a natural vasodilator, nitric oxide (NO). However, during the production of BRJ, a considerable amount of beetroot pomace (BRPOM) is leftover which is normally taken to landfills or used as feed in animal agriculture. Beetroot pomace not only contains $NO_3^-$ but is also a good source of dietary fibre - which plays a significant role in BG regulation.

The aim of this pilot study was to evaluate and compare the acute effects of BRPOM, BRJ and placebo on postprandial BP and BG in healthy adults in an acute randomized, cross-over trial.

The first objective of this study was to investigate and compare the changes in BP in the 3-h oral glucose tolerance test (OGTT) following the ingestion of BRPOM, BRJ or an isocaloric placebo. The amount in which BRPOM decreased SBP is considered clinically meaningful (>2 mmHg)(Hess et al., 2016) which was similar to the amount in which BRJ decreased systolic blood pressure (SBP) in this study. The clinically relevant results were also supported by a large effect size. The results however, showed no statistically significant difference between the three groups, but there was a trend towards significance ($p = 0.075$) in the differences in changes in SBP between the three groups.

The second objective of this study was to investigate and compare the changes in BG in the 3-h OGTT following the ingestion of BRPOM, BRJ, or placebo. Despite having large effect sizes for some of the BG markers, there were no significant differences between the three groups for mean glucose incremental area under the curve (iAUC), peak time, peak value, 2-h and 3-h values, and percentage increment.

We hypothesised that BRPOM would have a similar decrease in BP (SBP and diastolic blood pressure; DBP) compared to BRJ post-supplementation, but a greater decrease compared to PLA. In our findings, both BRJ and BRPOM had similar non-significant reductions in SBP, -2.3 and -3 mmHg respectively. Diastolic BP had similar non-significant increase for both BRJ and POM (0.3 and 0.4

mmHg, respectively). Furthermore, BRPOM also had a greater non-significant decrease compared to PLA (-3 mmHg and 5.1 mmHg, respectively). Therefore, our results support the hypothesis.

The second hypothesis was that BRPOM would lead to greater decreases in BG markers compared to BRJ and PLA due to its additional fibre content. Our results did not support this hypothesis because in all BG markers, BRPOM did not lead to greater decreases compared to BRJ and PLA.

**4.2 Strengths**

To date, no research has investigated the health benefits of consuming BRPOM specifically in relation to its effects on BP and BG levels in humans. Furthermore, there is limited research looking at dietary $NO_3^-$ and fibre together. This study builds on existing research looking at the cardioprotective mechanisms of dietary $NO_3^-$ and the BG regulating properties of fibre, individually and synergistically.

A further strength of this study was the use of three different treatments which were BRJ, BRPOM and PLA, to investigate the differences in BP and BG responses. Beetroot juice is widely researched for its vasodilating properties, therefore investigating whether BRPOM would have similar or better outcomes was able to highlight the relative effect of BRJ compared to BRPOM intake.

Furthermore, each treatment contained 75 g carbohydrates, ensuring uniformity amongst each treatment, creating a controlled setting that minimizes potential interactions that could affect carbohydrate digestion.

Another strength of this study was that the BRPOM had a dietary $NO_3^-$ dosage that was above the recommended minimum threshold (Ashworth & Bescos, 2017) and a similar dosage to Stanaway et al. (2019) who found that BRJ consumption decreased SBP and DBP in both older and younger adults. Using a similar dosage to Stanaway et al. (2019) study helped create similar testing conditions thus making it easier to compare the differences and similarities in the results.

Lastly, the utilisation of a single-blind, randomised, crossover design made the study more robust and eliminated potential biases. For example, the randomised order of treatment helped reduce the risk of selection bias, and researchers were blinded to the treatment, therefore, bias in the allocation of treatment and assessment of results were reduced. Furthermore, the crossover design allowed each participant to serve as their own control.

**4.3 Limitations**

Although the study had its strengths, it is also important to acknowledge the limitations. Firstly, as a pilot study with a small sample size, means that further studies would need to be conducted before any findings could be generalized to a wider population, and any population-based recommendations could be made. Additionally, the participants were generally healthy adults therefore results may not be transferrable to a hypertensive and/or hyperglycaemic population. Our secondary analysis findings showed those who had higher baseline SBP values, saw more non-significant reductions post-supplementation compared to those who had lower baseline SBP values in both the BRPOM and BRJ group. Therefore, the findings show greater potential in a hypertensive population. Furthermore, the sample size was based on previous studies using similar methodologies (Huang et al., 2019; Stanaway et al., 2019; Webb et al., 2008). Despite having a small, sample size of healthy volunteers this study was able to detect clinically relevant results for BP markers.

Another limitation was the consumption of BRPOM. Some participants found it challenging to consume the given volume. This was maybe due to the high fibre content of the BRPOM, which induced early satiety and resulted in some participants' inability to finish the treatment meal. However, this limitation may lead to further exploration of different methods of consumption for future research.

Another limitation was that we did not measure participants' plasma $NO_3^-$ or $NO_2^-$ levels. This made it challenging to determine whether the consumption of BRJ or BRPOM effectively increased plasma $NO_3^-$ and $NO_2^-$ levels and identifying potential non-responders within the sample. Furthermore, this study did not analyse the polyphenol content of BRPOM, which may have given more insight into the findings as to whether the presence of polyphenols would play a role in the results. However, this was only a pilot study which involved non-invasive procedures, and primary focus was on dietary $NO_3^-$ and fibre; therefore, future studies can delve into further analysis of the effects of BRPOM.

Lastly, this study only examined the acute effects of BRPOM consumption and not chronic effects. Some studies showed long-term effects such as significant reductions to BP and BG associated with other pomace consumption and BRJ (Bondonno et al., 2015; Jajja et al., 2014; Taladrid et al., 2022; Urquiaga et al., 2015). Therefore, chronic intake could give a more comprehensive understanding of the effects of BRPOM.

### 4.4 Future directions and recommendations

Given the high incidence of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) in New Zealand, it is imperative to find interventions that can actively manage and reduce the risks of developing CVD and T2DM. This study showed no statistical significance for any of the BP or BG markers. Nonetheless, there are several factors that may have influenced the lack of statistical significance including the sample's population size, average age and health state. Additionally, based on a previous paper, (Floyd et al., 2019), the researchers suggested that the acute elevations in BG may hinder the actions of $NO_3^-$. This may have occurred in this study, given that measurements of BP and BG were taken during an OGTT. Furthermore, studies suggest that chronic daily intake of fibre was associated with better BG control in the long term (Kabisch et al., 2021). Based on these factors, we have formed recommendations for future studies:

**Recommendations**

1. Further research is required on a larger sample size to allow extrapolation of findings to a population.

2. For this study, the majority of the participants were younger than 35 years who were normotensive and normoglycaemic. Future research is required to investigate the effects of BRPOM on an older population and/or populations at risk of CVD and T2DM to produce applicable findings for at-risk populations.

3. Future research is required to create a BRPOM product that is easier to consume while containing the required $NO_3^-$ and TDF similar to this study.

4. Future research investigating plasma changes to $NO_3^-$ and $NO_2^-$ after BRPOM consumption to determine whether BRPOM is affecting plasma $NO_3^-$ and $NO_2^-$ levels to elicit changes to BP.

5. Future research is required to investigate the effects of chronic supplementation of BRPOM, as CVD and T2DM is a prolonged condition that requires long-term management.

6. Future research is required to investigate the effects of BRPOM supplementation on BP not part of an OGTT where there is an acute BG elevation.

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## Appendices

### Appendix A: Recruitment material/poster

# Interested in being part of Scientific Research?

The School of Health Sciences at Massey University is conducting a study on blood pressure and blood sugar responses to beetroot pulp (produced after juicing) in people aged between **20-60 years**, living in **Auckland.**

**All participants will receive**

- **FREE body composition, blood pressure and blood sugar assessments**
- **FREE snacks and drinks for three visits**
- **$45 on completion of three trial visits***

*** in form of Westfield voucher**

## Can you help?

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

- Make **three*** visits from **7:00-8:30am** to the Massey University Nutrition Laboratory in Albany, Auckland
- Each trial takes **3.5 hours** to complete.
- Finger prick blood samples and blood pressure measurements will be taken.
- Eat three different foods/beverages. We will see if these will help control blood pressure and blood sugar levels

* Screening visit and 3 trial vists

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 21/10. 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.

For further information or to express interest, email egorbani@massey.ac.nz or phone 0226708797
Please pass this information on to anyone you know who could be interested.

Beetroot pulp Study
Email:
egorbani@massey.ac.nz
Phone: 0226708797

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Appendix B: Participant information sheet

Participant Information Sheet

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

| | | | |
|---|---|---|---|
| Study title: | **Pilot study to investigate consumption of beetroot pomace on acute blood pressure and post prandial blood glucose responses** | | |
| Location: | **Massey University Albany Campus, Auckland** | Ethics committee ref.: | |

**Researcher Introduction and invitation to take part**

My name is Elnaz Gorbani, and I am a dietetic Masters student at Massey University, School of Health Sciences. My supervisors are A/Prof Kay Rutherfurd-Markwick, A/Prof Rachel Page, Dr Cheryl Gammon, Prof Marie Wong and Prof Ajmol Ali

You are invited to take part in a pilot study to investigate consumption of beetroot pomace on acute blood pressure and post prandial blood glucose responses. 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.

## WHAT IS THE PURPOSE OF THE STUDY?

Diabetes and cardiovascular disease (CVD) are leading causes of death both worldwide and in New Zealand. High blood pressure is a key risk factor for both CVD and type 2 diabetes (T2D). Individuals with T2D have problems maintaining their blood sugar levels within normal levels.

Beetroot juice has been shown to reduce blood pressure and blood sugar responses in some studies. This may be due to the high levels of inorganic nitrate and other substances such as antioxidants and fibre. The process of juicing beetroot leaves a pulpy by-product called pomace. There have been no studies thus far looking at whether beetroot pomace provides benefits to blood pressure and blood sugar responses. Therefore, this study aims to determine whether blood pressure and blood sugar levels in healthy participants can be improved when consuming beetroot pomace alongside a typical breakfast meal.

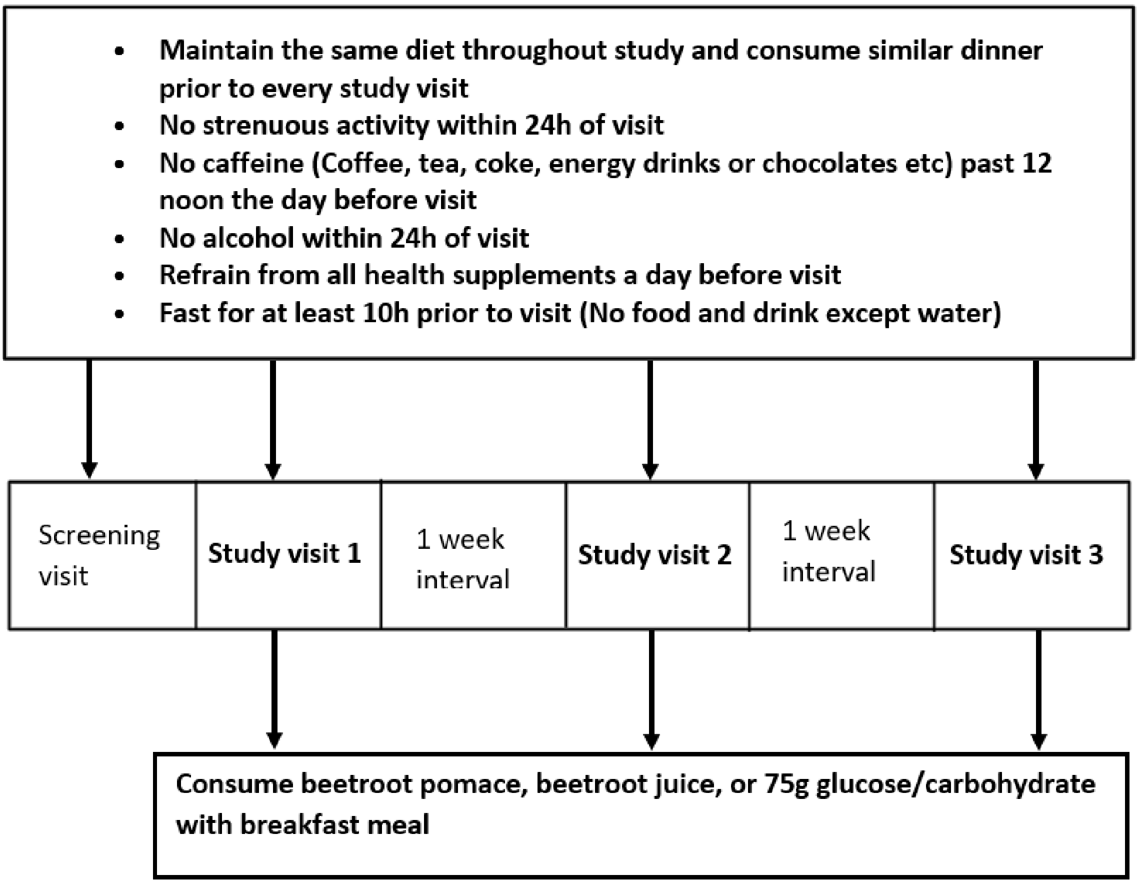
## WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

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

- 20-60 years of age
- BMI 18.5-30.0 kg/ $m^2$
- Fasting glucose < 5.7 mmol/ L
- HbA1c <40 mmol/mol
- Blood pressure >120/80 mmHg
- Not taking any medications that include blood glucose/ sugar lowering prescriptions
- Not taking nitrate-based dietary supplements
- Not pregnant or breastfeeding
- Not allergic to beetroot
- Non-smoker
- Generally healthy and not suffering from cardiovascular disease or diabetes
- Able to communicate well in English

The study will involve you coming to the Massey University Nutrition Research Facility, located at the Albany campus. If you are eligible after the screening visit, you will need to be available for **THREE** additional mornings during a three week study period. Each of these three visits will take approximately 3 hours from 7-7:30 – 9:30-10:00 am in the morning 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**

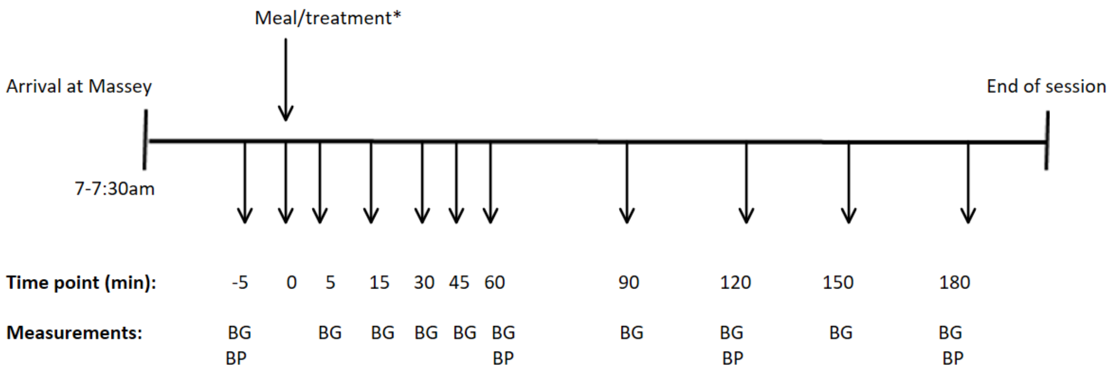



**<u>Screening visit</u>**

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 after at least a 10-hour overnight fast, to have a screening fasted blood sample collected. The session should take less than one hour. Trained personnel will measure your fasting blood glucose, and HbA1c levels (the latter is a measure of how stable your blood sugar levels have been over the last three months) using a finger prick blood sample. If your results indicate that you have glucose tolerance issues you will NOT be able to participate.

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 1 to 3**




Study visit protocol
*Meal/treatment comprises of either BRP, BJ, or glucose drink.
BRP= beetroot pomace; BJ = beetroot juice; BG = blood glucose; BP = Blood pressure.

During the study visits (visits 1-3), one or two finger-prick blood samples will be taken to measure your baseline blood sugar level. You will then consume a meal containing 75g of carbohydrate in total and including either placebo, beetroot juice or beetroot pomace . Further finger prick blood samples will be taken over the next 180 minutes (see diagram) after consuming the meal, to measure your blood sugar levels. Blood pressure (BP) will also measured hourly. You will need to remain in the research facility during this study period. 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?

Research is a big part of medicine and healthcare and being involved can be very rewarding. You can learn a lot about the processes that are involved in research by actively participating and it can be satisfying to know that you are contributing to knowledge. Furthermore, by participating in the study, you will also obtain information about your general health status, HbA1c, body composition and blood pressure measurement entirely at no cost to you. In addition, there will be a reimbursement for involvement in the study.

It is highly unlikely that you will be injured during this study. Finger prick blood sampling is safe and routinely used practices. Qualified personnel and a 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 sampling.

## WHO PAYS FOR THE STUDY?

In recognition of your time and participation in this study, you will be reimbursed $15 for completion of each trial session. You will be reimbursed a total of $45 for completing the whole study (3 study visits) in the form of vouchers. If for any reason you are unable to complete the study, you will be reimbursed for the visits you completed.

## 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, without experiencing any disadvantage.

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 are able to withdraw from the study at any time and will be compensated accordingly for your time. 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 pull out of the study all of the data that was related to you will be shredded.

The treatment intervention (beetroot pomace) 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:

**Elnaz Gorbani:** Master dietetic student, School of Health Sciences, Massey University, Albany

Phone: +64 226708797

Email: e.gorbani@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:** Professor, School of Sport, Exercise and Nutrition, Massey University, Albany

Phone: 09 213 6414

Email: A.Ali@massey.ac.nz

**Dr Cheryl Gammon:**

Phone: 09 213 6437

Email: C.Gammon@massey.ac.nz

**Prof Marie Wong:**

Phone: 09 213 36656

Email: M.Wong@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 C: Consent form**

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UNIVERSITY OF NEW ZEALAND

## <u>Consent for Participation</u>

**Pilot study to investigate consumption of beetroot pomace on acute blood pressure and post prandial blood glucose responses**

☐ 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 D: Health screening questionnaire**

**Participant Screening Questionnaire**

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UNIVERSITY OF NEW ZEALAND

# Pilot study to investigate consumption of beetroot pomace on acute blood pressure and post prandial blood glucose responses

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

**PRINCIPAL INVESTIGATORS:**

**Elnaz Gorbani**

**Assoc Prof Kay Rutherfurd-Markwick**

**Assoc Prof Rachel Page**

**Prof Ajmol Ali**

**Dr Cheryl Gammon**

**Prof Marie Wong**

## Inclusion Criteria:

1. Are you aged 20-60? 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. Are you taking any medications that include blood pressure lowering prescriptions? YES / NO
5. Are you taking any nitrate-based dietary supplements? YES / NO
6. Do you have any allergies to beetroot? YES / NO

7. 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:

Appendix E: Case record sheet

# CASE RECORD FORM

# Pilot study to investigate consumption of beetroot pomace on acute blood pressure and post prandial blood glucose responses


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

**PRINCIPAL INVESTIGATORS:**

Elnaz Gorbani

Assoc Prof Kay Rutherfurd-Markwick

Prof Ajmol Ali

Assoc Prof Rachel Page

Dr Cheryl Gammon

Prof Marie Wong


**Participant Initials:**

| | | |
|---|---|---|
| | | |

**Participant ID Number:**

| | | |
|---|---|---|
| | | |

**Date of Birth:**

______________________________________

DD MM YYYY

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

## Inclusion Criteria:

1. Is the participant aged 20-60? YES / NO
2. Does the participant have a BMI between 18.5-30.0? 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. Not taking any medications that include blood pressure lowering prescriptions? YES / NO
5. Not taking nitrate-based dietary supplements? YES / NO
6. Does the participant have any allergies to beetroot? YES / NO
7. 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: ______________________ **FASTING GLUCOSE** ___________________

Time of Collection: ______________________ **HbA1c** ________________________

Date of Collection: ______________________

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)

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 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Intervention | Date | -5 | 5 | 15 | 30 | 45 | 60 | 120 | 150 | 180 |
| Placebo | | | | | | | | | | |
| Beetroot pomace | | | | | | | | | | |
| Beetroot powder Juice | | | | | | | | | | |

## Blood Pressure

| Intervention | Date | -5 | 60 | 120 | 180 |
|---|---|---|---|---|---|
| Placebo | | | | | |
| Beetroot pomace | | | | | |
| Beetroot powder Juice | | | | | |

**Remarks:**

________________________________________________________________________

________________________________________________________________________

## Appendix F: Detailed beetroot pomace development

Beetroots were washed, and had the leaves and tails removed on the day of harvest. The beetroot was then juiced in a juicer (Atlas Pro Whole Slow Juicer, Biochef, Australia). The pomace was collected from the juicer and spread out onto plastic sheets and frozen overnight at -23°C. The pomace was then freeze dried at 25°C for 80 to 100 hours (Labconco Freeze Dryer, USA). After drying, the pomace was grounded to produce a fine powder (Coffee and spice grinder, Breville, Australia) then packed into foil vacuum bags and heat sealed. The sealed bags of powder were then heated to 90°C for 5 mins in a hot water bath to sterilise/pasteurise the powder (Grant Instruments, USA), cooled to room temperature and stored at -23°C. The microbial safety of the pomace was determined by carrying out aerobic plate count, presence of yeast and mould, lactic acid bacteria, Staphylococcus aureus, and spores' tests. The enumeration of yeast and mould, lactic acid bacteria and aerobic plate counts were achieved by plating serial-diluted samples on appropriate selective media-yeast extract glucose chloramphenicol agar (YGC), standard plate count (SPC), and deMan Rogosa and Sharpe (MRS) media. All the experiments were done in triplicate with aseptic techniques. The media was prepared according to the manufacturer's instruction.

## Appendix G: Standard of operation – fingerprick samples

UNIVERSITY OF NEW ZEALAND

COLLEGE OF HEALTH

TE KURA HAUORA TANGATA

# SCHOOL OF SPORT, EXERCISE AND NUTRITION RESEARCH STUDIES LABORATORY PROCEDURE

# Finger Prick

**Always use universal safety precautions.**

1. Collect supplies.

2. Position hand palm-side up. Choose whichever finger is least calloused.

3. Apply intermittent pressure to the finger to help the blood flow.

4. Clean the fingertip with alcohol. Start in the middle and work outward to prevent contaminating the area. Allow the area to dry.

5. Hold the finger and firmly place a new sterile lancet off-centre on the fingertip.

6. Firmly press the lancet to puncture the fingertip.

7. Wipe away the first drop of blood with a sterile gauze pad or cotton ball.

8. Collect the specimen. Blood may flow best if the finger is held lower than the elbow.

9. Apply a gauze pad or cotton ball to the puncture site until the bleeding stops.

10. Properly dispose of all contaminated supplies.

Use of trade names and commercial sources is for identification only and does not imply endorsement by WHO, the Public Health Service, or by the U.S. Department of Health and Human Services (2005).

WHO

**Appendix H: Standard of operation – Anthropometry**

# Beetroot Pomace Study (2021)

## Protocol for all anthropometric measures

**(adapted from International Society for the Advancement of Kinanthropometry (ISAK) protocol)**

- Written informed consent must be obtained from all subjects
- Subject should wear suitable clothing (no shoes, light clothing).
- All measurements should be taken in privacy.
- An explanation of the procedures should be given to each subject before measurements are taken including what measurements will be taken and how.
- Take measurements from the side or behind where possible.
- Record measurements on a sheet. Where possible a recorder should be used to assist the measurer and enter data.
- For girth measurements two measurements should be taken at each site. A $3^{rd}$ measure should be taken where the $2^{nd}$ measure is not within 1cm of the first. The mean value is used in any further calculations if two measurements are taken. If three measurements are taken the median value is taken.
- Obtain a complete set of data before repeating the measurements for the second and third time
- Take all measurements to 1 decimal place (eg. 67.6kg, 161.4cm)
- Order of measurements:
    - Measure height using stadiometer once (ensure calibrated prior to the measurement – dot needs to line up at 156.3cm)
    - Weight using Wedderburn scales once (these are the large scales plugged into the wall)
    - Measure waist and hip circumferences using a tape measure (Lufkin) (twice or 3 times if more than 1cm difference)

**Height**

Measure height using a standard procedure (check hair, feet, looking straight ahead, breathing normally – then read measurement).

**Weight**

Ensure participant is lightly clothed (remove heavy jackets, shoes, keys from pockets, etc). Ensure scales are placed on lino or a wooden board (not on carpet, if relevant). Use Wedderburn scales.

**Waist and hip circumferences**

**Equipment needed -** Lufkin W600PM flexible steel tape

**Measurement -** Use ISAK anthropometric protocol for both waist and hip measurements (ensure tape is upright and that you are measuring in cm)

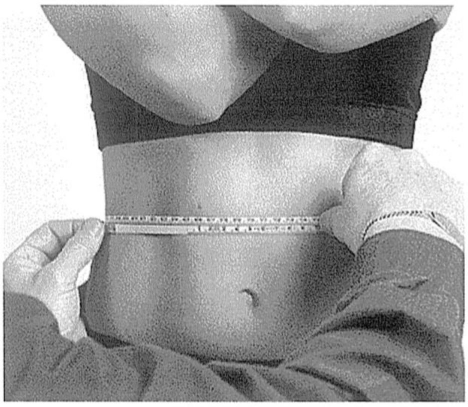

Figure 54. Waist girth.

### 19 Waist®

*Definition:* The circumference of the abdomen at its narrowest point between the lower costal (10th rib) border and the top of the iliac crest, perpendicular to the long axis of the trunk.

*Subject position:* The subject assumes a relaxed standing position with the arms folded across the thorax.

*Method:* The anthropometrist stands in front of the subject who abducts the arms slightly allowing the tape to be passed around the abdomen. The stub of the tape and the housing are then both held in the right hand while the anthropometrist uses the left hand to adjust the level of the tape at the back to the adjudged level of the narrowest point. The anthropometrist resumes control of the stub with the left hand and using the cross-hand technique positions the tape in front at the target level. The subject should breathe normally and the measurement is taken at the end of a normal expiration (end tidal). If there is no obvious narrowing the measurement is taken at the mid-point between the lower costal (10th rib) border and the iliac crest.

Figure 55. Gluteal (hip) girth.

## 20 Gluteal (hip)®

*Definition:* The circumference of the buttocks at the level of their greatest posterior protuberance, perpendicular to the long axis of the trunk.

*Subject position:* The subject assumes a relaxed standing position with the arms folded across the thorax. The subject's feet should be together and the gluteal muscles relaxed.

*Method:* The anthropometrist passes the tape around the hips from the side. The stub of the tape and the housing are then both held in the right hand while the anthropometrist uses the left hand to adjust the level of the tape at the back to the adjudged level of the greatest posterior protuberance of the buttocks. The anthropometrist resumes control of the stub with the left hand, and using the cross-hand technique, positions the tape at the side, checking that it is held in a horizontal plane at the target level, before taking the measurement.

**Appendix I: Standard of operation – BIA**

BIA Scales (Inbody 230)

**Purpose:** The purpose of the Bioelectrical Impedance Analyser (BIA) is to measure a participant's body composition, specifically their body fat.

**Advantages:** The advantages of using this particular BIA, is that it is easy to use, compactible, and can fold away. It also has an advantage as it is simple and quick to perform.

**Disadvantages:** Other equipment such as DEXA will give more accurate results as these results are not as accurate. It also cannot be taken outside in the field, as it needs to be plugged in to a power source and is not waterproof.

**Method:**

1. Switch on the machine from the back (on/off switch).
2. Wait until the screen starts up.

3. Stand on the electrodes, bare feet only (see step 9 picture).
4. Wait until the weight (kg) calculates.
5. Enter ID number.
6. Then enter your age and height (cm) into the machine.
7. Enter your gender by pressing the male/female button.
8. Once this information has been entered you then press enter.
9. Next step you must hold rods with thumb on the electrodes.

10. Stand with arms away from body and wait until the machine is 100% calibrated.


11. Now you can take your hands off the rods but feet must stay on the machine.
12. Take down your recordings of your proposed BMI (Body Mass Index), SMM (Skeletal Muscle Mass), FAT, WHR (Weight to height ratio) and Body mass (Weight in kg).
13. Now you can come off the machine and it will be ready for the next person.
14. To calculate your body fat percentage, divide the FAT recording by the Body Mass recording and times this number by 100.

e.g FAT/Body Mass= X x 100= Y

e.g 16.7/60.7= 0.275 x 100= 27.5% Body Fat.