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Repeatability of ultrasound parameters in measuring blood flow in the lower limb arteries of asymptomatic diabetic patients with early-stage peripheral artery disease

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Abstract

The objectives of the study were to i) To determine the repeatability of ultrasound parameters in measuring lower limb blood flow in diabetic patients with early-stage peripheral artery Disease (PAD) and ii) To determine whether any differences in dependent variables between sessions are significant or meaningful. Ultrasound parameters consisting of peak systolic velocity (PSV), pulsatility index (PI), resistive index (RI) and vessel diameter inner to inner (VDI) were assessed for repeatability in measuring blood flow in the popliteal arteries (PA), anterior tibial arteries (ATA) and posterior tibial arteries (PTA) of 10 asymptomatic Black-African diabetic patients [*3 males, 7 females; mean age - 49.5 (13.8) years; mean glycated haemoglobin levels (HbA_{1c}) - 5.9 (0.7)%; mean Ankle Brachial Index (ABI) 1.1 (0.1)and median Body Mass Index (BMI - 29.5 (24-33.7)]* with early stage peripheral artery Disease (PAD). Within and between sessions reliability intraclass correlation coefficients [ICC], percentage coefficient of variation [%CV]), measurement error (standard error of measurement [SEM] and smallest detectable difference [SDD] were calculated for Ultrasound parameters which include peak systolic velocity (PSV), resistive index (RI), pulsatility index (PI), and vessel diameter inner to the inner (VDI) to establish their repeatability in measuring blood flow in the popliteal arteries [PA], anterior tibial arteries [ATA] and posterior tibial arteries [PTA] of diabetic patients with early-stage peripheral artery disease [PAD]. Paired t-tests were performed and effect sizes calculated to establish if the differences between sessions were significant or meaningful. PSV, PI and RI showed very good *(ICC ≥0.8; 0.6 – 0.9, 95% CI)* to excellent *(ICC ≤1.0; 1.0 -1.0, 95% CI)* reliability and acceptably low variability (≤5.6%CV) both within and between sessions. The SEM was only high for VDI-PTA (SDD = 13.6%). PSV, PI and RI were repeatable in measuring blood flow in the lower limbs of diabetic patients with early-stage PAD.

Keywords: Doppler ultrasound imaging, Intraclass correlation coefficient, measurement error, B-mode vascular imaging, repeatability of ultrasound parameters, lower limb arteries, asymptomatic diabetic patients, early-stage Peripheral Artery Disease.

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1. INTRODUCTION

1.1 Aetiology of peripheral artery disease in Diabetic patients.

In the endothelial cells of arterial walls under normal circumstances insulin stimulates the expression and activity of endothelial nitric oxide synthase, resulting in increased production of nitric oxide which is critical for the process of vasodilation, thus maintaining stable blood pressure in the human body (Kiboki et al., 2000; Zeng et al., 2000; Kim et al., 2001). Endothelial nitric oxide is also part of the antioxidant defence system responsible for clearing reactive oxygen species, low-density lipoproteins and free radicals which are mostly produced during a host of defence and immunologic reactions by activated macrophages, thus retarding the rate of atherogenesis (Steinberg, 2009; Steinberg and Wizturn, 2010).

Insulin also promotes and maintains vascular smooth muscle cells in a well-differentiated and contractile state, thus reducing the chances of proliferation due to poor differentiation by these cells in its absence (Kiboki et al., 2000; Zeng et al., 2000; Kim et al., 2001). These stimulatory effects of insulin on Endothelial Nitric Oxide Synthase and nitric oxide production are therefore equally important in preventing endothelial dysfunction and

early pro-atherosclerotic changes which lead to Peripheral Artery Disease (PAD) (Kiboki et al., 2000; Zeng et al., 2000; Kim et al., 2001).

PAD is thus defined as atherosclerosis of the distal aorta and lower limb arteries causing arterial narrowing and impairment of blood circulation to the legs and diabetes is one of the main risk factors for causing PAD besides smoking hypertension and dyslipidaemia (Sun et al., 2013; Type 2 Diabetes in adults: management (NG 28), NICE, 2015; Sun et al., 2013; Jude, 2004; Peihua, 2003).

The chronic absence of insulin in diabetic patients also leads to a chronic absence of nitric oxide resulting in high blood pressure, dyslipidaemia, high levels of Reactive Oxygen Species, Low-Density Lipoproteins and free radicals in the circulation resulting in a gradual build-up of plaque and narrowing of arterial walls (Steinberg, 2009; Steinberg and Wizturn, 2010; Boaz et al., 2000), thus diabetes accelerates and worsens the occurrence of atherosclerosis leading to narrowing of arterial lumens and impairment of blood circulation increasing the risks of cardiovascular complications such as stroke, retinopathy, nephropathy and peripheral artery disease (PAD) to mention a few. Such complications constitute the main causes of poor prognosis amongst diabetic patients if left untreated (Seino et al., 2010).

The process of PAD progresses gradually in diabetic patients, even though most of them may be asymptomatic within the early stages (Sun et al., 2013; Peihua, 2003). However, lack of symptoms may not always be linked with early-stage PAD for evidence has shown lack of symptoms in diabetic patients with late-stage PAD especially if they experience neuropathy or lead lives of inactivity (Sun et al., 2013; Jude, 2004).

According to a study by Fowkes et al., (2013), PAD was shown as the third leading cause of atherosclerotic cardiovascular morbidity after coronary artery disease and stroke both in high income and low to medium-income countries. Gender-specific prevalence rates of PAD were found to increase with age and the prevalence in high-income countries in men at 45 – 49 years was 5.28% (95% CI, 3.38 - 8.17) while in women it was 18.83% (95% CI, 12.03 - 28.25%), though prevalence was higher in men from low to medium income countries than men from high-income countries.

In the rating of risk factors for PAD, diabetes was rated second after smoking and a prevalence of 1.88% (95% CI, 1.60 - 2.14) of diabetes was noted in high-income countries versus a 1.47% (95% CI, 1.29 - 1.68) prevalence in low to medium income countries, and this is the income band for Zimbabwe as well (Human Development Indices and Indicators: 2018).

Chronically, the arterial walls will be gradually stenosed starting with small diameter arteries below the knees such is the case with early-stage PAD in asymptomatic diabetic patients (Sun et al., 2013; Peihua, 2003). However, lack of symptoms may not always be linked with early-stage PAD for evidence has indicated lack of symptoms in diabetic patients with late-stage PAD especially if they experience neuropathy or lead a sedentary lifestyle (Sun et al., 2013; Jude, 2004).

From the early asymptomatic stage, mild PAD then manifests as intermittent claudication, which is a pain in the calf which manifests on walking but is relieved by rest. The peripheral pulses will be mostly normal to mildly decrease while the skin of the lower legs and feet will still be normal. Mild intermittent claudication, in this case, occurs due to ischaemic pain in the leg musculature when the patients walk (Type 2 Diabetes in adults: management (NG28), NICE, 2015; Macleod et al., 2008).

1.2 Classification of PAD

Classification systems for PAD must be put in place to allow accurate diagnosis of

its symptoms in each patient and this enables mapping how each patient will be treated. This consistent grading of patients enables objective criteria of treating patients with a clinical follow up (Hardman et al., 2014). Prior evidence has indicated that several classification systems have been put in place for utilisation in the classification of PAD in clinical settings, direct patient management and research (Hardman et al., 2014; Rutherford et al., 1997). According to the classification by Rutherford et al., (1997), the asymptomatic grade zero (early-stage PAD) is the category where the patient will not be experiencing symptoms of claudication even though the detected asymptomatic PAD warrants early treatment to slow its progression into critical limb ischaemia.

Prior evidence classified asymptomatic PAD as stage 1 by Fontaine et al., (1954) which refers to incomplete occlusion of arteries (Hardman et al., 2014), while Rutherford et al., (1997) classified asymptomatic PAD as grade zero with additional objective data of being confirmed by a normal reactive hyperaemic test or treadmill test (Hardman et al., 2014). However, the classification for asymptomatic PAD by Fontaine et al., (1954) had a weakness of lacking objective data to rigorously rule out the probability of symptomatic PAD (Hardman et al., 2014). Prior evidence (Hirsch et al., 2005; Rooke et al., 2011; Gerhard-Herman et al., 2016), has recommended the utilisation of Ankle Brachial Index to quantify PAD and an Ankle Brachial Index value of greater or equal to 0.9 concluded to indicate symptomatic PAD. However, a numerical value of the Ankle Brachial Index is not able to provide the clinicians with the objective information on the clinical severity of the disease. This investigation aimed to contribute to the filling of this gap in the literature by determining the repeatability of duplex ultrasound parameters in measuring blood flow in the lower limbs of diabetic patients with early-stage PAD (asymptomatic grade zero). The findings of this investigation could provide evidence for the drafting of management guidelines for diabetic patients with duplex ultrasound parameters whilst still in the secondary care of the Zimbabwean health delivery system.

In this investigation, ultrasound parameters including peak systolic velocity (PSV), pulsatility index (PI), resistive index (RI) and vessel diameter inner to inner (VDI) were combined into a diagnostic protocol and tested for repeatability in measuring blood flow in the lower limbs of asymptomatic diabetic patients with early-stage PAD. Although there is evidence showing that these ultrasound parameters have been widely used in the assessments of lower limb arteries blood flow for late-stage PAD (Eiberg et al., 2010; Andersen, 2010; Chen et al., 2015) this is the first time that these parameters have been combined this way. No prior studies which utilised ultrasound parameters to assess blood flow in asymptomatic diabetic patients with early-stage PAD were established during the writing up of this thesis. This investigation, therefore, aimed to provide evidence justifying the utilisation of duplex ultrasound parameters to assess blood flow in Black/African asymptomatic diabetic patients with early-stage PAD, which was objectively confirmed as asymptomatic grade zero PAD through a normal reactive hyperaemic test (Rutherford et al., 1997; Hardman et al., 2014).

2.0 MATERIALS AND METHODS

2.1 Design

Ultrasound parameters measurements were made by the same instrument (same sonar machine), same rater (principal investigator) under identical conditions in the two sessions. The measurements were made within a short lapse period of only 1 week (Scanlon, 2012), to avoid any probable changes in the participants' health state which could have altered the responses they elicited during first session

measurements (Kimberlin, 2008). In this case, the lapse period of one week between the measurements sessions would not have allowed a significant accumulation of PAD which could alter basal blood flow in the participants' lower limb arteries. This measure enabled minimisation of measurement error between sessions.

2.2 Participants

Both within and between sessions reliability and measurement error were assessed to determine if ultrasound parameters could repeatably measure blood flow in thirty arterial segments of the right lower limbs of 10 Black-African diabetic patients [3 males, 7 females; mean age - 49.5± (13.8) years; mean HbA_{1c} - 5.86± (0.7) %; mean - ABI 1.1 ± (0.1) and median BMI - 29.5 (24-33.7)] with early-stage PAD.

2.2.1 Ethical Considerations:

All the participants in this investigation provided written informed consent for participation and the Medical Research Council of Zimbabwe (MRCZ /A/2036) and Salford ethics board (HSR1617-32) approved the study.

2.2.2 Inclusion and Exclusion Criteria:

The participants with early stage asymptomatic PAD were recruited from the Black-African ethnic group from Mpilo Central Hospital diabetic clinic. The justification being that there is a noted higher incidence of diabetes and its complications in this population (Parirenyatwa and Gwinji, 2016-2020).

A control for the age limit for the recruited adult participants was 18 - 70 years, since the consenting age for adults in Zimbabwe is 18 years and also the fact that type 2 diabetes usually starts manifesting from adolescence onwards (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 70 years and above (Macleod et al., 2008; Type 2 Diabetes in adult's management (NG 28) NICE, 2015; Klabunde, 2007; Hernando and Conejero, 2007).

Pregnant women and those in the child bearing age but unsure of their last menstrual period were excluded because prior evidence has shown that blood pressure decreases while systemic blood flow increases as a result of systemic vasodilation in pregnancy (Mahendru et al., 2014; Sanghavi and Rutherford, 2014), therefore there were bound to be inconsistencies in blood flow measurements of the lower limbs of pregnant participants thus incurring measurement error.

Smokers and ex-smokers were excluded because there is a strong correlation between tobacco smoking and PAD (Hernarndo and Conejero, 2007; Klabunde, 2007), therefore it would not have been possible to get a representative sample of diabetic subjects with early-stage PAD amongst smokers and ex-smokers. Therefore, bias from misclassification of exposure and outcomes which would have emanated if smokers having different stages of PAD were to be included.

2.2.3 Sampling

Convenience sampling (Glen, 2015) was utilized in recruiting the ten patients for this study despite its inability to generalize study results to a wider population this sampling method appeared the most ideal with the limited resources and budget which were allotted to this study. Again, this sampling method had a weakness of selection bias since the participants selected were those who were available at the diabetic clinic when recruitment was being carried out.

2.2.4 Patient Preparation:

The diabetic patients 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 patients were advised not to take their prescribed diabetic and high blood pressure medications but to bring them on their appointment day. The justification for absconding morning medication was because the patients were made to fast 6 hours before undertaking the blood tests, blood flow and blood pressure measurements, thus they had a high chance of sliding into hypoglycaemia if they could have taken medication. 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 patients 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 since the patients needed to have a constant basal blood flow which was not influenced by the external factors controlled above. Thus, allowing the effects of early-stage PAD on blood flow to be assessed with the reduced measurement error.

The patients 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 blood pressure reducing medications. The patients were observed for about 20 minutes before being dismissed to go home.

2.3 Data collection procedures

Demographic data of participants was collected blindly using a validated Qdiabetes risk calculator in-order to minimise recall bias.

Body Mass Index for the participants was calculated to enable documentation of their health status by measuring their body fat. However, Bell et al., (2018) showed that body mass index is not an accurate measure of total body fat since it does not distinguish fat from muscle or locate where the stored fat is in the body. Despite this weakness Body Mass Index was utilised to establish the general health status of participants in this study since the limitations of body mass index are mostly associated with athletic populations (Mitchell et al., 2014; Dickerson et al., 2011).

2.3.1 Reactive Hyperaemic testing

The recruited volunteering patients underwent reactive hyperaemic testing in the physician's room at the diabetic clinic. Anonymous identification codes were issued to volunteering participants and the participants were allowed ten minutes' rest in the diabetic clinic to enable them to get a stable heart rate before undergoing hyperaemic testing.

Ankle Brachial Index was performed as a parallel test to the Doppler ultrasound parameters measurements and the upper arms and ankles blood pressure measurements were taken at a similar site on each participant.

Reactive hyperaemic testing was performed on the right leg of each of the patients. However, despite its documented weakness of causing mild discomfort in diabetic patients, the equipment for the reactive hyperaemic test was affordable within the budget compared to the treadmill test equipment (Higashi et al., 2001; Philpott and Anderson, 2007).

The classification for asymptomatic grade zero or early-stage PAD by Rutherford et al., (1997) (Hardman et al., 2014), was utilised during the recruitment of the ten diabetic patients. The patients who did not illicit a decrease or who elicited a small decrease in ankle blood pressure at rest

following reactive hyperaemia tests were included. All the diabetic patients who did not qualify to be categorised as having early-stage/asymptomatic grade zero PAD no longer eligible for this investigation but were left to continue with their care with the physician in the diabetic clinic. Therefore, this important instruction was highlighted in the information sheets given to the patients before consenting to participate in this investigation to ensure that non-qualifying patients would not be confused and discontinue their routine treatments.

This rigorous screening for diabetic patients with early stage PAD (the exposure) enabled minimisation of bias due to misclassification of exposure and outcomes because those patients in later stages of PAD were excluded.

2.3.2 Blood testing for renal function and glycaemic control

Blood tests were done first at 8 0'clock in the morning of day five in the laboratory and ultrasound measurements were done soon after blood tests downstairs in the same building. The patients' 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 to establish whether the recruited patients indeed had minimal renal damage as supposedly expected in early-stage PAD.

Blood tests were used as medical history demographic markers confirming that the prior reactive hyperaemic test had been effectively utilised to recruit diabetic patients with early-stage PAD as reflected by effective glycaemic control and minimal renal damage.

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

2.3.3 Body Mass Index and Ankle Brachial Index measurements

The automated blood pressure machine (*CareVue, Shenzhen, China)* was calibrated before utilised in the measurements for ankle brachial Index and Blood pressure.

All the recruited ten participants were escorted from the laboratory to the ultrasound room downstairs on day five. In the ultrasound room, the participants were 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 1 as follows;

$$Body\ Mass\ Index = \frac{weight}{height^2} \quad [1]$$

Ankle Brachial Index was performed on the participants after a supine rest of about 10 minutes and the highest ipsilateral ankle pressure was recorded and was subsequently divided by the highest ipsilateral upper arm pressure. Ankle Brachial Index were calculated for each participant and then collated in Microsoft excel sheets with anonymised identification codes.

Ankle Brachial Index measurements were done on day 5 together with the ultrasound measurements soon after the undertaking of blood tests in the laboratory which was located upstairs from the ultrasound private rooms. Blood tests to determine glycaemic control and renal function were important to establish the general health of the participants.

2.3.4 Duplex Ultrasound measurements

The quality control tests which were undertaken for the ultrasound scanner

included internal grid assessment for testing lateral and axial resolution for the 7.5-10.0 MHz probe of an ultrasound machine (Mindray model Z5, Shenzhen, China).

Ultrasound B-mode imaging was performed in the measurements of vessel diameter inner to inner followed by Doppler ultrasound parameters measurements for peak systolic velocity (PSV) and end diastolic velocity (EDV) which enabled automatic calculation of resistive index (RI) and pulsatility index (PI).

The linear array probe utilised has short wavelengths and high frequency which enabled high-resolution images during the scanning of superficial structures in this case blood vessels (Hamments, 2014; Hwang, 2017). This justification was deducted from the wave equation (Hamments, 2014; Hwang, 2017) which relates wavelength to the speed and frequency of the ultrasound wave as shown in equation 2;

$$ultrasound\ beam\ wavelength = \frac{Speed\ of\ sound\ in\ soft\ tissue}{ultrasound\ beam\ frequency} \quad [2]$$

Deducting from the equation it can be seen that the wavelength of the ultrasound beam is directly proportional to the speed of sound in soft tissue but inversely proportional to the frequency of the ultrasound beam.

To ensure consistency the ultrasound parameters measurements were taken by the same rater holding more than 5 years of experience in vascular ultrasound scanning in a bid to minimise performance bias. The ultrasound parameters which included PSV, EDV, RI and PI were measured from the still image of the spectral Doppler waveforms while the B-mode parameter which included vessel diameter inner to inner was measured from the still image of the longitudinal section of the popliteal arteries, anterior tibial arteries and posterior tibial arteries (Delis et al., 2000; Leoniuk et al., 2014).

In a bid to minimise measurement error these measurements were performed three times for each participant and the mean value was recorded.

Ultrasound gel was placed over the linear probe and over each artery for transverse scanning and then rotated 90^0 for the longitudinal scanning to enable the undertaking of Doppler and B-mode measurements (Hwang, 2017; Eiberg et al., 2010).

Blood flow was sampled for the popliteal arteries, anterior tibial arteries, the posterior tibial arteries and the dorsalis pedis 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 flowing in the arteries thus enabling manoeuvring for a Doppler angle of less or equal to $60°$ which gives maximum Doppler shifts interpreted as the blood velocity on the Doppler spectral display (Hamments, 2014; Hwang, 2017). Blood sampling was not done in the transverse section since it makes the ultrasound beam to be at $90°$ angle which gives zero Doppler shifts as the ultrasound beam will be traversing the arterial blood flow at right angles (Hamments, 2014; Hwang 2017). The Doppler equation was utilised in the scanning technique since it 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 an arterial 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 parameters including PI and RI were calculated automatically after the measurements for PSV and EDV were done on the displayed spectral Doppler waveform in each arterial segment for each participant.

Participants were given some refreshments before being dismissed to go home with bookings and prior preparation instructions to be followed before retesting by the after 1 week. Personalised contact was maintained with patients through prompt text messages and telephone calls to remind patients of the prior dietary preparations before their appointment for session 2 duplex ultrasound measurements. This measure enabled the minimisation of transfer bias (Pannucci and Wilkins, 2010) between the two sessions.

The quality control tests for the ultrasound scanner which were done before the undertaking of session 1 were repeated before the undertaking of session 2.

During the second session, the demographic data and Ankle Brachial Index findings of the diabetic patients were simply imported from the first session, while the blood tests were not repeated since they were simply demographic markers confirming early-stage PAD which had already been confirmed through prior reactive hyperaemic testing.

Duplex ultrasound measurements which were done in session 1 were repeated in session 2 after 1 week (Scanlon, 2012) to avoid incurring any probable changes in the patients' health state which could alter the responses which they had initially elicited during session 1 measurements (Kimberlin, 2008), such as progression of PAD if re-testing were to be done after a long-time frame. Second session measurements were blinded to the results of the first session measurements. The participants were

scanned and the data which was stored in the archives of the sonar machine was later collected and inputted it into simple excel tables tallying the scan findings of each patient with their session 1 findings by a different person to the one who scanned. This process minimised recall bias which could have occurred if one person collated the data findings (Pannucci Wilkins, 2010).

In this investigation, the shorter time frame between sessions 1 and 2 minimised the likeliness of patients moving away from the research site in pursuit of other personal reasons

During the arterial blood flow measurements, it was discovered that the longitudinal section of the dorsalis pedis arteries borders was not so clear or definite to allow repeatable and consistent measurements of vessel diameter inner to inner when compared to the posterior tibial artery, anterior tibial artery and popliteal artery vessels. Therefore, a decision was made to exclude the assessment of the dorsalis pedis artery preliminarily since all the ultrasound parameters making up the diagnostic protocol in this investigation were to be assessed for repeatability in measuring blood flow in the lower limb arteries. The data collection process then focussed on assessing the popliteal arteries, anterior tibial arteries and posterior tibial arteries.

2.3.5 Statistical analyses

The Shapiro-Wilks test (Shapiro and Wilks, 1965) was utilised to check for normality of the demographic data which included the diabetic patients' characteristics before further analysis of the data from the ultrasound parameters measurements. The justification for utilising the Shapiro-Wilks test was because statistical methods are more precise in detecting normality than graphical methods since actual probabilities that the sample was drawn from a normal population are calculated besides also being more sensitive in detecting non-normality in smaller samples of n < 100 (Zaiontz, 2013-2017). See table 2 for information on the demographic data for the first investigation. All the analyses of data was conducted using SPSS (Version 16.0; SPSS, Inc., IL, USA). Firstly, computation of the Intraclass correlation coefficient (ICC) values from within sessions single measurements for each ultrasound parameter which had been performed thrice was done. This then enabled the calculation of ICC for within-session reliability with the associated 95% confidence interval.

Secondly, the mean of the three trials of day one and the mean of the three trials on day two were calculated and computed to enable the calculation of ICC for between-sessions reliability with its associated 95% confidence interval.

ICC was then classified as follows; i) good = 0.60 - 0.74; ii) very good = 0.75 - 0.89 and iii) excellent ≥90, based on the lower bound confidence interval (Koo and Li, 2016). An *a priori* alpha level was set at $p \leq 0.05$ (Pannucci and Wilkins, 2010; Karras, 1997). The was done in a bid to try and answer the first research question for this investigation which sought to establish if the ultrasound parameters could repeatably measure blood flow in the diabetic lower limb arteries with early-stage PAD.

The ICC parameter used as a within and between sessions reliability correlation to quantify repeatability of the ultrasound parameters in this investigation had a weakness of showing a correlation only within sessions not between sessions (days), again if a measure increased or decreased by the same magnitude in all subjects then the ICC value may indicate that the measure is reliable, even though there could be a significant and meaningful change between sessions (days) (Pannucci and Wilkins, 2010; Karras, 1997). Therefore, the paired *t*-test (XU et al., 2017; Pannucci and Wilkins, 2010) was utilised to determine whether there was statistical evidence suggesting if the mean difference between the paired measurements of the two sessions was significantly different from zero or not to enable demonstration of the robustness of the measuring instrument. The hypothesis utilised here was that there was no significant difference between the paired measurements of the two sessions (days). Therefore, a statistically significant difference in the ultrasound parameters measurements between sessions would thus mean that the measurements were not stable over the two sessions. This was done in a bid to try and answer the second research question of this investigation which sought to determine if there were any significant differences in dependent variables (ultrasound parameters) between sessions.

Cohen's *d* weighting was not utilised since it was the same sample of diabetic patients measured under the same conditions over the two sessions, thus the samples were dependent. In this investigation, variability which could have been due to physiological differences between sessions/ days was quantified as a percentage coefficient of variation (%CV). To establish the variability of the ultrasound parameters amongst individuals, the mean and standard deviations (SDs) across the three trials for each individual were calculated then percentage coefficient of variation (%CV) for each individual was calculated and

expressed as an average for the 10 participants.

However, to establish the variability of the ultrasound parameters measurements between the two visiting sessions, the mean (SDs) across the average for session one and two were calculated leading to the calculation of the %CV for session one and session two. Thus good reliability was put at an upper limit of <10%CVwhen compared with previously established %CV values reporting good reliability (Thomas et al., 2015; Cormack et al., 2008; Sheppard et al., 2011).

The standard error of measurement (SEM) between sessions was calculated using the following formula in equation 4;

$SEM = SD\ (first\ observed)\ x\ \sqrt{1} -$ICC (first observed), 4

Accordingly, the smallest detectable difference (SDD) between sessions was calculated to determine the associated magnitude of measurement error using the following formula in equation 5;

$SDD =$ Z score (95% CI) x SEM x $\sqrt{2}$ thus SDD = 1.96 x SEM x $\sqrt{2}$ 5

(Lee et al., 2013; Thomas et al., 2015; Sheppard et al., 2011).

3. RESULTS

3.1 Demographic findings

In a cohort of 10 Black-African participants with early-stage PAD, 3 (30%) were males and 7 (70%) were females (Table 2). The demographic results showed that glycated haemoglobin levels, age and the Ankle Brachial Index were normally distributed, thus these were analysed as means (SD)s, while the Body Mass Index and Estimated

Glomerular Filtration rate were not normally distributed thus they were analysed as median interquartile ranges (IQR)s (Table 1).

Table 1 Descriptive statistics for demographic findings for a cohort of diabetic patients with early-stage PAD, (n = 10)

Variable	Normality test p-value	Mean ± (SD)	Median (IQR)
EGFR	0.0		105.0 (93.0 -116.0) ml/min/1.73 m^2
HbA_{1c}	0.6	6.0 (0.7)%	
BMI	0.0		30.0 (24.0 – 34.0)
Age	0.8	50.0 (14.0) years	
ABI	0.9	1.1 (0.1)	

EGFR-estimated glomerular filtration rate; HbA_{1c}-Glycated haemoglobin levels; BMI-Body mass Index; ABI-Ankle Brachial Index

3.2 The Popliteal artery (PA) findings

Peak systolic velocity, pulsatility index, resistive index and vessel diameter inner to inner showed very good to excellent reliability both within and between sessions with acceptably low variability both within and between sessions and an acceptably low Smallest Detectable Difference (SDD%) (Table 2a) Additionally, the difference between sessions for measurements for all the ultrasound parameters was equal to zero and not significant (Table 2b).

Table 2a: Descriptive and reliability statistics of ultrasound parameters in the popliteal artery (PA) (n= 10)

	Mean± (SD)		**ICC (95% CI)**		**% CV**		**Measurement error**	
Variable	**Session 1**	**Session 2**	**Within session**	**Between sessions**	**Within sessions**	**Between sessions**	**SEM**	**SDD (SDD %)**
PSV - PA	59.3 (9.0) cm/s	60.0 (8.4) cm/s	1.0 (1.0 - 1.0)	1.0 (1.0 – 1.0)	0.1%	0.3%	0.0	0.0 (0.0%)
PI -PA	6.0 (1.4)	6.0 (1.3)	0.9 (0.8 - 1.0)	1.0 (1.0 - 1.0)	0.1%	0.9%	0.1	0.4 (6.6%)
RI-PA	1.0 (0.0)	1.0 (0.0)	0.9 (0.7 - 1.0)	1.0 (1.0 - 1.0)	0.3%	0.5%	0.0	0.0 (3.0%)
VDI-PA	0.5 (0.1) cm	0.5 (0.1) cm	1.0 (1.0 - 1.0)	1.0 (1.0 - 1.0)	0.1%	0.5%	0.0	0.0 (0.0%)

SD = standard deviation; ICC = intraclass correlation coefficient; %CV = percentage coefficient of variation; SDD = smallest detectable difference; SEM = standard error of measurement; n = sample size. PSV = peak systolic velocity; PI = pulsatility index; RI = resistive index; VDI = vessel diameter inner to inner; PA = popliteal artery

Table 2b: Descriptive and between sessions comparisons of ultrasound parameters in the Popliteal Arteries (n = 10)

Vessel parameter	**Mean± (SD) session 1**	**Mean±(SD) session 2**	**Mean difference**	***t*- value**	**Degrees of freedom**	**2 tailed *p*-value**	**95% CI day 1**	**95% CI day 2**
PSV-PA	59.3 (8.0) cm/s	60.0 (9.0) cm/s	-0.3	-2.1	9	0.1	53.0; 66.0	53.3; 66.0
PI – PA	6 (2.0)	5.0 (1.3)	0.1	0.3	9	0.8	4.9; 7.0	5; 7.0
RI-PA	1.0 (0.0)	1.0 (0.0)	0.0	1.4	9	0.2	1.0; 1.0	1.0; 1.0
VDI – PA	0.5 (0.1) cm	0.5 (0.1) cm	0.0	1.5	9	0.2	0.4; 0.6	0.4; 0.6

SD= standard deviation; *t*-value = *t*- test statistic; CI = confidence interval; n = sample size; PSV = peak systolic velocity; PI = pulsatility index; RI = resistive index; VDI = vessel diameter inner to inner; PA = popliteal artery

3.3 The anterior tibial artery (ATA) Findings

Peak systolic velocity, pulsatility index, resistive index and vessel diameter inner to inner showed very good to excellent reliability both within and between sessions and acceptably low variability both within and between sessions and the associated Smallest Detectable Difference was acceptably low (Table 3a). Additionally, the difference between days of measurements for all the ultrasound parameters was equal to zero and not significant except vessel diameter inner to inner which showed a significant difference between sessions (Table 3b).

Table 3a: Descriptive statistics and within and between sessions reliability of ultrasound parameters in the anterior tibial artery (ATA) (n = 10)

	Mean± (SD)		ICC (95%CI)		%CV			
Variable	**Session 1**	**Session 2**	**Within session**	**Between sessions**	**Within sessions**	**Between sessions**	**SEM**	**SDD (SDD %)**
PSV - ATA	44.4 (9.2) cm/s	44.4 (9.0) cm/s	1.0 (1.0 - 1.0)	1.0 (1.0 - 1.0)	1.1%	0.1%	0.0	0.0 (0.0%)
PI - ATA	7.9 (2.1)	7.8 (1.8)	1.0 (1.0 - 1.0)	1.0 (1.0 - 1.0)	0.7%	0.6%	0.0	0.0 (0.0%)
RI - ATA	1.0 (0.1)	1.0 (0.1)	1.0 (0.9 - 1.0)	1.0 (1.0 - 1.0)	0.3%	0.9%	0.0	0.0 (3.0%)
VDI - ATA	0.2 (0.0) cm	0.2 (0.0) cm	0.8 (0.6 – 0.9)	1.0 (0.9 - 1.0)	5.6%	3.6%	0.00	0.0 (0.0%)

SD = standard deviation; ICC = intraclass correlation coefficient; %CV = percentage coefficient of variation; SDD = smallest detectable difference; SEM = standard error of measurement; PSV = peak systolic velocity, PI = pulsatility index; RI = resistive index; VDI = vessel diameter inner to inner

Table 3b: Descriptive statistics and between sessions comparisons of ultrasound parameters in the anterior tibial arteries (n = 10)

Vessel parameter	**Mean (SD) Session 1**	**Mean (SD) session 2**	**Mean difference**	***t*- value**	**Degrees of freedom**	**2 tailed *p*-value**	**95% CI session 1**	**95% CI session 2**
PSV - ATA	44.4 (9.6)	44.4 (9.3)	-0.0	-0.2	9	0.9	37.5; 51.2	37.7; 51.1
PI – ATA	7.9 (2.1)	7.8 (1.9)	0.1	0.5	9	0.6	6.4; 9.4	6.5; 9.2
RI - ATA	1.0 (0.1)	1.0 (0.1)	0.0	1.3	9	0.2	0.9; 1.1	0.9; 1.1
VDI - ATA	0.2 (0.0)	0.2 (0.0)	-0.0	**-2.9**	9	**0.0**	0.2; 0.2	0.2; 0.2

SD= standard deviation; *t*-value = *t*- test statistic; CI = confidence interval; n = sample size; PSV = peak systolic velocity, PI = pulsatility index; RI = resistive index; VDI = vessel diameter inner to inner.

3.4 The posterior tibial artery (PTA) findings

Peak systolic velocity, pulsatility index, resistive index and vessel diameter inner to inner showed very good to excellent reliability and acceptably low variability both within and between sessions and the associated Smallest Detectable Difference was acceptably low except for vessel diameter inner to inner where Smallest Detectable Difference was unacceptably high (Table 4a). Additionally, the difference between days of measurements for all the ultrasound parameters was equal to zero and not significant except for vessel diameter inner to inner which showed a significant difference between sessions (Table 4b).

Table 4a Descriptive statistics and within and between sessions reliability of ultrasound parameters in the posterior tibial artery

	Mean (SD)		ICC (95% CI)		%CV			
Variable	**Session 1**	**Session 2**	**Within session**	**Between sessions**	**Within sessions**	**Between sessions**	**SEM**	**SDD (SDD %)**
PSV-PTA	39.8 (11) cm/s	39.7 (11) cm/s	0.9 (0.8 - 1.0)	1.0 (1.0 - 1.0)	1.7%	0.1%	1.1	3.0 (7.6%)
PI - PTA	5.8 (1.4)	5.8 (1.3)	1.0 (0.9 - 1.0)	1.0 (1.0 - 1.0)	3.9%	0.3%	0.1	0.4 (6.6%)
RI - PTA	1.1 (0.2)	1.1 (0.1)	0.9 (0.8 - 1.0)	1.0 (1.0 - 1.0)	3.6%	0.6%	0.0	0.1 (5.9%)
VDI - PTA	0.2 (0.1) cm	0.2 (0.0) cm	1.0 (0.9 - 1.0)	1.0 (1.0 - 1.0)	4.8%	2.3%	0.0	**0.0 (13.6%)**

SD = standard deviation; ICC = intraclass correlation coefficient; %CV = percentage coefficient of variation; SDD = smallest detectable difference; SEM = standard error of measurement; PSV = peak systolic velocity, PI = pulsatility index; RI = resistive index; VDI = vessel diameter inner to inner.

(PTA) (n = 10).

Table 4b Descriptive statistics and between sessions comparisons of ultrasound parameters in the posterior tibial arteries (n = 10)

Vessel parameter	**Mean (SD) Session 1**	**Mean (SD) Session 2**	**Mean difference**	***t*-value**	**Degrees of freedom**	**2 tailed *p*-value**	**95% CI session 1**	**95% CI session 2**
PSV-PTA	39.8 (11.3) cm/s	39.7 (11.1) cm/s	-0.1	0.3	9	0.8	31.7; 47.9	31.8; 47.6
PI-PTA	5.9 (1.4)	5.8 (1.4)	0.0	0.3	9	0.8	4.9; 6.8	4.8; 6.8
RI-PTA	1.1 (0.2)	1.1 (0.1)	0.0	0.5	9	0.6	1.0; 1.2	1.0; 1.1
VDI - PTA	0.2 (0.1)	0.2 (0.1)	-0.0	**-2.5**	9	**0.0**	0.2; 0.3	0.2; 0.3

SD = standard deviation; *t* - value = *t* - test statistic; CI = confidence interval; n = sample size; PSV = peak systolic velocity, PI = pulsatility index; RI = resistive index; VDI = vessel diameter inner to inner.

4.0 DISCUSSION

In this investigation, the ultrasound parameters consisting of peak systolic velocity, pulsatility index, resistive index and vessel diameter inner to inner showed good repeatability in measuring blood flow in the lower limbs of diabetic patients with early-stage PAD which reflected as good *(ICC ≥0.8; 0.6 – 0.9, 95% CI)* to excellent *(ICC ≤1.0; 1.0 – 1.0, 95% CI)* reliability, low variability *(≤5.6% CV)*, small measurement error *(SEM ≤1.09)* and small magnitude of measurement error *(SDD <10%)* with the exclusion of vessel diameter inner to inner for the posterior tibial artery *(SDD% = 13.6%)*. Additionally, the difference between the two sessions of measurements for all the ultrasound parameters was not significant, except for vessel diameter inner to inner for the posterior tibial arteries which showed a higher magnitude of measurement error *(SDD% = 13.6%)* and a significant difference between sessions *(p = 0.0; t = -2.5)*. Similarly, the anterior tibial arteries which showed a significant difference between sessions *(p = 0.0; t = -2.9)*.

Deducting from the SEM equation; $SEM = SD\ (first\ observed)\ x\ \sqrt{1} -$ICC (first observed), it can be seen that measurements show an SEM closer to zero, thus SDD% ≤10% when reliability is 1.0 thus there will be no errors of measurement with a perfectly reliable test while a set of errors all equal to zero have no variability (Gitomer et al., 2019). Similarly, these findings were obtained by Thomas et al., (2015); Sheppard et al., (2011) in their studies with different populations as well. The findings of this investigation reflected a low SEM of ≤1.1 (SDD ≤10%) in all the measurements of the ultrasound parameters except for vessel diameter inner to inner for the posterior tibial arteries which showed an unacceptably high SDD% *(13.6%)*.

No prior evidence was found justifying the utilisation of ultrasound parameters to quantify or screen for early-stage PAD in asymptomatic diabetic patients or non-diabetic participants during the writing up of this thesis. One study by Leoniuk et al., (2014), utilised Doppler and B-mode ultrasound parameters to compare blood flow in the posterior tibial arteries and dorsalis pedis arteries of diabetic Polish participants with early-stage PAD with a non-diabetic control group and they established no significant difference in the measurements for the Doppler ultrasound between the two groups ($p > 0.05$). In their study, Leoniuk et al., (2014) did not show evidence which tested the robustness of their ultrasound tool before utilising it in measuring lower limb blood flow in a larger sample of their study to minimise on measurement error. The prior assessment of measurement error of a measurement tool is important to show the evidence that the tool can be able to accurately measure clinically significant changes which can show effects of an intervention on patients (Lee et al., 2013). Again, a small magnitude of the measurement error in a tool increases the confidence of using this tool to screen for pathology in larger sample populations (Lee et al., 2013). The generalisability of the findings of the study by Leoniuk et al., (2014) was not clear based on the fact that there was no clear description of the population studied in Poland i.e. whether the population was homogeneous or heterogeneous.

In this investigation, the ultrasound parameters including peak systolic velocity, resistive index, and pulsatility index showed a small SEM and a small SDD% except for vessel diameter inner to inner for the anterior tibial arteries and the posterior tibial arteries. These findings reflected the evidence that peak systolic velocity, resistive index and pulsatility index can detect a clinically significant change in the lower limb blood flow resulting from early-stage PAD with the exclusion of vessel diameter inner to inner due to more measurement errors.

Again, bias due to the misclassification of exposure and outcomes was not minimised in the methodology of the study by Leoniuk et al., (2014) for no controls

were put in place to limit the effects of nitrate enriched diets, blood pressure reducing medications or alcohol and this could have affected basal blood flow in the participants before the undertaking of Doppler ultrasound measurements and this could have contributed to error in their measurements.

The categorisation for early-stage by Leoniuk et al., (2014), utilised PAD grading undertaken by Fontaine et al., (1954) which classified early stage (asymptomatic) PAD in the category of incomplete blood vessel obstruction. However, the weakness of the PAD classification by Fontaine et al., (1954) is that it does not provide objective data which rules out the probability of symptomatic PAD. This was accordingly revealed in the findings of Leoniuk et al., (2014) which then showed 41 out of 148 arterial segments which had Doppler ultrasound waveforms with the biphasic flow and 4 out of 148 arterial segments which had Doppler waveforms with monophasic flow reflecting the presence of haemodynamically significant changes of symptomatic PAD amongst their participants.

In this investigation, the grading for PAD was done utilising the classification by Rutherford et al., (1997) which classified early-stage PAD as asymptomatic grade zero which is confirmed by objective data of a normal reactive hyperaemic test or treadmill test. All the diabetic patients in this investigation underwent reactive hyperaemic testing to strengthen the objectivity of grading for early-stage PAD and all the ultrasound Doppler waveforms of the participants' arterial segments showed normal triphasic flow reflecting evidence of non-haemodynamically significant changes in early-stage PAD.

4.1 Strengths and Limitations

The findings of this investigation had limited external validity because due to a limited budget, for then it was not possible to draw a wider heterogeneous sample size representing the Zimbabwean population such that these findings will only be generalised to Zimbabwean Black/African diabetic patients with early-stage PAD.

In this investigation, Body Mass Index was utilised to establish the health status of the diabetic patients though it had a weakness of not being able to differentiate weight from fat or muscle. This was because the budget of the investigation was limited and could not afford better tools for assessing body fat e.g. from skinfold thickness measurements. Thus, in this investigation Body Mass Index was simply used as a screening tool for body fatness in the participants but not as a diagnostic tool.

Reactive hyperaemic testing was utilised for screening for early-stage PAD in the asymptomatic diabetic patients despite prior evidence of it causing mild discomfort in the participants, this was because of the limited budget which could not afford a treadmill.

During the gathering of data, a decision was made to drop the measurements of the dorsalis pedis arteries since the measurements for vessel diameter inner to inner were not consistently reproducible.

The strength of this investigation was that it was carried out with some controls put in place to minimise recall, performance and misclassification of exposure and outcomes bias as well as measurement error (Pannucci and Wilkins, 2010). Again, the other control put in place in this investigation was the objective screening for early-stage PAD using reactive hyperaemic testing, HbA1c, EGFR and ABI to reduce measurement error by recruiting diabetic patients with a similar stage of PAD.

The other strength of this investigation was that the repeatability of the measurement method was determined under controlled settings to establish its robustness before utilisating it with bigger sample of participants in the second and third investigations of this thesis.

5.0 CONCLUSIONS

Based on the findings of this investigation, it was concluded that ultrasound parameters which include peak systolic velocity, pulsatility index and resistive index were repeatable in measuring the effects of early-stage PAD on the lower limb blood flow of asymptomatic Zimbabwean Black/African diabetic patients with no significant or meaningful differences between sessions except vessel diameter inner to inner.

5.1 Recommendations

In this investigation, it was therefore recommended that peak systolic velocity, pulsatility index and resistive index be utilised side by side with Ankle Brachial Index in screening and quantifying early-stage PAD in asymptomatic diabetic patients.

5.2 Implications

The deducted implications of this investigation was that the ultrasound parameters such which include peak systolic velocity, pulsatility index and resistive index form a robust diagnostic protocol for demonstrating the effects of early-stage Peripheral Artery Disease in lower limb blood flow of asymptomatic Black/African Zimbabwean diabetic patients.

5.3 Decision making for the second investigation

Regarding the outcomes of this investigation, a decision was then made to import peak systolic velocity, pulsatility index and resistive index into the second investigation to compare blood flow in the lower limb arteries of non-diabetic participants and asymptomatic diabetic patients with early-stage PAD due to their robustness shown in this investigation. The second decision made was to drop vessel diameter inner to inner from the diagnostic protocol of ultrasound parameters which was utilised in the second investigation due to more errors in measurements and instability between sessions.

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REFERENCES

Andersen, C.A. (2010) Non-invasive assessment of lower extremity haemodynamics in individuals with diabetes mellitus. Journal of Vascular Surgery, 52, 765-805.

Chen, Y-W, Wang, Y-Y, Zhao, D, Yu, et al. (2015) High Prevalence of Lower Extremity Peripheral Artery Disease in Type 2 Diabetes Patients with Proliferative Diabetic Retinopathy. PLoSONE; 10(3), e0122022. DOI: 10.1371/journal.pone.0122022.

Cormack, S. J., Newton, R. U., McGuigan, M. R., and Doyle, T. l. (2008) Reliability of measures obtained during single and repeated countermovement jumps, International Journal of Sports Physiology and Performance 3(2), 131-144 PubMed.

Eiberg, J.P., Gronvall-Rasmussen, J.B., Hansen, M.A. and Schroeder, T.V. (2010) Duplex Ultrasound scanning of peripheral arterial disease of the lower limb, European Society of Vascular Surgery, Elsevier, DOI: 10.1016/j.ejvs.2010.06.002.

Gerhard-Herman, M. D., Gornik, H.L., Barret, C., Barshes, N.R., et al., (2016), Clinical practice guideline, AHA/ACC Guideline on the Management of Patients With Lower Extremity Peripheral Artery Disease, Journal of the American College of Cardiology, Elsevier, 69 (11, http://dx.doi.org/10.1016/j.jacc.2016.11.007.

Gitomer, D.H, Martinez, J, Battey, D. and Hyland, N.E.(2019), Assessing the Assessment: Evidence of Reliability and Validity in the edTPA, American Educational Research Journal, https://doi.org/10.3102/0002831219890008

Higashi, Y., Sasakis, S., Nakagawa, K., Matsuura, H., Kajiyama, G. and Oshima, T. (2001) A non-I nvasive measurement of reactive hyperaemia that can be used to assess resistance artery endothelial function in humans. American Journal of Cardiology, 87,121-125. A129.

Hardman, R. L., Jazaeri, O., Yi, O., Smith, M. and Gupta, R. (2014) Overview of classification systems in Peripheral Artery Disease, Seminars in Interventional Radiology, 31 (4).

Kimberlin, C.L. and Winterstein, A. G. (2008) Validity and Reliability of measurement instruments used in Research. American Journal of Health-Systems Pharmacists, 65, 2276-84

Lee, P., Liu, C., Fan, C., Lu, C., Lu, W. and Hsieh, C. (2013) The test-retest reliability and the minimal detectable change of the Purdue Pegboard test in Schizophrenia, Journal of Formoson Medical Association,112, 332-337

Pannucci, C.J. and Wilkins, E.G (2010), Identifying and Avoiding Bias in Research, Plastic and Reconstructive surgery, 126 (2).

Philpott, A. and Anderson, T. J., (2007) Reactive hyperaemia and cardiovascular risk, Arteriosclerosis Thrombosis, Vascular Biology, Journal of the American Heart Association, 27, 2065-2067 http://atv.ahajournals.org. DOI: 10.1161/ATVBAHA.107.149740

Rooke, T.W., Hirsch, A.T., Misra, S., et al., (2011) ACCF/AHA focused update of the guideline for the management of patients with peripheral artery disease (updating the 2005 guideline): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Journal of American College of Cardiology, 58, 2020–45.

Rutherford, R.B.; Baker, J.D. and Ernst, C (1997), recommended standards for reports dealing lower extremity ischaemia: revised version, Journal of Vascular Surgeons, 26 (3), 517-538.

Scanlon, C., Park, K., Mapletoft, D., Begg, L. and Burns, J. (2012) Interrater and Intrarater reliability photoplethysmorgraphy for measuring toe blood pressure and toe brachial index in people with diabetes mellitus, Journal of Foot and Ankle Research, 5, 13.

Sheppard, J. M., Chapman, D. and Taylor, K. L. (2011) An evaluation of strength qualities assessment method for the lower body, Journal of Australian Strength and Conditioning, 19 (2) 4-10

Thomas, C., Jones, P.A. and Comfort, P. (2015) Reliability of the dynamic strength index in College Athletes, International Journal of Sports Physiology and Performance, 542-545, http://dx.doi.org/10.1123/ijspp.2014-0255

Type 2 diabetes in adults: management, (NG 28) NICE, (2018). www.nice.org.uk/guidance/ng28 [accessed February 2018].