Acute effects of beetroot juice ingestion on blood flow and blood pressure in diabetic patients with early-stage peripheral artery disease

Josephine. S. Tityiwe[1, 2] Gillian. Crofts[2] Anne, Newton-Hughes[2] Godfrey. Azangwe[1] Paul. Comfort[2]

[1]Radiography Department, National University of Science and Technology, Bulawayo, Zimbabwe
[2] School of Health and Society, University of Salford, Greater Manchester, UK

Email: josephine.tityiwe@nust.ac.zw

ABSTRACT

The objective of the study was to determine the acute effects of beetroot juice ingestion on blood flow and blood pressure in diabetic patients with early-stage peripheral arterial disease compared to non-diabetic controls. In this quasi-experimental cohort study of 35 Black-African diabetic patients and 36 non-diabetic controls, peak systolic velocity (PSV), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were utilised to assess blood flow in the popliteal arteries (PA) basally, 90 minutes, 150 minutes, and 210 minutes-post-ingestion of beetroot juice both between and within groups. A two-way analysis of variance with Benferroni post-hoc analysis were performed to compare the two groups across 4-time points after the intake of beetroot juice. One sample and two-sample t-tests with Cohen's *d* effects sizes were performed to determine whether any changes in dependant variables were significant and meaningful within and between groups. Within groups, PSV, SBP and DBP reduced significantly and meaningfully during baseline to 90 minutes and 150 minutes-210 minutes' time points *(P ≤0.02; d ≤1.70)*. However, no significant or meaningful change *(P ≤0.9; d ≤0.29)* occurred in PSV, SBP and DBP during the 90 minutes to 150 minutes' time point. Between groups, PSV and DBP were significantly and meaningfully higher *(P ≤0.04; d ≤1.95)* in diabetic patients at baseline. At 90 minutes and 150 minutes PSV remained higher in diabetic patients *(P ≤0.04; d ≤1.30)* unlike SBP *(P ≤0.8; d ≤0.34)*. At 210 minutes, PSV and SBP did not change significantly or meaningfully *(P ≤0.59; d ≤0.18)* between groups while DBP showed no significant or meaningful difference *(P ≤0.7; d ≤0.33)* between the groups at all the time points. The combined group effects were significant for PSV *(diff ≤ 20.0 cm/s; P <0.0001)* across all the time points except between 90 minutes to 150 minutes *(diff = 0.4cm/s; P = 1.0)* The combined group effects were significant for SBP *(diff ≤ 22.01mmHg; P <0.0001)* amongst all the time points except 90 minutes to 150 minutes time point *(diff = 1.2 mmHg; P = 1.00)* and finally the combined group effects for DBP were significant and meaningful *(diff ≤13.4 mmHg; P <0.0001)* amongst all the time points except 90 minutes to 150 minutes *(diff = 1.34 mmHg; P = 1.00)* after beetroot juice ingestions. The acute effects of beetroot juice on the blood flow of the popliteal artery were reflected as lowered PSV, SBP and DBP during the 150-210 minutes' time point in both groups.

Keywords: peripheral arterial disease, nitrite, nitric oxide, peak systolic velocity, systolic blood pressure, diastolic blood pressure.

Received: 10.01.21 **Accepted:** 18.03.21

1. Introduction

Type 2 Diabetes mellitus is a risk factor for cardiovascular complications which include peripheral arterial disease (PAD) and microvascular complications which include retinopathy, nephropathy, cerebrovascular disease erectile dysfunction to mention a few. This is mainly due to atherosclerosis/plaque build-up which occurs in the endothelium of the blood vessels of these diabetic patients (Steinberg, 2009; Steinberg and Wizturn, 2010). Chronically as atherosclerotic lesions are progressively deposited in the lumen of arteries in diabetic patients the process then becomes PAD and usually starts manifesting significantly in small diameter lower limb arteries below the knees. This gradual arterial stenosis due to plaque build-up in the endothelium manifests following endothelial cells injury by diabetes. This injury of the endothelium impairs the nitric Oxide-L-arginine

pathway thus reducing the bioavailability of nitric oxide which forms the anti-oxidation defence system to clear away reactive oxygen species, low-density lipoproteins and free radicals which are mostly produced during a host of defence and immunologic reactions by activated macrophages, preventing them from continually aggregating in the endothelium (Steinberg, 2009; Steinberg and Wizturn, 2010). This altered L-arginine-nitric oxide pathway and impaired nitric oxide bioavailability when uncontrolled it contributes to an acceleration of complications such as PAD, Hypertension and Cardiovascular diseases (Umans and Levi, 1995; Davignon and Ganz, 2004; Bahadoran et al., 2015; Siervo et al., 2013).

The UK and USA guidelines on adult Diabetes management outline the prescription of Aspirin/Clopidogrel as antiplatelet therapy, besides advising on smoking cessation, healthy eating of foods with high fibre, and foods with low glycaemic index sources of carbohydrate, increasing physical activity and exercise and self-monitored feet care (Eisenstein et al., 2017; Type 2 Diabetes in adults: management (NG 28), NICE, 2015). Diets containing natural inorganic nitrate have were found to be exogenous sources for the much-needed nitric oxide in patients suffering from highly inflammatory and oxidative diseases like Type 2 diabetes and the Evidence has shown that these diets rich in inorganic nitrate are associated with inhibition of platelet aggregation, preservation, and improvement of endothelial dysfunction which may be caused by diabetes in the arterial walls, these include beetroot, usually in the form of a juice, green leafy vegetables such as spinach, rocket and lettuce were also found to contain large sources of inorganic nitrate (Clements et al., 2014; Lundberg et al., 2008; Doel et al., 2005; Hyde et al., 2014). Prior evidence has proven beetroot juice as a popular vasodilator and has been used successfully in the treatment and reduction of blood pressure, in subjects with cardiovascular disease and Type 2 Diabetes as well (Clifford et al., 2015; Siervo et al., 2013; Bahadoran et al., 2015; Gilchrist et al., 2013; Kenjale et al., 2011), Therefore, it was justifiable to administer beetroot juice to diabetic patients with early-stage PAD in this study to assess its effects on the lower limb blood flow with ultrasound peak systolic velocity and blood pressure. Nitric oxide gas is produced endogenously from the amino acid L-arginine pathway by three isoforms of nitric oxide synthases in the endothelium of blood vessels, and it is useful as an anti-oxidation defence system and an antiplatelet thus inhibiting the acceleration of atherosclerosis (Stamler et al., 1989; Steinberg, 2009, Steinberg and Wizturn, 2010, Cooke, 1996; Stamler, 1989). Beetroot contains inorganic nitrate as the main bioactive component behind the reduction of blood pressure (Webb et al., 2008) and endurance exercise interactions (Vanhatalo et al., 2011) as well. In another study, Gilchrist et al., (2014), administered beetroot juice (nitrate content 7.5 mmol) versus beetroot juice placebo (nitrate content 0.002 mmol) to type 2 diabetic patients and noted a significant improvement in simple reaction time *(P <0.05)* in individuals who had blindly ingested beetroot juice with 7.5 mmol inorganic nitrate content compared to those who had blindly ingested placebo, thus strengthening the evidence that inorganic nitrate was the main bioactive component responsible for the noted change.

Following oral consumption of foods rich in inorganic nitrate such as beetroot juice, the nitrate is quickly absorbed in the stomach, duodenum and jejunum and availed in the circulation. Later about 25% is excreted in the oral cavity where commensal bacteria anaerobes (via nitrate reductive enzymes) mainly found under the back of the tongue bio-activate nitrate and reduce it to nitrite in saliva (the entero-salivary circulation) and about 75% of the nitrate is excreted via kidneys (Kapil et al., 2010; Vanhatalo et al., 2010). When this nitrite is swallowed into the acidic stomach, some of it is bio-activated into nitric oxide then both the nitric oxide and nitrite are rapidly absorbed into the circulation peaking this bioavailability from 2.5 – 3 hours (Kapil et al., 2010; Vanhatalo et al., 2010; Webb et al., 2008).

The main purpose of the availed nitric oxide is to maintain endothelial function in the inner walls of the arteries thus maintaining vascular homeostasis through maintaining the oxidative defence system, platelet function, vascular tone and the delicate balance between vasodilation and vasoconstriction (Clifford et al., 2015; Hobbs et al., 2012; Davignon and Ganz, 2004), thus a depletion in nitric oxide availability has been concluded as the main cause of endothelial dysfunction, a risk factor of cardiovascular disorders and in the pathogenesis of hypertension and atherosclerosis (Lidder et al., 2013; Joris and Mensik, 2013). Beetroot juice has been well

researched and is being considered as a promising therapy in a range of clinical pathologies associated with oxidative stress and inflammation (Clifford et al., 2015), Being a source of inorganic nitrate, ingestion of beetroot juice increases the bioavailability of nitric oxide to manage these pathologies associated with diminished nitric oxide availability, such as diabetes, hypertension, dyslipidaemia to mention a few, thus diminishing the rate of atherosclerosis (Kapil, 2010; Clements et al., 2014; Clifford et al., 2015; Kannady et al., 2012), and in all these studies no known adverse reactions were encountered besides short term effects such as beeturia + red stools, reduction in blood pressure and gastrointestinal discomfort (Kenjale et al., 2011, Webb et al., 2008, Vanhatalo et al., 2010; Bahadoran et al., 2015; Gilchrist et al., 2013).

Zimbabwe is experiencing the same chronic global problem of the prevalence of diabetes mellitus in its population as well the increased death risk from the complications of diabetes such as PAD in these patients (Parirenyatwa and Gwinji, 2016; Hakim et al., 2005). The aim of this study was to determine if there were acute effects in blood flow post beetroot juice ingestion in diabetic lower limb arteries with early-stage PAD and the non-diabetic controls using peak systolic velocity (PSV), systolic blood pressure (SBP) and diastolic blood pressure (DBP). Evidence from this study may be used in the formation of an affordable and cheap therapeutic pathway for managing early-stage PAD in diabetic patients.

2 MATERIALS AND METHODS

2.1 Design

In this prospective experimental study, the first aim was to determine if consumption of beetroot juice could result in meaningful changes in lower limb blood flow both within and between two groups of participants consisting of diabetic patients with early stage PAD and non-diabetic controls using PSV, SBP and DBP. Secondly this study aimed to identify the magnitude of any changes in blood flow after beetroot juice ingestion between individual time points within and between groups and also between the two groups across four time points using PSV and blood pressure.

2.2 Participants

Convenience sampling (Glen, 2015), was utilised to recruit a predetermined sample. The sample size for this study was obtained through power calculation for the reliability justification of a diagnostic tool using Schuman's two sided *t*-test procedure to determine the minimum sample size $(n = 71)$. The power calculation for the sample size was sixty-two and after considering a probable drop out of 10%, the sample size was now sixty-eight, however for this study seventy-one subjects were feasibly recruited thus thirty-six for healthy arteries group and thirty-five for the diabetic arteries group.

the predetermined samples of the two groups of participants consisting of 35 Black-African diabetic patients *(25 females and 10 males)* with early-stage PAD *median age 54 (IQR, 47 – 61) years; median glycated haemoglobin (HbA_{1c}) 6.3 (IQR, 5.7 – 8.0)%; mean Body Mass Index (BMI) 29.2 (± 6.7); mean Ankle Brachial Index (ABI) 1.1 (± 0.1)* and the controls were 36 non-diabetic participants (*28 females and 8 males); median age 38 (IQR,33 - 54) years; median HbA_{1c} 5.6 (IQR, 5.1-6.0)%, mean BMI 29.2 (±6.7); mean ABI 1.1 (± 0.1).*

Both groups of participants for this study were drawn from a homogenous Zimbabwean Black/African population which has a noted higher incidence of diabetes and its complications (Parirenyatwa and Gwinji, 2016-2020). A homogenous Black/African population enabled the eradication of the potential counteracting variables which could have emanated from a sample of participants from different ethnic groups. The sample for diabetic patients was drawn from the diabetic clinic at Mpilo Central Hospital while the control group was recruited from the National University of Science and Technology in Bulawayo.

A control for the age limit for the recruited adult participants for both groups was 18 - 70 years (Kaku, 2010; Bhatia et al., 2014). However, the age of the recruited participants was limited up to 70 years since there is evidence that there is an increased risk of late-stage PAD in subjects of above 70 years (Macleod et al., 2008; Klabunde, 2007; Hernando and Conejero, 2007).

Pregnant women were excluded (Mahendru et al., 2014; Sanghavi and Rutherford, 2014), in a

bid to avoid inconsistencies in blood flow measurements in which occur with pregnancy.

Smokers and ex-smokers were excluded because there is a strong correlation between tobacco smoking and PAD (Hernando and Conejero, 2007; Klabunde, 2007), Thus limiting bias which could have emanated from misclassification of exposure and outcomes.

Ethical approval was granted by the Medical Research Council of Zimbabwe (MRCZ /A/2036) and Salford ethics board (HSR1617-32).

2.3 Participants' Preparation

The recruited participants were instructed to adopt a low nitrate vegetable diet and no meat or fish for three days and they were told to fast six to twelve hours before undertaking the blood tests and ultrasound measurements on day 5.

The participants were instructed to avoid alcohol at least forty-eight hours before the blood tests and ultrasound measurements which were booked at eight o'clock in the morning at a private laboratory and private ultrasound imaging centre in town.

The participants were advised abscond their morning dose of prescribed diabetic and or high blood pressure medications but to bring them on their appointment day. The justification for this abscorndment was because the participants were made to fast 6 hours before undertaking the blood tests, blood flow and blood pressure measurements, such that the diabetic group participants had a higher chance of sliding into hypoglycaemia if they could have taken morning medication.

Prior evidence has shown beetroot juice as effective in reducing blood pressure, (Clifford et al., 2015; Gilchrist et al., 2013; Bahadoran et al., 2015; Kenjale et al., 2011; Vanhatalo et al., 2010; Webb et al., 2008), thus the participants were instructed not to take blood pressure reducing medications on the morning of the examination day to avoid masking the true effects of beetroot juice on blood pressure. However, the participants were only advised to take their blood pressure medications if their last blood pressure measurement at 210 minutes' post beetroot juice ingestion did not fall in the normal range shown in equation 1.

$$[\frac{120-130}{80-90}\,\mathrm{mmHg}\text{ or slightly lower.}] \quad (1)$$

All these preparation measures were put in place in-order to minimise the effects of a nitrate-rich diet, a recent meal, alcohol and medication on the basal blood flow of the participants before the undertaking of blood flow measurements and also to enable the measurement of accurate glycated haemoglobin levels in their blood. To check on compliance to prior preparation instructions, the participants were instructed to diarise all the foods they had eaten three days before the undertaking of measurements and those who had failed to comply were rebooked. All the controlled measures done enabled minimisation of measurement error during the assessing of the effects of beetroot juice ingestion on blood flow and blood pressure.

The participants were given a refreshment of 100% fruit juice and a low sugar biscuit after completing the measurements then they were allowed to take their prescribed diabetic and or blood pressure reducing medications under observation for about 20 minutes before being dismissed to go home.

2.4 Recruitment strategy

Demographic data of participants for both groups was collected blindly and anonymous identification codes were issued to all volunteering participants.

All the recruited 35 diabetic participants underwent reactive hyperaemic testing with thigh blood flow occlusion (Higashi et al., 2001; Philpott and Anderson, 2007) and were categorised as having early stage PAD. The recruited classification of early stage PAD was the asymptomatic grade zero by Rutherford et al., (1997) (Hardman et al., 2014).

The non-diabetic participants were blindly recruited through a mass advert and the Qdiabetes risk calculator (2015) was utilised to screen them until a predetermined sample size of 36 achieved. Good contact was maintained with participants through text messages and telephoning.

2.5 Data collection procedures

- **Blood testing for renal function and glycaemic control**

Blood tests were done first at 8 0'clock in the morning of the booked day five for each participant in the laboratory. The participants' blood was tested for glycated haemoglobin

levels to establish glycaemic control. Again, the blood was tested for Urea and Creatinine levels which were utilised to calculate the Estimated Glomerular Filtration Rate (EGFR) to establish whether the recruited patients indeed had minimal renal damage as supposedly expected in early-stage PAD.

The blood test results were availed by the laboratory the following day (Day 6) and they were stratified to the recruited anonymised coded participants while this was blinded to the rater to minimise recall bias.

- **Body Mass Index and Ankle Brachial Index measurements**

On their booked day five, each participant was escorted from the laboratory to the ultrasound room downstairs. In the ultrasound room, each participant was assessed in quiet, calm conditions at standard room temperature of about 23 - 25℃ by a thermometer.

Weight and height of the participants was measured and then stratified with the participants' anonymised codes on Microsoft Excel sheets. Body mass index was calculated for the recorded weight and height of each patient and stratified with the anonymised codes using equation 2 as follows;

$$Body\ Mass\ Index = \frac{weight}{height^2} \quad 2$$

Ankle brachial index was performed as a parallel test using a calibrated automated blood pressure machine (*CareVue, Shenzhen, China)* and the upper arms and ankles blood pressure measurements were taken at a similar site on each participant.

- **Duplex Ultrasound and Blood pressure measurements**

In-order to determine the validity of the ultrasound scanner, quality control tests were done before duplex ultrasound measurements were undertaken and these included the internal grid assessment for testing lateral and axial resolution for the 7.5-10.0 MHz probe of the ultrasound machine (Mindray model Z5, Shenzhen, China).

Each participant was instructed to lie down and relax on the examination bed for about 10 minutes in a supine position to allow a stable heart rate for a stable basal blood flow in the lower limbs arteries. The participants' blood pressure at rest was then measured from the non-dependent upper arm and the basal systolic and diastolic blood pressure values were recorded and later blindly collated to each participant's anonymised code to minimise recall bias. Concurrently the basal PSV of the popliteal artery was done in the longitudinal section of the popliteal artery. In a bid to minimise measurement error and performance bias, the ultrasound PSV measurements were taken three times from a still image of the Pulsed Spectral Doppler waveform and the mean value recorded (Delis et al., 2000; Leoniuk et al., 2014), and the mean value recorded for each participant by a rater holding more than 5 years of experience in vascular ultrasound imaging.

Blood flow was sampled for the popliteal arteries with B- mode imaging, colour and then Doppler in the longitudinal section. The longitudinal section enabled the manipulation of the ultrasound beam from the probe to be parallel to the blood flow and enabling manoeuvring for a Doppler angle of less or equal to $60°$ which gave maximum Doppler shifts (blood velocity) on the Doppler spectral display (Hamments, 2014; Hwang, 2017).

The Doppler equation utilised in the scanning technique identified all factors which affected the magnitude of the Doppler shift as follows in equation 3;

$$FD = \frac{2ftv(cos\ \theta)nx}{c} \quad 3$$

From equation 3, FD refers to Doppler shift frequency (positive in arteries and negative in veins),

ii) *2* is a constant and can be ignored,

iii) Transmitted frequency *(FT)* is directly proportional to Doppler shift frequency *(FD).*

iv) velocity of blood *(v)* is directly proportional Doppler shift frequency *(FD)* and *(C)* speed of sound which is 1.540 m/s and a constant (Hamments, 2014; Hwang 2017).

Deducting the Doppler equation above the Doppler angle *(θ)* was maintained at less or equal to 60 to enable a cosine value that was high and which was directly proportional to high Doppler shifts which were also directly proportional to high blood velocity.

The colour box was made as small as possible and the sample volume cursor was placed within the popliteal artery lumen to enable recording of more accurate and maximum Doppler shift frequencies. (Harrington, 2012; Chavhan et al., 2008). The blood flow velocities were then displayed in the *y-axis* in

cm/s against time in seconds in the *x-axis* on the Doppler spectrum. The pulsed spectral Doppler PSV was measured on the displayed spectral Doppler waveform.
After completion of the basal measurements, each participant was instructed to sit in the waiting area and was administered 500 ml of beetroot juice (7.38 mmol beetroot juice nitrate) for oral ingestion. However, the diabetic patients were allowed to take their diabetes medication together with beetroot juice to enable them to metabolise the small amount of carbohydrates in the ingested beetroot juice to avoid destabilising their blood sugar levels.

Each participant was instructed to relax for the initial 80 minutes to allow for the digestion and processing of beetroot juice in the stomach (Kapil et al, 2010; Vanhatalo et al, 2010). After the initial 80 minutes of relaxing following beetroot juice ingestion, each participant was assisted back on the couch and allowed another 10 minutes' supine rest to enable them to achieve a stable heart rate. Each participant's blood pressure was measured followed by PSV measurements at 90 minutes, 150 minutes and 210 minutes' post beetroot juice intake respectively.

PSV and blood pressure measurements were started at 90 minutes after beetroot juice ingestions to allow the capturing of the earlier effects of beetroot juice on blood flow even though prior evidence has shown that presumed vasodilation from orally administered beetroot juice occurred after about 180 minutes (Kapil et al, 2010; Vanhatalo et al, 2010).

For each participant, the archived PSV measurements were later blindly collated with the blood pressure, demographic data and the anonymised codes for each participant in Microsoft Excel sheets. After the measurement of blood pressure for each participant at 210 minutes' post beetroot juice ingestion, if the blood pressure was found to be within the normal range values (Equation 1; Eisenstein et al., 2017) then the participant was advised not to take their blood pressure reducing medications on that day but to resume on the following day to avoid reducing the blood pressure below the normal range.

After the all the measurements were completed, each participant was given a refreshment of 100% pure juice and low sugar biscuit and instructed to relax in the ultrasound department for about 20 minutes before being dismissed home.

2.6 Statistical analyses

Normality testing was performed using the Shapiro-Wilks test on the demographic data and the normal findings were reported in mean (SDs) while non-normal findings were reported in median interquartile ranges. (Table 1).
A two-way analysis of variance (ANOVA) (2 x 4; group x time) and post - hoc analysis using the Benferroni test was performed to compare the two groups across 4-time points enabling the comparison of combined groups effects at specific time points after the ingestion of beetroot juice. Two samples *t*-tests were performed and Cohen's *d* effect sizes calculated to compare the means of dependent variables between groups at each specific time point to establish if any differences were significant or meaningful. An *a priori* alpha level was set at 0.05 and effect sizes were interpreted as <0.20 = trivial, 0.20-0.49 = small, 0.50-0.80 =moderate >0.80 = large.
Again, one-sample *t*-test and Cohen's d effect sizes were performed to compare the means of dependent variables within each group between specific time points to establish if these differences were significant and meaningful. One sample t-test and two samples *t*-test were performed to determine any change in blood flow within groups and between groups at specific time points respectively as determined by the dependent variables (PSV, SBP and DBP) after beetroot juice ingestion.

3 Results

3.1 Demographic findings

In a cohort of 71 Black-African participants, 36 (51%) were non-diabetic controls and 35 (49%) were diabetic participants with early-stage PAD. The median for age was significantly higher in diabetic patients compared to non-diabetic patients (Table 1). The *means (SD)* for BMI were neither significantly nor meaningfully different between diabetic patients and the non-diabetic controls *(Table 1),* however, the *median* $HbA_{1c,}$ levels were significantly higher in diabetic patients when compared to do the non-diabetic controls (Table 1). There were neither significant nor meaningful differences in ABI between diabetic patients and non-diabetic controls (Table 1).

Table 1: Comparison of subject characteristics between 35 (49%) diabetic patients and 36 (51%) non-diabetic patients where BMI, ABI and EGFR did not show a significant or meaningful difference between groups while a significant difference was observed between groups in age and HbA_{1c}.

Non-normal demographic data				
Parameter	**Control Median (IQR)**	**Diabetic Median (IQR)**	**Two sample t-test p-value**	***T*-test** ***p*- value**
AGE	37.5 (33 – 54) yrs.	54 (47 – 61) yrs.	0.01	0.01
EGFR	108 (95.5 – 127.5) ml/min/1.73 m^2)	112 (96.0 - 126.0) ml/min/1.73 m^2)	0.8	0.8
HbA_{1c}	5.6 (5.1 – 6.0)%	6.3 (6.0 – 8.0)%	0.0	0.0
Normal demographic data				
Parameter	**Mean (sd) non-diabetics**	**Mean (sd) Diabetics**	**Two sample t-test p-value**	**Cohen's d effect sizes**
BMI	29 (7.0)	29.2 (7.0)	0.7	0.1
ABI	1.1 (0.1)	1.1 (0.1)	0.8	0.1
EGFR-Estimated glomerular filtration rate; **HbA_{1c}**; **BMI**-Body Mass Index; **ABI**-Ankle Brachial Index				

3.2 Combined groups PSV changes after beetroot juice ingestion at specific time points.

Combined group effects for PSV showed a significant change *(diff ≤ 12.3 cm/s; P ≤0.0001)* between the baseline and 90 minutes' time point, the baseline and 150 minutes' time point *(diff; 14.0 cm/s; P ≤0.0001)*, the baseline and 210 minutes' time point *(diff = 20.0 cm/s; P ≤0.0001)* and the 150 minutes and 210 minutes' time point *(diff = 6.0 cm/s; P <0.0001).* However, there was no significant change in PSV *(diff = 0.4cm/s; P = 1.00)* between the 90 minutes and 150 minutes' time point after beetroot juice ingestions (table 2).

Table 2 Combined groups' peak systolic velocity changes after beetroot juice ingestion at specific time points (Benferroni).

	baseline	**90 minutes**	**150 minutes**
90 minutes	-12.3 cm/s (diff) *P = 0.000*		
150 minutes	-14.0 cm/s (diff) *P = 0.000*	-2.0 cm/s (diff) *P =1.000*	
210 minutes	-20.0 cm/s (diff) *P = 0.000*	-8.0 cm/s (diff) *P = 0.000*	-6.0 cm/s (diff) *P = 0.000*
Diff = mean difference; p = value			

3.3 Peak systolic velocity comparisons between groups at specific time points.

Between groups, PSV was significantly and meaningfully higher *(P <0.0001; d = 1.95)* in diabetic patients *(73.0 ± 11.0 cm/s)* compared to non-diabetic patients *(56.3 ± 5.3 cm/s)* basally. After beetroot juice ingestions at 90 minutes PSV was again significantly and meaningfully higher *(P <0.0001; d = 1.30)* in diabetic patients *(57.0 ± 8.1 cm/s)* compared to non-diabetic patients *(48.0 ± 5.2 cm/s)*. At 150 minutes PSV was still significantly and meaningfully higher *(P <0.0001; d = 1.10)* in diabetic patients *(54.1 ± 8.0 cm/s)* compared to non-diabetic patients *(47.0 ± 5.0 cm/s)*. However, at 210 minutes there was neither a significant nor meaningful difference *(P = 0.4; d = 0.18)* in PSV between diabetic patients *(45.2 ± 9.0 cm/s)* compared to non-diabetic patients *(44.0 ± 4.0 cm/s)* (Table 3; figure 1).

Table 3 showing PSV comparisons between groups at specific time points

Parameter	**Mean (sd)** **Non-diabetics**	**Mean (sd)** **diabetics**	***T-test p*-value**	**%mean difference**	**Cohen's *d*** **Effect**
PSV baseline	56.3 (5.3)cm/s	73.0 (11.0)cm/s	<0.0001	16.2%	1.95
PSV 90 minutes	48.0 (5.1)cm/s	57.0 (8.1)cm/s	<0.0001	9.0%	1.30
PSV 150 minutes	47.0 (5.0)cm/s	54.1 (8.0)cm/s	<0.0001	7.2%	1.10
PSV 210minutes	44.0 (4)cm/s	45.2 (9)cm/s	0.4	1.2%	0.18

1.4.2.2 PSV change both within and between groups at specific time points

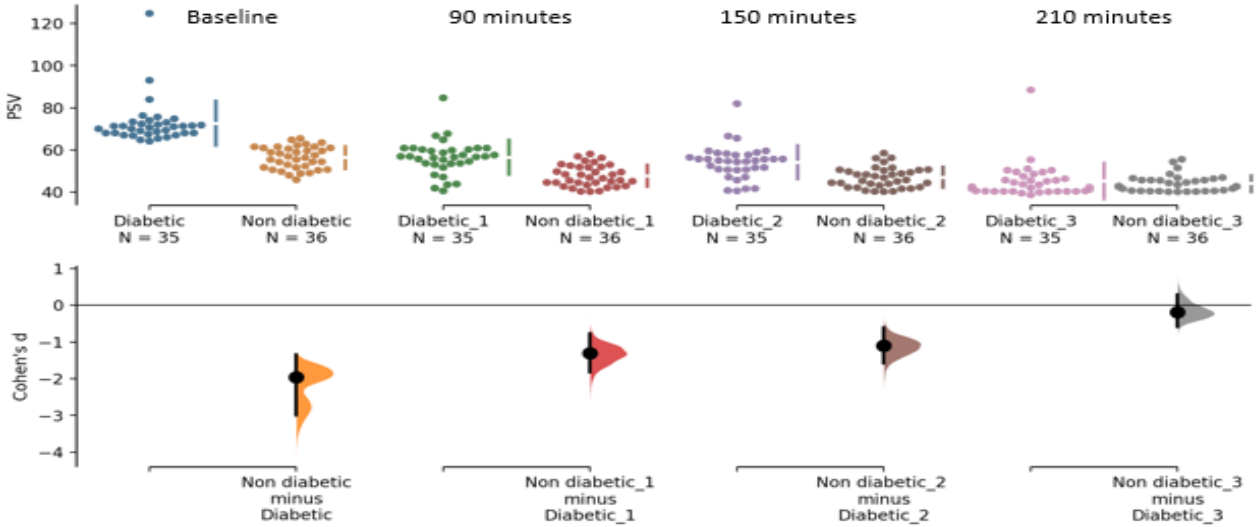

Figure 1: Change in peak systolic velocity after beetroot ingestions between groups, at each time point.

3.4 Comparison of PSV at specific time points within groups.

Within groups, PSV decreased significantly and meaningfully *(P ≤ 0.01; d ≤ 1.70)* at *90* minutes and 210 minutes after beetroot juice ingestions within diabetic patients and non-diabetic patients similarly. However, no significant or meaningful decrease *(p ≤ 0.5; d ≤ 0.29)* occurred at 150 minutes' time point after beetroot juice intake within both groups (Table 4; Figure 1).

Table 4 Comparison of peak systolic velocity changes at specific time points within groups.

Diabetic patients					
Parameter	**baseline**	**90 minutes**	**% mean difference**	***T-test* p-value**	**Cohen's *d* effect size**
PSV	73.0 (11.0) cm/s	57.0 (8.1) cm/s	16.1%	<0.0001	1.70
	90 minutes	**150 minutes**			
	57.0 (8.1) cm/s	54.1 (8.0) cm/s	3.0%	0.2	0.29
	150 minutes	**210 minutes**			
	54.1 (8.0) cm/s	46.0 (9.0) cm/s	9%	<0.0001	1.08
Non - diabetic participants					
PSV	**baseline**	**90 minutes**	**% mean Difference**	**T-test p-value**	**Cohen's d effect size**
	56.3(5.3) cm/s	47.0 (5.1)	9%	<0.0001	1.66
	90 minutes	**150 minutes**			
	47.0 (5.1) cm/s	47.0 (5.0)	1.0%	0.5	0.15
	150 minutes	**210 minutes**			
	47.0 (5.0) cm/s	44.0 (4.0) cm/s	3%	0.01	0.67

3.5 Combined groups systolic Blood pressure changes to beetroot juice ingestion at specific time points.

Combined group effects for SBP showed a significant change between the baseline and 90 minutes' time point *(diff = 13.0 mmHg; P <0.0001)*, baseline and 150 minutes' time point *(diff = 14.3 mmHg; P = 0.0001)*; baseline and 210 minutes' time point *(diff = 22.0; P <0.0001)* and between the 150 minutes to 210 minutes' time point *(diff = 8.0 mmHg; P = 0.01)* after beetroot juice ingestions. However, there was no significant change in SBP at the 90 minutes and 150 minutes' time point *(diff = 1.2 mmHg; P = 1.00)* (Table 5).

Table 5: Combined group effects for SBP changes after beetroot juice ingestion at specific time points (Benferroni).

	Baseline	**90 minutes**	**150 minutes**
90 minutes	-13.0 mmHg (diff) *P = 0.000*		
150 minutes	-14.3 mmHg (diff) *P = 0.000*	-1.2 mmHg (diff) *P = 1.000*	
210 minutes	-22.1 mmHg (diff) *P = 0.000*	-9.1 mmHg (diff) *P = 0.001*	-8.0 mmHg (diff) P = 0.009
Diff = mean difference; p = value			

3.6 Comparison of SBP changes between groups at specific time points.

SBP was significantly and meaningfully higher *(P = 0.01; d = 0.68)* in the diabetic patients *(156.0 ± 20.3 mmHg)* compared to non-diabetic patients *(143.1 ± 16.0 mmHg)* basally. At 90 minutes after beetroot juice intake there was no significant/meaningful difference *(P = 0.2; d = 0.34)* in SBP between diabetic patients *(139.0 ± 16.0 mmHg)* and non-diabetic patients *(134.0 ± 13.0 mmHg).* At 150 minutes after beetroot
juice intake, there was no significant or meaningful difference in SBP *(P >0.05; d = 0.06)* between diabetic patients *(135.4 ± 14.4 mmHg)* and non-diabetic patients *(135.0 ± 13.4 mmHg).* At 210 minutes after beetroot juice intake again there was no significant or meaningful difference *(P >0.05; d = 0.13)* between diabetic patients *(128.0 ± 9.0 mmHg)* and non-diabetic patients *(127.0 ± 9.0 mmHg)* (Table 6; Figure 2).

Table 6 Comparison of SBP changes between groups at specific time points

Parameter	**Non - diabetics**	**Diabetics**	***T*-test *p* value**	**% mean difference**	**Cohen's *d* effect sizes.**
SBP baseline	143.1 (16.0) mmHg	156.0 (20.3) mmHg	0.01	12%	0.68
SBP 90 minutes	134.0 (13.0) mmHg	139.0 (16.0) mmHg	0.2	5.0%	0.34
SBP 150 minutes	135.0 (13.4) mmHg	135.4 (14.4) mmHg	1.0	1%	0.06
SBP 210 minutes	127.0 (9.0) mmHg	128.0 (9.0) mmHg	1.0	1.2%	0.13

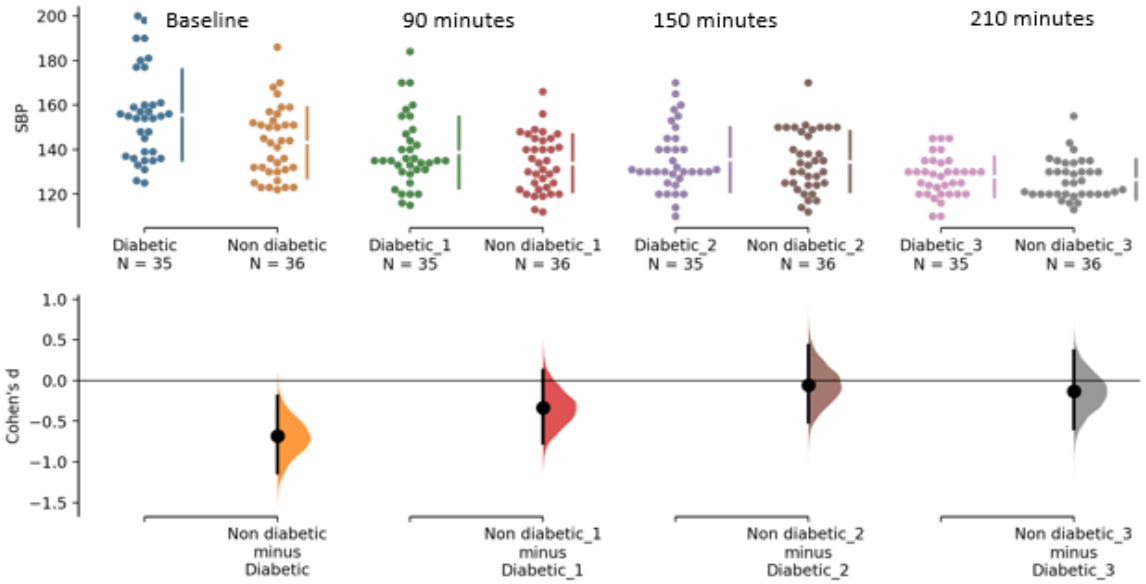

Figure 2: Changes in systolic blood pressure after beetroot juice ingestions between groups.

Key: SBP- Systolic Blood pressure

3.7 SBP change within groups and specific time points

Within groups, SBP reduced significantly and meaningfully *(P ≤0.01; d ≤0.92)* at 90 minutes and similarly at 210 minutes' time point after beetroot juice ingestions within both the diabetic and the non-diabetic groups.

However, no significant or meaningful decrease *(P >0.05; d ≤ 0.22)* occurred at 150 minutes after beetroot juice ingestions within both groups (Figures 2; Table 7).

Table 7: SBP change within groups at specifictime points

Diabetic participants					
SBP	**baseline**	**90 minutes**	**%mean difference**	**T-test p-value**	**Cohen's d effect sizes**
	156.0 (20.3) mmHg	139.0 (16.0) mmHg	17%	<0.001	0.92
	90 minutes	**150 minutes**			
	139.0 (16.0) mmHg	135.4 (14.4) mmHg	3.3%	0.4	0.22
	150 minutes	**210 minutes**			
	135.4 (14.4) mmHg	128.0 (9.0) mmHg	8.0%	0.01	0.64
Non-diabetic participants					
SBP	**baseline**	**90 minutes**	**%Mean difference**	***T*-test p-value**	**Cohen's d effect sizes**
	143.1(16.0) mmHg	134.0 (13.0) mmHg	9.3%	0.01	0.65
	90 minutes	**150 minutes**			
	134.0 (13.0) mmHg	135.0 (13.4) mmHg	0.8%	0.8	0.06
	150 minutes	**210 minutes**			
	135 (13.4) mmHg	127.0 (9.0) mmHg	8%	<0.0001	0.70

3.8 Combined groups DBP changes after beetroot juice ingestion at specific time points.

Combined group effects for DBP showed a significant change between the baseline and 90 minutes' time point *(diff = -7.1 mmHg; P ≤0.0001)*, baseline and 150 minutes' time point *(diff = 8.4 mmHg; P = 0.0001)*; baseline and 210 minutes' time point *(diff = 13.4 mmHg; P = 0.0001)* and between the 150 minutes and 210 minutes' time point *(diff = 5.0 mmHg; P = 0.001)*. However, there was no significant change in DBP between the 90 minutes and 150 minutes' time point *(diff = 1.3 mmHg; P = 1.00)* after beetroot juice ingestions (Table 8).

Table 8 Combined groups' diastolic blood pressure changes after beetroot juice ingestion at specific time points (Benferroni).

	baseline	**90 minutes**	**150 minutes**
90 minutes	-7.1 mmHg (diff) *P = 0.000*		
150 minutes	-8.4 mmHg (diff) *P = 0.000*	-1.3 mmHg (diff) *P = 1.000*	
210 minutes	-13.4 mmHg *P = 0.000*	-6.3 mmHg (diff) *P = 0.000*	-5.0 mmHg (diff) *P = 0.001*
Diff = mean difference; p = value			

3.9 Comparison of DBP change at specific time points within groups.

Between groups, basal DBP did not show a significant/meaningful difference *(P>0.05; d = 0.33)* between diabetic patients *(101.0 ± 10.2 mmHg*) and non-diabetic patients *(98.0 ± 8.0 mmHg)*. At 90 minutes after beetroot juice intake there was no significant or meaningful difference (P >0.05; d = 0.08) between diabetic patients (*92.0 ± 8.2 mmHg)* and non-diabetic patients *(92.4 ± 7.2 mmHg)*. At 150 minutes after beetroot juice intake there was no significant or meaningful difference *(P >0.05; d = 0.08)* between diabetic patients *(91.0 ± 8.0 mmHg)* and non-diabetic patients (91.1 ± 7.0 mmHg). At 210 minutes after beetroot juice intake there was no significant or meaningful difference *(P >0.05; d = 0.23)* between diabetic patients *(86.4 ± 6.0 mmHg)* and non-diabetic patients *(85.2 ± 4.4 mmHg)* (Table 9; Figure 3).

Table 9 Comparison of DBP change between groups at specific time points.

Parameter	**Non - diabetics**	**Diabetics**	**T-test *p*-value**	**% Mean difference**	**Cohen's *d* effect sizes**
DBP baseline	98.0 (8.0) mmHg	100.7 (10.2) mmHg	0.2	3.1%	0.33
DBP 90mins	92.4 (7.2) mmHg	92.0 (8.2) mmHg	0.7	1.0%	0.08
DBP 150mins	91.1 (7.0) mmHg	90.5 (8.0) mmHg	0.7	0.6%	0.08
DBP 210mins	85.2 (4.4) mmHg	86.4 (6.0) mmHg	0.3	1.1%	0.23

1.4.4.2 DBP changes between groups at specific time points.

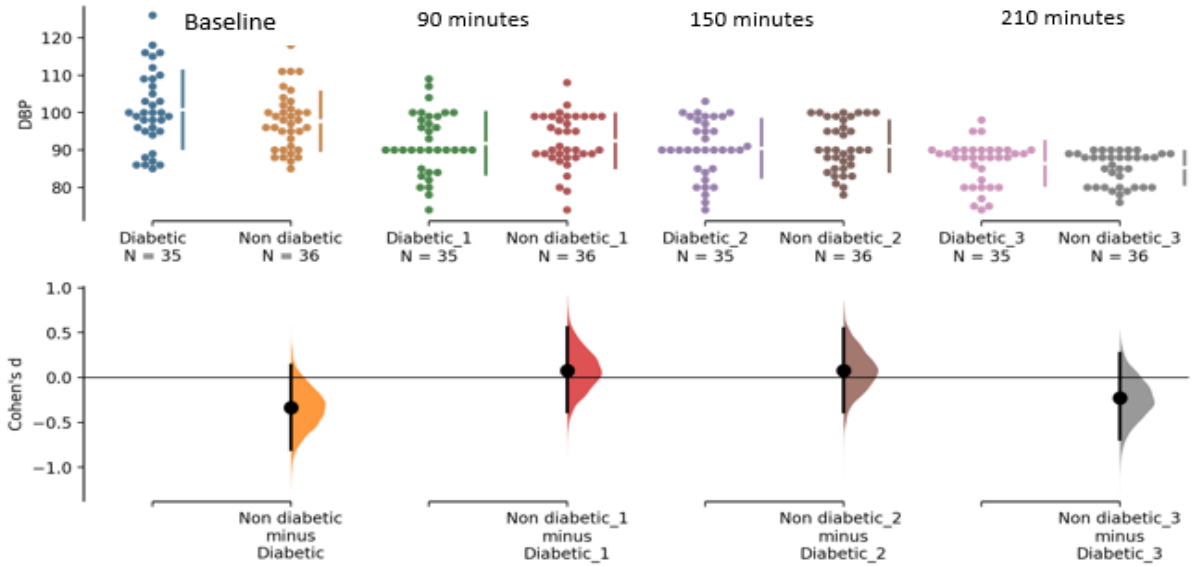

Figure 3: DBP change within and between groups

Key: *DBP – Diastolic Blood Pressure*

3.9.1 Comparison of DBP changes at specific time points within groups.

Within groups, DBP decreased significantly and meaningfully *(P ≤0.02; d ≤ 1.02)* at 90 minutes and 210 minutes *after* beetroot juice ingestions within both groups. However, no significant/meaningful decrease *(P >0.05; d ≤ 0.19)* was seen at 90 minutes to 150 minutes after beetroot juice ingestion by both groups (figure 3; table 10).

Table 10: shows a comparison of DBP changes at specific time points within groups.

Non – diabetic participants					
DBP	**baseline**	**90 minutes**	**% mean difference**	***T*-test *p*-value**	**Cohen's *d* effect sizes**
	98.0 (8.0) mmHg	92.4 (7.2) mmHg	5.3%	<0.0001	0.70
	90 minutes	**150 minutes**			
	92.1 (7.2) mmHg	91.1 (8.0) mmHg	1.4%	0.4	0.19
	150 minutes	**210 minutes**			
	91.0(8.0) mmHg	85.2 (4.4)mmHg	6.0%	<0.0001	1.02
Diabetic participants					
DBP	**baseline**	**90 minutes**	**% mean difference**	***T*-test *p*-value**	**Cohen's *d* effect sizes**
	101.0(10.3) mmHg	92.0(8.3) mmHg	9.0%	<0.0001	0.96
	90 minutes	**150 minutes**			
	92.0 (8.3) mmHg	90.5 (8.0)mmHg	1.3%	0.5	0.16
	150 minutes	**210 minutes**			
	90.5 (8.0)mmHg	86.4 mmHg	4.1%	0.02	0.60

4. Discussion

In this investigation, within groups, peak systolic velocity, systolic blood pressure and diastolic blood pressure reduced significantly and meaningfully from baseline to 90 minutes and from 150 minutes 210 minutes (*P ≤0.01; d ≤1.70)* after beetroot juice ingestion. However, no significant or meaningful change *(P >0.05; d ≤0.29)* occurred in peak systolic velocity, systolic blood pressure and diastolic blood

The findings in this investigation suggested that short term effects of beetroot juice ingestion resulted in improved blood flow as reflected by reduced peak systolic velocity, systolic blood pressure and diastolic blood pressure in both groups across all time points after beetroot juice ingestion. Prior studies have been undertaken on assessing the short term effects of beetroot juice within an intervention period ranging from 3 hours to about 15 days and the acute effects established were on blood pressure and

exercise endurance, while no prior studies were yet done on the effects of beetroot juice on blood flow using peak systolic velocity during the writing up of this thesis.

A study by Bahra et al., (2012), showed similar findings of improved vascular compliance signalling improved blood flow when conducted in hypertensive patients after ingestion of nitrate salts. Improved vascular compliance was reflected by a reduction in aortic pulse wave velocity and reduction in systolic blood pressure, while this investigation showed improved blood flow through reduced peak systolic velocity and reduction of blood pressure in diabetic patients and non-diabetic controls after ingestion of beetroot juice. These concurring findings justify the presence of a similar ingredient in beetroot juice and potassium nitrate salts which resulted in desirable improved blood flow effects in both hypertensive and diabetic patients.

The findings in this study showed improved blood flow in the asymptomatic diabetic patients and non-diabetic controls after 3 hours post beetroot juice ingestion as a clinically significant benefit and these findings prompted for long term assessment of chronic beetroot juice intake by diabetic patients with early-stage PAD as a post-doctoral study. Such improvements in blood flow if the long term could result in the reduction of medications needed to relieve impaired blood flow and those needed to reduce elevated blood pressure in diabetic patients. This improved blood flow after ingestion of beetroot juice carries the benefit of adaptation to exercise (Vanhatalo et al., 2010; Webb et al., 2008) and physical activity by patients with early-stage PAD thus improving their health management.

Between groups, PSV and SBP were significantly and meaningfully higher *(P ≤0.04; d ≤1.95)* in diabetic patients at baseline. However, at 90 minutes and 150 minutes PSV remained higher in diabetic patients *(P ≤0.04; d ≤1.30)* unlike SBP (*P >0.05; d ≤0.34)*. At 210 minutes after beetroot juice ingestions PSV and SBP showed no significant or meaningful change in diabetics and non-diabetics *(P >0.05; d ≤0.18)*. DBP showed no significant or meaningful difference *(P >0.05; d ≤0.33)* between the groups at all the time points after beetroot juice ingestions.

PSV and SBP showed a clinically significant change in blood between groups across all time points after intake of beetroot juice since their percentage (%) mean difference *(less or equal to 22.1%)* was higher than the smallest detectable difference (SDD %) from the first investigation *(9.2%)*. However, DBP did not show a clinically significant change in blood flow between groups across all the time points since its percentage (%) mean difference *(less or equal to 3.1%))* was lower than the smallest detectable difference (SDD %) from the first investigation *(9.2%).*

In the comparison for combined effects, PSV showed a significant change *(diff ≤ 19.7 cm/s; P ≤0.0001)* between the baseline and 90 minutes, baseline and 150 minutes, baseline and 210 minutes, and finally 150 minutes and 210 minutes' time points. However, there was no significant change in PSV *(diff = 0.4 cm/s; P = 1.00)* between the 90 minutes and 150 minutes' time point after beetroot juice ingestions. The combined effects for SBP showed a significant change *(diff ≤ 22.0 mmHg; P <0.0001)* between baseline and 90 minutes, baseline and 150 minutes, baseline and 210 minutes and finally 150 minutes to 210 minutes' time points after beetroot juice ingestions. However, there was no significant change in systolic blood pressure during the 90 minutes to 150 minutes' time point *(diff = 1.2 mmHg; P = 1.00)*. DBP pressure showed a significant change *(diff ≤13.4 mmHg; P <0.0001)* between baseline and 90 minutes, baseline and 150 minutes, baseline and 210 minutes, and 150 minutes and 210 minutes' time points after beetroot juice ingestions. However, there was no significant change in diastolic blood pressure between the 90 minutes and 150 minutes' time point *(diff = 1.3 mmHg; P = 1.00)* after beetroot juice ingestions.

These findings are in line with the research question of this study which sought to determine if the peak systolic velocity alongside systolic blood pressure and diastolic blood pressure were capable of demonstrating the acute effects of beetroot juice on blood flow within groups and between groups across time points while a significant and meaningful difference in blood flow was interpreted as the capability to show the acute effects of beetroot juice across the time points. The statistically significant and meaningful difference reflected by peak systolic, systolic blood pressure and diastolic blood pressure at 90 minutes after beetroot juice intake could be due to an increment in volume of blood after ingestion of 500 ml of beetroot juice while at 150 minutes

after beetroot juice ingestions there was no significant change in peak systolic velocity, systolic blood pressure and diastolic blood pressure probably due to the continual flow of blood which brought back its volume to the baseline level. However, the significant and meaningful differences/ changes later noted in peak systolic velocity, systolic blood pressure and diastolic blood pressure during the 150 minutes – 210 minutes' time point after beetroot juice ingestion could have been being the ones showing the true acute effects of the beetroot juice on the blood flow of the popliteal arteries of diabetic patients with early-stage PAD and non-diabetic controls.

Prior studies (Web et al., 2008; Bailey et al., 2009; Vanhatalo et al., 2010; Gilchrist et al., 2011; Lansely et al., 2011) have shown the effects of beetroot juice intake as reduced systolic blood pressure at least 3 hours' post-ingestion and most of the studies were done in healthy normotensive individuals. During the writing up of this thesis, there was no prior study which showed the acute effects of beetroot juice ingestion on blood flow by utilising duplex ultrasound peak systolic velocity in individuals who were at greater cardiovascular risk (Siervo et al., 2013; Ogbonmwan et al., 2012). This investigation has shown the evidence of improved blood flow as reflected by reduced peak systolic velocity and reduced systolic blood pressure and diastolic blood pressure during the 2 ½ -3 ½ hours' time point in individuals at a greater risk of cardiovascular disease (diabetic patients with early-stage PAD) after ingestion of beetroot juice.

Beetroot juice studies have confirmed the bioavailability of nitrite and nitric oxide from the nitrate in beetroot juice as being at less or equal to 3 hrs (180 minutes) -post-ingestion (Kapil et al., 2010; Kenjale et al., 2011; Webb et al., 2008; Vanhatalo et al., 2010). Similarly, in this investigation, peak systolic velocity, diastolic blood pressure and systolic blood pressure showed a significant difference in blood flow both in diabetic patients and non-diabetic patients from 150 minutes to 210 minutes (2 ½ - 3 ½ hours) time point after beetroot juice ingestion.

In this investigation, the fact that no significant or meaningful difference was found between groups after 210 minutes (3 ½ hours) could also mean the peak presence of the acute effects of beetroot juice in the blood for both groups. These findings could be owed to the peak bioavailability of nitrite and nitric oxide in the bloodstream after digestion and excretion of the beetroot juice nitrate (Kapil et al., 2010, Kenjale et al., 2011).

This study noted a reduction in the peak systolic velocity of the popliteal arteries in diabetic patients *(73.0 ± 11.0 cm/s pre to 45.2 ± 9.0 cm/s* during 2 ½ to 3 ½ hrs *post 500ml beetroot juice ingestion; p< 0.05)* and a reduction of peak systolic velocity in the popliteal arteries of non-diabetic patients (*57.0 ± 5.3 cm/s pre to 44 ± 4.0 cm/s during 2 ½ to 3 ½ hours post beetroot juice ingestion)*. Similarly, this investigation also noted a reduction in systolic blood pressure in diabetic patients with early-stage PAD *(156.0 ± 20.3 mmHg pre to 128.0 ± 9.0 mmHg during 2 ½ -3 ½ hours post-Beetroot Juice intake; p < 0.05)* and a reduction in non-diabetic controls *(143.4 ± 16.0 mmHg pre to 127.0 ± 9.0 mmHg during 2 ½ -3 ½ hours post-Beetroot Juice ingestion, p < 0.05)*. Concurring with these findings, other studies (Hobbs et al., 2012; Kapil et al., 2015) also noted a reduction in systolic blood pressure *(% mean difference = 20.5%)* and a reduction in diastolic blood pressure *(% mean difference = 14.6%)* at about 2 - 3 hours' post-ingestion of 5.7 mmol beetroot juice. However, a reduction in pulse wave velocity in hypertensive patients after dietary nitrate consumption by 0.59 m/s *(95% CI 0.2 - 0.9; p < 0.01)* compared to baseline values and 0.6 m/s *(95% CI 0.1 - 1.1; p <0.05)* compared to placebo was shown by Kapil et al., 2015).

4.1 Strengths and limitations.

The strength of this study was that it was carried out under controlled settings which limited transfer, recall, selection and misclassification of exposure factors bias and measurement error. An example was the objective selection of diabetic patients with early-stage PAD using reactive hyperaemic testing and also the prior preparations by participants before the undertaking of blood pressure and blood flow measurements and the beetroot juice intervention. Prior studies assessing the effects of beetroot juice (Kenjale et al., 2011; Kapil et al., 2010; Vanhatalo et al., 2011; Webb et al., 2008; Bailey et al., 2009) accordingly put such similar measures and controls in place.

Performance bias was limited during the gathering of data in this study since the rater holding more than 5 years' experience in vascular ultrasound imaging performed the

duplex ultrasound and blood pressure measurements. Again, recall bias was limited in this study because the rater measured the peak systolic velocity and the blood pressure while blinded to the final collation of these values with the participants' anonymous codes. The control measures (Kapil et al., 2010; Vanhatalo et al., 2011) utilised in this study also contributed to effective screening for the sample of asymptomatic diabetic patients with early-stage PAD thus minimising bias due to misclassification of exposure and outcomes, while prior patient preparations also allowed basal blood flow to be similar in all participants before the ingestion of the beetroot juice intervention to reduce measurement error.

In a bid to enhance rigor of the methodology for this study, some decisions were made basing on the findings of the preceding first and second studies as follows;

i) Dropping further assessment of the dorsalis pedis artery due to lack of reproducibility of the vessel diameter inner to inner measurements in the preceding first study.

ii) Dropping the utilisation of duplex ultrasound pulsatility index due to reasons of increased variability displayed in the preceding second study.

iii) Dropping, the utilisation of duplex ultrasound resistive index despite it coming out as a robust parameter in the preceding second study in a bid to enable a more focussed assessment of peak systolic velocity in assessing acute effects of beetroot juice in the popliteal arteries only since the blood flow effects quickly waned away in this study. Duplex ultrasound resistive index will be utilised in assessing the chronic effects of beetroot juice intake on the anterior tibial arteries by diabetic patients in follow up studies.

4.2 Internal and external validity

The budget for this study was limited such that it could not afford to draw a wider heterogeneous sample which could include other populations resident in Zimbabwe to justify its external validity and generalisability to the whole Zimbabwean population. However, the tight inclusion and exclusion criteria put in place for this study increased the internal validity of the study and the findings could be generalised to the Zimbabwean Black/African population of asymptomatic diabetic patients with early-stage PAD and the non-diabetic controls. However, external validity was limited in this study since the sample was not heterogeneous and did not include other populations resident in Zimbabwe besides Black/Africans.

4.3 Conclusions and Recommendations

From the findings of this study, it was concluded that the acute effects of beetroot juice in the popliteal arteries of Black/African asymptomatic diabetic patients with early-stage PAD and non-diabetic controls reflected as reduced peak systolic velocity, systolic blood pressure and diastolic blood pressure during the 150 - 210 minutes' time point. However, peak systolic velocity also showed a clinically significant change in blood flow between groups which was not shown by systolic blood pressure and diastolic blood pressure.

The ingestion of beetroot juice by asymptomatic diabetic patients with early-stage PAD and non-diabetic participants was recommended to enable improved blood flow and better management of blood pressure. However, the findings of this study will be augmented after the undertaking of studies assessing the long term effects of beetroot juice ingestion by diabetic patients who are at a greater risk for cardiovascular diseases.

The utilisation of duplex ultrasound peak systolic velocity was also recommended to monitor long term effects of beetroot juice ingestion by asymptomatic diabetic patients after it demonstrated a clinically significant and meaningful change in blood flow between groups in this study.

The findings of this study implied that beetroot juice ingestion has short term therapeutic effects in both diabetic patients as well as non-diabetic controls which may need pursuing in long term ingestion of beetroot juice.

REFERENCES

Bahadoran, Z., Ghasemi, A., Mirmiran, P., Azizi, F., and Hadaegh, F., (2015) Beneficial effects of inorganic nitrate/nitrite in type II diabetes and its complications, Review, *Nutrition and Metabolism*, 12: 16 Doi:10.1186/512986-015-0013-6.

Bahra, M., Kapil, V., Pearl, V., Ghosh, S., and Ahlawalia, A. (2012) Inorganic nitrate ingestion improves vascular compliance but does not alter flow-mediated dilatation in healthy volunteers, *Nitric Oxide*, 26, 197-202.

Bailey, S. J., Winyard, P., Vanhatalo, A., Blackwell, J. R., Dimenna, F.J., Wilkerson, D. P., Tarr, J., Benjamin, N., and Jones, A.M. (2009) Dietary nitrate supplementation reduces the oxygen cost of low-intensity exercise and enhance tolerance to high-density exercise in humans, *Journal of Applied Physiology*; 107:1144-55.

Clements, W.T., Lee, S-R., and Bloomer, R. J., (2014) Nitrate ingestion: A Review of Health and physical performance effects, *Nutrients*, 6, 5224-5264 DOI: 10.3390/nu6115.

Clifford, T., Howatson, G., West, D.J., and Stevenson, E. J. (2015) The Potential benefits of red beetroot supplementation in health and disease, *Nutrients*, 7, 2801-2822.

Doel, J. J.; Benjamin, N.; Hector, M. P.; Rogers, M.; Allaker, R. P. (2005) Evaluation of bacterial nitrate reduction in the human oral cavity. *European Journal of Oral Sciences*, *113*, 14–19.

Hobbs, D. A, Kaffa. N, George, T. W, Methven, L, Lovegrove, J. A. (2012) Blood pressure-lowering effects of beetroot juice and Novel beetroot enriched bread products in normotensive male subjects. *British Journal of Nutrition;* 108:2066-74.

Hord, N.G., Tang, Y., Bryn, N. S. (2009) Food sources of Nitrates and Nitrites, The Physiological Context for Potential health benefits, *American Journal of Clinical Nutrition*, 90:1-10.

Huang, Z., Shiva, S., Kim-Shapiro, D. B., Patel, R. P., Ringwood, L. A., Irby, C. E., Huang, K.T., Ho, C., Hogg, N., Schechter, A.N and Gladwin, M.T. (2005) Enzymatic Function of Haemoglobin as a nitrite reductase that produces NO under allosteric control, *Journal of Clinical Investigations*,115: 2099-2107.

Gilchrist, M., Winyard, P.G., Aizawa, K., Anning, C., Shore, A and Benjamin, N. (2013) Effect of dietary nitrate on blood pressure, endothelial function and insulin sensitivity in type II diabetes, *Free Radical Biological Medicine,* 60, 89 – 97.

Gilchrist, M., Winyard, P.G., Fulford, J., and Benjamin, N. (2014) Dietary Nitrate Supplementation improves reaction time in Type 2 Diabetes: Development and application of a novel nitrate depleted beetroot juice placebo, *Nitric Oxide*, 40: 67-74, DOI: 10. 101615.niox.2014.05.003

Hakim, J. G., Mujuru, N., Rusakaniko, S., and Gomo, Z. A. R. (2005) Zimbabwe Non-Communicable Diseases Risk Factors Surveillance report, Ministry of Health and Child Care.

Hobbs, D. A, Kaffa. N, George, T. W, Methven, L, and Lovegrove, J. A (2012) Blood pressure-lowering effects of beetroot juice and Novel beetroot enriched bread products in normotensive male subjects. *British Journal of Nutrition;* 108:2066-74.

Hyde, E. R.; Andrade, F.; Vaksman, Z.; Parthasarathy, K.; Jiang, H.; Parthasarathy, D. K.; Torregrossa, A.C.; Tribble, G.; Kaplan, H.B.; Petrosino, J. F. (2014), Metagenomic analysis of nitrate-reducing bacteria in the oral cavity: Implications for nitric oxide homeostasis. *PLoS One*, *9*, e88645.

Joris, P. J., and Mensinki, R. P. (2013) Beetroot juice improves in overweight and slightly obese men postprandial endothelial function after consumption of a mixed meal. *Atherosclerosis,* 231, 78- 83.

Kannady, J. A., Aruni, A.W, Ninnis, J. R. (2012) Nitrate reductive activity of bacteria in the saliva of term and preterm infants. PMC 3466899, *Nitric Oxide*, 27(4): 191-200 Doi: 10:1016/j: niox.2012.07.004 epub 2012.

Kapil, V., Milson, A.B., Okorie, M., Maleki-Toyserkani, S., Akram, F., Rehman, F., Arghandawi, S., Pael, V., Benjamin, N., Loukogeorgakis, S., MacAllister, R, Hobbs, A. J., Webb, A. J., and Ahluwani, A. (2010) Inorganic Nitrate Supplementation lowers blood pressure in humans, Role of Nitrite Derived NO, *Hypertension,* 56,274-281

DOI: 10.1161/hypertenstionAHA.110.153536.

Kapil, V., Khambata, R.S., Robertson, A., Caulfield, M. J., and Ahluwalia, A. (2015) Dietary nitrate provides sustained blood pressure lowering in hypertensive patients: a randomised phase 2, double-blind placebo-controlled study, *Hypertension*, 65 (2): 320-327.

Doi:10.1161/HYPERTENSIONAHA.114.04675

Kenjale, A. A., Ham, K. L., Stabler, T., Robbins, J. L, Johnson, J. L., Van Bruggen, M., Privette, G., Yim, e., Kraus, W. E., and Allen, J. D. (2011) Dietary Nitrate supplementation enhances exercise performance in peripheral arterial disease, *Journal of Applied Physiology*, 110:1582-1591

DOI: 10.1152/J Appl Physiol.00071.2011.

Kiboki, K., Jiang, Z., Y., Takahara, N., Ha, S.W., Igarashi, M., Yamauchi, T., Feener, E. P., Herget, T. P., Rhodes, C. J., King, G. I. (2000) Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and *in vivo*, *Circulation,* 101:676-681

Lidder, S., and Webb, A. J. (2013) Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway, *British Journal of Pharmacology*, 75, 677-696.

Lundberg, J.O.; Weitzberg, E.; Gladwin, M. T. (2008) the nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nature. Review. Drug Discovery, 7*, 156–167.

Mcdonagh, S.T.J, Wylie, L.J., Thompson, C., Vanhatalo, A. (2019) Potential benefits of dietary nitrate ingestion in healthy and clinical populations, A brief review, *European Journal of Sports Science,* 19 (1).

Ogbonmwan, I., Siervo, L.M., Lara, J., Mathers, J.C (2012), Effect of inorganic nitrate and beetroot juice supplementation on blood pressure: a

systematic review, *Proceedings of the Nutrition Society*, 71 (OCE2), E33

Siervo, M., Lara, J., Ogbonmwan, I., and Mathers, J.C. (2013) Inorganic Nitrate and Beetroot Juice supplementation reduces blood pressure in adults: A systematic review and meta-analysis, *Journal of Nutrition*, 143: 818-826.

Sun, N. F.; Tian, A. L.; Hu, S.Y.; and Xu, L (2013) the interventional therapy for diabetic peripheral artery disease *Biomedical Central Surgery; 13*: 32.

Umans, J.G and Levi, R. (1995) Nitric oxide in the regulation of blood flow and arterial pressure. *Annual Review of Physiology*; 57:771-90.

Vanhatalo, A., Bailey, S.J, Blackwell, J. R., Dimenna, F.J., Pavev, T.G., Wilkerson, D.P., Benjamin, N., Winyard, P.G and Jones, A.M (2010) Acute and chronic effects of dietary nitrate supplementation on blood pressure and physiological responses to moderate-intensity and incremental exercise. *American Journal of Physiology-Regulatory Integrative Comparative Physiology, 299: R1121-31.*

Vanhatalo, A., Fulford, J., Bailey, S. J., Blackwell, J. R., Winyard, P., Jones, A. M. (2011) Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia, *Journal of Physiology*,589 (22) 5517-5520.

Webb, A.J., Patel, N., Loukogeorgakis, S., Okorie, M., Aboud, Z., Misras, S., Rashid, R., Miall, P., Deanfield, J., and Benjamin, N. (2008) Acute blood pressure lowering Vasoprotective and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension*, 51, 784-790.