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Reliability of Popliteal Artery Flow-Mediated Dilation in the Seated Position

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RELIABILITY OF POPLITEAL ARTERY FLOW-MEDIATED DILATION IN THE SEATED POSITION

by

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ABSTRACT

RELIABILITY OF POPLITEAL ARTERY FLOW-MEDIATED DILATION IN THE SEATED POSITION

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Flow-mediated dilation (FMD) is a noninvasive measurement of endothelial function, which is a useful prognostic tool for cardiovascular disease risk. Despite its widespread use since 1992, the reproducibility of FMD varies widely between studies. This variability in reproducibility is especially significant in the case of the popliteal artery due to different methodological approaches. Studies perform popliteal FMD in various body positions, with the prone and seated positions most common. However, no studies have examined the reproducibility of both the seated and prone positions of the popliteal artery FMD. Therefore, the aim of this study is to examine the test-retest and visit-to-visit reliability of the popliteal artery FMD in the seated position and to see whether differences in % FMD exist between seated and prone positions. The popliteal artery FMD was measured on two occasions in twenty healthy young adults, both in seated and prone positions. Popliteal artery diameter was measured at baseline, during 5 minutes of cuff occlusion at 220 mmHg, and following cuff deflation. FMD was calculated as the percent change from baseline diameter to peak diameter. The reliability of FMD measures were assessed in the prone and seated positions via intraclass correlation coefficient (ICC). Further, differences in FMD measures between the prone and seated positions were assessed via the three-way repeated measures analysis of variance (body position x visit x trial). The results demonstrate that the popliteal artery %FMD is reliable in the seated position both within and between visits (ICC value from 0.67 to 0.89), whereas the prone position has poor-to-moderate reliability within and between visits (ICC value

from 0.25 to 0.74)**.** To conclude, the popliteal artery FMD has a good reliability when measured in the seated position which can contribute to the development of a standard protocol to measure the FMD in the seated position.

This thesis is dedicated to my daughter Tazmeen Binte Sadique, who has made me firmly believe that, “Verily, with every hardship comes ease”.

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NOMENCLATURE

ANOVA	Analysis of Variance
BMI	Body Mass Index
B-mode	Brightness-mode
Ca^{2+}	Calcium
cGMP	Cyclic guanosine monophosphate
CI	Confidence Intervals
CV	Coefficient of Variation
CVDs	Cardiovascular diseases
DBP	Diastolic Blood Pressure
ECG	Electrocardiogram
eNOS	Endothelial nitric oxide synthase
FMD	Flow-mediated dilation
GTP	Guanosine triphosphate
HR	Heart RSDate
ICC	Intraclass Correlation Coefficient
IRB	Institutional Review Board
NO	Nitric oxide
L-NMMA	N(G)-monomethyl L-arginine
SBP	Systolic Blood Pressure
SD	Standard deviation
VSM	Vascular smooth muscle

WHO	World Health Organization

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Background

According to the World Health Organization (WHO), cardiovascular diseases (CVDs) are the leading cause of death globally, claiming an estimated 17.9 million lives each year, which is about 32% of total deaths globally (World Health Organization, 2021). Individuals with hypertension, diabetes, advanced age, and physical inactivity are at a greater risk for developing CVDs (Dhingra & Vasan, 2012; Fletcher et al., 1996; Fuchs & Whelton, 2020). A hallmark sign of CVD is impaired endothelial function (Davignon & Ganz, 2004).

The endothelium is critical for regulating vascular tone in response to various stimuli (e.g., shear stress, circumferential wall strain) and maintaining vascular health (Green, Maiorana, O'Driscoll, & Taylor, 2004). One non-invasive method of measuring endothelial function is Flow-mediated dilation (FMD), which is used to determine endothelial-dependent vasodilatory function (Thijssen et al., 2019). This test measures the vasodilatory response of conduit arteries following post-occlusive reactive hyperemia of a distal limb using high-resolution doppler ultrasonography (Green, Dawson, Groenewoud, Jones, & Thijssen, 2014). Reflecting local nitric oxide (NO) bioavailability, FMD helps in identification of pre-clinical vascular disease initiation and progression and the impact of acute and long-term interventions (Deanfield, Halcox, & Rabelink, 2007; Inaba, Chen, & Bergmann, 2010). It was found that even a reduction of 1% in FMD is associated with up to 13% increased risk of developing future CVD (Inaba et al., 2010).

Thus, FMD testing is crucial as a surrogate marker of cardiovascular risk (Gokce et al., 2002; Kuvin et al., 2001; Neunteufl et al., 2000; Perticone et al., 2001; Vita & Keaney Jr, 2002).

The measurement of endothelial function using FMD is a popular research tool due to its non-invasive nature, ability to predict cardiovascular events (Inaba et al., 2010; Ras, Streppel, Draijer, & Zock, 2013; Thijssen et al., 2011; Vita & Keaney Jr, 2002) and correlation to coronary artery endothelial function (Anderson et al., 1995; Broxterman et al., 2019; Takase et al., 1998).

Statement of Problem

The application of FMD in peripheral conduit arteries such as the brachial, radial, superficial femoral, and popliteal arteries has been increasing both in clinical and physiological studies (Betik, Luckham, & Hughson, 2004; Green et al., 2010; Kooijman et al., 2008). It is important as a diagnostic tool for FMD to have high test-retest reliability within and between visits to predict the accuracy and consistency of FMD. There are several studies that found conflicting results regarding the reliability of FMD in brachial and radial arteries due to high variability between repeated measures (Brook et al., 2005; Magda, Ciobanu, Florescu, & Vinereanu, 2013; Peretz et al., 2007; Welsch, Allen, & Geaghan, 2002).

While collectively a number of pre-testing and testing requirements are established such as subject preparation, test protocol, doppler ultrasound technique, and data analysis, there appears to be a lack of consensus on body position when examining popliteal artery %FMD (Thijssen et al., 2019). Numerous guideline papers have been published describing the pretesting and testing requirements of the brachial artery (including that the subject should be lying in the supine position); however, these guidelines do not exist for the popliteal artery (Corretti et al., 2002; Thijssen et al., 2011; Thijssen et al., 2019).

Several studies on popliteal artery %FMD have been done where the body positions of participants are variable such as sitting, prone, supine, or semi-recumbent positions, and many of these studies have focused on the effects of prolonged sitting on popliteal arteries with or without physical activity or exercise interruptions (Broxterman et al., 2019; Kadoguchi, Horiuchi, Kinugawa, & Okita, 2020; Kruse, Hughes, Benzo, Carr, & Casey, 2018; Liu, O'Brien, Johns, & Kimmerly, 2021; Morishima et al., 2016; Morishima, Restaino, Walsh, Kanaley, & Padilla, 2017; Morishima, Tsuchiya, Ueda, Tsuji, & Ochi, 2020; Nakamura et al., 2019; Naylor et al., 2011; O'Brien, Johns, Al-Hinnawi, & Kimmerly, 2020; O'Brien, Johns, Williams, & Kimmerly, 2019; Padilla, Sheldon, Sitar, & Newcomer, 2009; Parker, Ridout, & Proctor, 2006; Peddie et al., 2021; Rakobowchuk et al., 2011; Restaino, Holwerda, Credeur, Fadel, & Padilla, 2015; Restaino et al., 2016; Teixeira, Padilla, & Vianna, 2017; Vranish et al., 2017; Wu, 2022). While in some studies, popliteal artery %FMD was measured while keeping the participants in a seated posture, in other studies, the researchers measured %FMD in the supine or prone position. Given the differences in hydrostatic pressure between supine or prone and seated positions (Restaino et al., 2015), it is likely that body position impacts FMD. However, to date, no studies have compared whether %FMD is different in the prone position compared to the seated position or the reliability of popliteal artery %FMD in the seated position.

Significance of Study

Lower limb arteries, especially popliteal arteries, are more vulnerable to developing atherosclerosis and vascular diseases, even though most of the FMD studies have used brachial arteries to predict the cardiovascular risks associated with endothelial dysfunction (Debasso et al., 2004; Inaba et al., 2010). An elevated risk of mortality from CVDs is linked to sedentary behavior (Katzmarzyk et al., 2019). Several studies have indicated a correlation between prolonged sitting and post-prandial cardiometabolic risk factors and vascular dysfunction (Dempsey et al., 2016; Grace et al., 2017; Morishima et al., 2016; Morishima et al., 2017).

A meta-analysis done by Paterson et. al (Paterson et al., 2020) states that lower limb arteries are more susceptible to developing endothelial dysfunction (via reduced %FMD) during prolonged sitting compared to the upper limb arteries. Also, atherosclerotic lesions develop first in the lower limb arteries before progressing to the upper limb arteries (Sanada et al., 2005). In addition, during the resting period, the shear rate decreases more in popliteal arteries than in brachial arteries (Nishiyama, Walter Wray, Berkstresser, Ramaswamy, & Richardson, 2007). Hence, the popliteal artery %FMD is increasingly utilized in clinical settings to evaluate CVD risk and in physiological research to evaluate endothelial function.

However, the difference in methodological approach in performing popliteal artery %FMD in different studies may impact the physiological responses. The inconsistent use of body position while measuring popliteal artery %FMD in different studies is an important methodological approach which may impact the reliability and comparison of results of different studies. So, there is a strong need for reliability of the popliteal artery FMD measures. This study has focused on the within and between visits reliability of popliteal artery %FMD in the seated position and has addressed whether differences in %FMD exist between seated and prone positions. This will provide greater insight into methodological approaches regarding body position when measuring popliteal artery %FMD.

Purpose of Study

Based on the author's knowledge, there have been no studies that have focused on the reliability of popliteal artery FMD measurements in the seated posture or studies examining differences in %FMD change between the seated and prone positions. This study has aimed to examine the test-retest reliability (variability between repeated trials within a day) and visit-to-visit reliability of popliteal artery FMD measurement in the seated position. Further, this study has also examined whether differences exist in case of percentage of FMD change between the prone and seated positions in the popliteal artery.

Hypothesis

It is hypothesized that:

a) There will be good reliability of popliteal artery %FMD in the seated position.

b) The percentage change of popliteal artery FMD in the seated position will be decreased compared to the prone position.

Limitations

a) This study has relied on self-reporting for co-morbidities.

b) Blood samples were not taken to assess the variables that may influence the arterial response to vasodilatation.

Delimitations

a) Standard anatomical position of the angle of the knee was between 60-70 degrees during the FMD test.

b) FMD is influenced by age, sex, and physical activity (Thijssen et al., 2019). But in this study design, the participants are not evaluated based on these factors. We recruited subjects who are apparently young adults (18-45 years old) and healthy, ignoring these confounding factors.

c) For measuring the popliteal artery FMD a resting period of 30 minutes was allowed for hemodynamic stability.

Operational Definitions

Prone position: Subjects lying flat, face down on the bed with their legs straight and knees at 0 degree.

Seated position: Subjects sitting with their knees bent at a 60–70-degree angle based on standard anatomical position.

Popliteal artery flow mediated dilation: Diameter in the popliteal artery was assessed before and after 5 minutes of cuff occlusion. The blood pressure cuff was placed ~10 cm distal to the popliteal fossa. During cuff inflation the pressure in the cuff was inflated to ~220 mmHg.

Popliteal artery blood flow: Blood flow in the popliteal artery was measured via duplex doppler ultrasound.

%FMD: the relative change (presented as a percentage) in artery diameter from baseline to peak. The formula is, %FMD = (maximal post deflation diameter − baseline diameter)/ (baseline diameter) × 100%.

Reliability: the consistency and stability of any measurement, research result, or test refers to reliability.

Conduit artery: an artery with many collagen and elastic filaments in the tunica media, which gives it the ability to stretch in response to each pulse.

Atherosclerosis: thickening or hardening of arteries due to plaque buildup.

Shear stress: tangential force of the flowing blood on the endothelial surface of the blood vessels.

Reactive hyperemia: transient increase in blood flow following an interval of arterial occlusion.

Significance/Rationale

Endothelial dysfunction is a significant predictor of CVD (Harris, Nishiyama, Wray, & Richardson, 2010). Popliteal artery FMD is used in various studies to determine the impact of different interventions on endothelial function due to its high susceptibility to develop atherosclerosis (Hotta et al., 2019; Liu et al., 2021; Morishima et al., 2016; Morishima et al., 2020; Peddie et al., 2021; Rakobowchuk et al., 2008; Restaino et al., 2016). There are several body positional approaches while performing popliteal artery FMD measurements, including seated, supine, prone, or semi-recumbent. As several studies are performing popliteal artery FMD measurements while keeping the participants in the seated position (Liu et al., 2021; O'Brien et al., 2020; O'Brien, Johns, Robinson, Mekary, & Kimmerly, 2019; O'Brien, Johns, Williams, et al., 2019), it is imperative that this methodological approach is reliable. Knowing the reliability of popliteal artery %FMD in the seated position will be useful in comparing results among popliteal artery %FMD in different body positions and evaluating future cardiovascular events based on endothelial dysfunction. However, there have not been any studies so far, according to the author's knowledge, which have focused on the reliability of popliteal artery FMD measurements in the seated position. Therefore, this study has determined the reliability of popliteal artery %FMD in the seated position and whether there are any differences in the percent change of popliteal artery FMD between the seated and prone positions.

CHAPTER II

LITERATURE REVIEW

Endothelium-dependent FMD is the vasodilatory response of a blood vessel due to increased blood flow-associated shear stress (Pyke & Tschakovsky, 2005). The methods of FMD measurement were described first in the literature in 1989 (Anderson & Mark, 1989). At present, FMD is a popular and widely used non-invasive tool for examining peripheral artery endothelium-dependent dilation, which was first developed in 1992 in a clinical research setting to test the risk of atherosclerosis of systemic arteries in young adults and children by using high-resolution ultrasonography (Celermajer et al., 1992). The FMD response is based on the introduction of the reactive hyperemia test (Anderson & Mark, 1989) that includes temporary occlusion of blood flow by applying a pressure cuff that creates ischemia and generates shear stress stimuli. When the pressure in the cuff is released, the resultant deflation induces reactive hyperemia. This increase in shear stress is a physiological stimulus resulting in vasodilation of blood vessels (Pyke & Tschakovsky, 2005).

This literature review includes the clinical significance of FMD, especially popliteal artery FMD, as an assessment tool of endothelial function and predictors of CVDs. This review also discusses the reliability of the FMD technique in the brachial artery; and how body position elicits hemodynamic changes which may alter FMD. Lastly, the influence of cuff placement, cuff occlusion timing, cuff occlusion pressure, and cuff deflation measures on FMD is also briefly discussed in the following review. The literature review also includes some technical approaches such as baseline diameter measurement, wall tracking, shear stimulus normalization, subject preparation, and ultrasonographic methods.

Physiological Aspects of FMD

Peripheral conduit arteries, which are often termed as muscular arteries because of the large relative proportion of vascular smooth muscle (VSM), are the primary arteries where FMD is measured. These peripheral arteries, including brachial, popliteal, and femoral arteries, are imperative for effective blood flow distribution (Smith & Fernhall, 2022). These conduit arteries are composed of three layers. The outermost layer containing blood supply for the artery is the tunica adventitia. The middle layer tunica media is primarily responsible for the maintenance of lumen diameter and vascular tone (Smith & Fernhall, 2022). The innermost layer, the tunica intima (i.e., endothelium), consists of a single layer of endothelial cells and lines the whole circulatory system. The endothelium is responsive to chemical and hemodynamic stimuli and its function is crucial to maintain vascular health and vascular tone (Cahill & Redmond, 2016). Studies demonstrate that when the aorta of a rabbit is stimulated with acetylcholine, vasodilation occurs. However, this only occurs in an intact endothelium (Furchgott & Zawadzki, 1980) which demonstrates the importance of the endothelium in the regulation of vascular homeostasis and vascular tone.

Several studies have focused on the fact that FMD is dependent on a nitric oxide (NO)-mediated pathway (Doshi et al., 2001; Jiang, Seddon, Fok, Donald, & Chowienczyk, 2011; Joannides et al., 1995; Mullen et al., 2001). For example, in the presence of the NO inhibitor-N(G)-monomethyl L-arginine (L-NMMA), brachial artery FMD was abolished, and vasoconstriction ensued (Joannides et al., 1995). This demonstrates the essential role of nitric oxide in FMD.

NO acts as a vasodilator by activating cyclic guanosine monophosphate (cGMP) in the vascular smooth muscle cell (Joyner & Dietz, 1997). In a FMD test, the NO-cGMP pathway is triggered when increased blood flow creates increased shear stress (Tousoulis, Antoniades, & Stefanadis, 2005). In response to increased shear stress, calcium (Ca^{2+})-activated potassium channels open. The opening of the potassium channels hyperpolarizes the endothelial cell, leading to entry of calcium ions (Corretti et al., 2002). Calcium increases the conversion of L-arginine to NO by activating endothelial nitric oxide synthase (eNOS) (Joannides et al., 1995; Pohl, Holtz, Busse, & Bassenge, 1986).

Other vasoactive agents like adenosine, bradykinin, serotonin, and substance P also increase calcium entry into the cell, thereby activating eNOS (Dawson et al., 2006; Deanfield et al., 2007). NO then diffuses through the endothelial cells into the vascular smooth muscle cells and influences the conversion of guanosine triphosphate (GTP) to cGMP by guanylate cyclase, leading to the conversion of GTP to cGMP and the phosphorylation of eNOS, which ultimately increases the production of NO and vasodilation (Corretti et al., 2002; Corson et al., 1996; Dimmeler et al., 1999). Cyclic GMP also produces vasodilatation by activating a cGMP-dependent protein kinase, inhibiting calcium entry into the VSM cells, and activating potassium channels (Archer et al., 1994). Although NO plays a prominent role in vasodilation produced in response to increases in shear stress, the endothelium also produces other vasodilators such as prostaglandin and endothelium-derived hyperpolarizing factor (Scotland et al., 2005; Sun et al., 1999). These vasodilators, when activated, bind with receptors on the endothelial cell surface and cause vasodilation (Stankevičius, Kėvelaitis, Vainorius, & Simonsen, 2003).

Clinical Significance of FMD

Non-invasive methods for assessment of endothelial function are helpful for the early detection, risk stratification, therapeutic strategies, and preventive measures for reversing the pathophysiology of various CVDs (Sen, Chandran, Jaryal, & Deepak, 2020).

Endothelial dysfunction indicates a reduction in the ability of the endothelium to vasodilate in response to endothelium-dependent vasodilators (Hadi, Carr, & Al Suwaidi, 2005). This reduction in endothelial function can predict and precede the development of CVD (Stanhewicz, Wenner, & Stachenfeld, 2018). Endothelial dysfunction can be detected at an early stage of atherosclerosis which facilitates earlier detection of CVD (Deanfield et al., 2007; Takase et al., 1998). This atherosclerosis can be a predisposing factor for myocardial infarction, stroke, unstable thrombotic events, and plaque instability (Bennett, Sinha, & Owens, 2016).

Endothelial dysfunction has been implicated not only in hypertension and diabetes but also in other pathophysiological processes, such as renal failure, coronary syndrome, microalbuminuria, thrombosis, intravascular coagulation, sickle cell anemia, bipolar disorder, preeclampsia, dyslipidemia, hyperhomocysteinemia, rheumatoid arthritis, periodontitis, low birth weight, mental stress, and sleep apnea syndrome (de Montalembert, Aggoun, Niakate, Szezepanski, & Bonnet, 2007; Félétou & Vanhoutte, 2006; Sandoo, Carroll, Metsios, Kitas, & Veldhuijzen van Zanten, 2011; Szijgyarto et al., 2013; Westman et al., 2013; Yinon et al., 2010). Endothelial dysfunction has also been associated with physiological states such as aging and menopause (Celermajer et al., 1994) and there may be some genetic predisposition as well (Chhabra, 2009). Moreover, cigarette smoking and a sedentary lifestyle may influence endothelial dysfunction (Félétou & Vanhoutte, 2006). In a 3-year follow-up study among hypertensive postmenopausal women, flow mediated dilation was significantly correlated with carotid intima-media thickness which is positively associated with atherosclerosis progression and cardiovascular events (Rossi, Nuzzo, Olaru, Origliani, & Modena, 2011). Also, impaired FMD can independently predict in-stent stenosis even after single-vessel coronary interventions (Patti et al., 2005).

FMD Reliability Studies

Reliability, indicating the consistency of measurements obtained within or between days is important for assessment of any measurement error in any technique. (Atkinson & Nevill, 1998; Meirelles, Leite, Montenegro, & Gomes, 2007). This is particularly important for FMD where methodological variations can have a significant impact on interpreting and comparing FMD data (Thijssen et al., 2011). There have been several studies on the reliability of conduit arteries (brachial, radial, popliteal) which demonstrate varying degrees of reliability. For example, in two different studies the researchers found upper arm cuff occlusion was associated with intraclass correlation coefficients (ICC) of 0.70 and 0.94 and coefficients of variation (CV) of 5.8% and 10%, respectively, for brachial artery FMD within-day measurements (Meirelles, Leite, Montenegro, & Gomes, 2007; Onkelinx et al., 2012). In another study, the brachial artery FMD performed with forearm cuff occlusion and between days, ICC was 0.92 (Welsch et al., 2002). Given that an ICC value above 0.5 demonstrates moderate reliability, these studies support the reliability of brachial artery FMD (Koo & Li, 2016). However, in one study (Malik et al., 2004) the brachial artery FMD ICC was 0.10 with upper arm cuff occlusion which indicates the poor reliability of this method. While some studies found good reliability of brachial artery FMD, there are studies that found variability in FMD due to different methodological approaches. For example, the reliability of manual and semi-automatic measurements of brachial artery FMD utilizing edge detection software had varied coefficients of variation of 24.8% and 6.7%, respectively (Woodman et al., 2001). The transducer can also impact the repeatability of brachial artery FMD. Also, a better outcome was discovered in one study with a 13-MHz transducer compared to a 7.5-MHz transducer (CV: 26.3% vs. 45.3%) of brachial artery FMD measurements on different days (Herrington et al., 2001). In another study, a 12-MHz transducer produced brachial artery FMD with a CV of 11% (Onkelinx et al., 2012). Cuff occlusion duration can impact the reliability of FMD. As cuff inflation extends from 30 seconds to 5 minutes, there is an increase in the brachial artery FMD (Corretti et al., 2002). Further, after 4 to 5 minutes of forearm occlusion, arteries dilate the most, and longer occlusion times have no additional effects (Leeson et al., 1997). It has been noted that adhering to modern rules stringently produces remarkably reproducible results with a CV ranging from 11.6 to 16.1% (Ghiadoni et al., 2012). Also, the variation increases when subsequent FMD measurements are spaced more apart in time such as when measurements are taken in more than 4 weeks apart there is significant variation in brachial artery %FMD measurement (van Mil et al., 2016). Thus, methodological approaches have a significant impact on the reliability of FMD measures. However, there is very little information about the reliability of popliteal artery %FMD. One study found poor inter-day and intra-day reliability of popliteal artery %FMD in the prone position (McLay, 2012). However, according to the knowledge of the author, there has been no study to date on the popliteal artery FMD reliability in seated posture or studies which compared reliability of popliteal artery across multiple body positions (such as seated and prone), which signifies the importance of the current study.

Body Positions and Hemodynamics

Different body positions impose significant effects on the hemodynamics of the body. For example, blood pressure can be affected by changes in body position. For instance, blood pressure is higher when lying down than when seated (Eşer, Khorshid, Yapucu Güneş, & Demir, 2007; Netea, Lenders, Smits, & Thien, 2003). Further, heart rate is lower in the supine position compared to the seated position (Li et al., 2019). Stroke volume is greater in the supine position compared to the seated position (Bevegård, Holmgren, & Jonsson, 1960). Further cardiac output is higher in the supine or prone positions compared to the upright position during rest (Frey, Tomaselli, &

Hoffler, 1994; Leyk et al., 1994). Table 1. outlines these changes in cardiovascular variables between the supine versus upright or seated positions.

Table 1. Impact of Body Positions on Cardiovascular Variables

Author	Variable	Response
Li et al., 2019	Heart Rate	↓supine position ↑ sitting position
Netea et al., 2003, Eser et al., 2007	Blood Pressure	↑supine position ↓sitting position
Bevegard et al., 1960	Stroke volume	↑supine position ↓upright position
Frey et al., 1994, Leyk et al., 1994	Cardiac output	↑supine position ↓upright position

Methodological Consideration of FMD

Since the introduction of FMD in 1992, several research groups worldwide have adopted this technique to assess conduit artery endothelial function. Over the years, however, this technique has been modified to improve reproducibility and reliability, with methodological guidelines being introduced and updated (Corretti et al., 2002; Thijssen et al., 2011; Thijssen et al., 2019).

FMD is measured non-invasively by high resolution duplex ultrasonography, which involves simultaneous recording of artery lumen diameter and red blood cell velocity via brightness-mode (B-mode) imaging and pulsed-wave doppler waveforms, respectively (Thijssen et al., 2011). An optimal image is achieved when the probe is perpendicular to the artery of interest, such that the signals are bisecting the vessel at an insonation angle of $\leq 60^{o}$, and this insonation angle is achieved via ‘steer’ angle (Thijssen et al., 2019). Following the recommended guidelines after 2 min of baseline imaging to obtain resting blood velocity and artery diameter, a 5-minute period of distal limb ischemia is induced by inflating a pneumatic cuff to suprasystolic level (~220mmHg) to occlude the blood flow to the upper or lower limb depending on the target artery (Thijssen et al., 2019). Following cuff pressure release, lumen diameter and blood velocity are continuously recorded for an additional 3 min and 5 min for the upper and lower limb, respectively. Subsequently, FMD is calculated as the relative (percentage) change in diameter from baseline to peak following cuff release.

Cuff Position

The cuff position (distal or proximal to artery of interest) can impact the magnitude, duration, nature, and even the clinical relevance of the FMD response (Green et al., 2014; Mullen et al., 2001; Naylor, Weisbrod, O'Driscoll, & Green, 2005; Patti et al., 2005). There are several studies where the FMD response is greater in proximal cuff occlusion compared to distal cuff occlusion due to higher shear stimulus during reactive hyperemia (Agewall et al., 2001; Berry, Skyrme-Jones, & Meredith, 2000; Betik et al., 2004; Mullen et al., 2001).

This leads to an idea to use proximal cuff position to be able to detect change in FMD response more precisely. However, in one study there was no difference in prognostic value between proximal and distal cuff occlusion (Green, Jones, Thijssen, Cable, & Atkinson, 2011). Moreover, a meta-analysis of cuff placement on the vasodilatory pathway of the endothelium reported a greater amount of NO-mediated vasodilation (~72%) during distal cuff occlusion compared to proximal cuff occlusion (Green et al., 2014). As a result, the standard positioning of cuff occlusion distal to the place on the artery that is being measured is recommended to ensure NO-mediated FMD response (Thijssen et al., 2019).

Cuff Occlusion Duration and Magnitude

Cuff occlusion period has a direct impact on the vasodilatory response of FMD (Leeson et al., 1997; Mullen et al., 2001; Naylor et al., 2005). According to one study, there was a similar stepwise rise in FMD response with a stepwise increase in cuff occlusion duration (0.5, 1.5, 2.5, 3.5, 4.5, and 8 minutes), although the plateau was observed between 4.5-8 (Leeson et al., 1997). As a result, 5 minutes of cuff occlusion duration is followed as standard guidelines. Furthermore, to prevent the inflow of arterial blood, the cuff occlusion pressure must remain at least 50 mmHg above the participant's systolic blood pressure. It is standard practice now to use an occlusion pressure of 220 mmHg as that ensures that the blood vessel is occluded in non-hypertensive individuals.

Cuff Deflation Measurement

The original study and guidelines for FMD in 2002 assessed the peak diameter of arteries at 60 and 120 seconds, respectively, following cuff occlusion (Corretti et al., 2002; Celermajer et al., 1992). However, using this time frame to measure peak artery diameter results in an underestimation of FMD. In one study, the researchers used various time windows, including 50-70, 70-90, 0-90, and 0-120 seconds, and they found low peak diameter compared to true peak FMD when measured at 60 seconds (Black, Cable, Thijssen, & Green, 2008). To make a successful estimation of true peak FMD, current guidelines support monitoring the peak diameter continuously for 3 minutes in upper limb arteries and 5 minutes in lower limb arteries following cuff deflation. As arteries in the legs have a significantly later peak compared to the arms, in case of popliteal/femoral arteries at least 5 minutes of observation is needed to ensure peak diameter (Tinken, Thijssen, Black, Cable, & Green, 2008).

Moreover, in early studies, the identification of peak diameter was based on electrocardiogram (ECG) based-gated analysis (Corretti et al., 2002). This manual analysis was highly operator-dependent leading to observer error and inaccurate FMD data (Harris et al., 2010; Mancini, Yeoh, Abbott, & Chan, 2002; Preik et al., 2000; Williamson, Bronas, & Dengel, 2008; Woodman et al., 2001). Now, continuous data analysis being more time efficient, accurate, and available makes ECG-based data analysis no longer a mandatory tool for determining the peak diameter (Kizhakekuttu et al., 2010). Given the current availability of several edge detection and wall-tracking analysis systems and its superiority in terms of validity and bias, validated, accurate, and reproducible edge-detection and wall tracking systems should be used where possible (Green & Reed, 2006; Williamson et al., 2008).

Baseline Diameter

As FMD is the relative change in diameter post occlusion, the correct assessment of baseline diameter plays a crucial role in FMD measurement (Thijssen et al., 2019). According to the guidelines, baseline diameter should be recorded for 1 minute with a minimum of 30s before occluding the limbs (Thijssen et al., 2011). Most guidelines and studies calculate the baseline diameter before the start of ischemia, as at the end of ischemia the blood-flow becomes low resulting in arterial constriction (Dawson et al., 2012; Donald et al., 2008; Gori et al., 2008; Magda et al., 2013). Moreover, there is a strong inverse relationship between the baseline diameter and FMD, thus, allometric scaling is proposed to adjust for the influence of baseline diameter on FMD (Atkinson & Batterham, 2013; Black et al., 2008).

Edge Detection and Wall Tracking

Manual analysis of edge detection and wall tracking of arteries has higher chances of operator error and is more time consuming. To overcome these limitations, automated edge detection and wall tracking analysis methods are recommended for use because of their time efficiency, greater validity and reproducibility as compared to manual analysis (Preik et al., 2000; Thijssen et al., 2011; Williamson et al., 2008; Woodman et al., 2001).

Sheer Stimulus Normalization

At present there is no clear statistical strategy to account for the eliciting shear rate stimulus during FMD (Thijssen et al., 2019). Ratio normalization is statistically flawed due to its lack of validity, capability to interpret results of endothelial function and cardiovascular risk. Current guidelines recommend reporting the relevant shear rate stimulus (i.e., shear area-under-the-curve) and the peak diameter, as these data seem to have clinical prognostic values since previous work found that the shear stimulus during reactive hyperemia was related to cardiovascular risk in healthy and diseased populations (Huang et al., 2007; Mitchell et al., 2004; Paine et al., 2016; Philpott et al., 2009; Thijssen et al., 2019).

Subject Preparation

To maintain the validity and reproducibility of FMD, importance should be given to properly following subject preparation guidelines (Thijssen et al., 2019). Several factors such as food, alcohol, smoking, caffeine, drugs, and physical exercise can influence FMD directly or indirectly (Dawson, Green, Timothy Cable, & Thijssen, 2013; Green, Hopman, Padilla, Laughlin, & Thijssen, 2017; Hijmering et al., 2002; Papamichael et al., 2005). Current recommendations advise adhering to some guidelines, such as the subject remaining fasted for at least 6 hours prior to the test, avoiding caffeine, vitamin C, polyphenols, alcohol, and supplements affecting the cardiovascular system for 12 hours prior to the test, and refraining from physical activity for about 24 hours prior to the test, to avoid confounding factors. Also, participants should not smoke for 6 hours prior to the measurement. To reduce the influence of mental stress, subjects should rest in a quiet, preferably darkened room for a period of 10-15 min before the test (Ghiadoni et al., 2000;

Hijmering et al., 2002). To avoid diurnal variation, it is recommended by guidelines ((Thijssen et al., 2019) that tests be conducted at a similar time of day repeat assessments. However, there is no specific guidelines currently that says how close repeated measurements should be in terms of the time of the day. Thus, the researchers should make every attempt to have repeated measures on the same time of the day. Due to hormonal effects during the menstrual period in premenopausal women, FMD measurements should be assessed in a standardized part of the menstrual cycle (Williams et al., 2001).

Ultrasound Assessment of Flow-Mediated Dilation

Vascular ultrasonography measures the size and volume of blood flowing through vessels in the body using ultra-high frequency sound waves. The ultrasound machine has two modes, which are referred to as "duplex." One of them is B-mode (brightness mode), which produces a two-dimensional image of the vessel under investigation. The second method, known as pulsed wave doppler, records sound waves that are reflected off moving objects either toward or away from the probe (e.g., red blood cells). Pulse wave doppler technologies are used to assess the direction and velocity of blood flow in the blood vessels (Revzin et al., 2019).

Summary

Recently, there have been several studies to see the effects of prolonged sitting or sedentary lifestyles, which have focused on popliteal artery FMD as an indicator of vascular dysfunction (Boyle et al., 2013; Liu et al., 2021; Morishima et al., 2016; Morishima et al., 2017; Peddie et al., 2021; Restaino et al., 2016; Shivgulam et al., 2022).

One recent meta-analysis provides evidence that prolonged uninterrupted sitting has a more detrimental effect on FMD in lower limb arteries than upper limb arteries (Paterson et al., 2020). Also, there have been several studies on popliteal FMD that used different postures to measure the

FMD such as supine, prone, semi-recumbent, and seated posture (Kadoguchi et al., 2020; Liu et al., 2021; Morishima et al., 2016; Morishima et al., 2017; Morishima et al., 2020; Nakamura et al., 2019; O'Brien, Johns, Robinson, et al., 2019; O'Brien, Johns, Williams, et al., 2019; Parker et al., 2006; Peddie et al., 2021; Rakobowchuk et al., 2011; Restaino et al., 2016). As there is no fixed body position for measuring FMD in popliteal arteries, it is important to make some special considerations regarding the reliability and precision of these findings.

FMD was first measured in the brachial artery, which was further validated by an invasive assessment of endothelial function using coronary arteries (Anderson et al., 1995; Celermajer et al., 1992). However, there is little information about the reliability of the popliteal artery, specifically when measured in the seated position. There are several studies that have followed the popliteal FMD measurement in the seated position (Liu et al., 2021; O'Brien et al., 2020; O'Brien, Johns, Williams, et al., 2019; Wu, 2022). These studies also have showed variability in knee angle positioning. In one study the knee angle was 90 degrees, in some other studies the knee angle was 60-70 degrees (Liu et al., 2021; O'Brien et al., 2020; O'Brien, Johns, Williams, et al., 2019). Lastly, one study examined the knee angle at 0, 45, and 90 degrees (Wu, 2022).

Moreover, in studies measuring the popliteal artery FMD after a long period of sitting time, the participants were moved back from the seated position to the lying position (Morishima et al., 2016; Restaino et al., 2015). This change in position is particularly important for the test assumptions and measurements. In the mechanism of FMD, shear stress plays a vital role during reactive hyperemia (Stoner, Tarrant, Fryer, & Faulkner, 2013). This shear stress stimulates the mechanoreceptors in the blood vessels, leading to the release of NO and a vasodilatory response occurs. This endothelial health is governed by the anti-atherogenic function of NO (Stoner et al., 2021). However, in the studies where there is a change in position from sitting to lying, especially in prolonged uninterrupted sitting studies, the hemodynamic instability resulting from lifting the subject from seated to supine position is challenging (Horiuchi & Stoner, 2021; Morishima et al., 2016; Restaino et al., 2016). To ensure hemodynamic stability it takes time, usually more than 10 minutes (Stoner et al., 2021), which makes the results from sitting interruption studies problematic by masking the true interaction effect between prolonged sitting with its effects and interruption strategies. Considering this evidence, the purposes of this study are to measure the reliability of popliteal FMD measurements in seated position and to examine whether popliteal artery %FMD is different between the two body positions (seated & prone).

CHAPTER III

METHODOLOGY

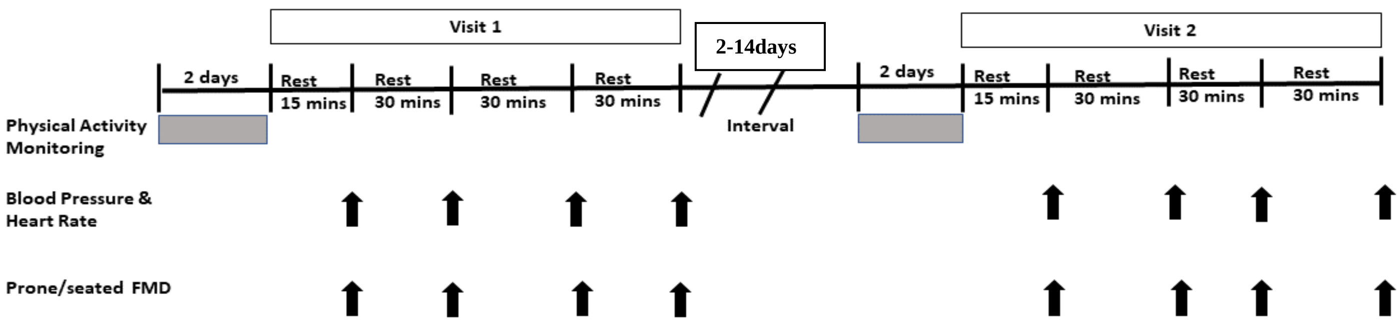
Participants

Based on previous studies related to FMD reliability, the number of participants was 20 (Liang et al., 1998; Pala et al., 2009; Sorensen et al., 1995). The inclusion criteria for participating included that participants were young (18-45 years of age), healthy, and nonobese (Body Mass Index (BMI) < 30 kg/m^2). Further, females were only studied in their early follicular phase to control for the effects of the menstrual cycle on endothelial function. Criteria for exclusion included the following: individuals with known CVD, hypertension, or metabolic diseases; on any medication affecting hemodynamic responses at the time of screening; and females who were pregnant, breastfeeding, or taking any hormonal contraception pills at the time of recruitment. The confirmation of eligibility to participate was determined by a health history questionnaire. Recruitment was conducted by using the university Institutional Review Board (IRB)- approved flyers and advertisements using internet sources such as university announcement emails, social media, and via word of mouth. All experimental procedures and measurements conformed to the Declaration of Helsinki, and the university IRB has approved the experimental protocols.

Study Design

This study used an experimental, randomized, and within-subject research design and was conducted at the Cardiometabolic Laboratory at Old Dominion University. Participants came to the lab for a “consent” meeting, where the details of the study were explained to them. If the subject chose to sign the consent form and participate in the study, then a health history questionnaire form was filled out and height and weight were measured using a physician’s scale (Detecto, Webb City, MO, USA) to determine the participant’s eligibility. Then, the participants had two, 3-hour visits to the laboratory within a period of 2 to 14 days. Further, body composition was assessed via air displacement plethysmography (Bod Pod, Cosmed USA Inc., Concord, CA) which took about 20 minutes. Moreover, participants were asked to track their physical activity levels for 2 days before beginning the study using an accelerometer (wGT3X-BT, ActiGraph, Pensacola, FL). Figure 1 demonstrates an example of the study design.

Figure 1. Experimental Design of the Study



Experimental Protocol

FMD of the popliteal artery was assessed according to the guidelines for the current standardized methodology (Corretti et al., 2002; Thijssen et al., 2019). To minimize confounding factors and their influence on FMD, participants were requested to refrain from engaging in any exercise 24 hours prior to the experiments and avoid the consumption of alcohol and caffeine for 12 hours beforehand. Smokers were required to abstain from smoking for at least six hours. Participants were given accelerometer (wGT3X-BT, ActiGraph, Pensacola, FL) to wear 48 hours prior to their study visit to monitor physical activity levels. Additionally, participants arrived at the lab after having refrained from eating for at least 6 hours, and the experiments were performed in a quiet, temperature-monitored (21-23°C) room after a period of rest for 15 minutes. Furthermore, as diurnal variation can affect FMD readings, the repeated measurements were performed at the same time of day (with a one-hour range) based on recommended guidelines (Thijssen et al., 2019). To minimize the potential confounding influence of exogenous sex hormones on vascular function, females were evaluated in the early-follicular phase (i.e., days 1-7 following the onset of menstruation) of their self-reported natural menstrual cycle (Thijssen et al., 2011).

During each visit, the subjects rested for 15 minutes in the seated or prone position and the body positions were randomized for the first trial on each visit to minimize the effect of order using the online randomization tool (www. Randomizer.org). For the subsequent 3 trials in each visit alternating body positions were assigned. For example, if the first trial was in the prone position based on randomization, the 2nd trial was performed in the seated position, 3rd trial in the prone position and finally, the 4th trial was performed in the seated position. Non-diagnostic 3 leads ECG were placed on the torso of the participant to monitor heart rate while continuing the procedure. After resting for 15 min, resting blood pressure was measured by an automated sphygmomanometer (Welch Allyn, Skaneateles Falls, New York, USA) with an appropriately sized cuff. To measure the popliteal artery FMD, the ultrasonography probe was placed immediately distal to the popliteal fossa, and a pneumatic pressure cuff attached to a reliable rapid cuff inflation system (E20 and AG101; Hokanson, Bellevue, WA, USA) was placed around the calf distal to the popliteal fossa (~10cm) of right lower limb (Shivgulam et al., 2022). In the seated position, the knee remained at an anatomical 60–70-degree angle for all measurements. In the prone position, the knee remained at a zero-degree angle. Baseline arterial lumen diameter and blood velocity were measured for 3 minutes. While continuing to record arterial lumen diameter and blood velocity, the pressure in the cuff was rapidly inflated to ~220 mmHg and remained at that occlusion pressure for 5 minutes. Then, following the deflation of the pressure cuff, another 5 minutes of continuous ultrasound recording was done. Vascular measurements were recorded via duplex ultrasonography using an 11-MHz multi-frequency linear array probe connected to a high-resolution ultrasound system (Logiq P9, GE Medical Systems, Milwaukee, WI) which is reliable and valid (Ratcliffe, Pawlak, Morales, Harrison, & Gurovich, 2017). Popliteal artery velocity was recorded at a pulsed frequency of 4.2 MHz and was corrected using an insonation angle of ~60° with the cursor set to mid-vessel. Offline analysis of vessel diameter and velocity was performed using Cardiovascular Suite FMD Studio (Quipu, Pisa, Italy). Participants then rested in the seated or prone position for 30 minutes to allow the blood flow and arterial dilatation to return to normal resting conditions, and the same procedure as described above was repeated 3 additional times (Harris, Padilla, Rink, & Wallace, 2007). Also, immediately before and after each FMD measurement blood pressure and heart rate were measured.

In the 2nd visit, all measurements were repeated utilizing the same pre-testing and procedural requirements as the first study visit.

Data Analysis

Ultrasound-derived vascular recordings were exported as video signals directly to a laptop that has reliable and valid automated commercial edge-detection and wall-tracking software combined with simultaneous doppler blood velocity waveform analysis (FMD Studio, Cardiovascular Suite, version 3.6, Quipu, Pisa, Italy) (Shivgulam et al., 2022). This software was used to measure the popliteal artery baseline diameter, peak diameter following cuff deflation, and minimum diameter during cuff inflation. Ultrasound images and video were recorded digitally for off-line analysis using the Elgato (Munich, Germany) video capture software. Following O'Brien et al. (O'Brien et al., 2020) %FMD was quantified in relative: [(post-cuff deflation peak diameter – baseline diameter) ÷ (baseline diameter) × 100%].

Statistical Analysis

All statistical analyses were performed using SigmaPlot software version 12.5 (Systat Software Inc., San Jose, CA), IBM SPSS Statistics 28 (IBM, Armonk, NY, USA), and Microsoft Excel 2205 (Microsoft, Seattle, Wash., USA). Descriptive statistics are used to describe the sample characteristics and are reported as the mean ± standard deviation (SD). The normality of the data was checked using the Shapiro-Wilk test, as the estimated sample size is less than 50. A p value of ≤0.05 is considered significant. A three-way (2 body position x 2 visit x 2 trial) repeated measures analysis of variance (ANOVA) was used to identify differences between baseline diameter, %FMD, minimum diameter during cuff inflation, and maximum diameter following cuff deflation with variances such as body position, visit number, and trials obtained during the tests and retests. The reliability of %FMD, baseline diameter, post-cuff deflation peak diameter, and the minimum diameter following cuff inflation between the trials were assessed using intraclass correlation coefficient (ICC) (Hendricks & Robey, 1936). These values were calculated using IBM

SPSS Statistics 28 based on absolute agreement and a 2-way mixed effects model. While interpreting ICC values, the following ranges were used where an ICC < 0.5 indicates poor reliability, an ICC value between 0.5 to 0.75 indicates moderate reliability, values between 0.75 and 0.9 indicate good reliability, and ICC > 0.90 indicates excellent reliability (Koo & Li, 2016).

CHAPTER IV

RESULTS

Characteristics of the Participants

An overview of characteristics of the 20 individuals (15 males & 5 females) who participated in the study is provided in Table 2.

Table 2. Subject Characteristics (n=20)

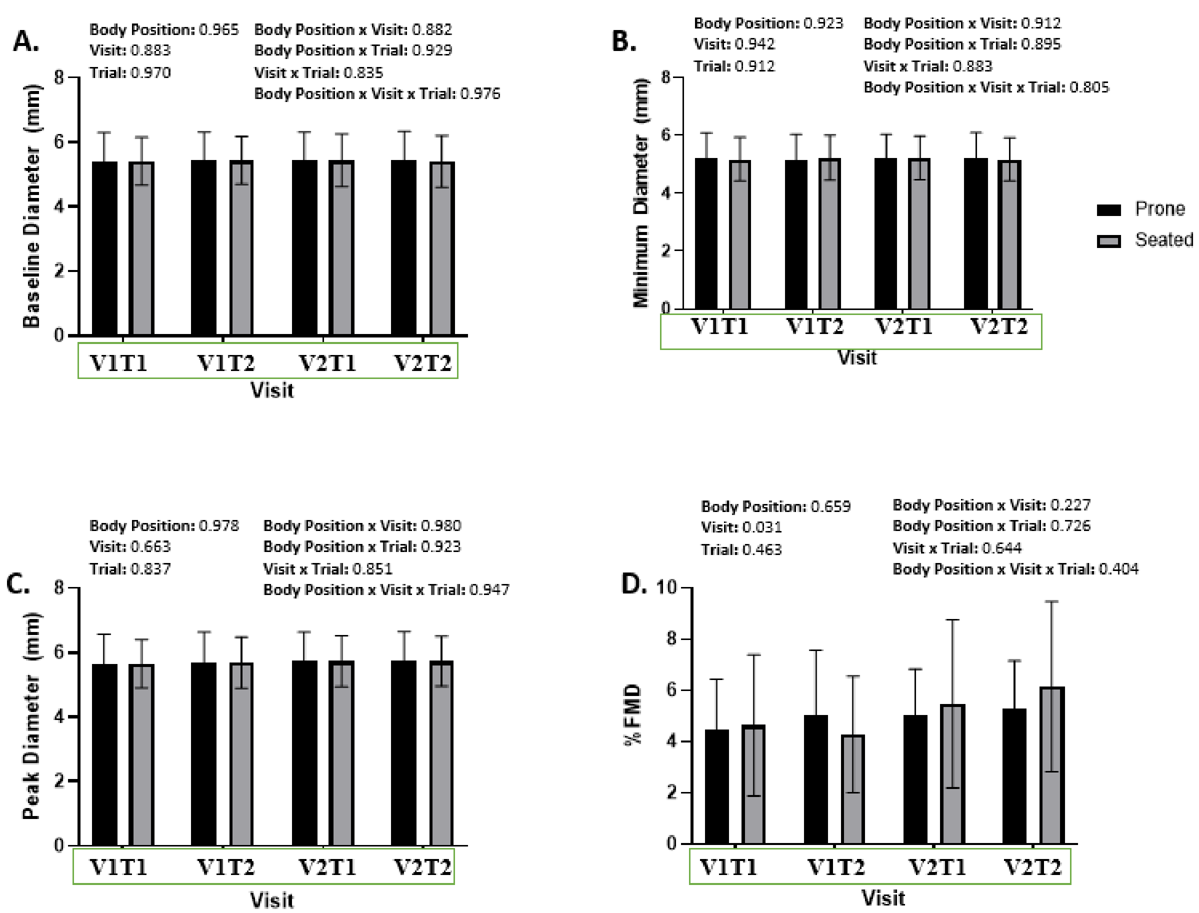
Variables	Data
Age (years)	29±5
Body mass (kg)	71.9±10.1
Height (cm)	168.2±9.1
BMI (kg/m^2)	25.5±3.0
Body fat (%)	29.4±10.1
Fat-free Mass (%)	70.6±10.1
Resting HR (bpm)	65±10
Resting SBP (mmHg)	110±8
Resting DBP (mmHg)	71±6

Notes: Values are expressed as mean ± SD (Standard deviation); BMI-Body Mass Index; SBP- Systolic Blood Pressure; DBP- Diastolic Blood Pressure; HR- Heart Rate; bpm- beats per minute

FMD Variables and Measurements

Baseline diameter, minimum diameter during cuff inflation, maximum diameter following cuff deflation, and %FMD during the seated and prone positions within visits and between visits are represented in Figure 2.

Figure 2. FMD Variables and Measures. 1st Visit 1st Trial (V1T1), 1st Visit 2nd Trial (V1T2), 2nd Visit 1st Trial (V2T1), and 2nd Visit 2nd Trial (V2T2)



Average baseline diameter and minimum diameter during cuff inflation of the popliteal artery were found to be normally distributed using Shapiro-wilk normality test ($p = 0.112$ and $p = 0.068$, respectively). However, peak diameter of the popliteal artery following deflation and %FMD were not normally distributed ($p < 0.05$). Natural log transformation and a log10 transformation were unsuccessful at normalizing these variables, thus we are reporting the non-transformed data (Brown-Forsythe test). No significant main effects for body position, visit , or trial on baseline diameter, minimum diameter during cuff inflation, or peak diameter post-cuff deflation were found. Further, no significant interactions existed. However, a significant main effect of visit ($p = 0.031$) was found for %FMD (Visit 1: 4.61±2.37 and Visit 2: 5.48±2.66). No other significant main effects or interactions were shown for %FMD ($p > 0.05$) (Figure 2).

Measures of Reliability of the Popliteal Artery FMD in the Prone Position

The ICC estimates and their 95% confidence intervals (CI) for baseline diameter, minimum diameter during cuff occlusion, peak diameter following cuff deflation, and %FMD in the prone position are shown in Table 3. Baseline diameter, minimum diameter during cuff inflation, and peak diameter following cuff deflation within and between visits were foundto have high ICC estimates (>0.90), indicative of excellent reliability. %FMD within visits ICC estimates demonstrated poor-to-moderate reliability (1^{st} visit ICC: 0.60, 2^{nd} visit ICC: 0.25). %FMD between visits also demonstrated poor-to-moderate reliability, with ICC estimates below 0.75 for all measures.

Table 3. ICC Values of FMD Measurements of Popliteal Artery in the Prone Position

Variable	Within-Visit ICC (95%CI)	Variable	Between-Visit ICC (95%CI)	
			Visit 2 Trial 1	Visit 2 Trial 2
%FMD (Visit 1)	0.60 (0.00-0.84)	%FMD (Visit 1 Trial 1)	0.68 (0.23-0.87)	0.35 (-0.53-0.73)
%FMD (Visit 2)	0.25 (-0.99-0.71)	%FMD (Visit 1 Trial 2)	0.48 (-0.36-0.80)	0.74 (0.33-0.90)
Baseline Diameter (mm) (Visit 1)	0.99 (0.98-1.00)	Baseline Diameter (mm) (Visit 1 Trial 1)	0.99 (0.97-1.00)	0.99 (0.98-1.00)
Baseline Diameter (mm) (Visit 2)	1.00 (0.99-1.00)	Baseline Diameter (mm) (Visit 1 Trial 2)	1.00 (0.99-1.00)	0.99 (0.98-1.00)
Peak Diameter (mm) (Visit 1)	0.99 (0.98-1.00)	Peak Diameter (mm) (Visit 1 Trial 1)	0.99 (0.97-1.00)	0.99 (0.95-1.00)
Peak Diameter (mm) (Visit 2)	0.99 (0.98-1.00)	Peak Diameter (mm) (Visit 1 Trial 2)	0.99 (0.99-1.00)	0.99 (0.98-1.00)
Minimum Diameter (mm) (Visit 1)	0.99 (0.97-1.00)	Minimum Diameter (mm) (Visit 1 Trial 1)	0.99 (0.97-1.00)	0.99 (0.98-1.00)
Minimum Diameter (mm) (Visit 2)	0.99 (0.97-1.00)	Minimum Diameter (mm) (Visit 1 Trial 2)	0.98 (0.96-0.99)	0.98 (0.96-0.99)

Measures of Reliability of the Popliteal Artery in the Seated Position

The ICC estimates and their 95% confidence intervals for baseline diameter, minimum diameter during cuff occlusion, peak diameter following cuff deflation, and %FMD in the seated position are shown in Table 4. Baseline diameter, minimum diameter during cuff inflation and peak diameter following cuff deflation within visits and between visits were found to have high ICC estimates >0.90 indicative of excellent reliability. %FMD within visit ICC estimates demonstrated good reliability (1st Visit ICC: 0.86, 2nd Visit ICC: 0.89). %FMD between visit ICC estimates demonstrated moderate-to-good reliability with ICC estimates between 0.67-0.87 for all measures.

Table 4. ICC Values of FMD Measurements of Popliteal Artery in the Seated Position

Variable	Within visit-ICC (95%CI)	Variable	Between-visit ICC (95%CI)	
			Visit 2 Trial 1	Visit 2 Trial 2
%FMD (Visit 1)	0.86 (0.64-0.94)	%FMD (Visit 1 Trial 1)	0.87 (0.66-0.95)	0.83 (0.39-0.94)
%FMD (Visit 2)	0.89 (0.72-0.96)	%FMD (Visit 1 Trial 2)	0.81 (0.47-0.93)	0.67 (0.09-0.87)
Baseline Diameter (mm) (Visit 1)	0.98 (0.96-0.99)	Baseline Diameter (mm) (Visit 1 Trial 1)	0.98 (0.96-0.99)	0.98 (0.96-0.99)
Baseline Diameter (mm) (Visit 2)	1.00 (0.99-1.00)	Baseline Diameter (mm) (Visit 1 Trial 2)	0.99 (0.97-0.99)	0.98 (0.96-0.99)
Peak Diameter (mm) (Visit 1)	0.98 (0.96-0.99)	Peak Diameter (mm) (Visit 1 Trial 1)	0.99 (0.97-1.00)	0.98 (0.96-0.99)
Peak Diameter (mm) (Visit 2)	1.00 (0.99-1.00)	Peak Diameter (mm) (Visit 1 Trial 2)	0.99 (0.99-1.00)	0.99 (0.97-1.00)
Minimum Diameter (mm) (Visit 1)	0.97 (0.93-0.99)	Minimum Diameter (mm) (Visit 1 Trial 1)	0.98 (0.95-0.99)	0.98 (0.95-0.99)
Minimum Diameter (mm) (Visit 2)	1.00 (0.99-1.00)	Minimum Diameter (mm) (Visit 1 Trial 2)	0.99 (0.98-1.00)	0.99 (0.98-1.00)

CHAPTER V

DISCUSSION

The main purpose of this study was to assess the reliability of the popliteal artery FMD measurement in the seated position within visits and between visits so that test-retest and visit-to-visit reliability of %FMD could be assessed. Moreover, this study also focused on investigating the difference in %FMD between the prone and seated positions. A total of eight trials across two visits (4 in the seated position and 4 in the prone position) were assessed. The findings indicate that the baseline diameter, peak diameter, minimum diameter, and %FMD of the popliteal artery had moderate to excellent reliability in the seated position both within and between visits (ICC > 0.67). Although the baseline diameter, peak diameter, and minimum diameter had excellent reliability within and between visits in the prone position, %FMD demonstrated poor-to-moderate reliability both within and between visits in the prone position. Furthermore, there were no significant differences in baseline diameter, peak diameter, or minimum diameter between body positions, visits, or trials. While %FMD was higher following the 2^{nd} visit compared to the 1^{st} visit, no other differences (body position or trial number) were shown.

Given that vascular shear rate is reduced in the seated position compared to the supine position (Trinity et al., 2014), and the important role that shear rate has on stimulating vasodilation of blood vessels (Smieško, Kožík, & Doležel, 1985), we expected that the seated position would result in a lower %FMD compared to the prone position. However, contrary to our hypothesis, popliteal artery %FMD was not found to be different between the seated and prone body positions. One other study (Soga et al., 2007) demonstrated similar findings in the brachial artery when comparing the seated versus supine %FMD by reporting no statistically significant difference. To our knowledge, this thesis is the first to report in the popliteal artery that there is no statistically significant difference in %FMD between the seated and prone positions.

This study reported ICC values to assess the reliability of popliteal artery FMD measures. The ICC values for baseline diameter, peak diameter following cuff deflation, and minimum diameter during cuff inflation all have excellent reliability (ICC > 0.9) in both positions (seated and prone position) within and between visits. These results confirm findings from McLay (McLay, 2012) in that popliteal artery FMD measurements made in the prone position demonstrate high reliability for baseline (ICC: 0.91) and peak (ICC: 0.86) diameters. This high reliability for the baseline and peak diameter has been supported in other studies examining other conduit arteries. For example, West et al. (West et al., 2004) found that the baseline and peak diameter of the brachial artery had a small coefficient of variation (2.7% and 2.5%, respectively) measured on different days, supporting the high reliability of the FMD technique in measuring the baseline and peak diameter of conduit arteries. The reliability of baseline and peak diameter measurement using the FMD technique was also supported by Harris et al. (Harris, Padilla, Hanlon, Rink, & Wallace, 2007), who reported a between-days ICC value of 0.96 and 0.93 for baseline and peak brachial artery diameter, respectively. Another study on brachial artery FMD showed an average ICC of 0.95 for baseline diameter and an average ICC of 0.93 for peak hyperemic diameter measured across testing sessions on different days (Richardson, 2016). These data also support the high reliability of the FMD technique in measuring the baseline and peak diameter of the conduit arteries. Collectively, these findings are comparable to the reliability that was found for the popliteal artery in the present thesis. However, we expand upon the current literature knowledge base by demonstrating that baseline diameter, peak diameter, and minimum diameter demonstrate excellent reliability between and within visits in both the seated and prone positions.

%FMD measured in the prone position demonstrated less reliable ICC values of 0.60 and 0.25 for within visit 1 and visit 2 trials, respectively. A range of ICC values from 0.35 to 0.74 was found in the prone position for between visits reliability, which indicates that reliability of the popliteal artery %FMD is poor-to-moderate (ICC<0.75) in this prone position. Collectively, these findings confirm the results from McLay (McLay, 2012) and McLay et al. (McLay, Nederveen, Pogliaghi, Paterson, & Murias, 2016) which found that the ICC values for popliteal artery %FMD in the prone position intra-day and inter-day were 0.36 & 0.25, respectively. Collectively, in the prone position within the popliteal artery, the present thesis found that baseline diameter and peak diameter had reliable ICC values; however, the %FMD was not found to be reliable. These results confirm data reported by McLay who also demonstrated reliable baseline and peak diameter ICC values but not reliable ICC values for %FMD in the prone position. Similarly, West et al. (West et al., 2004) demonstrated that baseline diameter had a small coefficient of variation while %FMD had a large coefficient of variation. Our data and others (McLay, 2012; West et al., 2004) suggest that baseline and peak diameter measurements are reliable. However, the difference in the change in diameter from baseline to peak is small. Thus, when calculating the % change in diameter, the error is amplified due to this small difference. This results in a worse ICC value for %FMD (McLay, 2012). On the other hand, the reliability for popliteal artery %FMD had moderate-to-good reliability in the seated position for both within and between visits. The mechanism behind why the seated position but not prone position had a good %FMD reliability is not known but should be investigated in future studies. To our knowledge, no studies have examined the reliability of the popliteal artery in the seated position. Thus, this study appears to be the first to demonstrate that popliteal artery %FMD has good reliability in the seated position.

This present study was developed following the standard protocol to control for different variables that may have a confounding impact on the findings. For example, all the tests were performed in a quiet and temperature-monitored room. To minimize the effect of diurnal variation, all the tests were performed at the same time with a 1-hour period range. The gap between two visits was 2-14 days, and a single sonographer performed all FMD data recording and analysis. To avoid the impact of different variables, participants were instructed to arrive in a fasted state for a minimum of six hours, avoid exercise 24 hours prior to each scheduled exam, and refrain from consuming tobacco and caffeine for 6 and 12 hours, respectively, prior to the visit. Female participants were assessed during the first 7 days of their menstrual cycle to avoid potential influences of female sex hormone variation on FMD measures. Adhering to this protocol likely improved the reliability measures of the FMD technique in both positions, as others have demonstrated remarkable reproducibility with a CV ranging from 11.6 to 16.1% (Ghiadoni et al., 2012).

Limitations

This study has some limitations including that it was only conducted on people who were apparently healthy and self-reported no relevant co-morbidities. Although the participants fasted for 6 hours prior to the visit, their diets before each visit were not standardized, which might have influenced the FMD measures. One study demonstrated that low salt consumption (50 mmol Na/d) resulted in a lower brachial artery FMD compared to the usual salt consumption (150 mmol Na/d) diet (Dickinson, Keogh, & Clifton, 2009). Another study demonstrated increased brachial artery FMD in subjects who consumed a low-carbohydrate diet for 12 weeks compared to a low-fat diet (Volek et al., 2009). Thus, the food that is consumed prior to the FMD test may impact %FMD.

Conclusion

To conclude, this study demonstrated that popliteal artery %FMD has moderate-to-good reliability for both test-retest and visit-to-visit in the seated position. However, poor-to-moderate reliability was demonstrated when %FMD was performed in the prone position. One of the implications of this study would be that the popliteal artery FMD can be performed in the seated position following recommended guidelines which will help researchers conduct studies related to prolonged sitting or a sedentary lifestyle in the seated position. Moreover, the popliteal artery %FMD demonstrating less reliability in the prone position warns caution while using this technique to predict cardiovascular disease risk in clinical trials.

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- Demonstrated practical use of exercise testing equipment to undergraduate students.
- Laboratory reports and skill tests evaluation and grading

Graduate Research Assistant, Cardiometabolic Research Laboratory, Department of Human Movement Sciences, January 2022 – May 2023

- Performing several tests, including resting metabolic rate measurement using indirect calorimetry, body composition assessment via air displacement plethysmography, and blood flow assessment via FMD ultrasonography for research subjects
- Collecting and analyzing data from research studies in the laboratory using different software, Subject recruitment

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PUBLICATIONS

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- Lutfor, A. B., Afroz, S., Mahmud, A. M., Selim, T., Akhter, T., Sultana, T., & Taskeen, S. (2021). Nocardia infection causing non-healing surgical wounds: A case series from Bangladesh. *International Journal of Infectious Diseases*, *110*, 272-278.
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