**The Thesis committee for Melanie Ann Heath**

**Certifies that this is the approved version of the following Thesis:**

**Validity of digital thermal monitoring techniques to assess vascular reactivity following finger and brachial occlusion.**

**SUPERVISING COMMITTEE:**

_____

Hirofumi Tanaka, Supervisor

_____

Sophie Lalande

# Validity of digital thermal monitoring techniques to assess vascular reactivity following finger and brachial occlusion.

**by**

**Melanie Ann Heath**

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## Dedication

This document is dedicated to my parents, Mike and Dianne, and my brother and sister-in-law, Jeff and Paige. Without my family's constant support, I would not be the person I am today. Thank you for always encouraging me to follow the right path, not the easy path. I love you all endlessly.

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## Abstract

**Validity of digital thermal monitoring techniques to assess vascular reactivity following finger and brachial occlusion.**

Melanie Ann Heath, M.S.KIN.

The University of Texas at Austin, 2020

Supervisor: Hirofumi Tanaka

Digital thermal monitoring (DTM) using the VENDYS-II device is an alternative, fully automated and noninvasive methodology to evaluate endothelial function using temperature change on finger as a surrogate measure of the magnitude of vascular reactivity index (VRI). Due to the simplicity, it could provide a more feasible technique to assess vascular endothelial function in the clinical setting. A most recent modification to the technique includes the application of occlusion cuff at the base of a finger. Therefore, the purpose of this study is to assess the validity of the VENDYS-II device compared with the standard flow-mediated dilation (FMD) protocol. Thirty-eight (22 males; 38±15 years) participants varying widely in age, health status, ethnicity, and socioeconomic status were studied. Occlusion cuff was placed over the right antecubital fossa or at the base of the right index finger. Temperature monitors were placed on bilateral index fingers to assess change in temperature throughout 5-minute occlusion and recovery phases. FMD was obtained simultaneously using high-resolution ultrasound. Shear rate total area under the curve ($SR_{AUC}$) was calculated for 180 cardiac cycles following cuff release. Mean brachial artery FMD was 7.5±2.2% and mean $SR_{AUC}$ was 43,924±10,256. $SR_{AUC}$ was significantly correlated with VRI obtained from brachial occlusion ($r=0.34$; $p<0.05$), but more




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strongly correlated with finger occlusion VRI ($r=0.43$; $p<0.05$) (Figure 1). Inversely, brachial FMD was more strongly correlated with brachial occlusion VRI ($r=0.69$; $p<0.05$), than finger occlusion VRI ($r=0.53$; $p<0.05$). VRI values obtained with the finger occlusion (1.58±0.29 AU) were not significantly different from VRI measured with the brachial artery occlusion (1.55±0.26 AU) ($p=0.47$), and both VRI values were moderately correlated with each other ($r=0.25$; $p=0.47$) Therefore, finger-based VRI may be a valid and novel alternative measure of endothelial function that is more suitable than the standard FMD or hyperemic shear rate for the assessment of endothelial function in the routine clinical setting.

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# LITERATURE REVIEW

## Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States, with 1 in every 4 deaths caused by CVD each year (CDC, 2019). Atherosclerosis is an inflammatory disease that occurs within the intima and surrounding areas of the internal lamina of arterial vessels (Akhtar et al., 2010). Associated with endothelial dysfunction, atherosclerosis is significant for arterial plaque development which hardens and narrows vessels resulting in reduced local blood flow and tissue perfusion (Durand & Gutterman, 2013). Patients with severe plaque calcification are at a higher risk of developing potentially lethal distal ischemia or thrombotic occlusion of major conduit arteries to the heart, brain, or legs (Akhtar et al., 2010).

Atherosclerosis often manifests in mid-life in the form of ischemic heart disease, carotid artery disease (CAD), peripheral artery disease (PAD), or chronic kidney disease (CKD) (Durand & Gutterman, 2013). However, multiple post-mortem studies performed on U.S. soldiers who died serving in Korea and Vietnam identified atherosclerotic changes within the vessel wall in persons under 30 years of age (Enos, Holmes, & Beyer, 1953; McNamera, Molot, & Stremple, 1971). Moreover, Tuzcu et al., (2001) identified atherosclerotic lesions in the coronary arteries of 51% of the 262 hearts donated for heart transplant from young, asymptomatic donors free of any CVD history. Aforementioned studies suggest that atherosclerotic lesions are likely present in 1 of 6 young adults (Tuzcu et al., 2001). Therefore, the purpose of this literature review is to discuss the underlying physiological mechanisms driving asymptomatic atherosclerosis progression and to also assess the clinical utility of techniques frequently utilized to assess endothelial function and CVD risk in the research setting.

**Endothelial Function and the Development of Atherosclerosis**

The endothelium is a monolayer of cells lining the luminal surface of the vascular system, which consists of large conduit arteries that branch into smaller vessels until they become arterioles and capillaries (Hartley & Tanaka, 2010). Large conduit arteries, referred to as the macrovasculature, provide compliance to mitigate pulsatile flow as it distributes blood away from the heart to various organs. Whereas small resistance vessels, referred to as the microvasculature, function to control regional flow by adjusting vascular resistance based on local demands (Hartley & Tanaka, 2010). Together they are governed by both endothelial-dependent and independent physiological regulatory mechanisms, as well as a number of biochemical agents.

Foundational work done by Furchgott & Zawadzki (1980) identified the role of the endothelium in regulating vascular tone by documenting a vasodilator response to infused acetylcholine (ACh) in the aorta of rabbits with intact endothelium. Conversely, rabbits whose aorta was stripped of endothelial cells had an attenuated vasodilatory response which suggest it was in some way endothelial-dependent (Furchgott & Zawadzki, 1980). The endothelium has since been identified to be a key moderator of vascular homeostasis. Additional functions of the endothelium being to provide an antithrombotic and nonadherent surface to maintain blood fluidity, act as a permeability barrier for regulating exchange and active transport of substances into the arterial wall, and to produce cytokines and adhesion molecules to regulate the inflammatory process (Libby, Ridker, & Maseri, 2002).

The endothelium's ability to regulate vascular tone is dependent on a balance in vasodilators such as; nitric oxide (NO), prostaglandin, and endothelial-derived hyperpolarizing factor (EDHF), and vasoconstrictors such as; endothelin-1, proteinoids, and angiotensin-II

(Widlansky, 2003). These vasoactive substances work synergistically to maintain the endothelial-dependent vasomotor response in human peripheral conduit arteries (Engelke et al., 2003). Specific emphasis has been placed on NO-bioavailability due to its role in supporting healthy vascular function via its antithrombotic and anti-inflammatory properties (Libby, Aikawa, & Jain, 2006). Increased hemodynamic shear stress along the arterial endothelium has been identified to be the primary stimulus resulting in endothelial NO synthase (eNOS) enzymatic activity and the subsequent release of NO within conduit arteries (Koller & Kaley, 1991). Shear stress is augmented during periods of reactive hyperemia, a term which refers to a transient influx in local blood flow that is triggered by the release of vasodilators and myogenic relaxation within the microvasculature in response to pharmacologic or physiologic stimuli (Carlsson et al., 1987). Additional stimuli, such as bradykinin and ACh, have also been shown to stimulate NO synthesis (Giannotti & Landmesser, 2007).

Endothelial dysfunction refers to the broad alteration in endothelial phenotype that may contribute to the development and clinical expression of atherosclerosis (Levine, Keaney, & Vita, 1995). Evidence for the link between endothelial dysfunction and early stage atherosclerosis was provided when patients with mild CAD were found to have similar endothelial dysfunction as patients with advanced CAD (Ludmer et al., 1986). Later studies expanded on this notion by reporting a strong association between endothelial dysfunction and atherosclerotic risk factors such as hypercholesterolemia, obesity, hypertension (HTN), sedentarism, smoking, diabetes, and family history of premature CAD (Reddy et al.,1994; Celermajer et al., 1994).

The underlying mechanism which unifies endothelial dysfunction and atherosclerosis was addressed in Ross and Glomset's (1976) "response to injury hypothesis", which states that the

development of atherosclerosis is due to injury of the endothelium stimulating platelet adherence to the subendothelial connective tissue at the site of the injury (Ross & Glomset, 1976). Atherosclerotic plaque then forms via a positive feedback loop in which inflammatory factors promote monocyte and T-cell adhesion, foam cell formation, extracellular matrix digestion, and vascular smooth muscle migration and proliferation (Libby, Ridker, & Maseri, 2002).

Additionally, oxidative stress has been identified to be the unifying mechanism for CVD risk factors to cause vessel damage and reduced vascular reactivity (Minor et al., 1990). Vascular reactivity refers specifically to the circulatory systems ability to respond to physiologic and pharmacologic stimuli that require adjustments of blood flow and alterations of vessel tone and diameter (Hartley & Tanaka, 2010). The role of oxidative stress in diminishing vascular reactivity is likely due to the negative impact of reactive oxygen species on NO-bioavailability (Minor et al., 1990). Evidence supporting this claim was shown in hypercholesterolemic rabbits who were found to have severely impaired vascular reactivity, which suggests a lack of NO (Minor et al., 1990). Paradoxically, eNOS signaling pathways appeared to be intact due to NO production increasing as expected when stimulated with ACh (Minor et al., 1990). Findings which led researchers to conclude that the reductions in vascular reactivity were likely due to rapid NO degradation caused by reactive oxygen species (Minor et al., 1990). The greater implications of reduced NO-bioavailability being increased oxidative modification of low-density lipoproteins, platelet and leukocyte adhesion, inflammation, and proliferation of vascular smooth muscle cells (Libby, Aikawa, and Jain, 2006). Altogether resulting in damage to the endothelium which is worsened by the negative effect of CVD risk factors on the number and functionality of circulating bone marrow derived endothelial progenitor cells which typically function to repair damage to the endothelium (Schmidt-Lucke et al., 2005).

Microvascular function has been historically overlooked by researchers assessing atherosclerotic risk because inhibition of NO-synthase using $N^G$-monomethyl-L-arginine (L-NMMA) appears to have little effect on the reactive hyperemic response (Engelke et al., 1996). Therefore, assessments of microvascular function are unsuitable for assessing NO-bioavailability which, as previously discussed, directly influences atherosclerosis development. Nevertheless, microvascular function directly impacts atherosclerotic plaque progression just through different physiological mechanisms. For example, the laminar shear stress within conduit arteries is less likely to cause vessel damage compared to the turbulent shear stress in resistance vessels caused by significant branching (Mitchell et al., 2004). Therefore, resistance vessels are at higher risk of facing vessel damage and consequently developing arterial plaque which altogether result in reduced limb perfusion (Mitchell et al., 2004). A vicious loop is then triggered with reduced limb perfusion causing reductions in the clearance of atherogenic lipids and lipoproteins which further stimulates plaque development and subsequently less tissue perfusion (Lind & Lithell, 1993). Therefore, both macro- and microvasculature function significantly contribute to the development of atherosclerosis.

Fortunately, adopting healthier lifestyle habits may reduce CVD risk factors and improve endothelial function. Aerobic exercise has been shown to improve endothelial function in healthy adults (Clarkson et al., 1999) as well as those with hypertension (HTN) (Higashi et al., 1999) and CAD (Hambrecht et al., 2000). Additionally, diets high in monounsaturated fats (Fuentes et al., 2001), flavonoids-containing beverages such as tea (Duffy et al., 2002), and antioxidants (Heitzer et al., 1999) have also been shown to improve vascular reactivity. Nevertheless, the majority of the general public are unaware of the endothelium's existence, let alone its impact on atherosclerosis development. Additionally, due to the current techniques utilized to assess

endothelial function very few people in the general public are aware that their endothelium is not functioning optimally. This lack of knowledge fuels a false sense of security which may lead to unmotivated behavior and reduced engagement in the lifestyle habits necessary to limit endothelial damage and dysfunction. Therefore, early risk factor stratification paired with direct assessments of the endothelium in the routine clinical setting is necessary for early detection of its dysfunction. In the hopes that early detection may provide the necessary time and motivation for the adoption of healthier lifestyle habits which have been shown to be able to halt or even reduce preclinical atherosclerotic development (Markus et al. 1997).

**Clinical Assessments of Cardiovascular Risk Factors**

Traditional methods for early assessment of cardiovascular risk in the clinical setting often focus solely on risk factor measurement, such as the Framingham Risk Score (FRS). Using epidemiological data, the FRS provides guidelines for predicting 10-year risk of developing CVD in the general population making it a useful tool for risk factor management in large populations (Brindle, 2003). Moreover, FRS provides reassurance to those in low risk populations and motivation to make healthier lifestyle choices for those in higher risk categories.

Despite the FRS being an efficient risk assessment tool, it does not come without a series of limitations. Clinical data reviewed from patients hospitalized for myocardial infarction (MI) over a three-year period showed the FRS to underestimate risk for CVD in young adults (< 55 years old for men and < 65 years old for women) (Akosah et al., 2003). Specifically, when the authors relied solely on the National Cholesterol Education Program (NCEP) to classify the CVD risk for the 222 patients hospitalized for acute MI, only 18% of the women and 41% of men met risk assessment criteria to receive pharmacotherapy (Akosah et al., 2003). Additionally,

Michos et al. (2005) compared FRS with coronary artery calcium (CAC) shown by numerous histopathologic studies to be linearly correlated with atherosclerotic plaque burden. Of the 2,447 asymptomatic females studied, 45% of the women classified as low risk by the FRS had significant CAC. Moreover, of the women with CAC >75$^{th}$ percentile for their age and gender, 84% of them were classified as being low risk by the FRS (Michos et al., 2005). Therefore, exclusive use of the FRS for risk factor assessment may result in under identification of those with asymptomatic atherosclerosis.

Researchers have validated methodologies such as coronary artery calcium (CAC), high-sensitivity C-reactive lipoprotein (hs-CRP), carotid intima-media thickness (CIMT), and ankle-brachial index (ABI) to be used in tandem with the FRS for more direct risk factor assessment Goff et al., 2013). Yet ACC/AHA guidelines (2013) reported insufficient evidence of their routine clinical assessment providing additional clinical utility. Consequently, routine assessment of carotid IMT in clinical practice is not recommended by the ACC/AHA, and assessments of CAC, ABI, and hs-CRP are only recommended for those whose risk-based treatment decision is uncertain (Goff et al., 2013). Moreover, these techniques have inherently high costs and fail to address underlying functional changes that occur within the vasculature. Therefore, due to all of the aforementioned reasons these techniques are highly impractical for routine risk-factor screening in the general public.

**Invasive Assessments of Endothelial Function**

Assessment of intracoronary endothelial function can be done invasively by using quantitative angiography to analyze the dose–response curves to infused endothelial antagonists and agonists (Tousoulis, 2005). Specifically, based on foundational studies conducted by Furchgott & Zawadzki (1980), ACh stimulates endothelial-dependent vasodilation in vessels

with intact endothelium and vasoconstriction in vessels with endothelial dysfunction due to its direct effect on the smooth muscle. Using a similar protocol, infusion of nitroprusside or glyceryl trinitrate, instead of ACh, can be used for evaluation of endothelial-independent vasodilation due to their role in stimulating vascular smooth muscle dilation (Winquist et al., 1984). While researchers often infuse L-NMMA to block eNOS signaling pathways so they may directly assess NO-bioavailability and basal endothelial function (Tousoulis, 2005). Thus intracoronary infusion techniques are considered to be versatile and highly reliable, making them the gold-standard assessment of endothelial function within the coronary circulation (Tousoulis, 2005).

The primary constraint of this technique is its invasive nature and associated health risk. Risks inherently associated with coronary artery catheterization include stroke, MI, infection, and vascular injury (Widlansky et al., 2003). Additionally, researchers have detected a nonuniform response to infused ACh measured within the coronary arteries (Horio et al., 1986). Discoveries which suggests that the response of the endothelium and smooth muscle to ACh infusion is variable along the course of the vessel or among patients (Horio et al., 1986). Moreover, vasoconstriction following infused ACh has been shown to occur in normal segments of the coronary artery while vasodilation was seen in segments with diffuse atherosclerotic development (Nishimura et al.,1995). Outcomes which have caused several researchers to question the implications of the vascular response to ACh infusion. Therefore, due to its questionable reliability, invasive nature, and inherently high cost intracoronary infusion techniques are impractical for use in the clinical setting.

Endothelial function within the coronary arteries has been shown to be closely related to that of the peripheral arteries (Anderson et al., 1995). Therefore, analyzing the dose-response curve to intrabrachial infusion of vasoactive agents can also be utilized to assess endothelial

function in the periphery. Specifically, use of strain-gauge plethysmography to assess changes in forearm blood flow (FBF) in response to infused vasoactive agents is considered to be the gold-standard assessment of endothelial function within the microvasculature (Higashi et al., 2001). Briefly, as described by Wilkinson & Webb (2001) a strain-gauge is first placed over the thickest part of the forearm to detect changes in forearm volume. A wrist cuff is then inflated to 50 mmHg above systolic blood pressure to exclude hand circulation due to skin blood flow sensitivity to temperature and its extensive arterio-venous shunting. A second upper arm cuff is then placed and inflated to 40 mmHg for 7 seconds during every 15 second cycling using a rapid cuff inflator. A technique which allows arterial inflow but impede venous outflow (Wilkinson & Webb, 2001). Consequently, any linear increases in forearm volume are directly proportional to arterial inflow into the limb which allows researchers to assess changes in FBF to vasoactive agents without influencing the systemic circulation (Wilkinson & Webb, 2001).

Intrabrachial infusion techniques have also been shown to be easily applied and highly reproducible with a variation of only 5-8% in short and long term evaluation (Lind, Hall, & Johanssan, 2002) Additionally, use of this technique has been able to demonstrate reduced endothelial function in the resistance vessels of several at risk populations such as those with HTN (Panza et al., 1990), diabetes (Vehkavaara et al., 2000), and smoking history (Kimura et al., 2003). Nevertheless, its invasive nature is associated with risk of injury to the median nerve and brachial artery which also makes this technique impractical for routine use in asymptomatic populations.

**Non-Invasive Assessments of Endothelial Function**

Due to the impracticality of using invasive techniques for assessing endothelial function in the clinical setting, researchers have validated several non-invasive techniques for assessing endothelial function of the conduit and resistance vessels of the periphery. Most techniques use brief periods of occlusion to assess vascular reactivity. The quantification of the reactive hyperemic response to occlusion representing microvascular function, and the changes in conduit artery diameter representing macrovascular function. The primary purpose of this review is to evaluate the feasibility of using digital thermal monitoring for assessing endothelial function in the clinical setting. For an assessment of pre-clinical disease to be feasible for the clinical setting the assessment must be incrementally predictive over risk factors, responsive to therapy, operator-independent and reproducible, low-cost and widely accessible in primary care settings, and posing no significant side effects (Naghavi et al., 2016). Therefore, this criterion will be utilized to compare digital thermal monitoring to other noninvasive techniques.

Tissue temperature has been shown to be a direct function of local blood perfusion (Hartley and Tanaka, 2010). Based on this association, digital thermal monitoring (DTM) presents as an alternative, non-invasive technique, which uses limb temperature change as a surrogate measure of blood perfusion and subsequently vascular reactivity (Ahmadi et al., 2011). During periods of proximal blood flow occlusion, fingertip temperature drops in the occluded limb due to heat loss to the cool external environment. After occlusion release, a reactive hyperemic influx in blood flow stimulates an increase in finger skin temperature often above the baseline skin temperature. The rate and intensity of temperature rebound is dependent on the ability for arteries to vasodilate to and restore normal circulation (Hartley & Tanaka, 2010). By measuring temperature rebound, normalized with baseline and core temperatures, researchers are

able to identify the reactive hyperemic response with good sensitivity (Ley et al., 2008; Akhtar et al., 2010).

The VENDYS-II device is the first to use this temperature-based approach to provide instantaneous, operator independent assessments of participants vascular reactivity index (VRI) (Ahmadi et al., 2011). VRI values are ranked as being poor (0.0 to <1.0), intermediate (1.0 to <2.0), or good (≥2.0) so that they may be easily interpreted by clinicians and participants (Naghavi et al., 2016). Additionally, they are incrementally predictive of CVD risk with poor VRI assessments shown to be significantly associated high FRS (Ahmadi et al., 2008), subclinical atherosclerosis measured by CAC (Ahmadi et al., 2009), and CAD measured by CT angiography (Ahmadi et al., 2009). Additionally, VRI assessments have been shown to be responsive to treatment, with 1-year dietary intervention shown to improve CAC and temperature rebound in 65 intermediate risk patients (Budoff et al., 2009). Shown to be easily applied, reproducible, and inexpensive by a cohort study of 6,084 participants, DTM presents as a feasible technique for assessment of asymptomatic populations in the clinical setting (Ahmadi et al., 2011; Naghavi et al., 2016).

Currently, the most widely accepted and utilized noninvasive technique to assess vascular reactivity in the research setting is flow-mediated dilation (FMD). Technically the term FMD can be used to describe any vasodilation of an artery following an increase in luminal flow and internal-wall shear stress. However, most use it to describe a technique introduced by Celermajer et al. (1994) which involves direct assessments of the peripheral artery diameter and blood flow velocity via non-invasive ultrasound following a period of ischemia.

Brachial artery FMD, with occlusion cuff placement around the forearm distal the ultrasound probe, is considered to be “standard” FMD protocol and is most frequently referenced

throughout the literature (Thijssen et al., 2019). By calculating the percent change in lumen diameter before and after arterial occlusion, researchers are able to calculate FMD as an assessment of endothelial-dependent vasodilation in the macrovasculature (Thijssen et al., 2019). Additionally, vessel diameter and blood velocity can be used to calculate reactive hyperemia and shear rate area under the curve (Shear $rate_{AUC}$), which is representative of microvascular function (Llewellyn et al., 2012).

The mechanism which drives the FMD response is thought to be primarily NO-mediated due to infusion of NO synthase blockers resulting in an abolished vasodilatory response to increased shear stress (Engelke et al., 1996; Eskurza et al., 2001). However, the physiological mechanism which underlie the FMD response has not been well characterized. For example, mice with the eNOS knockout gene still convey an FMD response to shear stress; indicating redundancies in the vasodilatory response to stimuli (Sun et al., 1999). Increased sympathetic outflow has also been shown to significantly attenuate the FMD response via endothelial-independent vasoconstriction (Hijmering et al., 2002). Lastly, FMD responses have not been correlated with intrabrachial ACh infusion techniques which suggest the underlying stimulus may differ between the two assessments (Eskurza et al., 2001). When considering these discoveries altogether the interpretation of FMD responses becomes much less straight-forward.

The clinical utility of FMD assessments has also been questioned due to findings from the Framingham Study which indicated that measurements of microvascular function are more correlated with cardiovascular risk factors than FMD (Mitchell et al., 2004). A discovery which could indicate that reductions in FMD may not be due to impaired local endothelial function as previously assumed, and are instead due to microvasculature dysfunction resulting in reduced hyperemic shear stress and consequently NO synthesis (Mitchell et al., 2004). Paradoxically,

FMD has not been shown to be significantly related with reactive hyperemic flow or hyperemic shear stress (Dhindsa et al., 2008). A finding which may be explained by Poiseuille's law being only applicable to long, straight, and rigid tubes with steady laminar flow, Newtonian fluid, and a parabolic velocity profile. All of which are physiological circumstances which are never realized in the in vivo conditions in which FMD is measured.

Nevertheless, brachial FMD is frequently used in physiological studies to examine the mechanisms that underlie the impact of CVD risk factors and physiological stimuli, such as exercise or diet, on vascular function (Duffy et al., 2002; Heitzer et al., 1999; Higashi et al., 1999; Hambrecht et al., 2000; Fuentes et al., 2001). FMD has also become increasingly popular for clinically oriented studies due to its ability to provide independent prognostic information for those with CVD or CVD risk (Patti et al., 2005; Meyer et al., 2005; Fischer et al., 2004; Fathi et al., 2004; Chan et al., 2003; Neunteufl et al., 2000) and for asymptomatic healthy subjects (Yeboah et al., 2009). Specifically, brachial FMD has been shown to be more closely related to carotid intima-media thickness progression in populations without CVD when compared to conventional risk factors (Halcox et al., 2009).

Although FMD is widely accepted and utilized within the research setting, it is highly impractical for the clinical setting for a variety of reasons. The first reason being a protocol which is highly sensitive to variations in methodology. Any change in occlusion placement, duration, and intensity triggering distinct shear stress profiles which may result in different vasodilatory mechanisms driving FMD that are affected differently by disease (Peretz et al., 2007; Pyke & Tschakovsky, 2005). Due to this, reproducibility has been a significant issue plaguing studies which have utilized FMD to assess vascular reactivity. Although protocol guidelines are in place to mitigate this issue, a meta-analysis which reviewed 48 studies utilizing

FMD protocol identified considerable variation on protocol adherence (ranging from 2.4 to 9.2 out of 10; mean 5.3) (Greyling et al., 2016). Protocol adherence shown to be inversely associated with FMD reproducibility (Greyling et al., 2016). In an attempt to make assessments more reliable, many researchers use expensive ultrasound equipment and medical imaging software capable of detecting arterial edge. The time commitment for data analysis per subject still being ~1 hour when using edge detection software. Nevertheless, several researchers still avoid using FMD protocols because of large-intertester variability that is often still present even when using edge detection software (Hardie et al., 2008). While labs that do frequently use FMD protocols do so by relying heavily on skilled and well-trained ultrasound technicians for reliable data acquisition. Therefore, due to its high expense, dependence on highly trained technicians, and high inter- and intratester variability FMD is an impractical assessment for use in the clinical setting.

Laser Doppler flowmetry (LDF) is a noninvasive assessment also utilized to measure reactive hyperemia within the skin microcirculation. Briefly, a laser beam is fixed 15 cm above the center of the proximal phalanx of the third digit. Researchers can then obtain a relative measure of cutaneous blood flow based on the Doppler shift of the scattered laser light following arterial occlusion or infused ACh (Shamim-Uzzaman et al., 2002). A technique which is capable of detecting reductions in reactive hyperemia in women with high CV risk factors (Vuilleumier et al., 2002), as well as in patients with CAD (Shamim-Uzzaman et al., 2002), HTN (Carberry, Shepard, & Johnson, 1992), and hypercholesteremia (Hayoz et al., 1995). Therefore, LDF is a valid, noninvasive, technique to assess vascular reactivity in the research setting. Yet use of LDF in the clinical setting is also impractical due to it providing only relative perfusion

measurements, the inherently high cost of equipment, and its dependence on optimal properties of the tissue which vary greatly depending on anatomical location (Hartley & Tanaka, 2010).

Lastly, the technique most similar to DTM for assessing endothelial function is peripheral artery tonometry using the EndoPAT 2000 device (Itamar Medical, Israel). A technique which uses specially designed finger plethysmograph to assess beat-to-beat changes in finger arterial pulse wave amplitude (PWA) following 5-minutes of brachial artery occlusion (Kuvin et al., 2003). Hyperemia-induced finger PWA changes, expressed as reactive hyperemia index (RHI), have been shown to have a low intra-individual variability of 13% when using the EndoPAT device (Moreland et al., 2012).

Studies utilizing the EndoPAT to assess vascular function have identified reduced RHI in patients with peripheral artery disease (Igari et al., 2016), hypertension, hyperlipidemia, CAD, and family history of CAD (Kuvin et al.,2003). Additionally, reduced RHI has been linked to the occurrence of adverse cardiovascular events over a 7-year follow-up period (Rubinshtein et al., 2010). However, the EndoPAT was unable to detect differences in RHI between healthy controls and participants with diabetes, renal dysfunction, and acute cigarette use (Moerland et al., 2002). Gordon, et al., (2009) also reported that pulse contour analysis obtained by finger plethysmography, a method comparable to EndoPAT, may not be suitable for measuring endothelial function in subjects with extensive CAD. Although assessments of RHI using the EndoPAT have been significantly correlated with brachial FMD and reactive hyperemic flow (Dhindsa et al., 2008). FMD has been shown to be more sensitive than EndoPAT for evaluating the effect of atherosclerotic risk factors on endothelial function (Wilk et al., 2013). Additionally, its inability to detect endothelial dysfunction in certain populations may indicate that the

physiological mechanisms assessed by the EndoPAT might be different from endothelial function as measured by conventional techniques.

Assessments of RHI using EndoPAT and VRI using VENDYS-II both obtain operator-independent assessments of the microvascular response to 5 minutes of brachial occlusion within the finger. Both shown to be correlated with brachial FMD, suggesting that they may be used as an alternative approach for assessing endothelial function. Additionally, both techniques are low-cost, low-risk, and have been shown to have low intra-individual variability which makes them feasible for the clinical setting (Ahmadi et al., 2011; Kuvin et al., 2003). However, no significant correlation has been shown between VRI and RHI values which may be due to a difference in underlying physiological mechanisms driving the endothelial response (Dhindsa et al., 2008). In this context, because brachial arterial blood flow is directed to both muscle and skin and RHI is more strongly associated with skin vascular reactivity as assessed by LDF, the hyperemic response assessed using the EndoPAT may be affected to a greater extent by skin blood flow changes and/or its correlates (Dhindsa et al., 2008). However, more research is required to confirm this hypothesis.

Based on this review, DTM using the VENDYS-II device appears to be a practical technique for assessing vascular reactivity in the asymptomatic general public due to its low-cost, low-risk, operator independent protocol (Ahmadi et al., 2011). The current DTM technique, which involves placement of occlusion cuff on the upper arm, has been shown to be significantly correlated with brachial FMD (Dhindsa et al., 2008) and reactive hyperemic stimulus (Ahmadi et al., 2011). However, these findings have been variable and unconfirmed by follow-up studies. Additionally, a most recent alteration to the technique includes the application of occlusion cuff at the base of the finger instead of the upper arm. A modification which provides users with a

more comfortable testing option than the standard full arm brachial artery occlusion. However, evidence supporting the validity of this finger-based assessment is lacking within the current literature.

# MANUSCRIPT

## INTRODUCTION

Endothelial dysfunction results in reduced vascular reactivity and proinflammatory pathways which stimulate gradual, asymptomatic, plaque development within the arterial system (Libby, Ridker, & Maseri, 2002; Minor et al., 1990). Assessments of vascular reactivity have been shown to be more predictive of cardiovascular disease (CVD) risk compared to other risk factor assessments such as the Framingham risk score (FRS) (Mitchell et al., 2004). Nevertheless, clinical settings often rely solely on the FRS to assess for atherosclerotic risk in large populations due to its simplicity and low expense (Brindle, 2003). Consequently, a considerable number of at-risk patients go unidentified on the basis of these conventional risk factors. Thus, discovering a methodology which assesses vascular reactivity and may be incorporated into the routine clinical setting is a serious epidemiological issue (Akosah et al., 2003; Michos et al., 2005).

Several methodologies have be utilized to assess vascular reactivity within the research setting, the most popular being brachial artery flow-mediated dilation (FMD). FMD is useful for its ability to provide independent prognostic information for those with CVD or CVD risk (Thijssen et al., 2011). However, for an assessment of pre-clinical disease to be feasible for the clinical setting, the assessment must be incrementally predictive over risk factors, responsive to therapy, operator-independent and reproducible, low-cost and widely accessible in primary care settings, and posing no significant side effects (Naghavi et al., 2016). Based on this criterion, FMD is highly impractical for the clinical setting due to its large intra- and intertester variability and reliance on expensive equipment and highly skilled research technicians (Greyling et al., 2016; Hardie et al., 2008).

Changes in tissue temperature following blood flow occlusion are a direct function of blood perfusion and are dependent on the arteries ability to dilate (Hartley & Tanaka, 2010). Based on this association, digital thermal monitoring (DTM) uses temperature change on finger following 5 minutes of blood flow occlusion as a surrogate measure of tissue perfusion and subsequently vascular reactivity. The VENDYS-II is the first device to use this temperature-based approach to provide instantaneous, operator independent, assessments of vascular reactivity index (VRI) (Ahmadi et al., 2011). Assessments of VRI following brachial artery occlusion shown to be significantly correlated with brachial FMD (Dhindsa et al., 2008) and reactive hyperemic stimulus (Ahmadi et al., 2011). Additionally, assessments of poor VRI have been associated with subclinical atherosclerosis measured by CAC and the presence and extent of CAD measured by CT angiography (Ahmadi et al., 2009). Associations which reveal the clinical utility of DTM assessments.

A most recent modification to the technique includes the application of an occlusion cuff at the base of the index finger instead of the upper arm, which provides users a more comfortable testing option than the standard full arm brachial artery occlusion. Due to the simplicity, this temperature-based assessment may provide a more feasible technique to assess vascular endothelial function in the clinical setting. However, evidence supporting its validity is lacking. Therefore, the purpose of this study is threefold; 1) to determine if VRI assessments are mediated by changes in shear stress, 2) to compare both finger and brachial based VRI to FMD which is a more established assessment of vascular reactivity, 3) to determine the association between VRIs derived from finger occlusion compared to those derived from brachial occlusion.

## METHODS

### Subjects

Adults between the ages of 18 and 80 were recruited through flyers and online advertisements posted in Austin, Texas. Emphasis was placed on recruiting participants varying widely in age, health, ethnicity and socioeconomic status to ensure that the sample was representative of the general population. Participants were excluded from participation if they were pregnant or had undergone a surgical procedure or medical intervention within 48 hours of study participation. All participants completed a Health History Questionnaire prior to initiating testing. The study was in accordance with the Declaration of Helsinki and was approved by the local institutional review committee. Thirty-eight adults participated in the study, providing written informed consent before enrollment.

### Digital Thermal Monitoring Assessments

All DTM assessments were performed in the morning in a quiet, dimmed laboratory room at a controlled ambient temperature between 22.5 and 25.0° C. Studies were conducted after an overnight fast of at least 8 hours (water was permitted) and abstinence from alcohol, tobacco, caffeine, vasoactive medications, and exercise. Measurements were obtained after 15-minutes of supine rest. Participants underwent two independent DMT assessments, finger occlusion or brachial artery occlusion, with a 30-minute resting period between each assessment. The order in which the two assessments were conducted was randomly assigned. While resting, all participants were placed under a heated blanket to ensure they reached the required baseline fingertip temperature (>27° C) needed for accurate DTM measurements (Akhtar et al., 2010).

Prior to testing, an automated blood pressure measurement was taken to determine the necessary occlusion pressure. Each assessment followed an operator-independent protocol (VENDYS-II, Endothelix Inc., Houston, TX) that was 15 minutes in duration. There were three stages including 5 minutes of temperature stabilization, 5 minutes of blood flow occlusion at 50 mmHg above systolic blood pressure, and 5 minutes of post-occlusion reactive hyperemia. Thermal changes were continuously monitored in the fingertips of both the occluded and non-occluded arm using VENDYS-II temperature monitors placed bilaterally on pulp of the participants' index fingers. Following test completion, participants' VRI value was automatically generated via VENDYS-II software. VRI values ranged from 0.0 to 3.5 and were classified as being indicative of poor (0.0 to <1.0), intermediate (1.0 to <2.0), or good (≥2.0) vascular reactivity. For further information regarding VRI calculation by the VENDYS-II, see Naghavi et al. (2016).

**Finger-based Measurement**

Finger occlusion assessments utilized a neonatal blood pressure cuff placed at the base of the right index finger to obtain an automated blood pressure measurement and administer the 5-minutes of arterial occlusion. Due to the variation in participants' index finger diameter, width at the base of the index finger was measured to determine the optimal cuff size. If the diameter of the participants' index finger was smaller than 6cm then Neonatal Disposable Cuff #3 (Medline Industries, Northfield, IL) was used, and if it was greater than 6cm then Neonatal Disposable Cuff #4 (Medline Industries, Northfield, IL) was used. If a reliable blood pressure reading was unable to be obtained from the finger site because of small finger diameter, an automated blood pressure was obtained from the upper arm and manually entered into the VENDYS-II device. Participants remained supine for the assessment, with their hands placed comfortably on an

insulated pad located on their lap so that constant pressure could be applied to the temperature sensors and to ensure body-temperature did not interfere with temperature measures.

**Brachial-based Measurement and Doppler Ultrasound Assessment**

Brachial artery occlusion assessments began with an automated blood pressure and heart rate measurement obtained with a standard blood pressure cuff placed on the right upper arm. To allow for simultaneous ultrasound of the right brachial artery, participants' right arm was abducted to a 90-degree angle and placed onto an arm support. The occlusion cuff was moved to the ipsilateral forearm distal to the antecubital fossa (Thijssen et al., 2019). Tape was placed around the extending right index finger to ensure constant pressure on the temperature sensor. Participants were instructed to hold the remaining fingers on their right hand in a fisted position to limit heat radiation onto the temperature sensor. Participants' left hand rested comfortably on the insulated pad placed on the participant's lap.

High-resolution ultrasound (iE 33 Ultrasound System, Philips, Bothell, WA) was used to measure brachial artery diameters and blood flow velocity. Brachial artery images were obtained in a longitudinal orientation located 5–10 cm proximal to the antecubital fossa (Thijssen et al., 2019). Once an acceptable ultrasound image had been obtained, the 15-minute DTM protocol commenced. One minute of baseline arterial diameter and blood flow velocity was acquired during the 5-minute temperature stabilization period. Following the release of arterial occlusion, ultrasound-derived blood velocity and diameter data were recorded for the entire 5-minute recovery period.

**Data Analyses**

Ultrasound images were saved as DICOM format and transferred to a computer using a digital image viewing software (Access Point 2004, Freeland Systems; Westminster, CO). All ultrasound brachial images were analyzed by the same investigator using image analysis software (Vascular Research Tool Brachial Analyzer, Medical Imaging Applications, Coralville, IA). FMD was expressed as the percent change in brachial artery diameters recorded during the pre and post occlusion phases and was calculated using the equation: (maximum diameter – baseline diameter)/baseline diameter x 100 (Thijssen et al., 2019). The average of brachial artery diameters for one minute before blood flow occlusion was used for baseline diameters, and the peak diameter during the reperfusion phase was used for maximum brachial artery diameter. Vessel diameter and blood velocity were used to calculate the shear rate area under the curve (Shear $rate_{AUC}$) stimulus. Shear rate ($s^{-1}$) was calculated using the following equation: (8 x blood viscosity (assumed to be 0.035 $dyn \times s/cm^2$) x peak blood velocity / brachial artery diameter for 180 cardiac cycles following the release of cuff occlusion (Parkhurst et al., 2012). Shear $rate_{AUC}$ was then calculated by summing the area under the curve from cuff release until 180 cardiac cycles had occurred.

**Statistical Analyses**

Statistical analyses were performed using GraphPad Prism version 8.3.1 for Mac (GraphPad Software, San Diego California USA, www.graphpad.com). All group data was presented as group means (±SD). Statistical difference between the two assessments was evaluated by using Student's t-tests for paired data. Associations of interest were analyzed by Pearson correlational analyses and stepwise regressional analyses. A p-value $<0.05$ was

considered statistically significant. Statistical procedure proposed by Bland and Altman was used to compare the two different methods (Bland & Altman, 1986). First, the correlation and regression analyses were conducted between measured values. The initial step served as the evaluation of the degree of agreement between the two methodologies. Second, the relative differences within each pair of measurements were plotted against the mean of the pair.

## RESULTS

Of the forty-one participants recruited for participation, thirty-eight were included in final statistical analyses (Table 1). Rationale for the exclusion of three participants were an inability to obtain a finger temperature >27°C, extreme discomfort expressed by a participant with brachial artery occlusion causing a sympathetic response, and a difference in VRIs greater than three standard deviations from the group mean difference suggestive of statistical outlier. Mean values (± SD) for brachial artery vascular reactivity measures are displayed in Table 2. As depicted in Figure 1, Shear $rate_{AUC}$ was significantly correlated with VRI obtained from brachial occlusion ($r=0.34$; $p<0.05$), and finger occlusion VRI ($r=0.43$; $p<0.05$). Additionally, Figure 2 depicts significant correlations between brachial FMD and brachial occlusion VRI ($r=0.69$; $p<0.05$), and VRI obtained from the finger ($r=0.53$; $p<0.05$). FMD and Shear $rate_{AUC}$ were not significantly correlated with each other ($p=0.08$). VRI values obtained from finger (1.58±0.29) and brachial (1.55±0.26) occlusion were not significantly different ($p=0.47$) with a mild ($r=0.25$) correlation that was not significant ($p=0.14$). This mild agreement is consistent with the results of the Bland-Altman plot (Figure 3).

**Table 1: Participant Demographics**

| | |
|---|---|
| Sex (M/F) | 22/16 |
| Age (years) | 38±15 |
| Min-max age (years) | 21 - 67 |
| Height (cm) | 175.3±8.8 |
| Min-max height (cm) | 160.1 - 193.3 |
| Body Weight (kg) | 82.5±20.9 |
| Min-max body weight (kg) | 54.1 - 156.9 |
| BMI ($kg/m^2$) | 26.7±6.4 |
| Min-max BMI ($kg/m^2$) | 19.4 - 48.8 |
| Obese (BMI > $30kg/m^2$), n (%) | 8 (20%) |
| Supine Brachial Systolic BP (mmHg) | 112±13 |
| Min-max supine brachial systolic BP (mmHg | 95 - 156 |
| Supine Brachial Diastolic BP (mmHg) | 63±12 |
| Min-max supine brachial diastolic BP (mmHg) | 45 - 92 |
| Supine Finger Systolic BP (mmHg) | 108±12 |
| Min-max supine finger systolic BP (mmHg) | 94 - 130 |
| Supine Finger Diastolic BP (mmHg) | 57±10 |
| Min-max supine finger diastolic BP (mmHg) | 36 - 79 |
| **Self-Reported Health History** | |
| Hypertension, n (%) | 7 (18%) |
| Antihypertensive medication, n (%) | 4 (11%) |
| Family history of hypertension, n (%) | 16 (42%) |
| Hypercholesteremia, n (%) | 4 (11%) |
| Smoking, n (%) | 8 (21%) |
| Sedentary, n (%) | 8 (21%) |
| **Ethnicity** | |
| Caucasian, n (%) | 28 (74%) |
| African American, n (%) | 3 (8%) |
| Hispanic or Latino, n (%) | 2 (5%) |
| Asian, n (%) | 5 (13%) |
| **Highest Level of Education** | |
| High school diploma or equivalent, n (%) | 6 (16%) |
| Associate degree, n (%) | 2 (5%) |
| Bachelor's degree, n (%) | 14 (37%) |
| Master's degree or higher, n (%) | 16 (42%) |

All values are means ± SD. BMI=Body mass index, BP = Blood pressure.

**Table 2:** Brachial artery measures

| | |
|---|---|
| Brachial Occlusion VRI (AU) | 1.55±0.26 |
| Finger Occlusion VRI (AU) | 1.58±0.29 |
| Resting Brachial Diameter (mm) | 4.2±0.6 |
| Brachial artery FMD (%) | 7.5±2.2 |
| Resting Blood Flow Velocity (cm/s) | 100.5±21.7 |
| Peak Blood Flow Velocity (cm/s) | 208.6±37.5 |
| Shear rate $_{rest}$ ($s^{-1}$) | 196.1±50.7 |
| Shear rate $_{peak}$ ($s^{-1}$) | 428.7±124.9 |
| Shear rate $_{AUC}$ (AU) | 43,924±10,256 |

All values are means±SD. VRI = Vascular reactivity index. FMD = Flow-mediated dilation.

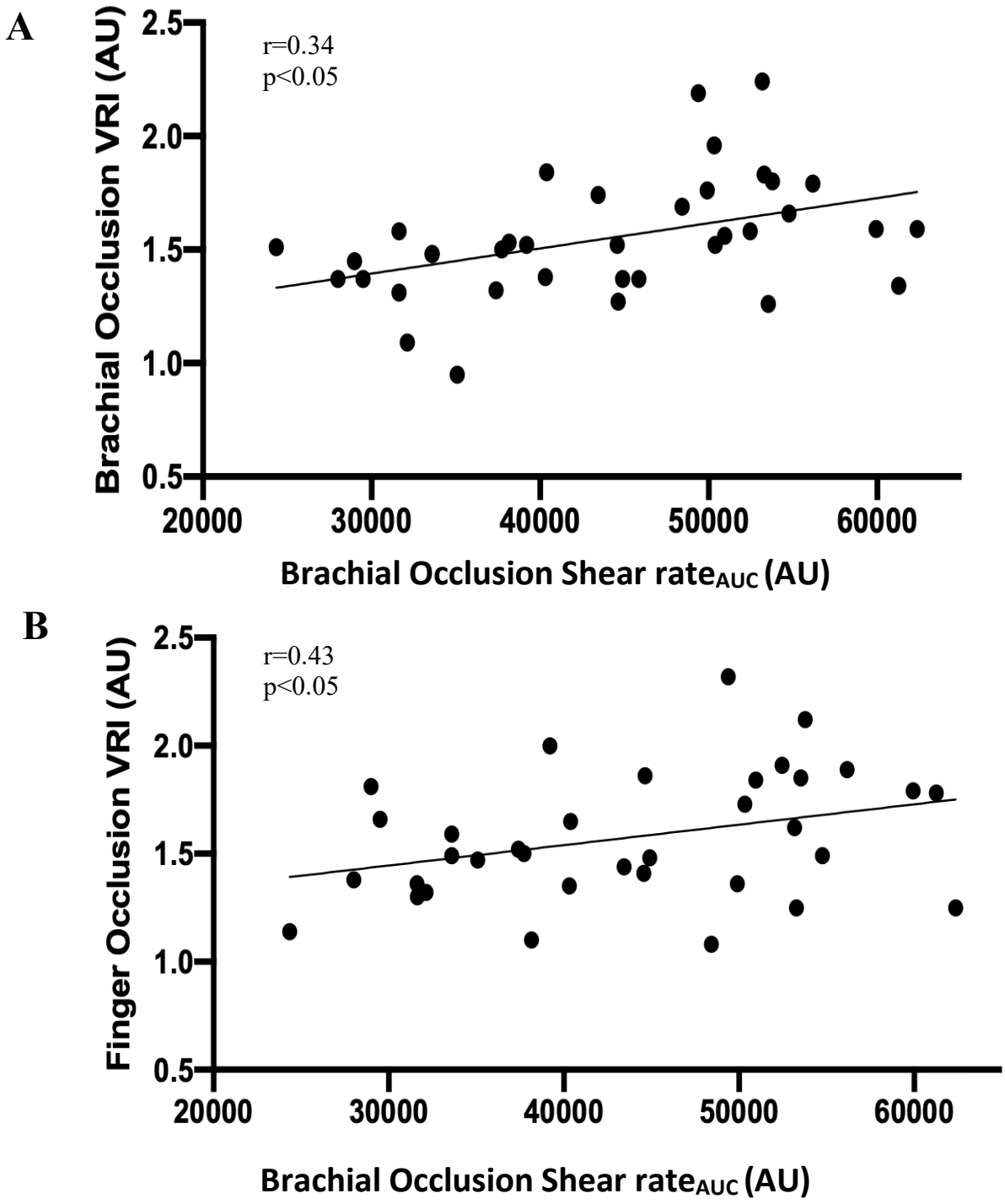


**Figure 1:** Relation between Shear rate$_{AUC}$ and brachial (A) and finger (B) occlusion VRI.

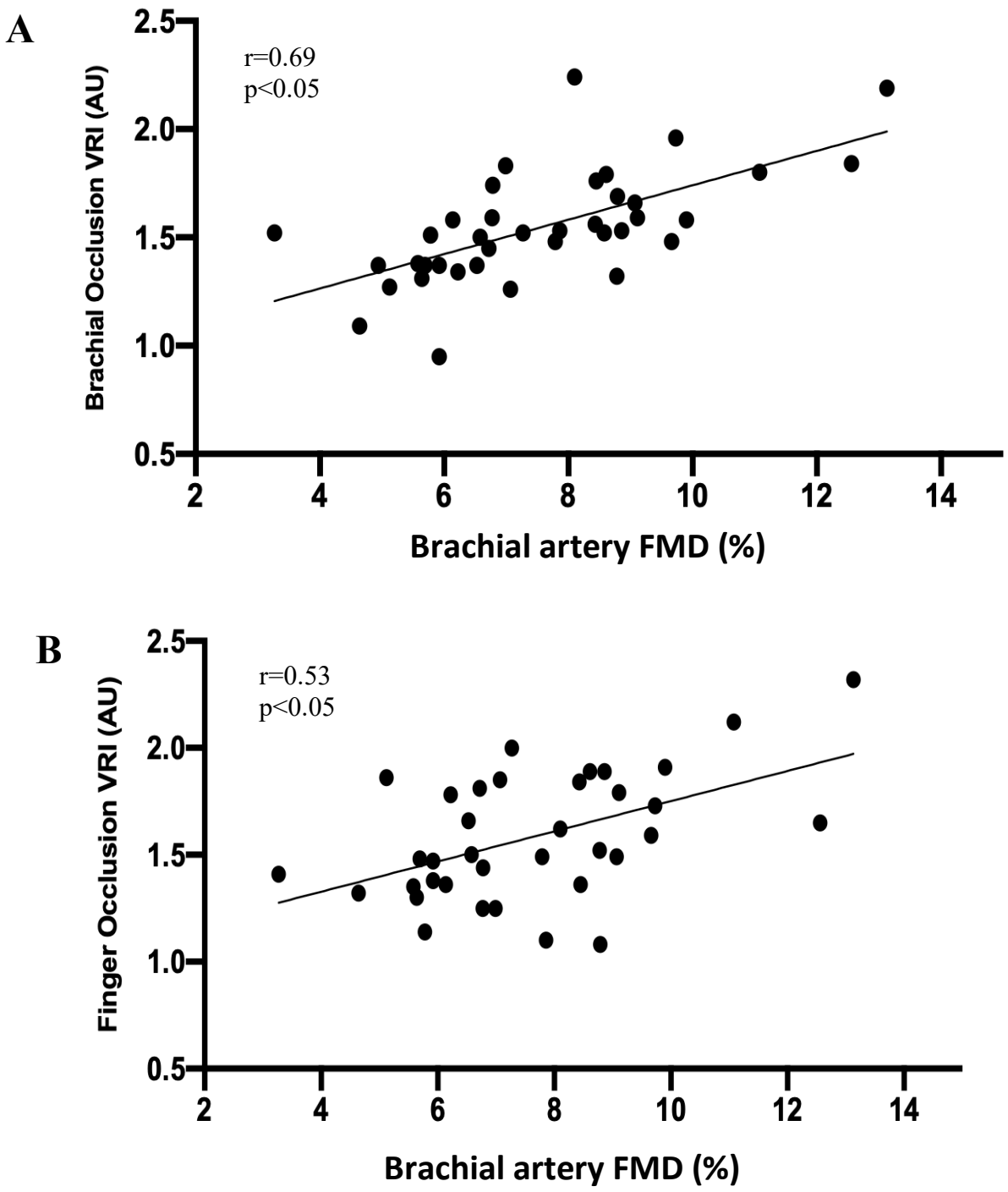


**Figure 2:** Relation between brachial artery FMD and brachial (A) and finger (B) occlusion VRI.

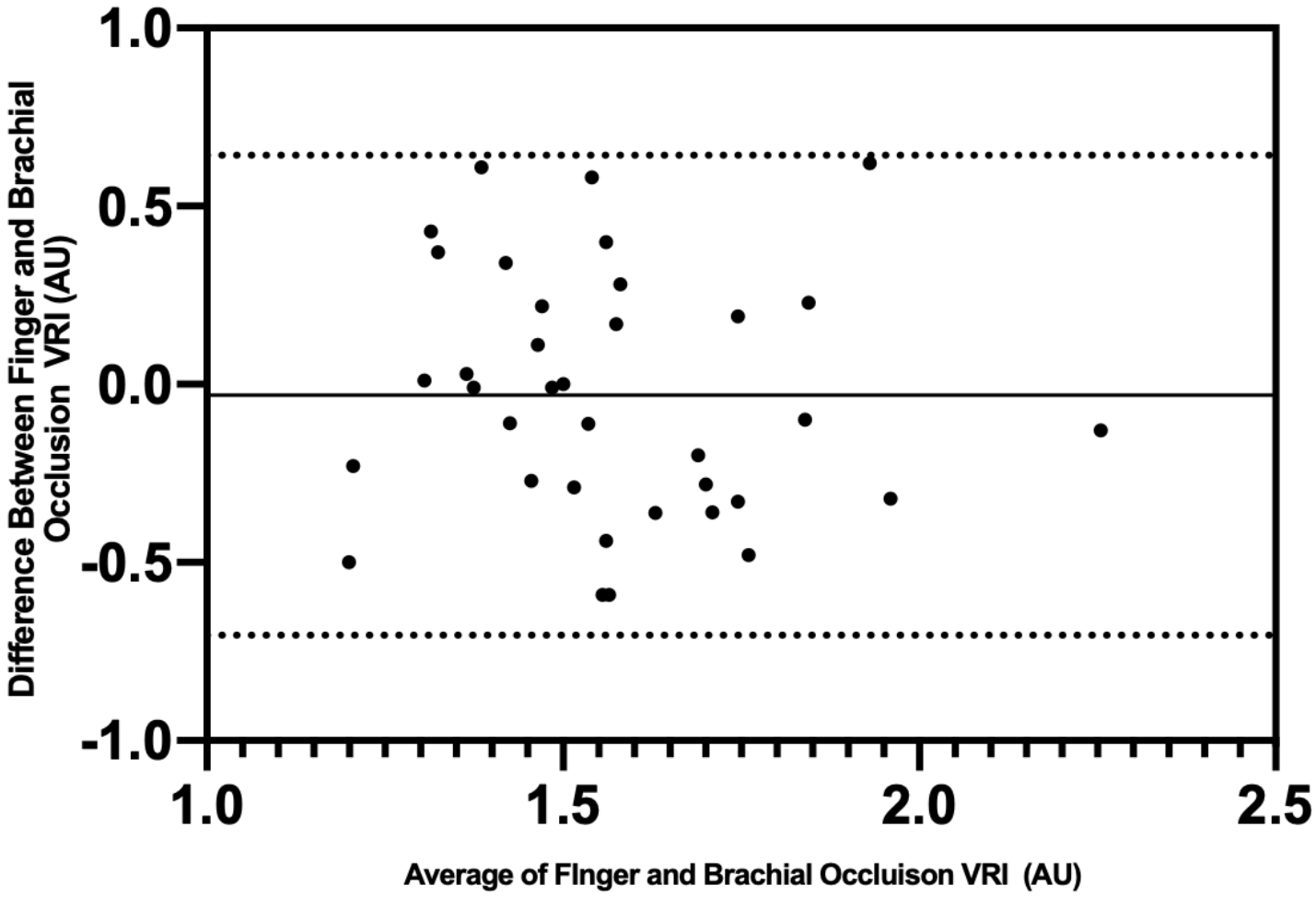


**Figure 3:** Bland-Altman plot with average for individual participants' finger and brachial VRI plotted against the difference between the two assessments. Dotted lines indicate ± 2 SD from the group mean difference.

## DISCUSSION

The purpose of the present study was to determine the associations between VRI obtained from finger and brachial occlusion, and to compare both assessments to the more established FMD protocol. Additionally, we assessed whether VRI assessments were mediated by changes in shear stress. The primary novel finding of the present study is the significant correlations between Shear $rate_{AUC}$ and both finger and brachial based VRI assessments. Discoveries which suggest that both assessments are mediated by changes in shear stress. An additional noteworthy finding is the significant associations between both VRI assessments and brachial FMD, which suggest that either may be used to assess vascular reactivity instead of standard FMD. Lastly, brachial and finger based VRI assessments were shown to be only mildly correlated. However, group means were not significantly different and the difference between the two assessments for each participant was not greater than two standard deviations.

Similarities in the underlying physiological mechanisms mediating FMD and DTM assessments provide useful context for the significant correlations found in the present study. Brachial FMD uses noninvasive ultrasound to assess changes in brachial artery diameter and blood flow velocity following 5 minutes of arterial occlusion (Thijssen et al.,2019). During occlusion, distal ischemia within the microvasculature stimulates a reactive hyperemic response to accelerate local blood flow to deliver oxygen and remove metabolic byproducts following occlusion release (Carlsson et al., 1987). Augmented local blood flow stimulating increased shear stress along the endothelium which is the primary stimulus for NO synthase (eNOS) enzymatic activity and NO-mediated vasodilation within the conduit arteries (Koller & Kaley, 1991). Comparably, DTM uses temperature, a direct function of blood perfusion within the tissue, instead of ultrasound to assess vascular reactivity following arterial occlusion (Hartley &

Tanaka, 2010). During occlusion, fingertip temperature drops due to reductions in limb perfusion but once occlusion is released a temperature rebound occurs due to a reactive hyperemic influx in blood flow. The rate and intensity of temperature rebound is dependent on the arterioles ability to vasodilate and restore normal circulation (Hartley & Tanaka, 2010). Therefore, by assessing temperature rebound, normalized with baseline and core temperatures, researchers are able to identify the reactive hyperemic response with good sensitivity (Ley et al., 2010; Akhtar et al., 2010). Based on this understanding, the significant correlations found in the present study between both VRI assessments and Shear $rate_{AUC}$ and brachial FMD are supported by physiological rationale.

The primary novel findings of the present study are that both finger and brachial based VRI assessments are mediated by changes in shear stress and therefore reflect microvasculature function. A conclusion which has been supported by Ahmadi et al. (2011) and the aforementioned physiological rationale. Conversely, Dhindsa et al. (2008) failed to detect a significant association between brachial VRI and hyperemic shear stress. However, their lack of significant findings may be attributable to the use of an outdated DTM technique which did not normalize temperature round with baseline and core temperatures, which is now known to be necessary for accurate temperature rebound assessments (Ley et al., 2008).

The significant association between brachial based VRI and brachial FMD has also been supported by Dhindsa et al., (2008). However, this study is the first to provide evidence for the significant association between finger based VRI and brachial FMD. The greater implications of this outcome being that either brachial or finger occlusion VRI may be used to assess vascular reactivity instead of standard brachial FMD. This is of significant clinical importance because of the impractically of using FMD assessments in the clinical setting due to their inherently high

cost and reliance on highly trained ultrasound technicians. Additionally, FMD methodologies have been shown to be highly sensitive to even slight changes protocol or motion artifact (Greyling et al., 2016). Due to this, reproducibility has been a significant issue plaguing studies which have utilized FMD assessments. Thus, the use of FMD in the clinical setting is extremely impractical.

Conversely, DTM assessments using the VENDYS-II device provide clinicians with an inexpensive, operator independent, protocol which is more feasible for assessing vascular reactivity within the clinical setting. The validation of finger-occlusion VRI in the current study offering an even easier and more comfortable testing option than the standard full arm brachial artery occlusion. Additionally, assessments of microvasculature function may be more indicative of CVD risk than FMD (Mitchell et al., 2004), with poor VRIs shown to be significantly associated with subclinical atherosclerosis (Ahmadi et al., 2009) and the presence and extent of CAD (Ahmadi et al., 2009). VRI assessments may also be used to assess the effectiveness of therapeutic interventions, shown to be responsive to a 1-year diet intervention which improved CAC and temperature rebound in 65 intermediate risk patients (Budoff et al., 2009). All of which suggest that DTM assessments provide a more feasible methodology for assessing vascular reactivity within the clinical setting.

In the present study, FMD was not significantly associated with hyperemic shear stress. Findings consistent with a previous study conducted within our laboratory which assessed the interrelationships among noninvasive assessments of vascular reactivity (Dhindsa et al., 2008). A paradoxical outcome considering that reactive hyperemia and shear stress are thought to be stimuli for FMD. Causing some researchers to argue that assessments taken at the fingertip can be used solely to assess microvascular function and are not representative of responses within the

macrovasculature. However, such discrepancies in the literature may be due, at least in part, to a failure of Poiseuille's law to accurately capture in vivo shear rate/stress. Poiseuille's Law is applicable only to long, straight, and rigid tubes with steady laminar flow, Newtonian fluid, and a parabolic velocity profile. Physiological conditions which can never be realized in the in vivo condition in which FMD is measured. Additionally, the findings of the present study support the use of VRI obtained from either finger or brachial occlusion as an alternative assessment of both reactive hyperemia and brachial FMD.

Brachial occlusion VRI was shown to be only mildly correlated with finger based VRI in the present study. However, group means were not significantly different and the difference between the two assessments for each participant was not greater than two standard deviations. Outcomes which make it difficult to make conclusive interpretations for the relationship between brachial and finger based VRI assessments. A likely reason for this being the lack of repeated measures for each VRI assessment on each participant. Therefore, a future study which assesses the association between repeated measures of both brachial and finger based VRI is necessary to provide clarification to the current results. Additionally, while brachial based VRI assessments have been shown to have low day-to-day variability (Ahmadi et al., 2011), future research is necessary to address the reproducibility of finger-based assessments.

Several limitations of this study deserve comments. The primary limitation being the required baseline fingertip temperature >27°C, which may prevent the use of DTM assessments in places that experience extreme external temperatures or for patients with poor limb perfusion secondary to severe CVD. To ensure all subjects reached the threshold temperature in the current study, a heated blanket was placed over the participants. Acute heating shown to influence the endothelial response, which may explain the only mild association between the two assessments

Romero et al., 2016). However, we attempted to mitigate this cofounding stimulus by randomizing the order of the two assessments to prevent systemic heating from significantly impacting one assessment over the other. The current study was also conducted with a relatively small sample size. Therefore, a community-based study with a larger sample and repeated measures is necessary to further assess the sensitivity of DTM assessments and the association between brachial and finger based VRI assessments.

In summary, VRI obtained following finger and brachial occlusion are mildly correlated with each other, but both are significantly correlated with standard brachial FMD and hyperemic shear stress. Outcomes which indicate that changes in shear stress mitigate changes in finger temperature and VRI assessments may be a valid alternative to standard FMD. Therefore, due to its operator independent protocol that is low-cost and easily applied, DTM using the VENDYS-II device following finger occlusion may be a promising methodology for assessment of endothelial dysfunction and atherosclerotic risk in the routine clinical setting.

## REFERENCES


Ahmadi, N., et al., (2008). Relations between digital thermal monitoring of vascular function, the Framingham risk score, and coronary artery calcium score. *Journal of Cardiovascular Computed Tomography*, *2*(6), 382–388. doi: 10.1016/j.jcct.2008.09.001

Ahmadi, N., et al., (2009). Low fingertip temperature rebound measured by digital thermal monitoring strongly correlates with the presence and extent of coronary artery disease diagnosed by 64-slice multi-detector computed tomography. *The International Journal of Cardiovascular Imaging*, *25*(7), 725–738. doi: 10.1007/s10554-009-9476-8

Ahmadi, N. et al., (2011). Reproducibility and variability of digital thermal monitoring of vascular reactivity. *Clinical Physiology and Functional Imaging*, *31*(6), 422–428. doi: 10.1111/j.1475-097x.2011.01037.x

Akhtar, M., et al., (2010). Sensitivity of Digital Thermal Monitoring Parameters to Reactive Hyperemia. *Journal of Biomechanical Engineering*, *132*(5). doi: 10.1115/1.4001137

Akosah, K., et al., (2003). Preventing myocardial infarction in the young adult in the first place: how do the national cholesterol education panel iii guidelines perform? *Journal of the American College of Cardiology*, *41*(9), 1475–1479. doi: 10.1016/s0735-1097(03)00187-6

Anderson, E., & Mark, L. (1989). Flow-mediated and reflex changes in large peripheral artery tone in humans. *Circulation*, *79*(1), 93–100. doi: 10.1161/01.cir.79.1.93



Anderson, E., & Mark, L. (1989). Flow-mediated and reflex changes in large peripheral artery tone in humans. *Circulation*, *79*(1), 93–100. doi: 10.1161/01.cir.79.1.93



Bland, J., & Altman, D. (2010). Statistical methods for assessing agreement between two methods of clinical measurement. *International Journal of Nursing Studies*, *47*(8), 931–936. doi: 10.1016/j.ijnurstu.2009.10.001

Brindle, P. (2003). Predictive accuracy of the Framingham coronary risk score in British men: prospective cohort study. *Bmj*, *327*(7426), 1267–0. doi: 10.1136/bmj.327.7426.1267

Budoff, M., et al., (2009). Aged garlic extract supplemented with B vitamins, folic acid and l-arginine retards the progression of subclinical atherosclerosis: A randomized clinical trial. *Preventive Medicine*, *49*(2-3), 101–107. doi: 10.1016/j.ypmed.2009.06.018

Carberry, P., Shepherd, A., & Johnson, J. (1992). Resting and maximal forearm skin blood flows are reduced in hypertension. *Hypertension*, *20*(3), 349–355. doi: 10.1161/01.hyp.20.3.349

Carlsson, I., et al., (1987). The role of myogenic relaxation, adenosine and prostaglandins in human forearm reactive hyperemia. *The Journal of Physiology*, *389*(1), 147–161. doi: 10.1113/jphysiol.1987.sp016651

CDC (2019) Heart disease facts. https://www.cdc.gov/heartdisease/facts.htm

Celermajer, D., et al., (1994). Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *Journal of the American College of Cardiology*, *24*(6), 1468–1474. doi: 10.1016/0735-1097(94)90141-4

Chan, S., et al., (2003). The prognostic importance of endothelial dysfunction and carotid atheromaburden in patients with coronary artery disease. *Journal of the American College of Cardiology*, *42*(6), 1037–1043. doi: 10.1016/s0735-1097(03)00927-6

Dhindsa, M., et al., (2008). Interrelationships among noninvasive measures of postischemic macro- and microvascular reactivity. *Journal of Applied Physiology*, *105*(2), 427–432. doi: 10.1152/japplphysiol.90431.2008

Durand, M., & Gutterman, D. (2013). Diversity in mechanisms of endothelium-dependent vasodilation in health and disease. *Microcirculation (New York, N.Y. : 1994)*, *20*(3), 239–247. doi:10.1111/micc.12040

Duffy, S., et al., (2002). Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. *ACC Current Journal Review*, *11*(1), 28. doi: 10.1016/s1062-1458(01)00554-2

Engelke, K., et al., (1996). Contribution of nitric oxide and prostaglandins to reactive hyperemia in the human forearm. *Journal of Applied Physiology*, *81*(4), 1807–1814. doi: 10.1152/jappl.1996.81.4.1807

Enos W., Holmes R., & Beyer J. (1953) Coronary disease among United States Soldiers Killed in Action in Korea: Preliminary Report. *The Journal of the American Medical Association*;152:1090.

Eskurza, I., et al., (2001). Pharmacologic versus flow-mediated assessments of peripheral vascular endothelial vasodilatory function in humans. *The American Journal of Cardiology*, *88*(9), 1067–1069. doi: 10.1016/s0002-9149(01)01997-x

Fathi, R., et al., (2004). The relative importance of vascular structure and function in predicting cardiovascular events. *Journal of the American College of Cardiology*, *43*(4), 616–623. doi: 10.1016/j.jacc.2003.09.042

Fischer, D., et al., (2004). Endothelial dysfunction in patients with chronic heart failure is independently associated with increased incidence of hospitalization, cardiac transplantation, or death. *European Heart Journal*, *26*(1), 65–69. doi: 10.1093/eurheartj/ehi001

Fuentes, F., et al., (2001). Mediterranean and Low-Fat Diets Improve Endothelial Function in Hypercholesterolemic Men. *Annals of Internal Medicine*, *134*(12), 1115. doi: 10.7326/0003-4819-134-12-200106190-00011

Furchgott, R., & Zawadzki, J. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, *288*(5789), 373–376. doi: 10.1038/288373a0

Giannotti, G., & Landmesser, U. (2007). Endothelial Dysfunction as an Early Sign of Atherosclerosis. *Herz Kardiovaskuläre Erkrankungen*, *32*(7), 568–572. doi: 10.1007/s00059-007-3073-1

Goff et al., (2013). ACC/AHA Guideline on the Assessment of Cardiovascular Risk. *Circulation*, *129*(25 suppl 2). doi: 10.1161/01.cir.0000437741.48606.98

Gordon, S., et al., (2009). Investigation of endothelial function by pulse contour analysis: a protocol for drug administration and timing of pulse contour assessment. *British Journal of Clinical Pharmacology*, *68*(4), 630–633. doi: 10.1111/j.1365-2125.2009.03482.x

Greyling, A., et al., (2016). Adherence to guidelines strongly improves reproducibility of brachial artery flow-mediated dilation. *Atherosclerosis*, *248*, 196–202. doi: 10.1016/j.atherosclerosis.2016.03.011

Halcox, J., et al., (2009). Endothelial Function Predicts Progression of Carotid Intima-Media Thickness. *Circulation*, *119*(7), 1005–1012. doi: 10.1161/circulationaha.108.765701

Hambrecht, R., et al., (2000). Effect of Exercise on Coronary Endothelial Function in Patients with Coronary Artery Disease. *New England Journal of Medicine*, *342*(7), 454–460. doi: 10.1056/nejm200002173420702

Hardie, K., et al., (1997). Reproducibility of brachial ultrasonography and flow-mediated dilatation (FMD) for assessing endothelial function. *Australian and New Zealand Journal of Medicine*, *27*(6), 649–652. doi: 10.1111/j.1445-5994.1997.tb00992.x

Hartley, C., & Tanaka, H. (2010). Assessment of Macro- and Microvascular Function and Reactivity. *Asymptomatic Atherosclerosis*, 265–275. doi: 10.1007/978-1-60327-179-0_19

Hayoz, D., et al., (1995). Postischemic Blood Flow Response in Hypercholesterolemic Patients. *Hypertension*, *26*(3), 497–502. doi: 10.1161/01.hyp.26.3.497

Heitzer, T., et al., (1999). Effect of vitamin E on endothelial vasodilator function in patients with hypercholesterolemia, chronic smoking or both. *Journal of the American College of Cardiology*, *33*(2), 499–505. doi: 10.1016/s0735-1097(98)00584-1

Higashi, Y., et al., (1999). Regular Aerobic Exercise Augments Endothelium-Dependent Vascular Relaxation in Normotensive As Well As Hypertensive Subjects. *Circulation*, *100*(11), 1194–1202. doi: 10.1161/01.cir.100.11.1194

Higashi, Y., et al., (2001). A noninvasive measurement of reactive hyperemia that can be used to assess resistance artery endothelial function in humans. *The American Journal of Cardiology*, *87*(1), 121–125. doi: 10.1016/s0002-9149(00)01288-1

Hijmering, M., et al., (2002). Sympathetic activation markedly reduces endothelium-dependent, flow-mediated vasodilation. *Journal of the American College of Cardiology*, *39*(4), 683–688. doi: 10.1016/s0735-1097(01)01786-7

Horio, Y., et al., (1988). Effects of intracoronary injection of acetylcholine on coronary arterial hemodynamics and diameter. *The American Journal of Cardiology*, *62*(13), 887–891. doi: 10.1016/0002-9149(88)90887-9

Igari, K., et al., (2016). Endothelial Dysfunction of Patients with Peripheral Arterial Disease Measured by Peripheral Arterial Tonometry. *International Journal of Vascular Medicine*, *2016*, 1–6. doi: 10.1155/2016/3805380

Kimura, M., et al., (2003). PDE5 Inhibitor Sildenafil Citrate Augments Endothelium-Dependent Vasodilation in Smokers. *Hypertension*, *41*(5), 1106–1110. doi: 10.1161/01.hyp.0000068202.42431.cc

Koller, A., & Kaley, G. (1991). Endothelial regulation of wall shear stress and blood flow in skeletal muscle microcirculation. *American Journal of Physiology-Heart and Circulatory Physiology*, *260*(3). doi: 10.1152/ajpheart.1991.260.3.h862

Kuvin, J., et al., (2003). Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *American Heart Journal*, *146*(1), 168–174. doi: 10.1016/s0002-8703(03)00094-2

Levine, G., Keaney, J., & Vita, J. (1995). Cholesterol Reduction in Cardiovascular Disease — Clinical Benefits and Possible Mechanisms. *New England Journal of Medicine*, *332*(8), 512–521. doi: 10.1056/nejm199502233320807

Ley, O., et al., (2010). Use of temperature alterations to characterize vascular reactivity. *Clinical Physiology and Functional Imaging*, *31*(1), 66–72. doi: 10.1111/j.1475-097x.2010.00981.x

Libby, P., Ridker, P. M., & Maseri, A. (2002). Inflammation and Atherosclerosis. *Circulation*, *105*(9), 1135–1143. doi: 10.1161/hc0902.104353

Libby, P., Aikawa, M., & Jain, M. (2006). Vascular Endothelium and Atherosclerosis. *The Vascular Endothelium II Handbook of Experimental Pharmacology*, 285–306. doi: 10.1007/3-540-36028-x_9

Lind, L., Hall, J., & Johansson, K. (2002). Evaluation of four different methods to measure endothelium-dependent vasodilation in the human peripheral circulation. *Clinical Science*, *102*(5), 561. doi: 10.1042/cs20010200

Lind, L., & Lithell, H. (1993). Decreased peripheral blood flow in the pathogenesis of the metabolic syndrome comprising hypertension, hyperlipidemia, and hyperinsulinemia. *American Heart Journal*, *125*(5), 1494–1497. doi: 10.1016/0002-8703(93)90446-g

Llewellyn, T., et al., (2012). The relationship between shear rate and flow-mediated dilation is altered by acute exercise. *Acta Physiologica*, *205*(3), 394–402. doi: 10.1111/j.1748-1716.2012.02417.x

Ludmer P., et al., (1986) Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *New England Journal of Medicine. 315*: 1046–1051.

Markus, et al., (1997). Influence of lifestyle modification on atherosclerotic progression determined by ultrasonographic change in the common carotid intima-media thickness. *The American Journal of Clinical Nutrition*, *65*(4), 1000–1004. doi: 10.1093/ajcn/65.4.1000

McNamara J., Molot M., & Stremple J. (1971) Coronary Artery disease in Combat Casualties in Vietnam. *The Journal of the American Medical Association*;216:1185-118

Meyer, B., et al., (2005). Flow-Mediated Vasodilation Predicts Outcome in Patients with Chronic Heart Failure. *Journal of the American College of Cardiology*, *46*(6), 1011–1018. doi: 10.1016/j.jacc.2005.04.060

Michos, E. et al., (2005). Women with a low Framingham risk score and a family history of premature coronary heart disease have a high prevalence of subclinical coronary atherosclerosis. *American Heart Journal*, *150*(6), 1276–1281. doi: 10.1016/j.ahj.2005.02.037

Minor, R., et al., (1990). Diet-induced atherosclerosis increases the release of nitrogen oxides from rabbit aorta. *Journal of Clinical Investigation*, *86*(6), 2109–2116. doi: 10.1172/jci114949

Mitchell, G., et la., (2004). Local Shear Stress and Brachial Artery Flow-Mediated Dilation. *Hypertension*, *44*(2), 134–139. doi: 10.1161/01.hyp.0000137305.77635.68

Moerland, M., et al., (2012). Evaluation of the EndoPAT as a Tool to Assess Endothelial Function. *International Journal of Vascular Medicine*, *2012*, 1–8. doi: 10.1155/2012/904141

Naghavi, M., et al., (2016). New Indices of Endothelial Function Measured by Digital Thermal Monitoring of Vascular Reactivity: Data from 6084 Patients Registry. *International Journal of Vascular Medicine*, *2016*, 1–8. doi: 10.1155/2016/1348028

Neunteufl T, et al., (2000) Late prognostic value of flow-mediated dilation in the brachial artery of patients with chest pain. *American Journal of Cardiology*; *86*: 207–210.

Nishimura, R. et al., (1995). Epicardial vasomotor responses to acetylcholine are not predicted by coronary atherosclerosis as assessed by intracoronary ultrasound. *Journal of the American College of Cardiology*, *26*(1), 41–49. doi: 10.1016/0735-1097(95)00142-m

Panza, J., et al., (1990). Abnormal Endothelium-Dependent Vascular Relaxation in Patients with Essential Hypertension. *New England Journal of Medicine*, *323*(1), 22–27. doi: 10.1056/nejm199007053230105

Parkhurst, K. et al., (2012). Contribution of blood viscosity in the assessment of flow-mediated dilation and arterial stiffness. *Vascular Medicine*, *17*(4), 231–234. doi: 10.1177/1358863x12450095

Patti, G., et al., (2005). Impaired Flow-Mediated Dilation and Risk of Restenosis in Patients Undergoing Coronary Stent Implantation. *Circulation*, *111*(1), 70–75. doi: 10.1161/01.cir.0000151308.06673.d2

Peretz, A., et al., (2007). Flow mediated dilation of the brachial artery: an investigation of methods requiring further standardization. *BMC Cardiovascular Disorders*, *7*(1). doi: 10.1186/1471-2261-7-11

Pyke, K., & Tschakovsky, M. (2005). The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *The Journal of physiology*, *568*(Pt 2), 357–369. doi:10.1113/jphysiol.2005.089755

Reddy K., et al., (1994) Evidence that selective endothelial dysfunction may occur in the absence of angiographic or ultrasound atherosclerosis in patients with risk factors for atherosclerosis. J Am Coll Cardiol. 1994; *23*: 833–843

Romero, S., et al., (2017). Acute limb heating improves macro- and microvascular dilator function in the leg of aged humans. *American Journal of Physiology-Heart and Circulatory Physiology*, *312*(1). doi: 10.1152/ajpheart.00519.2016

Ross, R., & Glomset, J. (1976). The Pathogenesis of Atherosclerosis. *New England Journal of Medicine*, *295*(7), 369–377. doi: 10.1056/nejm197608122950707

Rubinshtein, R., et al., (2010). Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. *European Heart Journal*, *31*(9), 1142–1148. doi: 10.1093/eurheartj/ehq010

Schmidt-Lucke, C., et al., (2005). Reduced Number of Circulating Endothelial Progenitor Cells Predicts Future Cardiovascular Events. *Circulation*, *111*(22), 2981–2987. doi: 10.1161/circulationaha.104.504340

Shamim-Uzzaman, Q., et al., (2002). Altered cutaneous microvascular responses to reactive hyperaemia in coronary artery disease: a comparative study with conduit vessel responses. *Clinical Science*, *103*(3), 267–273. doi: 10.1042/cs1030267

Sun, D., et al., (1999). Enhanced Release of Prostaglandins Contributes to Flow-Induced Arteriolar Dilation in eNOS Knockout Mice. *Circulation Research*, *85*(3), 288–293. doi: 10.1161/01.res.85.3.288

Thijssen, D. et al., (2011). Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *American Journal of Physiology-Heart and Circulatory Physiology*, *300*(1). doi: 10.1152/ajpheart.00471.2010

Thijssen, D. et al., (2019). Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *European Heart Journal* , *40*(30), 2534–2547. doi: doi: 10.1093/eurheartj/ehz350.

Tousoulis, D. (2005). Evaluating endothelial function in humans: a guide to invasive and non-invasive techniques. *Heart*, *91*(4), 553–558. doi: 10.1136/hrt.2003.032847

Tuzcu, E., et al., (2001). High Prevalence of Coronary Atherosclerosis in Asymptomatic Teenagers and Young Adults. *Circulation*, *103*(22), 2705–2710. doi: 10.1161/01.cir.103.22.2705

Vehkavaara, S., et al., (2000). Insulin Therapy Improves Endothelial Function in Type 2 Diabetes. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *20*(2), 545–550. doi: 10.1161/01.atv.20.2.545

Vuilleumier, P., et al., (2002). Post ischemic forearm skin reactive hyperemia is related to cardiovascular risk factors in a healthy female population. *Journal of Hypertension*, *20*(9), 1753–1757. doi: 10.1097/00004872-200209000-00018

Widlansky, M., et al., (2003). The clinical implications of endothelial dysfunction. *Journal of the American College of Cardiology*, *42*(7), 1149–1160. doi: 10.1016/s0735-1097(03)00994-x

Wilk, G., et al., (2013). Endothelial function assessment in atherosclerosis. Comparison of brachial artery flow-mediated vasodilation and peripheral arterial tonometry. *Polish Archives of Internal Medicine*, *123*(9), 443–452. doi: 10.20452/pamw.1879

Wilkinson, I., & Webb, D. (2002). Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *British Journal of Clinical Pharmacology*, *52*(6), 631–646. doi: 10.1046/j.0306-5251.2001.01495.x

Winquist, R. et al., (1984). Atrial natriuretic factor elicits an endothelium-independent relaxation and activates particulate guanylate cyclase in vascular smooth muscle. *Proceedings of the National Academy of Sciences*, *81*(23), 7661–7664. doi: 10.1073/pnas.81.23.7661

Yeboah, J., et al., (2009). Predictive Value of Brachial Flow-Mediated Dilation for Incident Cardiovascular Events in a Population-Based Study. *Circulation*, *120*(6), 502–509. doi: 10.1161/circulationaha.109.864801