COMPARISON OF NON-INVASIVE PERIPHERAL VASCULAR FUNCTION TO INVASIVE MEASURES OF CORONARY FUNCTION IN PATIENTS WITH SUSPECTED CORONARY MICROVASCULAR DYSFUNCTION

MASSIMO NARDONE

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTERS OF SCIENCE

GRADUATE PROGRAM IN KINESIOLOGY AND HEALTH SCIENCE

YORK UNIVERSITY

TORONTO, ONTARIO

JULY 2019

ABSTRACT

This thesis examined whether reactive hyperemia peripheral arterial tonometry (RH-PAT) and flow-mediated dilation (FMD), non-invasive measures of peripheral endothelial function, are associated with coronary microvascular function following multiple pharmacological stimuli in patients with suspected coronary microvascular dysfunction (CMD). Patients with suspected CMD completed peripheral vascular assessments using concurrent RH-PAT and FMD, while coronary microvascular function was measured using the index of microvascular resistance (IMR) and the coronary flow reserve (CFR) during endothelial independent (adenosine), endothelial-dependent (acetylcholine), and sympathetically-mediated (dobutamine) hyperemia. Any abnormality in the IMR and/or CFR during the adenosine and/or acetylcholine trials defined patients with CMD. RH-PAT and FMD were attenuated in patients with CMD ($P<0.05$). RH-PAT was correlated with the dobutamine IMR and CFR ($P<0.05$), while FMD was correlated with the adenosine ($P<0.05$) and acetylcholine IMRs ($P<0.05$), but not the CFRs. Therefore, this thesis suggests that both RH-PAT and FMD can identify patients with CMD in clinical settings.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my family for their continuous support throughout my educational journey. You've always pushed me to follow my passions, and have instilled hard work and dedication into all tasks of life. You've taught me to never limit my full potential, and to continuously strive to be the best I can be. You have all been fundamental to my current successes. Thank you.

To my advisor, Dr. Heather Edgell. I appreciate all of the time and energy you have put towards making me realize my full potential as a future researcher. I am forever grateful of the skills you have taught me, and the opportunities you have provided me over the past 2 years. I thank you for constantly pushing me to pursue new ideas, new challenges, and to uncover new solutions.

Thank you to Dr. Steven Miner and Mary McCarthy for providing me with the opportunity to gain invaluable research experience from the clinical perspective.

I would also like to thank my committee member, Dr. Chris Ardern for supporting me throughout this process. I am honoured to have had the opportunity to learn from such an incredible mentor.

A special thanks to my lab mates, Hitesh, Ismina, Elnaz, Sara, Ilana, and Baithat. Thank you for supporting me in this project every step of the way. I enjoyed working with you all and look forward to our future success.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Term
AI	Augmentation index
AI@75bpm	Augmentation index at 75 beats per minute
CASS	Coronary Artery Surgery Study
CFR	Coronary flow reserve
CMD	Coronary Microvascular Dysfunction
CSX	Cardiac Syndrome X
CVD	Cardiovascular disease
EDHF	Endothelium-derived hyperpolarizing factor
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
ER	Estrogen receptors
FMD	Flow mediated dilation
IMR	Index of microvascular resistance
L-NMMA	N-monomethyl-L-arginine
LAD	Left Anterior Descending Artery
LnRHI	Natural logarithm of the reactive hyperemic index
NO	Nitric Oxide
Pa	Proximal coronary pressure
Pd	Distal coronary pressure
RHI	Reactive hyperemia index
RH-PAT	Reactive hyperemia peripheral arterial tonometry
Tmn	Transit time
VSMCs	Vascular smooth muscle cells
WISE	Women’s Ischemia Syndrome Evaluation

CHAPTER 1.0 GENERAL INTRODUCTION

Patients with exertional chest pain and a positive exercise stress test are routinely referred for coronary angiography to assess for obstructive coronary artery disease. The presence of a blockage in the coronary arteries often elicits inadequate oxygen delivery to cardiac muscle, explaining the observed chest pain. However, when obstructive coronary arteries are not observed, physicians are often faced with challenges in determining the cause of chest pain. Importantly, the observation of non-obstructive coronary arteries have been apparent since the introduction of coronary angiography (1, 2), while more recently, approximately one third of patients referred for coronary angiography have no significant coronary obstructions (3).

One limitation of coronary angiography is the inability to visualize the microcirculation, the compartment of the vascular system that plays an important role in regulating blood flow to cardiac muscle (4). In particular, the inability to dilate the coronary microcirculation, a condition known as coronary microvascular dysfunction (CMD) (5), is gaining appreciation as a significant contributor to increased adverse cardiac events and mortality (6, 7). However, screening patients with CMD is clinically challenging, and often results in increased emergency department visits and multiple coronary angiographies without reaching a confirmed diagnosis (8, 9). Since the procedures required to diagnose patients with CMD are invasive, expensive, and require highly trained medical personnel, determining clinically available non-invasive measures to screen this cohort is important to help patients with chest pain and non-obstructive coronary arteries reach a diagnosis faster.

In 1992, the use of ultrasound to measure vascular function in the human arm using a technique called flow mediated dilation (FMD) was first reported (10), while later in 2003, vascular function was measured in the finger microvasculature using reactive hyperemia peripheral arterial tonometry (RH-PAT). However, it is currently unclear if these measures are related to vascular function within the coronary microvasculature. The relationship between peripheral and coronary vascular function could have important clinical implications and can help screen patients with non-obstructive coronary arteries and suspected CMD. Therefore, the aim of the current thesis is to determine if vascular function in the coronary microvasculature is associated with vascular function in the periphery.

CHAPTER 2.0 LITURATURE REVIEW

2.1. General Introduction

The pulsatile nature of blood flow through the circulation is governed by the contracting heart. During each heartbeat, the left ventricle propagates blood peripherally to meet the metabolic demands of tissue. Myocardial oxygen delivery, however, primarily occurs during diastole, as ventricular contraction impedes blood delivery to the myocardium (11, 12). Given that cardiac muscle extracts ~70-80% of oxygen from the arterial coronary vasculature, augmenting coronary blood flow is the primary mediator of matching myocardial oxygen supply to demand, both at rest and during exercise (reviewed by Duncker and colleagues (4)). This is accomplished by both increases in perfusion pressure and reductions in coronary resistance (4). In 1971, Holmberg and colleagues noted that the reduction in coronary resistance primarily facilitated the increase in coronary flow during cycle ergometer exercise (13). Further, coronary resistance decreases during exercise by ~20-30% in humans (14), accounting for ~90% of the coronary vasculature's ability to augment blood flow (4).

Matching myocardial oxygen supply and demand through changes in coronary resistance fundamentally relies on healthy functioning coronary arteries. In 1989, Gordon and colleagues examined the epicardial coronary vasomotor response to supine cycle ergometer exercise in participants with healthy and diseased coronary epicardial vessels (15). In healthy epicardial coronary arteries, the response to exercise was vasodilation, however, in arterial segments with epicardial stenosis (i.e. vessel narrowing), the response to exercise was vasoconstriction (15). In chronically instrumented dogs, experimentally attenuating endothelial function independent of coronary stenosis, also caused epicardial coronary vasoconstriction during treadmill exercise (16). These concepts highlight the importance of vascular health in mediating the appropriate vascular responses to increased metabolic demand (i.e. exercise) in order to adequately match myocardial oxygen supply to demand.

2.2. Understanding Blood Flow in the Circulation

2.2.1. Blood Vessel Anatomy

Blood vessels, viscoelastic in nature, are comprised of three layers: the adventitia, media and intima (17). The proportion of each vascular layer varies largely throughout the arterial tree,

and given that all three layers contain separate viscoelastic properties (18), the stiffness of vessels can therefore vary. The adventitia, the outer layer lining blood vessels, is composed primarily of collagen and fibroblasts, and contains sympathetic nerve endings that are responsible for mediating vasoconstriction (17). The media layer is composed of vascular smooth muscle cells (VSMC), as well as collagen and elastin (17). In young healthy humans, elastin is predominant compared to collagen, which enables compliant vessels. However with aging, collagen content begins to increase (19, 20), concurrently increasing the overall stiffness of the vasculature, which can negatively impact myocardial performance (explained in subsequent section). Lastly, the intima is composed of endothelium cells, which physically contacts adjacent blood flow and is responsible for mediating a range of cellular responses. These cellular responses will be discussed in detail in Section 2.3.

2.2.2. Blood Flow in the Macrocirculation

The arterial circulation can be divided into the macrocirculation (i.e. large arteries, and conduit arteries) and the microcirculation (i.e. arterioles) (17). Large arteries are primarily composed of elastin, thus facilitating high compliance (21). This is important given that the primary role of these arteries are to: 1) dampen the magnitude of pressure caused by blood ejection from the left ventricle, and 2) store blood within the vessel during systole and expel it during diastole (22). Termed the Windkessel function, approximately half of stroke volume is propagated peripherally during systole, while the remaining stroke volume is circulated during diastole, a process that allows for continuous blood flow through the large vessels (22). Further, high central artery compliance decreases left ventricular work (i.e. reduced afterload) (23), and increases left ventricular relaxation (24). In an animal model, chronically increasing aortic stiffness using aortic bandaging to constrict the aorta, the brachiocephalic trunk and the left subclavian artery, resulted in higher resting coronary blood flow relative to control animals (25), due to the increased ventricular afterload.

With aging, increased collagen deposition in both large and conduit arteries increases artery stiffness (19, 20), with large arteries being more susceptible to increases in vascular stiffness compared to conduit arteries (26). During systole, forward wave pulsation through the arterial tree collides at vascular bifurcations systemically, causing pressure wave reflections that propagate retrograde to the heart (27). Increases in arterial stiffness impact this process by

augmenting both retrograde pressure wave velocity and magnitude, due to less distensibility, and therefore less Windkessel effect (20, 28). This increased wave reflection propagation and amplitude causes blood pressure to arrive back at the heart during late systole, opposed to diastole, which augments blood pressure and left ventricular afterload (28).

Conduit arteries, predominantly composed of smooth muscle, actively modulate vascular tone and diameter depending on the physiological stressor (28). Fairfax and colleagues observed in resting young healthy men that spontaneous sympathetic discharges increased mean arterial pressure and decreased vascular conductance in the brachial, femoral, and popliteal arteries (29, 30). Additionally, increasing metabolic activity (i.e. changes in CO_2, O_2, lactate, and hydrogen ions) can cause conduit arteries to dilate (31). For example, Tremblay and Pyke highlighted that rhythmic handgrip exercise elicits an intensity dependent increase in brachial artery diameter (31), important for matching blood flow to metabolic demands. Together, these studies demonstrate that sympathetic activation can constrict conduit arteries, while exercise can dilate conduit arteries in both active and inactive muscles to augment blood flow to match metabolic demand.

2.2.3. Blood Flow in the Microcirculation

Arterioles, primarily composed of endothelial cells and several layers of VSMCs, vary in size depending on their proximity to distal capillaries (32). Similarly to conduit arteries, these vessels are highly innervated by sympathetic nerve fibers (33, 34), which elicit vasoconstriction. Arterioles contain a large capacity to modulate diameter, and therefore total vascular resistance and overall blood flow (35). Specifically, increasing metabolic activity can cause arterioles to dilate up to 50% of their original diameter (36), leading to a 100-fold increase in blood flow to muscles (37). Contrarily, sympathetic stimulation can lead to significant vasoconstriction and significantly limit blood flow. *In vitro* sympathetic nerve stimulation innervating distal arterioles and terminal arterioles resulted in ~35% and ~55% reductions in diameter respectively (38).

During exercise, sympathetic activation and the production of metabolites concurrently increase (39), yet have conflicting vascular responses. In order to preserve blood delivery to exercising muscles, local metabolites attenuate the response of the sympathetic nervous system by a process called functional sympatholysis (40–42). In 1962, Remensnyder and colleagues noted that sympathetic stimulation caused vasoconstriction in the dog's hindlimb at rest,

however, the same stimulus during exercise resulted in an attenuated vasoconstrictor response (40). Similar findings later found that during dynamic exercise of the hamster retractor muscle, distal arterioles were the most sensitive to preserving their diameter upon sympathetic stimulation, relative to conduit arteries and proximal arterioles (41). In humans, Tschakovsky and colleagues investigated the process of functional sympatholysis by infusing a low, medium, and high dose of tyramine (i.e. pharmacological sympathoexcitatory agent) into the brachial artery during rest, moderate and heavy exercise (42). Tyramine elicited a dose-dependent decrease in forearm vascular conductance and flow, while exercise elicited an intensity-dependent attenuation of the tyramine response. Together, these select studies highlight the utility of functional sympatholysis in preserving blood delivery to exercising muscles.

2.3. The Endothelium and Nitric Oxide

2.3.1. Definition and Discovery

The endothelium is a single layer of cells that lines the inside of blood vessels systemically (43) and physically interacts with adjacent blood flow (44). The endothelium has the capacity to synthesize and release a variety of substances, however, one of the most potent substances aiding in the maintenance of overall vascular health is nitric oxide (NO). NO has been extensively researched since its discovery in 1980 by Robert F. Furchgott and John V. Zawadzki (45). In their seminal experiment, Furchgott and colleague noted that acetylcholine-induced dilation of the rabbit abdominal aorta was abolished following removal of the endothelial cells, but not removal of the tunica adventitia, suggesting that the interaction between the endothelial cells and acetylcholine caused the release of a substance not fully identified (45). Initially termed endothelial-derived relaxing factor, the substance was later identified in 1987 as NO (46, 47).

2.3.2. Synthesis of Nitric Oxide

Nitric oxide (NO) is a soluble gas with a half-life of ~6-30 seconds (48), and is synthesized by a family of nitric oxide synthases (49). In particular, NO is synthesized in the endothelium primarily by the enzyme endothelial nitric oxide synthase (eNOS), located in specialized folds within the endothelial cell membrane, termed caveolae (48, 50–52). Influx of calcium into endothelial cells causes the displacement of the protein caveolin-1 from calmodulin, activating eNOS, and subsequently leading to the synthesis of NO from the precursor L-arginine

(51). Further, several co-factors are involved in the conversion of L-arginine to NO, including tetrahydrobiopterin, and nicotinamide adenine dinucleotide phosphate (50). Intracellular endothelial NO then diffuses out of the endothelium and into the VSMCs, where it activates guanylate cyclase, the enzyme responsible for the conversion of guanosine triphosphate into cyclic guanosine monophosphate (50). Cyclic guanosine monophosphate causes smooth muscle relaxation by decreasing intracellular calcium, ultimately leading to vasodilation (50).

2.3.3. Endothelial Function and Dysfunction

NO synthesized and released from endothelial cells plays a large role in mediating vasodilation (48, 51). However, NO possesses further functions that help maintain vascular health, including the prevention of thrombosis formation (53–56), anti-inflammatory properties (53, 54), maintenance of blood pressure (57, 58), and limiting VSMC proliferation (59). Specifically, NO has been shown to inhibit key leukocyte adhesion molecules including vascular cell adhesion molecule-1 and intracellular adhesion molecule-1 *in vitro* (53) and *in vivo* (54, 56), in addition to limiting platelet deposition (55). Further, administration of the NO inhibitor N-monomethyl-L-arginine (L-NMMA) to anesthetized rabbits caused an increase in mean arterial pressure (58), indicating that NO plays a role in maintaining blood pressure. Exogenous nitrate consumption (i.e. beetroot juice extract) has also been shown to acutely decrease blood pressure in normotensive subjects (60). Lastly, several studies have highlighted that the endothelium and the sympathetic nervous system can interact and have a bidirectional effect on one another (61–65). For instance, nitric oxide blockade in young healthy humans caused an increase in sympathetic nerve activity (66), while sympathetic blockade with trimethaphan acutely improved the forearm blood flow responses to acetylcholine in obese hypertensive participants (67).

The endothelium releases a variety of additional factors that possess divergent effects on vascular health. For instance, prostacyclin, bradykinin, and endothelium-derived hyperpolarizing factor (EDHF) supplement NO in mediating vasodilation and inhibiting platelet aggregation (51, 68). Contrarily, endothelin-1 (ET-1) and thromboxane A_2 can stimulate vascular constriction and facilitate atherosclerotic plaque development (51, 68). Specifically, increased resting ET-1 often observed in patients with cardiovascular disease (CVD), can promote vascular inflammation and oxidative stress (69, 70).

In a healthy endothelium, there is a balance between vasodilator and vasoconstrictor factors produced, with sufficient basal production of NO mediating normal vascular responses (43). However, in dysfunctional endothelial cells, NO bioavailability becomes attenuated, while concurrent increases in vasoconstrictors and pro-inflammatory substances are produced by the endothelium (43). As traditional cardiovascular risk factors develop and progress, dysfunction of the endothelium increases with the gradual accumulation of inflammation and oxidative damage (71), which attenuates NO bioavailability; since NO must be redirected to scavenge free radicals (48). In support of this, endothelial function is attenuated in subjects with diabetes (72, 73), hypercholesterolemia (10, 74), hypertension (75), and smokers (76). Further, Celermajer and colleagues noted a strong negative correlation between endothelial function and the number of traditional cardiovascular risk factors in 500 apparently healthy subjects (77). Hashimoto and colleagues observed similar findings in 101 patients with one or more traditional cardiovascular risk factors compared to 40 control subjects (78). These studies highlight that traditional cardiovascular risk factors may influence endothelial dysfunction due to the accumulation of inflammation and oxidative damage, while the number of risk factors can further attenuate vascular function.

2.3.4. The Role of Shear Stress on Endothelial Function

Shear stress, defined as the frictional force between endothelial cells and adjacent blood flow (79), is the main stimulus for the release of NO (44, 80). Shear stress is proportional to the product of blood viscosity and blood velocity relative to arterial diameter (i.e. Shear stress = viscosity x velocity/diameter) (81). Viscosity is typically not measured and therefore assumed to be a constant of 4 for small arteries (82), such as the brachial artery. Shear stress causes the activation of a large number of mechanoreceptors located on endothelial cells (44, 80), which mediates the influx of calcium and subsequent signalling cascade resulting in NO production and vasodilation.

The magnitude and type of shear stress can modulate endothelial cell function and morphology, having large implications on atherosclerotic development (80, 83). Given the pulsatile nature of blood flow, the arterial tree experiences pulsatile shear stress, defined as high unidirectional shear stress with varying magnitudes (44). High shear stress can increase production and synthesis of NO, and upregulate endothelial transcription factors that positively

influence cell function (44). However, regions of the vasculature containing curves and bifurcations can experience oscillatory shear stress (i.e. multidirectional shear stress containing both positive and negative periods of shear stress over the cardiac cycle (44)) and/or low endothelial shear stress. In experimental models of the human carotid arteries, Ku and colleagues noted large oscillatory shear stress patterns in the internal carotid artery, which was strongly correlated to plaque deposition (84). Further, while experimentally inducing an acute stimulus designed to attenuate resting endothelial shear stress in the human forearm, Thijssen and colleagues observed a reduction in endothelial function (85). Animal studies suggest that chronic exposure to oscillatory and low endothelial shear stress can upregulate gene expression of pathways that ultimately increase oxidative damage, inflammation, and thrombosis (44). Thus, the type and magnitude of shear stress can play an important role in affecting acute and chronic adaptations to endothelial cell function and morphology.

2.4. Measures of Vascular Function

2.4.1. Flow Mediated Dilation

2.4.1.1. Definition and Discovery

First reported in the Lancet in 1992 by David S. Celermajer and John E. Deanfield, flow mediated dilation (FMD) is defined as the dilation of a blood vessel following a period of increased blood flow (i.e. hyperemia). The stimulus most commonly used to elicit a hyperemic response is 5 minutes of forearm ischemia (86). This test is utilized to assess endothelial function, given that hyperemia (i.e. shear stress) increases NO production (described in detail in former section). The procedure of these measures will be described in depth in Section 3.3.2.

2.4.1.2. Physiology of FMD

Forearm ischemia and subsequent reperfusion induces a hyperemic response that largely increases endothelial shear stress (81). Given the downstream signalling cascade previously described, several studies have evaluated the contributions of NO to the FMD response. Joannides and colleagues were the first to investigate the hyperemic dilatory response of the radial artery following infusion of the NO inhibitor L-NMMA (87). The radial artery had a normal dilatory response to hyperemia, however, following L-NMMA infusion, the radial artery constricted following hyperemia (87). Mullen and colleagues also observed that L-NMMA

completely abolished the FMD response of the radial artery (88). Investigating the dilatory response in the brachial artery, a more recognized approach to measuring the FMD response (86), showed similar results to Mullen and colleagues, such that L-NMMA completely abolished the FMD response following wrist occlusion (89). Further, measures from the brachial artery after forearm occlusion (the most standardized protocol for assessments of FMD) in participants that were young healthy (90), old healthy (91), and smokers (92), showed that L-NMMA attenuated the FMD response, but was not completely abolished. Lastly, a meta-analysis of 20 studies that included over 300 participants revealed that NO-inhibition blunted the FMD response by ~70% (93), highlighting that FMD is highly NO mediated.

2.4.1.3. Clinical Relevance of FMD

Several longitudinal cohort studies have assessed the capacity of FMD to predict the incidences of, and mortality attributed to, CVD (94–99). These studies have been conducted in population-based cohorts of apparently healthy individuals (98, 99), post-menopausal women (97), patients with chest pain (95, 96) and patients with coronary artery disease (94). In all populations, FMD was a strong predictor of cardiovascular events and mortality (100). These findings remain, regardless of the methodology employed (i.e. proximal or distal cuff location, varying ischemic time, etc.) (101). Taken together, FMD for the assessment of vascular function can strongly predict population risk of CVD, highlighting its relevance within clinical settings, and may therefore provide additive value to traditional risk factors for clinicians.

2.4.2. Reactive Hyperemia Peripheral Arterial Tonometry

2.4.2.1. Definition and Discovery

Reactive hyperemia peripheral arterial tonometry (RH-PAT) is a novel non-invasive method of measuring endothelial function. RH-PAT was first utilized by Robert P. Schnall and Peretz Lavie in 1999 to investigate obstructive sleep apnea. However in 2003, Jeffrey T. Kuvin and colleagues were the first to utilize this novel assessment for quantification of endothelial function (102). During RH-PAT, volume changes within the finger microvessels (i.e. pulse amplitude changes) are measured at rest and following a stimulus of ischemia-induced hyperemia, similarly to that of FMD. Then, the reactive hyperemia index (RHI) is calculated based on the hyperemic pulse amplitude relative to the baseline amplitude (discussed in further

detail in Section 3.3.2.). The natural log transformation of the RHI (LnRHI) is also calculated, as the monotonic transformation is more normally distributed compared to the RHI (103).

2.4.2.2. Physiology of RH-PAT

Resting digit blood flow is primarily regulated by the sympathetic nervous system (104), while NO seems to play a smaller role in maintaining resting microvascular tone (104, 105). Currently, there is limited data investigating the mediators of the dilatory response during RH-PAT. Nohria and colleagues (106) examined the effect of NO inhibition on RH-PAT, and demonstrated that L-NMMA infusion into the brachial artery attenuated the RHI by ~46%, indicating that NO plays a significant role in the microcirculatory hyperemic response. Other factors are likely to facilitate this response given that >50% of the hyperemic response remained. Further, NO inhibition also had no effect on resting pulse amplitude (106), confirming earlier studies that basal NO plays a minor role in regulating resting fingertip flow (104, 105).

2.4.2.3. Clinical Relevance of RH-PAT

RH-PAT has been shown to aid clinicians in risk prognosis in apparently healthy patients (107), and patients with coronary artery disease (108–110) or chest pain (111, 112). Specifically, in patients with unexplained chest pain, Rubinshtein and colleagues (112) observed higher rates of adverse events in patients with lower microvascular function, defined by an LnRHI<0.4, compared to patients with higher microvascular function following a 7 year follow-up (adverse events=48% vs 28%, respectively). Further, in high-risk patients undergoing coronary angiography due to chest pain, low microvascular function, defined by an RHI<0.53, also had significantly greater incidence of cardiovascular events compared to patients with greater microvascular function following a 5 year follow-up (cardiovascular events=32% vs 8%, respectively) (111). Both studies support the concept of utilizing RH-PAT as a measure of risk prognosis for CVD incidence and mortality.

2.4.3. Comparison of FMD and RH-PAT

Since both FMD and RH-PAT are non-invasive measures attempting to quantify endothelial function, several studies have compared the two measures to determine similarities. However, given that both measures are obtained from different vascular sites (i.e. conduit artery

vs. microvasculature) and are governed by relatively different contributions of NO (NO antagonists abolish the hyperemic responses of FMD and RH-PAT by >70% and <50%, respectively (93, 106)), several smaller studies (102, 113), and larger cross-sectional studies (114, 115) have highlighted that FMD and RH-PAT are weakly correlated. In a cohort from the Framingham Heart Study, Hamburg and colleagues have highlighted how FMD and RH-PAT are affected by different risk factors. Specifically, attenuated brachial artery FMD was associated with age, higher systolic blood pressure, and BMI, while the risk factors associated with abnormal RH-PAT were BMI, cholesterol, diabetes and smoking (114). Although FMD and RH-PAT are weakly correlated and appear to be influenced by different cardiovascular risk factors, a recent meta-analysis concluded that both FMD and RH-PAT equally contain a significant predictive value of cardiovascular events (116). Therefore, both FMD and RH-PAT provide clinically important information regarding patient risk stratification, regardless of physiological differences governing both measures.

2.5. Sex Differences in Cardiovascular Disease

2.5.1. History and Epidemiology

In the early 20th century, women were excluded from longitudinal studies, as CVD was once considered "a man's disease" (117). It was not until the 1950's that women were included in longitudinal studies, and subsequent reports indicated that the development of CVD in women significantly increased in later years of life, following menopause (118–120). A report from the American Heart Association in 2006 highlighted that: 1) CVD was the number one cause of death in women 65 years and older, and 2) more women have died from CVD compared to men (121). An updated report in 2017 showed that the prevalence of CVD was equal in men and women 40 years and older, with greater cardiovascular mortality in women compared to men aged 80 years and older (122). Despite this, there is still a lack of clinical trials and overall cardiovascular research targeted towards women (123). At both 1 year and 5 years following a myocardial infarction, women aged 45 years and older are more likely to die compared to men (122), highlighting the need for more research on improving clinical outcomes in women (123).

2.5.2. Sex Differences in Vascular Function

Premenopausal women have a low incidence of CVD compared to aged matched men (124). First highlighted by Kannel and colleagues in 1976, the loss of estrogen facilitated by the onset menopause was associated with an increase in CVD incidence rates (120). Subsequent studies have mechanistically highlighted that estrogen directly influences vascular function (125–127). Estrogen directly interacts with two estrogen receptors (ER) in the vasculature: ERα and ERβ, located within caveolae on the endothelium (128, 129). Activation of ERs can upregulate eNOS gene expression (130) and eNOS activity (131) *in vitro*; therefore increasing NO bioavailability (125, 128, 132, 133). In support of this mechanistic data, pre-menopausal women have greater FMD (134, 135) and RH-PAT (135) responses compared to age-matched men. However differences within resting brachial artery diameter may largely influence this sex difference, since women have smaller brachial arteries compared to men (134, 136). When allometrically scaling the FMD response (an analysis method designed to remove the influence of baseline diameter (137)) Shenouda and colleagues observed that men actually had a greater FMD response compared to age-matched women (138), however, the usefulness of allometric scaling is still in debate (139). Further, Heffernan and colleagues noted that women had higher RH-PAT responses compared to men, however, when utilizing baseline brachial artery diameter as a covariate, this observed sex difference was abolished (140). These results highlight that women may have larger dilatory responses compared to men, but this response may be intrinsically influenced by baseline brachial diameters, rather than a direct influence from estrogen.

In a cross-sectional design, Celermajer and colleagues investigated the effects of aging on vascular function, by conducting FMD assessments on 238 apparently healthy subjects between the ages of 15 to 72 years (136). Here, it was observed that FMD declined earlier in life for men, following the age of ~41 years, while women had a sharp reduction in FMD occurring later in life, at ~53 years of age (136). Interestingly, there was no age related reductions in VSMC function, assessed using the endothelial independent dilator glyceryl trinitrate, indicating that the age associated reduction in vascular function was specific to the endothelium (136). Similar findings were observed in a separate small cross-sectional cohort (141), highlighting that menopause mediated declines in estrogen likely attenuate endothelial function rapidly in women, while vascular function in men gradually declines with age.

2.6. Cardiac Microvascular Dysfunction

2.6.1. Definition and Discovery

Since the introduction of coronary angiography for the detection of coronary epicardial stenosis, the observation of chest pain (i.e. angina pectoralis) with normal coronary arteries was becoming apparent (1, 2). Arbogast and Bourassa in 1973 were one of the first groups to highlight the concept of myocardial ischemia despite normal coronary arteries (142). Subjects with electrocardiographic evidence of ischemia and normal coronary arteries (Group X) were compared with subjects with electrocardiographic evidence of ischemia and coronary artery obstruction (Group C) during atrial pacing. The authors observed similar changes in myocardial lactate production and myocardial ischemia (determined by ST segment depression on the electrocardiogram) in both groups, despite only the latter group having coronary obstruction. In a subsequent editorial, Harvey Kemp later termed "group X" as "syndrome X" (143). In 1981, Opherk and colleagues demonstrated that syndrome X patients had reduced coronary dilatory capacity to dipyridamole (i.e. an endothelial independent vasodilator) and subsequently, increased lactate production during atrial pacing compared to controls (144), highlighting that the inability of the coronary vasculature to dilate may explain the reduced myocardial blood delivery and observed ischemia. Later in 1988, Cannon and Epstein introduced the term "microvascular angina" to further describe patients with attenuated coronary flow and increased coronary resistance of the microvasculature (145).

Today, cardiac syndrome X (CSX) and coronary microvasculature dysfunction (CMD) are used to describe different patient groups. CSX is used to describe patients with exertional angina pectoralis, ST segment depression during exercise stress testing, and angiographically normal arteries (defined as <50% epicardial stenosis). CMD is diagnosed in patients with angina pectoralis, angiographically normal arteries, and abnormal coronary microvascular vasodilation during coronary reactivity testing (described in detail in Section 2.6.4.).

2.6.2. Prevalence

Approximately 10-30% of chest pain patients undergoing coronary angiography have no significant epicardial stenosis (3, 146, 147), while the prevalence of normal coronary arteries is higher in women compared to men. The Coronary Artery Surgery Study (CASS) observed that 11% and 39% of men and women undergoing angiography, respectively, had angiographically

normal coronary arteries (148), while more recently, the Women's Ischemia Syndrome Evaluation (WISE) study observed that 62% of women undergoing coronary angiography had no significant epicardial stenosis (149). Further, in 2001 the WISE study also demonstrated that approximately half of their cohort with chest pain and angiographically normal arteries had CMD (150) while Sara and colleagues observed a prevalence of ~66% in a mixed sex cohort (151). Originally, the prognosis of CSX and CMD was considered to be benign. However, the WISE study has also shown numerous times that CMD is associated with a poor prognosis (6, 7, 152–154). Specifically, the WISE study observed that abnormal coronary reactivity testing to adenosine (7, 154) or acetylcholine (6, 154) was independently associated with adverse cardiovascular events. Together, these studies highlight that women have a higher prevalence of CMD, a disease associated with a poor prognosis.

2.6.3. Pathophysiology

Patients with CSX have reduced coronary dilatory capacity and therefore attenuated blood delivery to the myocardium, leading to myocardial ischemia (144). Endothelial dysfunction is likely central to this abnormal response (155). For example, in patients with angiographically normal arteries, intracoronary infusions of L-NMMA caused a reduction in distal coronary artery diameter, highlighting that NO plays a role in maintaining basal vasculature resistance (156), in addition to facilitating epicardial dilation during scenarios of increased metabolic demand, such as atrial pacing (157). Further, there is evidence that endothelial dependent vasodilation in CSX has greater dysfunction in microvessels compared to epicardial vessels (158). In response to intracoronary acetylcholine infusions, CSX patients had a similar epicardial dilatory response compared to controls, however, demonstrated an attenuated increase in coronary blood flow (158). Since coronary microvessels primarily mediate augmentations in coronary blood flow (13, 14), the impaired coronary blood flow response in subjects with CSX suggests impaired microvascular dilation. Subsequent studies by Chauhan and colleagues observed both endothelial dependent and independent dilatory dysfunction in a larger sample of patients with CSX (159). Indeed, several studies have shown that coronary endothelial dysfunction was associated with myocardial perfusion impairments (160, 161). Additionally, CSX patients have a reduced brachial FMD response relative to healthy controls, and of a similar magnitude with patients with coronary obstruction (162), highlighting how endothelial

dependent vasodilation is systemically attenuated. Several other studies have additionally observed a reduced brachial artery FMD in patients with CSX (163–166).

A healthy endothelium regulates the balance between the secretion of vasodilators (i.e. NO, prostacyclin, etc.) and vasoconstrictors (i.e. ET-1) (68). As endothelial dysfunction progresses, this balance becomes disrupted, with increased release of vasoconstrictors, such as ET-1 (43). Indeed, Kaski and colleagues observed higher plasma concentrations of ET-1 in CSX compared to controls (167). Given that ET-1 causes vasoconstriction within the coronary vasculature (168), it is possible that this may further contribute to the reduced blood delivery to the myocardium, and subsequent myocardial ischemia. Together, these data demonstrate that endothelial dysfunction likely plays a central role in the microvascular abnormalities observed in CSX patients.

Arterial stiffness, often elevated in patients with CSX compared to healthy age-matched controls (169), is another vascular abnormality that can influence the coronary flow response. In an animal model, experimentally increasing aortic stiffness using a rigid aortic tube, designed to bypass the abdominal aorta, augmented coronary blood flow at rest (170). In humans with angiographically clear coronary arteries, subjects with elevated arterial stiffness (as measured by pulse wave velocity, augmentation indices (171) or pulse pressure (172)) also had increased resting coronary flow (171, 172) and an attenuated coronary vasodilatory capacity (171). Greater resting flow would inherently decrease vasodilatory capacity since coronary flow reserve (CFR) is calculated as the maximal coronary flow divided by baseline coronary flow (173). Further, the WISE study observed a strong negative correlation between arterial stiffness (measured using pulse wave velocity and augmentation index) and the coronary vasodilatory capacity in 50 women with suspected CMD (174). Together, these studies highlight how arterial stiffness can attenuate the coronary flow response and increase the susceptibility of the myocardium to ischemia.

2.6.4. Diagnosis and Screening

The assessment of the coronary microvasculature using coronary reactivity testing, which involves the quantification of microvascular vasomotion in response to pharmacological vasodilation (commonly during an endothelial-dependent and endothelial independent stimuli), is required to diagnosis CMD. The most common measure of microvascular function is the

coronary flow reserve (CFR), calculated as the ratio of coronary blood flow during pharmacological stimulation to coronary blood flow at rest (150). Different modalities can be used to evaluate CFR using either non-invasive measures (including cardiac PET scans (175, 176), cardiac MRI (177–179), and transthoracic or transesophageal echocardiography (180)) or invasive measures (intracoronary Doppler flow wire and thermodilution (7, 181)). A variety of different endothelial-dependent and independent pharmacological stimuli have been previously utilized to elicit hyperemia. For instance, endothelial dependent pharmacological stimuli include acetylcholine, bradykinin, atrial pacing, and adenosine triphosphate (6, 155, 157, 158, 182–184), while endothelial independent pharmacological stimuli include dipyridamole, sodium nitroprusside, and papaverine (154, 155, 159). Adenosine, a mainly endothelial independent stimulus in the coronary circulation, is also commonly utilized to assess coronary microvascular function (154, 159). Different CFR cut-offs have been utilized to diagnose CMD, ranging from <2.5 (150), <2.32 (7, 154) and <2.0 (175) for endothelial independent stimuli, while a CFR cut-off of <1.5 is generally utilized for endothelial-dependent stimuli (151, 185). Regardless of the cut-off used, patients with an abnormal CFR demonstrate increased rates of adverse cardiac outcomes (7, 154).

Another measure of microvascular function is the index of microvascular resistance (IMR), which utilizes thermodilution techniques to measure coronary flow, along with the pressure gradient across the microvasculature (186). Using Ohm's Law, the IMR is calculated as the product of the distal coronary artery pressure and coronary flow during pharmacological hyperemia. An IMR of >25 during intravenous adenosine has been utilized as a clinical cut-off for identifying patients with CMD (181). An IMR > 25 is also associated with increased adverse cardiac events and mortality in patients with CVD (187, 188). Lastly, several studies have shown that concurrently measuring the CFR and IMR can improve the capacity to predict patient outcomes (189, 190). The procedures involved in obtaining measures of the CFR and IMR during coronary reactivity testing will be described in detail in Section 3.3.

2.6.4.1. Non-Invasive Screening of Vascular Function

In 1987, Sax and colleagues were one of the first groups to demonstrate that impaired vasomotor function may be a systemic condition, not simply specific to the coronary vessels (191). Further, the magnitude of coronary vessel dysfunction was correlated with the magnitude

of forearm vascular dysfunction (191). Later, in 50 patients with angiographically clear arteries, Anderson and colleagues (192) observed modest correlations between coronary endothelial function, measured by quantitative angiography during intracoronary acetylcholine infusions, and brachial artery FMD. Other studies have measured the CFR from the left anterior descending coronary artery (LAD) using transthoracic echocardiography (a strong correlate to invasive Doppler wire measures (193)) and observed positive correlations between FMD and the CFR during intracoronary dipyridamole infusions (194, 195). However most recently, the iPOWER study observed no correlations between FMD and CFR using dipyridamole (196).

Utilizing RH-PAT as a predictive tool of coronary vascular function has also been previously investigated (197–199). Bonnetti and colleagues (197) conducted RH-PAT in patients with clear coronary arteries with (n=55) and without (n=39) coronary endothelial dysfunction. It was observed that the RHI was an independent predictor of the coronary blood flow response to acetylcholine, with an RHI<1.35 yielding highly sensitive results for the detection of coronary endothelial dysfunction. Similar findings were observed in women with ischemic heart disease, with RH-PAT yielding superior predictive results compared to the Reynolds Risk Score (198), a scoring system used to help predict the risk of future cardiovascular events. Contrarily, the iPOWER Study demonstrated that in 322 women with non-obstructive coronary artery disease, RH-PAT was not correlated to dipyridamole induced CFR (199). These results suggest that RH-PAT is associated with endothelial-dependent, but not endothelial independent coronary microvascular function.

CHAPTER 3 MANUSCRIPT

Flow-mediated dilation and peripheral arterial tonometry are associated with pharmaceutical stimulation of the coronary microvasculature

Massimo Nardone, BASc[a], Steven Miner, MD[a,b], Mary McCarthy, NP[b], Chris Ardern, PhD[a], Heather Edgell, PhD[a,b]

[a] School of Kinesiology and Health Science, York University, Toronto, Ontario, Canada.
[b] Southlake Regional Health Center, Newmarket, Ontario, Canada.

Brief Title: Relationship between peripheral and coronary vascular function

Word Count of Abstract: 250
Word Count of Manuscript: 5,140

Funding: This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), Canadian Foundation for Innovation (CFI), the Ontario Research Fund (ORF), the Heart of Gold Cardiac Research Fund, St. Jude Medical, and the Faculty of Health York University. M.N. was supported by a Canadian Institute of Health Research (CIHR) Fredrick Banting and Charles Best Canadian Graduate Scholarship.

Disclosures: None

To be submitted to Circulation: Cardiovascular Interventions

3.1. Abstract

Background: Reactive hyperemia peripheral arterial tonometry (RH-PAT) and flow-mediated dilation (FMD) are common measures of peripheral vascular function. Whether RH-PAT and FMD can be used to identify abnormalities in the coronary circulation, particularly in coronary microvascular dysfunction (CMD), is unclear.

Objectives: To compare RH-PAT and FMD to coronary microvascular function following endothelial independent, endothelial-dependent, and sympathetically-mediated pharmacological hyperemia.

Methods: Forty-seven patients with suspected CMD completed peripheral and coronary assessments. The reactive hyperemia index (LnRHI) was collected using the EndoPAT2000^{TM} device, while a subset of patients (n=28) completed FMD using Duplex ultrasound of the brachial artery. Coronary microvascular function was measured using the index of microvascular resistance (IMR) and the coronary flow reserve (CFR) during adenosine, acetylcholine, and dobutamine infusions. Any abnormality in the IMR and/or CFR during the adenosine and/or acetylcholine trials were used to define CMD.

Results: LnRHI and FMD were attenuated in patients with CMD (RH-PAT: No CMD: 0.88 ± 0.23 vs CMD: 0.63 ± 0.26; FMD: No CMD: 7.8 ± 2.2 vs CMD: 4.8 ± 2.8; $P<0.05$). RH-PAT was correlated with the dobutamine IMR and CFR ($\rho= -0.44$ and $\rho= 0.39$; $P<0.05$). FMD was correlated with the adenosine ($\rho= -0.48$; $P<0.05$) and acetylcholine IMRs ($\rho= -0.66$; $P<0.05$), but not the CFRs ($P>0.05$). RH-PAT and FMD cut-offs of 0.76 and 5.37% had sensitivities of 77% and 92%, and specificities of 76% and 69%, respectively, for identifying patients with CMD.

Conclusions: Peripheral endothelial function is attenuated in patients with CMD, while both LnRHI and FMD can identify patients with CMD.

Key Words: Coronary microvascular dysfunction, coronary reactivity testing

Condensed Abstract

The relationship between reactive hyperemia peripheral arterial tonometry (RH-PAT) or brachial artery flow-mediated dilation (FMD), common non-invasive measures of peripheral endothelial function, and coronary microvascular function is unclear in CMD. The current study highlights that peripheral conduit artery and microvascular endothelial function are attenuated in patients with CMD. Further, these results suggest that either RH-PAT or FMD can predict the presence of CMD in patients with chest pain and non-obstructive coronary arteries. Thus, these findings suggest that both RH-PAT and FMD may be important screening measures for identifying patients with CMD.

Abbreviations

CFR	Coronary Flow Reserve
CMD	Coronary Microvascular Dysfunction
FMD	Flow-mediated Dilation
IMR	Index of Microvascular Resistance
Pa	Proximal Coronary Pressure
Pd	Distal Coronary Pressure
RH-PAT	Reactive Hyperemia Peripheral Arterial Tonometry
LnRHI	Natural Logarithm of the Reactive Hyperemia Index
SR_{AUC}	Shear Rate area under the curve
Tmn	Mean Transit Time

3.2. Introduction

Coronary microvascular dysfunction (CMD) is emerging as an important contributor to chest pain despite non-obstructive coronary arteries (155). Chest pain in patients with CMD is often attributed to reduced coronary microvascular vasodilation, and is commonly assessed by quantifying the vasomotor responses to either an endothelial-dependent or independent pharmacological stimulus (154, 155). Attenuated microvascular dilation is associated with increased adverse cardiac events (7, 154, 185), and although multifactorial, is partially facilitated by coronary endothelial dysfunction (5, 155).

Several studies have attempted to assess the association between common non-invasive measures of peripheral endothelial function (i.e. reactive hyperemia peripheral arterial tonometry (RH-PAT) or brachial artery flow-mediated dilation (FMD) (10, 102)) to the coronary flow responses to pharmacological hyperemia, particularly in patients with chest pain and non-obstructive coronary arteries (195–199). However, several methodological considerations should be noted from previous work, making the relationship between invasive coronary measurements and non-invasive peripheral vascular function unclear. First, many studies have been limited to the assessment of coronary function through echocardiographic measures of coronary flow velocity (195, 196, 199). Although the coronary flow velocity may be clinically useful, newer techniques can more precisely quantify microvascular function in the coronary circulation (181, 186). For example, the index of microvascular resistance (IMR), which utilizes thermodilution to measure coronary flow with concurrent measures of coronary pressure, has been suggested to be a physiologically superior marker of microvascular function, given the reduced hemodynamic dependency and increased reproducibility (200). Second, patients with CMD often present with a complex interaction of endothelial independent and/or endothelial-dependent coronary abnormalities (151), however, most studies tend to only associate peripheral vascular function with one specific pharmacological measure of coronary microvascular function (195, 196, 199). The assessment of coronary microvascular function using multiple pharmacological stimuli may help to determine the mechanisms of CMD and the similarities between coronary and peripheral vascular function. Lastly, no previous study has assessed the relationship between peripheral vascular function and coronary microvascular function during a simulated sympathetic stimulus, important given that cardiac sympathetic activity has been shown to be elevated in patients with chest pain and non-obstructive coronary arteries (201). Particularly, dobutamine is a

sympathomimetic that augments heart rate, myocardial contractility, myocardial oxygen demand and subsequent coronary flow (202, 203).

The purpose of this study is to compare non-invasive peripheral vascular measures (RH-PAT and FMD) to invasive measures of coronary resistance and flow following endothelial independent (adenosine), endothelial-dependent (acetylcholine), and sympathetically-mediated (dobutamine) hyperemia in patients with suspected CMD. Given that the RH-PAT and FMD responses are mainly endothelial-dependent (101, 106), we hypothesized that RH-PAT and FMD will be associated with coronary resistance and flow during acetylcholine, but not with adenosine. Further, the endothelium has been shown to interact with the sympathetic nervous system (61), and therefore we hypothesized that RH-PAT and FMD will also be associated with coronary resistance and flow during dobutamine. The results of this study will identify non-invasive clinical measures for screening patients with suspected CMD.

3.3. Methods

Patient Selection

Patients referred to the Cardiovascular Integrated Physiology Clinic at Southlake Regional Health Centre for the suspicion of coronary microvascular dysfunction (CMD) were enrolled in this study. Patients were included in this study if they were experiencing typical or atypical chest pain with non-obstructive coronary arteries. Participants were excluded if there was evidence of: 1) coronary obstruction, based on a fractional flow reserve < 0.80, or coronary stenosis in any coronary vessel > 50%, 2) recent (<1 year) percutaneous coronary intervention or coronary artery bypass grafting, 3) exercise induced pulmonary hypertension, 4) heart failure with reduced ejection fraction (< 40%), or 5) hypertrophic cardiomyopathy. All procedures were approved by the Research Ethics Boards of Southlake Regional Healthcare Centre and York University.

Experimental Protocol

Peripheral Vascular Assessments

Fifty-seven patients underwent an assessment of peripheral microvascular function using reactive hyperemia peripheral arterial tonometry (RH-PAT) using the EndoPAT2000^{TM} device (EndoPat, Itamar Medical, Israel). A standard blood pressure cuff was positioned on the right

forearm, followed by two applanation tonometry cuffs placed on the index fingers of both hands. The applanation tonometry cuffs quantified the pulse amplitude of the finger microvasculature during 5 minutes of baseline, 5 minutes of forearm ischemia, and 5 minutes of hyperemia. Forearm ischemia was achieved via supra-systolic cuff inflation to either 200 mmHg or 50 mmHg above systolic blood pressure, while the hyperemic response was initiated via rapid deflation of the blood pressure cuff. The pulse amplitude recordings were stored, digitized and analyzed by an automated algorithm, which quantified microvascular function as the reactive hyperemic index. Due to the non-normal distribution of the reactive hyperemia index (103), the natural logarithm of the reactive hyperemia index (LnRHI) was calculated and utilized as the primary variable of interest. The EndoPAT device concurrently quantified arterial stiffness by measuring the augmentation index (AI) of the arterial waveforms during baseline, and subsequently calculated the resting heart rate and the augmentation index at a controlled heart rate of 75 bpm (AI@75bpm) (204).

To quantify conduit artery endothelial function, a subset of participants (n=28) completed an assessment of brachial artery flow-mediated dilation (FMD), conducted concurrently with RH-PAT. Using a linear array high resolution (9L-RS; 3-10 MHz) ultrasound transducer, the brachial artery was imaged in the longitudinal plane proximal to the blood pressure cuff. Duplex ultrasound (Vivid i, GE Healthcare Systems, Canada) was obtained for quantification of arterial diameter and shear measurements. Duplex ultrasound recordings were obtained for 2.5 minutes of baseline, 5 minutes of ischemia, and 2.5 minutes after cuff release.

Duplex ultrasound files were recorded in video format using a video grabber device (AV.io HD, Epiphan Video). Video files were analyzed using an automated software program (Cardiovascular Suite, Quipu, Italy). Following calibration, a specific area of interest within the B-mode was highlighted, and edge detection and wall tracking software continuously measured changes in brachial artery diameter over each cardiac cycle, while shear measurements were analyzed using automatic Doppler flow analysis. FMD was calculated as: FMD = [(maximal diameter–baseline diameter)/baseline diameter]*100. Shear rate was calculated as: SR = 4*(velocity/diameter). The shear rate area under the curve (SR_{AUC}) of the first 100 seconds of hyperemia was subsequently calculated using the following formula: $SR_{AUC} = \sum \{y_i (x_{i+1} - x_i) + ½ (y_{i+1} - y_i)(x_{i+1} - x_i)\}$, where x_i is initial time point, x_{i+1} is the second following x_i, y_i is initial shear rate, and y_{i+1} is the shear rate following y_i.

Coronary Reactivity Testing

Approximately 5 months following the peripheral vascular assessments, patients underwent coronary reactivity testing using the guidewire method, as previously described (181, 186). In brief, a guide catheter was advanced into the left anterior descending coronary artery, followed by the insertion of a 0.014-inch pressure-temperature sensor-tipped guidewire (Abbott Vascular). Simultaneously, the guidewire measured coronary pressure at a proximal arterial segment (Pa) and distal arterial segment (Pd), respectively. Coronary flow was measured using the thermodilution method, where a 3 mL room temperature saline bolus was infused from the catheter to the guidewire. The time taken for the saline to arrive at the guidewire (transit time) was used to determine coronary flow (205). This was repeated in triplicate and averaged to obtain the mean transit time (Tmn), while the inverse of the Tmn was used to estimate absolute flow. The fractional flow reserve, calculated as the Pd relative to the Pa, was collected to confirm patients did not have significant coronary epicardial stenosis. A fractional flow reserve < 0.80 was considered hemodynamically significant coronary epicardial stenosis, and if present, patients were excluded.

Measures of coronary pressure (i.e. Pa, Pd) and flow (i.e. Tmn) were collected during baseline and pharmacological hyperemia. Three pharmacological stimuli were utilized in the following order; intravenous adenosine (140μg/kg/min for at least 1 minute), intracoronary acetylcholine (20μg followed by a 100μg slow injection over 90 seconds), and intravenous dobutamine (increased by 10μg/kg/min every 3 minutes until a final dose of 40μg/kg/min was achieved after 12 minutes), each separated by rest and baseline measures. Measurements were obtained at the end of each infusion. Two measures were calculated during infusion to diagnostically determine coronary microvascular function; 1) index of microvascular resistance (IMR) and 2) coronary flow reserve (CFR). IMR was calculated as the product of the hyperemic Pd and the hyperemic Tmn. CFR was calculated as baseline Tmn divided by the hyperemic Tmn (206). Patients were considered to have CMD if any of the following criteria were observed: 1) adenosine IMR>25, 2) adenosine CFR<2.0, 3) acetylcholine IMR>30, 4) acetylcholine CFR<1.5 (151, 154, 175, 181).

Data and Statistical Analysis

Normal distribution was assessed using the Spiro-Wilks test of normality. Anthropometrics, peripheral and coronary vascular function were compared among patients with and without CMD using an independent sample t-test for normally distributed data, and a Mann–Whitney U test for skewed data. Risk factors and medication use were compared using the Chi-Square Test. Pearson bivariate correlations or Spearman's rank order correlations were conducted between the non-invasive measures of peripheral vascular function (i.e. LnRHI and FMD), and invasive measures of coronary microvascular function (i.e. IMR and CFR). Lastly, receiver operating characteristic (ROC) curve analysis was conducted to determine the ROC area under the curve (ROC_{AUC}) sensitivities and specificities for LnRHI and FMD to predict a diagnosis of CMD. The optimal thresholds for LnRHI and FMD to predict CMD were determined by maximizing the sum of the sensitivity and specificity (207). Statistical analyses were performed using IBM SPSS Statistics 23 (Armonk, NY). Normally distributed data are presented as Mean ± SD and skewed data are presented as Median (25^{th} to 75^{th} percentile); non-parametric data are presented as Count (%). Significance was defined as $P<0.05$.

3.4. Results

Between October 2017 and May 2019, vascular assessments were obtained in 57 patients. After excluding patients with an abnormal fractional flow reserve at the time of the coronary reactivity testing (n=7), technical difficulties during coronary reactivity testing (n=3) and technical difficulties during RH-PAT testing (n=1; though FMD was still obtained in this patient), 47 patients were included in this study. Baseline characteristics are shown in Table 1. Age, height, weight, BMI, systolic blood pressure, diastolic blood pressure, AI, and AI@75bpm were similar between patients with and without CMD ($P>0.05$; Table 1). Hypertension, dyslipidemia, diabetes, smoking status, medication use, time between invasive and non-invasive tests, and coronary stenosis were also similar between patients with and without CMD ($P>0.05$; Table 1).

Of the 47 patients who completed coronary reactivity testing, at least one coronary microvascular abnormality was observed in 64% of patients; 23% had an abnormal adenosine IMR, 26% had an abnormal adenosine CFR, 38% had an abnormal acetylcholine IMR, and 30% had an abnormal acetylcholine CFR. A single coronary abnormality or combinations of two,

three, or all four abnormalities were observed in 26%, 28%, 6% and 4% of patients, respectively. The adenosine and acetylcholine IMR and CFR responses were lower in patients with CMD compared to patients without CMD ($P<0.05$; Table 2) due to using these IMRs and CFRs as diagnostic criteria for CMD. The dobutamine IMR and CFR responses were similar between patients with and without CMD ($P>0.05$; Table 2).

The LnRHI and FMD measurements were lower in patients with CMD compared to patients without CMD ($P<0.05$; Table 3). Resting brachial artery diameter, SR_{AUC}, and time to maximal dilation were similar in patients with and without CMD ($P>0.05$; Table 3). When investigating the correlations between coronary microvascular function and the LnRHI, it was observed that the LnRHI did not correlate with the adenosine or acetylcholine IMR and CFR ($P>0.05$; Figure 1A-D). However, the LnRHI was correlated with the dobutamine IMR and CFR ($P<0.05$; Figure 1E and 1F). FMD was correlated with the adenosine IMR ($P<0.01$; Figure 2A), but not with the adenosine CFR ($P>0.05$; Figure 2B). FMD was also correlated with the acetylcholine IMR ($P<0.01$; Figure 2C), but not with the acetylcholine CFR ($P>0.05$; Figure 2D). Lastly, FMD was not correlated with the dobutamine IMR or CFR ($P>0.05$; Figure 2E and 2F).

The LnRHI was not associated with baseline coronary flow, or hyperemic coronary flow during the adenosine, acetylcholine, or dobutamine trials ($P>0.05$; Table 4). Lastly, while FMD did not correlate with all baseline coronary flows, or the hyperemic coronary flow during dobutamine ($P>0.05$; Table 4), FMD was correlated with the hyperemic flow response during both the adenosine and acetylcholine trials ($P<0.05$ Table 4).

The ROC_{AUC} for the LnRHI to predict CMD was 0.78 (95% CI: 0.64 – 0.92; $P<0.01$). Using an LnRHI cut-off of 0.76, the sensitivity and specificity of the LnRHI to predict CMD were 77% and 76%, respectively. The ROC_{AUC} for FMD to predict CMD was 0.81 (95% CI: 0.64 – 0.97; $P<0.01$). Using an FMD cut-off of 5.37%, the sensitivity and specificity of FMD to predict CMD were 92% and 69%, respectively.

3.5. Discussion

The relationship between LnRHI and FMD to the coronary microvascular responses to adenosine, acetylcholine and dobutamine was previously unclear. The main findings of the current study are: 1) both LnRHI and FMD are attenuated in patients with CMD, 2) the LnRHI is associated with the IMR and CFR responses to intravenous dobutamine, 3) FMD is associated

with the IMR response, but not the CFR response to endothelial independent and -dependent dilation, and 4) LnRHI and FMD can significantly identify patients with CMD.

Peripheral vascular function is attenuated in CMD

Patients with chest pain and non-obstructive coronary arteries often present with endothelial independent abnormalities, endothelial-dependent abnormalities, or both (151); highlighting the importance of measuring the coronary microvascular responses to both types of pharmacological stimuli when evaluating patients with suspected CMD. However, prior studies failed to consider this complex interaction, limiting the assessment of microvascular function to a single pharmacological stimulus (195, 196, 199). Further, the IMR has been suggested to be a superior marker of coronary microvascular function compared to the CFR (200), though most previous studies have only considered the CFR. When considering both the IMR and the CFR during endothelial independent and -dependent pharmacological hyperemia to define patients with CMD, our results highlight that RH-PAT and FMD are attenuated in CMD, while both RH-PAT and FMD can significantly predict the presence of CMD in patients with chest pain and non-obstructive coronary arteries. Previous work has shown that in women with chest pain and non-obstructive coronary arteries, the ROC_{AUC} for RH-PAT to predict non-obstructive coronary artery disease was greater than that observed here (198). However, we attribute the lower area under the curve in the current study to: 1) including male patients (representing 34% of the sample), given that sex differences have been previously observed in both peripheral and coronary microvascular function (103, 206), and 2) not including coronary epicardial responses to acetylcholine as a diagnostic criteria for CMD, given the *a priori* objective of investigating the relationship between RH-PAT and FMD to coronary microvascular function. Nonetheless, our findings support the concept that peripheral endothelial function can provide important information about overall vascular function in the coronary circulation.

Adenosine

Attenuated endothelial independent coronary vasodilation is associated with increased adverse cardiac events in patients with suspected CMD (7, 154), highlighting the clinical relevance of investigating the relationship between endothelial independent coronary vasodilation and peripheral vascular function (195, 196, 199). In the current study, no association

was observed between LnRHI and the IMR or CFR during intravenous adenosine, supporting prior work that observed no correlation between RH-PAT and the coronary flow velocity reserve during intravenous dipyridamole in women with chest pain and non-obstructive coronary arteries (199).

Studies associating FMD and endothelial independent coronary vasodilation have observed opposing results (195, 196). Specifically, the coronary flow velocity reserve during intravenous dipyridamole was associated with brachial artery FMD in men and women with chest pain and non-obstructive coronary disease (195), though more recent findings in women with non-obstructive coronary artery disease observed no association using the same measures (196). Considering that coronary function in previous work was measured indirectly using transthoracic echocardiography (195, 196), the results of the current study are superior to previous studies since thermodilution and measures of coronary pressure were obtained to more accurately assess the coronary microvasculature. In the current study, the FMD response was associated with hyperemic coronary flow during intravenous adenosine, yet it was not associated with coronary flow at baseline. Considering that the CFR is calculated as the ratio of hyperemic coronary flow to baseline coronary flow, FMD was also not associated with the CFR in the current study. However, we did find a significant correlation between FMD and IMR during intravenous adenosine, and given the proposed superiority of the IMR as a marker of coronary microvascular function (200), our results imply that brachial artery FMD is associated with endothelial independent coronary vasodilation in men and women with suspected CMD. In support of these findings, Al-Badri and colleagues observed that changes in femoral vascular resistance to acetylcholine was also associated with the change in coronary vascular resistance to adenosine (208).

Acetylcholine

Acetylcholine, an endothelial-dependent pharmacological stimulant, activates muscarinic receptors leading to the synthesis of nitric oxide and subsequent vasodilation, however, in dysfunctional coronary vasculature, acetylcholine can directly interact with vascular smooth muscle cells and cause paradoxical vasoconstriction (209). The nitric oxide dependency of RH-PAT and acetylcholine-mediated vasomotion (106, 210) suggests that these measures should assess vascular function via similar pathways. However, contrary to previous studies (197, 198),

the current study did not observe an association between LnRHI and the IMR, CFR, or baseline and hyperemic flow responses to intracoronary acetylcholine. Discrepancies between studies may be due to the location of the blood pressure cuff that elicited ischemia during the RH-PAT assessment. Prior studies that observed an association between RH-PAT and endothelial-dependent coronary function utilized upper arm ischemia (197, 198), while the current study utilized forearm ischemia to enable concurrent measures of brachial artery FMD. However, forearm occlusion tends to produce a smaller RH-PAT response compared to upper arm occlusion (211). Considering that upper arm ischemia elicits an RH-PAT response that is <50% nitric oxide mediated, it is possible that forearm ischemia may reduce the nitric oxide contribution, thereby reducing its association with an endothelial-dependent stimulus in the coronary vasculature.

FMD is well established to be highly endothelial-dependent, given the large contribution of nitric oxide to the vasodilatory response (101). Although several studies have shown that brachial artery FMD is associated with coronary epicardial vasomotion during endothelial-dependent pharmaceutical infusions (184, 192, 212), no study to date has assessed the relationship between FMD and endothelial-dependent cardiac microvascular vasodilation. In the current study, FMD was associated with the IMR and the hyperemic flow response to intracoronary acetylcholine, but not with baseline coronary flow or CFR, supporting previous studies associating FMD with coronary epicardial vasomotion during intracoronary acetylcholine (192, 212). Similar to the coronary microvascular responses to adenosine described above, FMD was not correlated with the acetylcholine CFR likely due to variability within the baseline flow. Our results suggest that FMD, but not RH-PAT, is associated with coronary microvascular function during endothelial-dependent vasodilation.

Dobutamine

Endothelial function can influence the vasomotor responses to sympathetic stimulation in the coronary circulation. For instance, sympathetic activation via the cold pressor test caused epicardial dilation in young healthy humans (213), yet caused epicardial vasoconstriction in patients with coronary endothelial dysfunction (214). Further, intravenous dobutamine also causes epicardial dilation in patients with normal coronary arteries, a response that is attenuated in patients with mildly atherosclerotic arteries and abolished in patients with significant

epicardial stenosis (215). Facilitated by β adrenergic receptor activation, dobutamine is a sympathomimetic that augments coronary flow due to the increase in myocardial oxygen demand produced by the enhanced cardiac inotropy and chronotropy (202) and the activation of vasodilatory β_2 adrenergic receptors (202). In the current study, RH-PAT was associated with the IMR and CFR response to dobutamine, implying that peripheral microvascular endothelial function can provide insight into the coronary vasodilatory responses to sympathetic stimulation by the sympathetic nervous system. Indeed, RH-PAT has been shown to be inversely associated with muscle sympathetic nerve activity in healthy participants (216).

FMD was not associated with coronary microvascular function during dobutamine infusions. Conduit arteries receive less sympathetic innervation and are less susceptible to sympathetic neurovascular transduction compared to the microvasculature (29, 34), implying that conduit artery endothelial function may be less influenced by sympathetic activation. Although prior studies have shown that sympathetic activity can attenuate brachial artery FMD (217–219), this is likely secondary to sympathetic activity altering conduit artery shear patterns (218, 220). Collectively, these results suggest that peripheral microvascular, but not conduit artery endothelial function, can provide insight into coronary microvascular function during sympathetic stimulation, likely due to microvascular endothelial function being under greater influences of sympathetic activity compared to conduit arteries.

Limitations

First, the time between the non-invasive vascular assessment and coronary reactivity testing was ~150 days due to clinical scheduling. Although medication regime was unaltered during this time, we cannot rule out that vascular function could have been altered by disease progression or lifestyle changes. Second, we recognize that no study to date has confirmed the acetylcholine IMR diagnostic cut-off of >30, and therefore its clinical relevance has yet to be confirmed. Third, this study did not assess peripheral endothelial independent dilation via nitroglycerin-mediated dilation. By adding this third non-invasive measurement we could have determined the endothelial independent relationship between peripheral and coronary vascular function.

Conclusions

LnRHI and FMD are lower in patients with CMD compared to patients without CMD, and either LnRHI or FMD can identify the presence of CMD in patients with chest pain and non-obstructive coronary arteries. Particularly, the LnRHI is associated with the IMR and CFR responses to intravenous dobutamine, while FMD is associated with the IMR responses to intravenous adenosine and intracoronary acetylcholine. These findings suggest that both RH-PAT and FMD can be used to screen patients with suspected CMD.

3.6. Clinical Perspectives

Clinical Competencies:

RH-PAT and FMD are attenuated in patients with CMD, and can significantly identify patients with CMD. These results suggest that non-invasively measuring peripheral vascular function can be an important clinical tool to help screen patients with chest pain and non-obstructive coronary arteries for the presence of CMD.

Translational Outlook:

Utilizing RH-PAT or FMD to screen patients with chest pain and non-obstructive coronary arteries and suspected CMD may help to identify patients who may require further testing. This may help patients reach a diagnosis faster, leading to improved quality of life and reduced medical healthcare costs. Additional studies should focus on how the combination of non-invasive vascular assessments and other traditional clinical variables can be concurrently integrated to help improve the capability to identify patients with CMD.

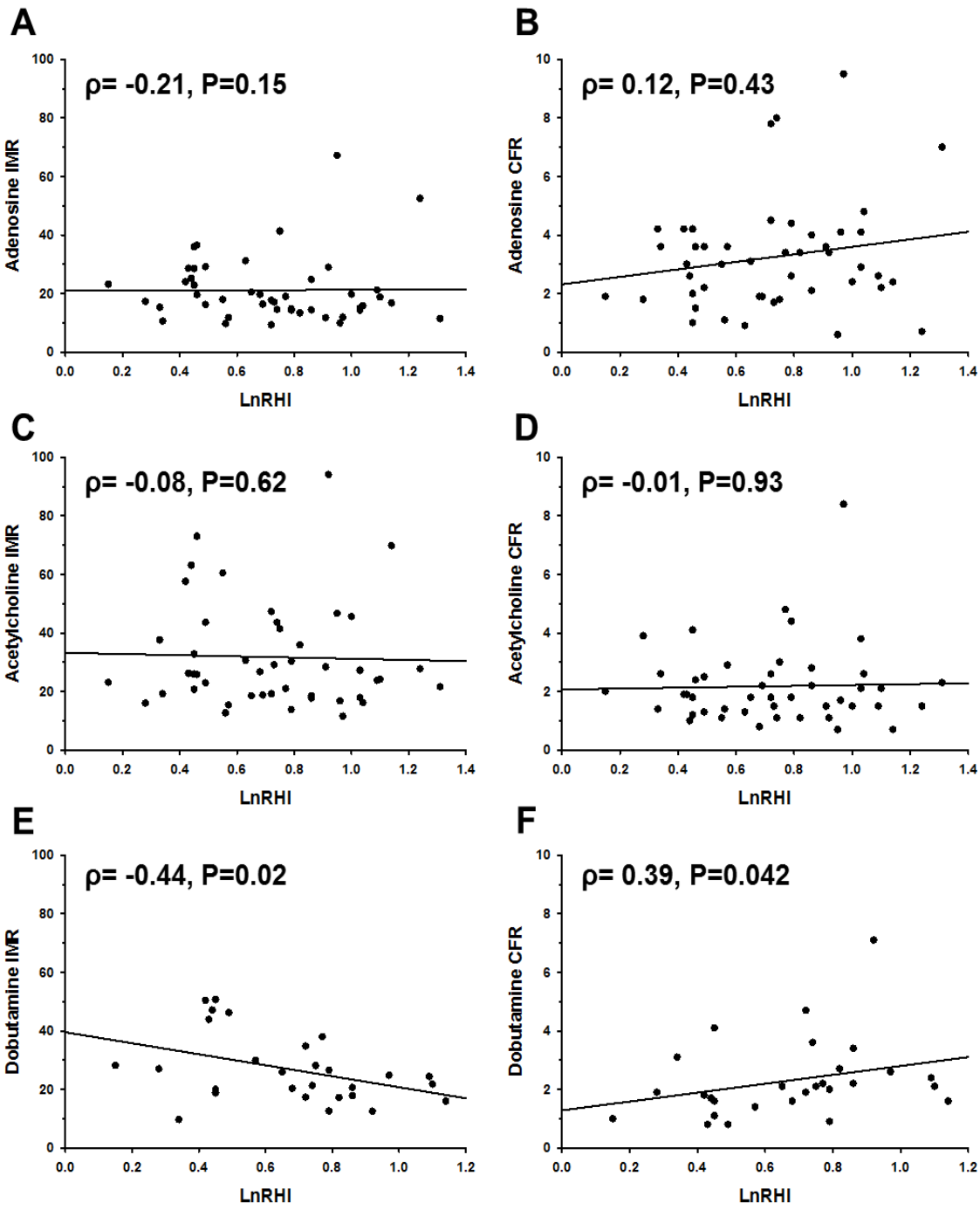
Figure Legends

Figure 1: RH-PAT and Coronary Microvascular Function

Correlations between the LnRHI and the IMRs (left panels) and the CFRs (right panels) to adenosine (A and B; n=46), acetylcholine (C and D; n=46), and dobutamine (E and F; n=28). CFR: Coronary Flow Reserve, LnRHI: Natural Logarithm of the Reactive Hyperemia Index, IMR: Index of Microvascular Resistance, ρ: Spearman's Rho.

Figure 2: FMD and Coronary Microvascular Function

Correlations between brachial artery FMD and the IMRs (left panels) and the CFRs (right panels) to adenosine (A and B; n=28), acetylcholine (C and D; n=28), and dobutamine (E and F; n=17). CFR: Coronary Flow Reserve, FMD: Flow-Mediated Dilation, IMR: Index of Microvascular Resistance, ρ: Spearman's Rho.


A
ρ= -0.21, P=0.15
Adenosine IMR
LnRHI
B
ρ= 0.12, P=0.43
Adenosine CFR
LnRHI
C
ρ= -0.08, P=0.62
Acetylcholine IMR
LnRHI
D
ρ= -0.01, P=0.93
Acetylcholine CFR
LnRHI
E
ρ= -0.44, P=0.02
Dobutamine IMR
LnRHI
F
ρ= 0.39, P=0.042
Dobutamine CFR
LnRHI

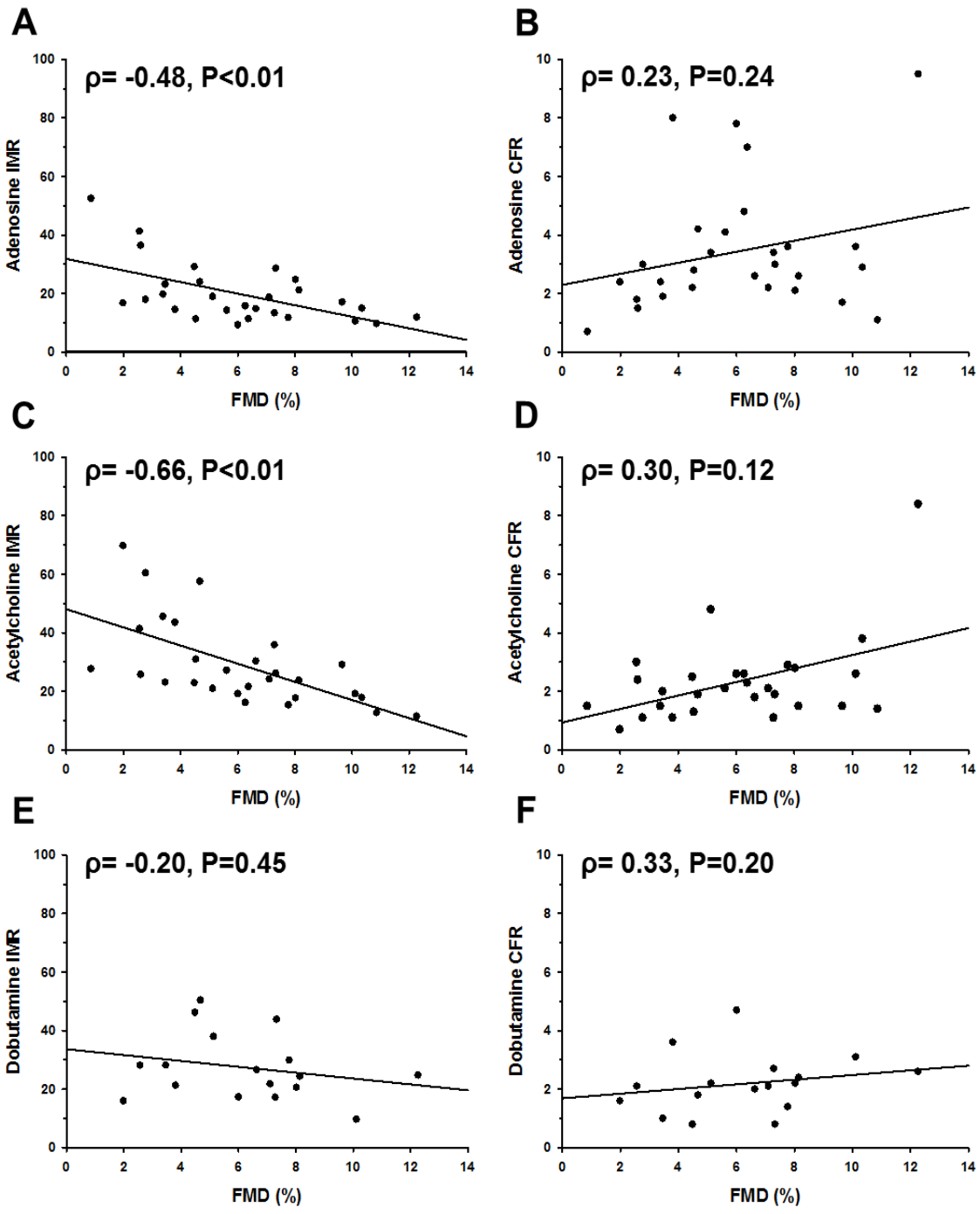

A
ρ= -0.48, P<0.01
Adenosine IMR
FMD (%)
B
ρ= 0.23, P=0.24
Adenosine CFR
FMD (%)
C
ρ= -0.66, P<0.01
Acetylcholine IMR
FMD (%)
D
ρ= 0.30, P=0.12
Acetylcholine CFR
FMD (%)
E
ρ= -0.20, P=0.45
Dobutamine IMR
FMD (%)
F
ρ= 0.33, P=0.20
Dobutamine CFR
FMD (%)

Table 1: Anthropometrics, hemodynamics, traditional cardiovascular risk factors, and medication use in patients with or without coronary microvascular dysfunction (CMD).

Variable	All Patients	No CMD	CMD
n	47	17	30
Sex (male)	16 (34)	5 (29)	11 (37)
Age (years)	60 ± 13	56 ± 12	62 ± 14
Weight (kg)	78.6 ± 16.2	77.4 ± 20.0	79.2 ± 14.0
Height (m)	1.67 ± 0.09	1.67 ± 0.10	1.67 ± 0.09
BMI (kg/m^2)	28.0 ± 4.7	27.7 ± 6.0	28.2 ± 3.8
SBP (mmHg)	125 ± 15	122 ± 18	126 ± 14
DBP (mmHg)	76 ± 10	76 ± 9	76 ± 10
AI	23.3 ± 21.4	24.4 ± 20.9	22.7 ± 22.1
AI@75bpm	16.6 ± 20.3	16.4 ± 18.4	16.7 ± 21.7
Risk Factors			
Hypertension	21 (45)	6 (35)	15 (50)
Dyslipidemia	22 (47)	7 (41)	15 (50)
Diabetes	9 (19)	1 (6)	8 (27)
Smoker	17 (36)	4 (24)	13 (43)
Medication use			
BB	18 (38)	5 (29)	13 (43)
ACE or ARB	21 (45)	6 (35)	15 (50)
Statins	28 (60)	9 (53)	19 (63)
CCB	15 (32)	6 (35)	9 (30)
Aspirin	29 (62)	12 (71)	17 (57)
Time between tests (days)	151 ± 53	153 ± 55	150 ± 53
Stenosis of LAD (%)	25 (0 – 30)	0 (0 – 30)	25 (0 – 25)

ACE: Angiotensin Converting Enzyme Inhibitor, AI: Augmentation Index, AI@75bpm: Augmentation Index at 75bpm, ARB: Angiotensin Receptor Blocker, BB: Beta-Blocker, BMI: Body Mass Index, CCB: Calcium Channel Blocker, DBP: Diastolic Blood Pressure, LAD: Left Anterior Descending Coronary Artery, SBP: Systolic Blood Pressure. Parametric data presented as Mean ± SD. Skewed parametric data presented as Median (25^{th} – 75^{th} percentile). Non-parametric data presented as Count (%).

Table 2: The index of microvascular resistance (IMR) and coronary flow reserve (CFR) responses to adenosine, acetylcholine, and dobutamine in patients with and without coronary microvascular dysfunction (CMD).

Variable	All Patients	No CMD	CMD
Adenosine			
n	47	17	30
IMR	17.7 (14.3 – 24.8)	14.3 (11.5 – 18.9)	19.8 (16.3 – 29.1)*
CFR	3.0 (1.9 – 4.1)	3.6 (3.0 – 4.6)	2.4 (1.8 – 3.5)*
Acetylcholine			
n	47	17	30
IMR	26.2 (18.8 – 41.4)	18.5 (16.5 – 22.7)	31.9 (25.9 – 46.8)*
CFR	1.8 (1.3 – 2.6)	2.6 (2.0 – 3.4)	1.5 (1.1 – 2.0)*
Dobutamine			
n	28	11	17
IMR	24.6 (18.0 – 33.6)	21.8 (17.3 – 25.9)	27.0 (19.4 – 45.0)
CFR	2.0 (1.6 – 2.7)	2.2 (2.1 – 3.1)	1.8 (1.3 – 2.4)

Skewed parametric data presented as Median (25^{th} – 75^{th} percentile). * indicates a significant difference between patients with and without CMD ($P<0.05$).

Table 3: Peripheral vascular function, obtained during reactive hyperemia peripheral arterial tonometry (RH-PAT) and brachial artery flow-mediated dilation (FMD) assessments in patients with and without coronary microvascular dysfunction (CMD).

Variable	All Patients	No CMD	CMD
RH-PAT			
n	46	17	29
LnRHI	0.72 ± 0.27	0.88 ± 0.23	0.63 ± 0.26*
FMD			
n	28	12	16
Resting Diameter (mm)	3.75 ± 0.68	3.89 ± 0.75	3.64 ± 0.60
FMD (%)	6.2 ± 2.9	7.8 ± 2.2	4.8 ± 2.8*
FMD (mm)	0.23 ± 0.12	0.30 ± 0.11	0.17 ± 0.09*
SR_{AUC} x 10^{-3} (sec^{-1})	48.2 ± 18.6	43.1 ± 15.5	51.6 ± 19.9
Time to Max Dilation (s)	44 (35 – 70)	42 (34 – 67)	47 (37 – 70)

LnRHI: Natural logarithm of Reactive Hyperemia Index, SR_{AUC}: Shear Rate Area under the Curve. Normally distributed parametric data presented as Mean ± SD. Skewed parametric data presented as Median (25^{th} – 75^{th} percentile). * indicates a significant difference between patients with and without CMD ($P<0.05$).

Table 4: Correlations coefficients when comparing the natural logarithm of the reactive hyperemia index (LnRHI) and brachial artery flow-mediated dilation (FMD) with baseline and hyperemic coronary flow responses to adenosine, acetylcholine, and dobutamine.

	Baseline Flow (1/Tmn)	Hyperemic Flow (1/Tmn)
Adenosine		
LnRHI	0.06	0.24
FMD	0.09	0.51*
Acetylcholine		
LnRHI	0.05	-0.01
FMD	0.15	0.63*
Dobutamine		
LnRHI	-0.09	0.30
FMD	0.17	0.35

Spearman's rho were calculated due to non-normal distribution of invasive vascular measures. * indicates significant association ($P<0.05$).

CHAPTER 4 EXTENDED DISCUSSION

RH-PAT and Sympathetic Activity: Role of β Adrenergic Receptors

In the current thesis, dobutamine, a β adrenergic receptor agonist, was utilized to assess coronary function during a sympathetic stimulus, and was compared with endothelial function in the peripheral circulation. We observed that peripheral microvascular, but not conduit artery endothelial function was associated with coronary microvascular function during intravenous dobutamine. Endothelial function and sympathetic activity likely have a greater interaction effect within the microcirculation due to greater sympathetic innervation and greater susceptibility to sympathetic neurovascular transduction, as previously mentioned (29, 34). However, despite the inverse association between RH-PAT and muscle sympathetic nerve activity (216), phenylephrine, an α_1 adrenergic agonist, does not attenuate the hyperemic finger pulse amplitude during RH-PAT (106), suggesting a potential role of β adrenergic receptors in the RH-PAT response. Particularly, β_2 adrenergic receptors are highly prevalent within the microcirculation (221), and possess a direct vasodilatory effect in both the coronary (202) and forearm circulation (222). Further, the vasodilatory responses to β adrenergic receptor mediated dilation were attenuated by NO blockade in both the coronary and peripheral microvascular circulation (221, 222). Collectively, these studies suggest that β adrenergic receptors may play a role in the RH-PAT response, though future studies are needed to confirm this hypothesis.

Arterial Stiffness and Coronary Microvascular Function

Increased arterial stiffness can augment blood pressure and left ventricular afterload, due to the increases in both retrograde blood flow velocity and amplitude (20, 28). To investigate if arterial stiffness can impact coronary microvascular function, the augmentation index at a controlled heart rate of 75 bpm (AI@75bpm) was compared with coronary microvascular function (Appendix A: Figure 1A-F). Contrary to previous reports (171, 174), the AI@75bpm was not associated with coronary microvascular function during adenosine, acetylcholine, or dobutamine mediated pharmacological hyperemia. Several studies observed that both the aortic augmentation index (174) and the carotid artery augmentation index (171) were associated with the CFR response to intravenous adenosine in patients with CMD, implying that arterial stiffness in the large, mainly elastic arteries, is associated with coronary function. However, in the current

study augmentation index was obtained from the finger microvasculature using the EndoPAT device, suggesting that arterial stiffness in the microvasculature may differ from those obtained in larger arteries, though no study has directly compared both measures. Interestingly, the AI@75bpm was positively associated with absolute baseline coronary flow (Appendix A; Figure 2A), implying that the myocardial oxygen demand at baseline was elevated in patients with increased arterial stiffness. In support of this, the baseline rate pressure product (RPP; the product of baseline heart rate and baseline systolic blood pressure that has previously been utilized as an indirect marker of myocardial oxygen consumption (223)) was also associated with the AI@75bpm (Appendix A: Figure 2B). Therefore, these data indicate that arterial stiffness is associated with both myocardial oxygen consumption and resting coronary flow, however, arterial stiffness obtained from the finger microvasculature is not associated with coronary microvascular function.

RH-PAT and Acute Exercise in Clinical Practice

Standard guidelines for conducting RH-PAT using the EndoPAT device have not been established. Although exercise has been shown to attenuate brachial artery endothelial function (224), the effect of exercise on RH-PAT within clinical setting has not been assessed, important considering that exercise stress testing is commonly conducted in clinical practice. Using a cross sectional design, we determined that exercise stress testing prior to EndoPAT testing significantly attenuated peripheral microvascular function in chest pain patients with and without CMD (Appendix B). To avoid the potential confounding effect of exercise on peripheral microvascular function, patients who exercised prior to EndoPAT testing were excluded from the current thesis paper. Additionally, these results suggest that clinicians who adopt RH-PAT testing in clinical settings should avoid exercise stress testing prior to assessing peripheral microvascular function.

Clinical Implications

Identifying patients with suspected CMD can be clinically challenging. Currently, exercise stress testing with 12 lead electrocardiography is commonly used in clinical practice to assess for myocardial ischemia, and a full examination of the coronary arteries is necessary to distinguish CSX from obstructive artery disease. Patients with an abnormal exercise stress test

and non-obstructive coronary arteries are diagnosed with CSX, as previously described. However a diagnosis of CSX does not necessarily imply that patients also have CMD, given that several studies have shown that ST segment depression observed during exercise stress testing does not adequately identify patients with CMD (151, 225). This may be due to the diffuse pattern of ischemia observed in CMD, as patients with CMD likely have myocardial ischemia in small, localized regions of the myocardium that likely do not generate detectable changes within the ST segment during exercise (226, 227). Additionally, dobutamine stress echocardiography has also been suggested as another potential screening measure for CMD (228, 229), however these tests are more technically and clinically challenging for large scale implementation. Therefore, current clinical tests do not adequately screen patients with chest pain and normal coronary arteries, suggesting that new non-invasive measures should be considered. The results of the current thesis, along with prior work (197, 198) collectively suggest that the assessment of endothelial function using the peripheral vasculature may help provide clinicians with a novel screening measure to estimate vascular function in the coronary microcirculation.

Future Studies

Despite the current thesis and other work showing moderate sensitivities of RH-PAT and FMD for identifying patients with CMD (197, 198), additional work is still needed to answer important knowledge gaps within the current literature. First, sex differences are highly prevalent in the diagnosis of CMD (151), and sex differences exist in both the coronary and peripheral vasculature (114, 206). Therefore, further studies should investigate if sex differences exist in the capacity for RH-PAT and FMD to predict a CMD diagnosis in addition to determining optimal sex specific thresholds for diagnosis using these non-invasive peripheral vascular measures. Additionally, future studies should investigate the synergistic effect of abnormal peripheral vascular function and other traditional cardiovascular risk factors, to determine if a combination of variables can improve the predictive capacity of RHPAT and FMD within clinical settings.

Limitations

Important limitations to the current thesis have been highlighted in the manuscript above. However, the effects of intravenous pharmacological infusions on the sympathetic nervous system should also be addressed. In particular, although dobutamine acts as a sympathomimetic,

dobutamine has been shown to decrease muscle sympathetic nerve activity (230), and cardiac norepinephrine spillover (231). Therefore, the reduced cardiac sympathetic nerve activity could enhance the vasodilatory response in the coronary vasculature. Indeed, the α_1 adrenergic receptor blocker phentolamine significantly improved coronary epicardial dilation during intravenous dobutamine in patients with severe coronary stenosis (215) highlighting that the α_1 adrenergic receptors influence the coronary vasomotor responses to dobutamine. Additionally, adenosine can increase muscle sympathetic nerve activity (232, 233) and cardiac sympathetic activity (234, 235), thereby potentially attenuating the endothelial independent dilatory response of adenosine. Therefore, modulations in cardiac sympathetic activity by either dobutamine or adenosine may confound the coronary vasodilatory responses, possibly reducing the association between peripheral and coronary microvascular function.

Conclusions

In conclusion, this thesis demonstrated that peripheral endothelial function is attenuated in CMD, while both the RH-PAT and FMD may provide important clinician information for identifying patients with suspected CMD. Particularly, brachial artery FMD is associated with coronary microvascular function during intravenous adenosine and intracoronary acetylcholine, while RH-PAT is associated with coronary microvascular function during intravenous dobutamine.

CHAPTER 5 REFRENCES

1. Neill WA, Kassebaum DG, Judkins MP. Myocardial Hypoxia as the Basis for Angina Pectoris in a Patient with Normal Coronary Arteriograms. N. Engl. J. Med. 1968;279:789–792.

2. Neill WA, Judkins MP, Dhindsa DS, Metcalfe J, Kassebaum G, Kloster FE. Clinically suspect ischemic heart disease not corroborated by demonstrable coronary artery disease. Physiologic investigations and clinical course. Am. J. Cardiol. 1972;29:171–179.

3. Patel MR, Peterson ED, Dai D, et al. Low Diagnostic Yield of Elective Coronary Angiography. N. Engl. J. Med. Boston 2010;362:886–95.

4. Duncker DJ, Bache RJ. Regulation of Coronary Blood Flow During Exercise. Physiol. Rev. 2008;88:1009–1086.

5. Lanza GA, Crea F. Primary Coronary Microvascular Dysfunction: Clinical Presentation, Pathophysiology, and Management. Circulation 2010;121:2317–2325.

6. von Mering GO, Arant CB, Wessel TR, et al. Abnormal coronary vasomotion as a prognostic indicator of cardiovascular events in women: Results From the National Heart, Lung, and Blood Institute–Sponsored Women's Ischemia Syndrome Evaluation (WISE). Circulation 2004;109:722–725.

7. Pepine CJ, Anderson RD, Sharaf BL, et al. Coronary microvascular reactivity to adenosine predicts adverse outcome in women evaluated for suspected ischemia: Results from the National Heart, Lung and Blood Institute WISE (Women's Ischemia Syndrome Evaluation) Study. J. Am. Coll. Cardiol. 2010;55:2825–2832.

8. Jespersen L, Abildstrom SZ, Hvelplund A, et al. Burden of Hospital Admission and Repeat Angiography in Angina Pectoris Patients with and without Coronary Artery Disease: A Registry-Based Cohort Study. PLOS ONE 2014;9:e93170.

9. Leise MD, Locke GR, Dierkhising RA, Zinsmeister AR, Reeder GS, Talley NJ. Patients Dismissed From the Hospital With a Diagnosis of Noncardiac Chest Pain: Cardiac Outcomes and Health Care Utilization. Mayo Clin. Proc. 2010;85:323–330.

10. Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. The Lancet 1992;340:1111–1115.

11. Katz SA, Feigl EO. Systole has little effect on diastolic coronary artery blood flow. Circ. Res. 1988;62:443–451.

12. Khouri EM, Gregg DE, Rayford CR. Effect of Exercise on Cardiac Output, Left Coronary Flow and Myocardial Metabolism in the Unanesthetized Dog. Circ. Res. 1965;17:427–437.

13. Holmberg S, Serzysko W, Varnauskas E. Coronary Circulation During Heavy Exercise in Control Subjects and Patients with Coronary Heart Disease. Acta Med. Scand. 1971;190:465–480.

14. Duncker DJ, Bache RJ, Merkus D. Regulation of coronary resistance vessel tone in response to exercise. J. Mol. Cell. Cardiol. 2012;52:802–813.

15. Gordon JB, Ganz P, Nabel EG, et al. Atherosclerosis influences the vasomotor response of epicardial coronary arteries to exercise. J. Clin. Invest. 1989;83:1946–1952.

16. Berdeaux A, Ghaleh B, Dubois-Randé JL, et al. Role of vascular endothelium in exercise-induced dilation of large epicardial coronary arteries in conscious dogs. Circulation 1994;89:2799–2808.

17. Nichols WW, O'Rourke MF, Vlachopoulos C. McDonald's Blood Flow in Arteries, Sixth Edition: Theoretical, Experimental and Clinical Principles. 6th ed.; 1990.

18. Gosling RG, Budge MM. Terminology for Describing the Elastic Behavior of Arteries. Hypertension 2003;41:1180–1182.

19. Laogun AA, Gosling RG. In vivo arterial compliance in man. Clin. Phys. Physiol. Meas. 1982;3:201.

20. Mitchell GF, Parise H, Benjamin EJ, et al. Changes in Arterial Stiffness and Wave Reflection With Advancing Age in Healthy Men and Women. Hypertension 2004;43:1239–1245.

21. Chirinos JA. Arterial Stiffness: Basic Concepts and Measurement Techniques. J Cardiovasc. Transl. Res. 2012;5:243–255.

22. Belz GG. Elastic properties and Windkessel function of the human aorta. Cardiovasc. Drugs Ther. 1995;9:73–83.

23. Simon AC, O'Rourke M, Levenson J. Arterial distensibility and its effect on wave reflection and cardiac loading in cardiovascular disease. Coron. Artery Dis. 1991;2:1111.

24. Ochi H, Shimada T, Ikuma I, Morioka S, Moriyama K. Effect of a Decrease in Aortic Compliance on the Isovolumic Relaxation Period of the Left Ventricle in Man. Am. J. Noninvasive Cardiol. 1991;5:149–154.

25. Watanabe H, Ohtsuka S, Kakihana M, Sugishita Y. Coronary circulation in dogs with an experimental decrease in aortic compliance. J. Am. Coll. Cardiol. 1993;21:1497–1506.

26. Bortolotto LA, Hanon O, Franconi G, Boutouyrie P, Legrain S, Girerd X. The Aging Process Modifies the Distensibility of Elastic but not Muscular Arteries. Hypertension 1999;34:889–892.

27. Nichols WW, Denardo SJ, Wilkinson IB, McEniery CM, Cockcroft J, O'Rourke MF. Effects of Arterial Stiffness, Pulse Wave Velocity, and Wave Reflections on the Central Aortic Pressure Waveform. J. Clin. Hypertens. 2008;10:295–303.

28. Nichols WW, Singh BM. Augmentation index as a measure of peripheral vascular disease state. Curr. Opin. Cardiol. 2002;17:543–551.

29. Fairfax ST, Padilla J, Vianna LC, Holwerda SH, Davis MJ, Fadel PJ. Influence of spontaneously occurring bursts of muscle sympathetic nerve activity on conduit artery diameter. Am. J. Physiol.-Heart Circ. Physiol. 2013;305:H867–H874.

30. Fairfax ST, Padilla J, Vianna LC, Davis MJ, Fadel PJ. Spontaneous bursts of muscle sympathetic nerve activity decrease leg vascular conductance in resting humans. Am. J. Physiol.-Heart Circ. Physiol. 2013;304:H759–H766.

31. Tremblay JC, Pyke KE. Flow-mediated dilation stimulated by sustained increases in shear stress: a useful tool for assessing endothelial function in humans? Am. J. Physiol.-Heart Circ. Physiol. 2017;314:H508–H520.

32. Rhodin JAG. The ultrastructure of mammalian arterioles and precapillary sphincters. J. Ultrastruct. Res. 1967;18:181–223.

33. Fuxe K, Sedvall G. The Distribution of Adrenergic Nerve Fibres to the Blood Vessels in Skeletal Muscle. Acta Physiol. Scand. 1965;64:75–86.

34. Stassen FR, Maas RG, Schiffers PM, Janssen GM, De Mey JG. A positive and reversible relationship between adrenergic nerves and alpha-1A adrenoceptors in rat arteries. J. Pharmacol. Exp. Ther. 1998;284:399–405.

35. Pries AR, Secomb TW. Blood Flow in Microvascular Networks. In: Tuma RF, Durán WN, Ley K, editors. Microcirculation (Second Edition). San Diego: Academic Press, 2008:3–36. Available at: https://www.sciencedirect.com/science/article/pii/B9780123745309000012. Accessed February 23, 2018.

36. Clifford PS, Hellsten Y. Vasodilatory mechanisms in contracting skeletal muscle. J. Appl. Physiol. 2004;97:393–403.

37. Segal SS. Regulation of Blood Flow in the Microcirculation. Microcirculation 2005;12:33–45.

38. Marshall JM. The influence of the sympathetic nervous system on individual vessels of the microcirculation of skeletal muscle of the rat. J. Physiol. 1982;332:169–186.

39. Joyner MJ, Casey DP. Regulation of Increased Blood Flow (Hyperemia) to Muscles During Exercise: A Hierarchy of Competing Physiological Needs. Physiol. Rev. 2015;95:549–601.

40. Remensnyder JP, Mitchell JH, Sarnoff SJ. Functional Sympatholysis During Muscular Activity: Observation on influence of carotid sinus on oxygen uptake. Circ. Res. 1962;11:370–380.

41. van Teeffelen JWGE, Segal SS. Interaction between sympathetic nerve activation and muscle fibre contraction in resistance vessels of hamster retractor muscle. J. Physiol. 2003;550:563–574.

42. Tschakovsky ME, Sujirattanawimol K, Ruble SB, Valic Z, Joyner MJ. Is sympathetic neural vasoconstriction blunted in the vascular bed of exercising human muscle? J. Physiol. 2002;541:623–635.

43. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial Function and Dysfunction Testing and Clinical Relevance. Circulation 2007;115:1285–1295.

44. Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. Role of Endothelial Shear Stress in the Natural History of Coronary Atherosclerosis and Vascular Remodeling: Molecular, Cellular, and Vascular Behavior. J. Am. Coll. Cardiol. 2007;49:2379–2393.

45. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373–376.

46. Ignarro LJ, Byrns RE, Buga GM, Wood KS. Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. Circ. Res. 1987;61:866–879.

47. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987;327:524–526.

48. Tousoulis D, Kampoli A-M, Stefanadis CTNP and C. The Role of Nitric Oxide on Endothelial Function. Curr. Vasc. Pharmacol. 2011. Available at: http://www.eurekaselect.com/89263/article. Accessed February 22, 2018.

49. Stuehr DJ. Structure-Function Aspects in the Nitric Oxide Synthases. Annu. Rev. Pharmacol. Toxicol. 1997;37:339–359.

50. Behrendt D, Ganz P. Endothelial function: From vascular biology to clinical applications. Am. J. Cardiol. 2002;90:L40–L48.

51. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation 2004;109:III27-32.

52. Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 1988;333:664–666.

53. Caterina RD, Libby P, Peng HB, et al. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. J. Clin. Invest. 1995;96:60–68.

54. Gauthier TW, Scalia R, Murohara T, Guo J, Lefer AM. Nitric Oxide Protects Against Leukocyte-Endothelium Interactions in the Early Stages of Hypercholesterolemia. Arterioscler. Thromb. Vasc. Biol. 1995;15:1652–1659.

55. Graaf JC de, Banga JD, Moncada S, Palmer RM, Groot PG de, Sixma JJ. Nitric oxide functions as an inhibitor of platelet adhesion under flow conditions. Circulation 1992;85:2284–2290.

56. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc. Natl. Acad. Sci. 1991;88:4651–4655.

57. Cherney A, Edgell H, Krukoff TL. NO mediates effects of estrogen on central regulation of blood pressure in restrained, ovariectomized rats. Am. J. Physiol.-Regul. Integr. Comp. Physiol. 2003;285:R842–R849.

58. Rees DD, Palmer RM, Moncada S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. Proc. Natl. Acad. Sci. U. S. A. 1989;86:3375–3378.

59. Cornwell TL, Arnold E, Boerth NJ, Lincoln TM. Inhibition of smooth muscle cell growth by nitric oxide and activation of cAMP-dependent protein kinase by cGMP. Am. J. Physiol.-Cell Physiol. 1994;267:C1405–C1413.

60. Webb AJ, Patel N, Loukogeorgakis S, et al. Acute Blood Pressure Lowering, Vasoprotective, and Antiplatelet Properties of Dietary Nitrate via Bioconversion to Nitrite. Hypertension 2008;51:784–790.

61. Amiya E, Watanabe M, Komuro I. The relationship between vascular function and the autonomic nervous system. Ann. Vasc. Dis. 2014;7:109–119.

62. Gamboa A, Okamoto LE, Diedrich A, et al. Sympathetic activation and nitric oxide function in early hypertension. Am. J. Physiol. - Heart Circ. Physiol. 2012;302:H1438–H1443.

63. Sakuma I, Togashi H, Yoshioka M, et al. NG-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo. A role for nitric oxide in the central regulation of sympathetic tone? Circ. Res. 1992;70:607–611.

64. Sander M, Hansen PG, Victor RG. Sympathetically Mediated Hypertension Caused by Chronic Inhibition of Nitric Oxide. Hypertension 1995;26:691–695.

65. Zanzinger J, Czachurski J, Seller H. Inhibition of basal and reflex-mediated sympathetic activity in the RVLM by nitric oxide. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 1995;268:R958–R962.

66. Young CN, Fisher JP, Gallagher KM, et al. Inhibition of nitric oxide synthase evokes central sympatho-excitation in healthy humans. J. Physiol. 2009;587:4977–4986.

67. Gamboa A, Figueroa R, Paranjape SY, Farley G, Diedrich A, Biaggioni I. Autonomic Blockade Reverses Endothelial Dysfunction in Obesity-Associated Hypertension. Hypertension 2016;68:1004–1010.

68. Drexler H. Factors involved in the maintenance of endothelial function. Am. J. Cardiol. 1998;82:3S-4S.

69. Pernow J, Shemyakin A, Böhm F. New perspectives on endothelin-1 in atherosclerosis and diabetes mellitus. Life Sci. 2012;91:507–516.

70. Sowers JR. Hypertension, Angiotensin II, and Oxidative Stress. N. Engl. J. Med. 2002;346:1999–2001.

71. Ross R. Atherosclerosis — An Inflammatory Disease. N. Engl. J. Med. 1999;340:115–126.

72. Clarkson P, Celermajer DS, Donald AE, et al. Impaired vascular reactivity in insulin-dependent diabetes mellitus is related to disease duration and low density lipoprotein cholesterol levels. J. Am. Coll. Cardiol. 1996;28:573–579.

73. McVeigh GE, Brennan GM, Johnston GD, et al. Impaired endothelium-dependent and independent vasodilation in patients with Type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 1992;35:771–776.

74. Casino PR, Kilcoyne CM, Quyyumi AA, Hoeg JM, Panza JA. The role of nitric oxide in endothelium-dependent vasodilation of hypercholesterolemic patients. Circulation 1993;88:2541–2547.

75. Panza JA, Quyyumi AA, Brush JE, Epstein SE. Abnormal Endothelium-Dependent Vascular Relaxation in Patients with Essential Hypertension. N. Engl. J. Med. 1990;323:22–27.

76. Celermajer DS, Sorensen KE, Georgakopoulos D, et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. Circulation 1993;88:2149–2155.

77. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. J. Am. Coll. Cardiol. 1994;24:1468–1474.

78. Hashimoto M, Kozaki K, Eto M, et al. Association of Coronary Risk Factors and Endothelium-Dependent Flow-Mediated Dilatation of the Brachial Artery. Hypertens. Res. 2000;23:233–238.

79. Slager CJ, Wentzel JJ, Gijsen FJH, et al. The role of shear stress in the generation of rupture-prone vulnerable plaques. Nat. Clin. Pract. Cardiovasc. Med. 2005;2:401–407.

80. Davies PF. Flow-mediated endothelial mechanotransduction. Physiol. Rev. 1995;75:519–560.

81. Pyke KE, Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. J. Physiol. 2005;568:357–369.

82. Harris RA, Nishiyama SK, Wray DW, Richardson RS. Ultrasound Assessment of Flow-Mediated Dilation. Hypertension 2010;55:1075–1085.

83. Chiu J-J, Chien S. Effects of Disturbed Flow on Vascular Endothelium: Pathophysiological Basis and Clinical Perspectives. Physiol. Rev. 2011;91:327–387.

84. Ku DN, Giddens DP, Zarins CK, Glagov S. Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low oscillating shear stress. Arterioscler. Thromb. Vasc. Biol. 1985;5:293–302.

85. Thijssen DHJ, Dawson EA, Tinken TM, Cable NT, Green DJ. Retrograde Flow and Shear Rate Acutely Impair Endothelial Function in Humans. Hypertension 2009;53:986–992.

86. Thijssen DHJ, Black MA, Pyke KE, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am. J. Physiol. - Heart Circ. Physiol. 2011;300:H2–H12.

87. Joannides R, Haefeli WE, Linder L, et al. Nitric Oxide Is Responsible for Flow-Dependent Dilatation of Human Peripheral Conduit Arteries In Vivo. Circulation 1995;91:1314–1319.

88. Mullen MJ, Kharbanda RK, Cross J, et al. Heterogenous Nature of Flow-Mediated Dilatation in Human Conduit Arteries In Vivo: Relevance to Endothelial Dysfunction in Hypercholesterolemia. Circ. Res. 2001;88:145–151.

89. Doshi SN, Naka KK, Payne N, et al. Flow-mediated dilatation following wrist and upper arm occlusion in humans: the contribution of nitric oxide. Clin. Sci. 2001;101:629–635.

90. Wray DW, Witman MAH, Ives SJ, et al. Does Brachial Artery Flow–Mediated Vasodilation Provide a Bioassay for NO? Hypertension 2013;62:345–351.

91. Agewall S, Hulthe J, Fagerberg B, Gottfridsson B, Wikstrand J. Post-occlusion brachial artery vasodilatation after ischaemic handgrip exercise is nitric oxide mediated. Clin. Physiol. Funct. Imaging 2002;22:18–23.

92. Heiss C, Kleinbongard P, Dejam A, et al. Acute Consumption of Flavanol-Rich Cocoa and the Reversal of Endothelial Dysfunction in Smokers. J. Am. Coll. Cardiol. 2005;46:1276–1283.

93. Green DJ, Dawson EA, Groenewoud HMM, Jones H, Thijssen DHJ. Is flow-mediated dilation nitric oxide mediated? Novelty and significance: A meta-analysis. Hypertension 2014;63:376–382.

94. Fathi R, Haluska B, Isbel N, Short L, Marwick TH. The relative importance of vascular structure and function in predicting cardiovascular events. J. Am. Coll. Cardiol. 2004;43:616–623.

95. Frick M, Suessenbacher A, Alber HF, et al. Prognostic Value of Brachial Artery Endothelial Function and Wall Thickness. J. Am. Coll. Cardiol. 2005;46:1006–1010.

96. Neunteufl T, Heher S, Katzenschlager R, et al. Late prognostic value of flow-mediated dilation in the brachial artery of patients with chest pain. Am. J. Cardiol. 2000;86:207–210.

97. Rossi R, Nuzzo A, Origliani G, Modena MG. Prognostic Role of Flow-Mediated Dilation and Cardiac Risk Factors in Post-Menopausal Women. J. Am. Coll. Cardiol. 2008;51:997–1002.

98. Yeboah J, Crouse JR, Hsu F-C, Burke GL, Herrington DM. Brachial Flow-Mediated Dilation Predicts Incident Cardiovascular Events in Older Adults: The Cardiovascular Health Study. Circulation 2007;115:2390–2397.

99. Yeboah J, Folsom AR, Burke GL, et al. Predictive Value of Brachial Flow-Mediated Dilation for Incident Cardiovascular Events in a Population-Based Study: The Multi-Ethnic Study of Atherosclerosis. Circulation 2009;120:502–509.

100. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. Int. J. Cardiovasc. Imaging 2010;26:631–640.

101. Green DJ, Jones H, Thijssen D, Cable NT, Atkinson G. Flow-mediated dilation and cardiovascular event prediction Does nitric oxide matter? Hypertension 2011;57:363–369.

102. Kuvin JT, Patel AR, Sliney KA, et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. Am. Heart J. 2003;146:168–174.

103. Hamburg NM, Keyes MJ, Larson MG, et al. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. Circulation 2008;117:2467–2474.

104. Coffman JD. Effects of endothelium-derived nitric oxide on skin and digital blood flow in humans. Am. J. Physiol.-Heart Circ. Physiol. 1994;267:H2087–H2090.

105. Noon JP, Haynes WG, Webb DJ, Shore AC. Local inhibition of nitric oxide generation in man reduces blood flow in finger pulp but not in hand dorsum skin. J. Physiol. 1996;490:501–508.

106. Nohria A, Gerhard-Herman M, Creager MA, Hurley S, Mitra D, Ganz P. Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. J. Appl. Physiol. 2006;101:545–548.

107. Kuznetsova T, Vlierberghe EV, Knez J, et al. Association of digital vascular function with cardiovascular risk factors: a population study. BMJ Open 2014;4:e004399.

108. Heffernan KS, Karas RH, Patvardhan EA, Jafri H, Kuvin JT. Peripheral Arterial Tonometry for Risk Stratification in Men With Coronary Artery Disease. Clin. Cardiol. 2010;33:94–98.

109. Ikonomidis I, Kadoglou NNP, Tritakis V, et al. Association of Lp-PLA2 with digital reactive hyperemia, coronary flow reserve, carotid atherosclerosis and arterial stiffness in coronary artery disease. Atherosclerosis 2014;234:34–41.

110. Matsue Y, Yoshida K, Nagahori W, et al. Peripheral microvascular dysfunction predicts residual risk in coronary artery disease patients on statin therapy. Atherosclerosis 2014;232:186–190.

111. Matsuzawa Y, Sugiyama S, Sumida H, et al. Peripheral endothelial function and cardiovascular events in high-risk patients. J. Am. Heart Assoc. 2013;2:e000426.

112. Rubinshtein R, Kuvin JT, Soffler M, et al. Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. Eur. Heart J. 2010;31:1142–1148.

113. Dhindsa M, Sommerlad SM, DeVan AE, et al. Interrelationships among noninvasive measures of postischemic macro- and microvascular reactivity. J. Appl. Physiol. 2008;105:427–432.

114. Hamburg NM, Palmisano J, Larson MG, et al. Relation of brachial and digital measures of vascular function in the community: the Framingham Heart Study. Hypertension 2011;57:390–396.

115. Schnabel RB, Schulz A, Wild PS, et al. Noninvasive Vascular Function Measurement in the Community Clinical Perspective: Cross-Sectional Relations and Comparison of Methods. Circ. Cardiovasc. Imaging 2011;4:371–380.

116. Matsuzawa Y, Kwon T-G, Lennon RJ, Lerman LO, Lerman A. Prognostic value of flow-mediated vasodilation in brachial artery and fingertip artery for cardiovascular events: A systematic review and meta-analysis. J. Am. Heart Assoc. 2015;4:e002270.

117. Thomas JL, Braus PA. Coronary Artery Disease in Women: A Historical Perspective. Arch. Intern. Med. 1998;158:333–337.

118. Beard MC, Kottke TE, Annegers JF, Ballard DJ. The Rochester Coronary Heart Disease Project: Effect of Cigarette Smoking, Hypertension, Diabetes, and Steroidal Estrogen Use on Coronary Heart Disease Among 40- to 59-Year-Old Women, 1960 Through 1982. Mayo Clin. Proc. 1989;64:1471–1480.

119. Epstein FH. Epidemiological Studies of Cardiovascular Disease in a Total Community—Tecumseh, Michigan. Ann. Intern. Med. 1965;62:1170.

120. Kannel WB. Menopause and Risk of Cardiovascular Disease: The Framingham Study. Ann. Intern. Med. 1976;85:447.

121. Thom T, Haase N, Rosamond W, et al. Heart Disease and Stroke Statistics—2006 Update: A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2006;113:e85–e151.

122. Benjamin EJ, Blaha MJ, Chiuve SE, et al. Heart Disease and Stroke Statistics—2017 Update: A Report From the American Heart Association. Circulation 2017;135:e146–e603.

123. Maas AHEM, Schouw VD, T Y, et al. Red alert for women's heart: the urgent need for more research and knowledge on cardiovascular disease in womenProceedings of the Workshop held in Brussels on Gender Differences in Cardiovascular disease, 29 September 2010. Eur. Heart J. 2011;32:1362–1368.

124. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A. Myocardial infarction and coronary deaths in the World Health Organization MONICA Project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. Circulation 1994;90:583–612.

125. Chakrabarti S, Morton JS, Davidge ST. Mechanisms of Estrogen Effects on the Endothelium: An Overview. Can. J. Cardiol. 2014;30:705–712.

126. Kim KH, Bender JR. Membrane-initiated actions of estrogen on the endothelium. Mol. Cell. Endocrinol. 2009;308:3–8.

127. Mendelsohn ME, Karas RH. The Protective Effects of Estrogen on the Cardiovascular System. N. Engl. J. Med. 1999;340:1801–1811.

128. Chambliss KL, Yuhanna IS, Mineo C, et al. Estrogen Receptor α and Endothelial Nitric Oxide Synthase Are Organized Into a Functional Signaling Module in Caveolae. Circ. Res. 2000;87:e44–e52.

129. Kim-Schulze S, McGowan KA, Hubchak SC, et al. Expression of an Estrogen Receptor by Human Coronary Artery and Umbilical Vein Endothelial Cells. Circulation 1996;94:1402–1407.

130. Tan E, Gurjar MV, Sharma RV, Bhalla RC. Estrogen receptor-α gene transfer into bovine aortic endothelial cells induces eNOS gene expression and inhibits cell migration. Cardiovasc. Res. 1999;43:788–797.

131. Caulin-Glaser T, García-Cardeña G, Sarrel P, Sessa WC, Bender JR. 17β-Estradiol Regulation of Human Endothelial Cell Basal Nitric Oxide Release, Independent of Cytosolic Ca2+ Mobilization. Circ. Res. 1997;81:885–892.

132. Haynes MP, Li L, Sinha D, et al. Src Kinase Mediates Phosphatidylinositol 3-Kinase/Akt-dependent Rapid Endothelial Nitric-oxide Synthase Activation by Estrogen. J. Biol. Chem. 2003;278:2118–2123.

133. Simoncini T, Mannella P, Fornari L, Caruso A, Varone G, Genazzani AR. Genomic and non-genomic effects of estrogens on endothelial cells. Steroids 2004;69:537–542.

134. Herrington DM, Fan L, Drum M, et al. Brachial flow-mediated vasodilator responses in population-based research: methods, reproducibility and effects of age, gender and baseline diameter. J. Cardiovasc. Risk 2001;8:319–328.

135. Schnabel RB, Biener MP, Wilde S, et al. Sex differences in noninvasive vascular function in the community. J. Hypertens. 2013;31:1437–1446.

136. Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J. Am. Coll. Cardiol. 1994;24:471–476.

137. Atkinson G, Batterham AM. Allometric scaling of diameter change in the original flow-mediated dilation protocol. Atherosclerosis 2013;226:425–427.

138. Shenouda N, Priest SE, Rizzuto VI, MacDonald MJ. Brachial artery endothelial function is stable across a menstrual and oral contraceptive pill cycle, but lower in premenopausal women than age-matched men. Am. J. Physiol.-Heart Circ. Physiol. 2018. Available at: https://www.physiology.org/doi/abs/10.1152/ajpheart.00102.2018. Accessed July 27, 2018.

139. McLay KM, Nederveen JP, Koval JJ, Paterson DH, Murias JM. Allometric scaling of flow-mediated dilation: is it always helpful? Clin. Physiol. Funct. Imaging 2018;38:663–669.

140. Heffernan KS, Karas RH, Mooney PJ, Patel AR, Kuvin JT. Pulse wave amplitude is associated with brachial artery diameter: Implications for gender differences in microvascular function. Vasc. Med. 2010;15:39–45.

141. Jensen-Urstad K., Johansson J. Gender difference in age-related changes in vascular function. J. Intern. Med. 2001;250:29–36.

142. Arbogast R, Bourassa MG. Myocardial function during atrial pacing in patients with angina pectoris and normal coronary arteriograms. Comparison with patients having significant coronary artery disease. Am. J. Cardiol. 1973;32:257–263.

143. Kemp HG. Left ventricular function in patients with the anginal syndrome and normal coronary arteriograms. Am. J. Cardiol. 1973;32:375–376.

144. Opherk D, Zebe H, Weihe E, et al. Reduced coronary dilatory capacity and ultrastructural changes of the myocardium in patients with angina pectoris but normal coronary arteriograms. Circulation 1981;63:817–825.

145. Cannon RO, Epstein SE. "Microvascular angina" as a cause of chest pain with angiographically normal coronary arteries. Am. J. Cardiol. 1988;61:1338–1343.

146. Hochman JS, Tamis JE, Thompson TD, et al. Sex, Clinical Presentation, and Outcome in Patients with Acute Coronary Syndromes. N. Engl. J. Med. 1999;341:226–232.

147. Humphries KH, Pu A, Gao M, Carere RG, Pilote L. Angina with "normal" coronary arteries: Sex differences in outcomes. Am. Heart J. 2008;155:375–381.

148. Davis KB, Chaitman B, Ryan T, Bittner V, Kennedy JW. Comparison of 15-year survival for men and women after initial medical or surgical treatment for coronary artery disease: A CASS registry study. J. Am. Coll. Cardiol. 1995;25:1000–1009.

149. Shaw LJ, Merz CNB, Pepine CJ, et al. The economic burden of angina in women with suspected ischemic heart disease: Results from the National Institutes of Health–National Heart,

Lung, and Blood Institute–Sponsored Women's Ischemia Syndrome Evaluation. Circulation 2006;114:894–904.

150. Reis SE, Holubkov R, Smith AJC, et al. Coronary microvascular dysfunction is highly prevalent in women with chest pain in the absence of coronary artery disease: Results from the NHLBI WISE study. Am. Heart J. 2001;141:735–741.

151. Sara JD, Widmer RJ, Matsuzawa Y, Lennon RJ, Lerman LO, Lerman A. Prevalence of coronary microvascular dysfunction among patients with chest pain and nonobstructive coronary artery disease. JACC Cardiovasc. Interv. 2015;8:1445–1453.

152. Gulati M, Cooper-DeHoff RM, McClure C, et al. Adverse Cardiovascular Outcomes in Women With Nonobstructive Coronary Artery Disease: A Report From the Women's Ischemia Syndrome Evaluation Study and the St James Women Take Heart Project. Arch. Intern. Med. 2009;169:843–850.

153. Johnson BD, Shaw LJ, Buchthal SD, et al. Prognosis in Women With Myocardial Ischemia in the Absence of Obstructive Coronary Disease: Results From the National Institutes of Health–National Heart, Lung, and Blood Institute–Sponsored Women's Ischemia Syndrome Evaluation (WISE). Circulation 2004;109:2993–2999.

154. Al-Badri A, Bairey Merz CN, Johnson BD, et al. Impact of abnormal coronary reactivity on long-term clinical outcomes in women. J. Am. Coll. Cardiol. 2019;73:684–693.

155. Bairey Merz CN, Pepine CJ, Walsh MN, Fleg JL. Ischemia and no obstructive coronary artery disease (INOCA): Developing evidence-based therapies and research agenda for the next decade. Circulation 2017;135:1075–1092.

156. Lefroy DC, Crake T, Uren NG, Davies GJ, Maseri A. Effect of inhibition of nitric oxide synthesis on epicardial coronary artery caliber and coronary blood flow in humans. Circulation 1993;88:43–54.

157. Quyyumi AA, Dakak N, Andrews NP, Gilligan DM, Panza JA, Cannon RO. Contribution of Nitric Oxide to Metabolic Coronary Vasodilation in the Human Heart. Circulation 1995;92:320–326.

158. Egashira K, Inou T, Hirooka Y, Yamada A, Urabe Y, Takeshita A. Evidence of Impaired Endothelium-Dependent Coronary Vasodilatation in Patients with Angina Pectoris and Normal Coronary Angiograms. N. Engl. J. Med. 1993;328:1659–1664.

159. Chauhan A, Mullins PA, Taylor G, Petch MC, Schofield PM. Both endothelium-dependent and endothelium-independent function is impaired in patients with angina pectoris and normal coronary angiograms. Eur. Heart J. 1997;18:60–68.

160. Hasdai D, Gibbons RJ, Holmes DR, Higano ST, Lerman A. Coronary Endothelial Dysfunction in Humans Is Associated With Myocardial Perfusion Defects. Circulation 1997;96:3390–3395.

161. Zeiher AM, Krause T, Schächinger V, Minners J, Moser E. Impaired Endothelium-Dependent Vasodilation of Coronary Resistance Vessels Is Associated With Exercise-Induced Myocardial Ischemia. Circulation 1995;91:2345–2352.

162. Lekakis JP, Papamichael CM, Vemmos CN, Voutsas AA, Stamatelopoulos SF, Moulopoulos SD. Peripheral Vascular Endothelial Dysfunction in Patients With Angina Pectoris and Normal Coronary Arteriograms. J. Am. Coll. Cardiol. 1998;31:541–546.

163. Bellamy MF, Goodfellow J, Tweddel AC, Dunstan FDJ, Lewis MJ, Henderson AH. Syndrome X and endothelial dysfunction. Cardiovasc. Res. 1998;40:410–417.

164. Masci PG, Laclaustra M, Lara JG, Kaski JC. Brachial Artery Flow-Mediated Dilation and Myocardial Perfusion in Patients With Cardiac Syndrome X. Am. J. Cardiol. 2005;95:1478–1480.

165. Naidu OA, Rajasekhar D, Latheef S. Assessment of endothelial function by brachial artery flow mediated dilatation in microvascular disease. Cardiovasc. Ultrasound 2011;9:40.

166. Tondi P, Santoliquido A, Di Giorgio A, et al. Endothelial dysfunction as assessed by flow-mediated dilation in patients with cardiac syndrome X: role of inflammation. Eur. Rev. Med. Pharmacol. Sci. 2011;15:1074–1077.

167. Kaski JC, Elliott PM, Salomone O, et al. Concentration of circulating plasma endothelin in patients with angina and normal coronary angiograms. Heart 1995;74:620–624.

168. MacCarthy PA, Pegge NC, Prendergast BD, Shah AM, Groves PH. The physiological role of endogenous endothelin in the regulation of human coronary vasomotor tone. J. Am. Coll. Cardiol. 2001;37:137–143.

169. Arroyo-Espliguero R, Mollichelli N, Avanzas P, et al. Chronic inflammation and increased arterial stiffness in patients with cardiac syndrome X. Eur. Heart J. 2003;24:2006–2011.

170. Saeki A, Recchia F, Kass DA. Systolic Flow Augmentation in Hearts Ejecting Into a Model of Stiff Aging Vasculature: Influence on Myocardial Perfusion-Demand Balance. Circ. Res. 1995;76:132–141.

171. Saito M, Okayama H, Nishimura K, et al. Possible link between large artery stiffness and coronary flow velocity reserve. Heart 2008;94:e20–e20.

172. Lembo M, Sicari R, Esposito R, et al. Association Between Elevated Pulse Pressure and High Resting Coronary Blood Flow Velocity in Patients With Angiographically Normal Epicardial Coronary Arteries. J. Am. Heart Assoc. 2017;6:e005710.

173. Hoffman JI. Maximal coronary flow and the concept of coronary vascular reserve. Circulation 1984;70:153–159.

174. Nichols WW, Denardo SJ, Davidson JB, Huo T, Bairey Merz CN, Pepine CJ. Association of aortic stiffness and wave reflections with coronary flow reserve in women without obstructive

coronary artery disease: An ancillary study from the National Heart, Lung, and Blood Institute–sponsored Women's Ischemia Syndrome Evaluation (WISE). Am. Heart J. 2015;170:1243–1254.

175. Murthy VL, Naya M, Taqueti VR, et al. Effects of sex on coronary microvascular dysfunction and cardiac outcomes. Circulation 2014;129:2518–2527.

176. Schindler TH, Cardenas J, Prior JO, et al. Relationship Between Increasing Body Weight, Insulin Resistance, Inflammation, Adipocytokine Leptin, and Coronary Circulatory Function. J. Am. Coll. Cardiol. 2006;47:1188–1195.

177. Lanza GA, Buffon A, Sestito A, et al. Relation Between Stress-Induced Myocardial Perfusion Defects on Cardiovascular Magnetic Resonance and Coronary Microvascular Dysfunction in Patients With Cardiac Syndrome X. J. Am. Coll. Cardiol. 2008;51:466–472.

178. Liu A, Wijesurendra RS, Liu JM, et al. Diagnosis of Microvascular Angina Using Cardiac Magnetic Resonance. J. Am. Coll. Cardiol. 2018;71:969–979.

179. Panting JR, Grothues F. Abnormal Subendocardial Perfusion in Cardiac Syndrome X Detected by Cardiovascular Magnetic Resonance Imaging. N. Engl. J. Med. 2002:6.

180. Mygind ND, Michelsen MM, Pena A, et al. Coronary Microvascular Function and Cardiovascular Risk Factors in Women With Angina Pectoris and No Obstructive Coronary Artery Disease: The iPOWER Study. J. Am. Heart Assoc. 2016;5:e003064.

181. Lee B-K, Lim H-S, Fearon WF, et al. Invasive evaluation of patients with angina in the absence of obstructive coronary artery disease. Circulation 2015;131:1054–1060.

182. Matsuo S, Matsumoto T, Takashima H, et al. The Relationship Between Flow-Mediated Brachial Artery Vasodilation and Coronary Vasomotor Responses to Bradykinin: Comparison with Those to Acetylcholine: J. Cardiovasc. Pharmacol. 2004;44:164–170.

183. Takase B, Hamabe A, Satomura K, et al. Close relationship between the vasodilator response to acetylcholine in the brachial and coronary artery in suspected coronary artery disease. Int. J. Cardiol. 2005;105:58–66.

184. Takase B, Uehata A, Akima T, et al. Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. Am. J. Cardiol. 1998;82:1535–1539.

185. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 2000;101:948–954.

186. Fearon WF, Kobayashi Y. Invasive assessment of the coronary microvasculature: The index of microcirculatory resistance. Circ. Cardiovasc. Interv. 2017;10:e005361.

187. Fearon WF, Low AF, Yong AS, et al. Prognostic Value of the Index of Microcirculatory Resistance Measured After Primary Percutaneous Coronary InterventionClinical Perspective. Circulation 2013;127:2436–2441.

188. Fearon WF, Shah M, Ng M, et al. Predictive Value of the Index of Microcirculatory Resistance in Patients With ST-Segment Elevation Myocardial Infarction. J. Am. Coll. Cardiol. 2008;51:560–565.

189. Lee JM, Jung J-H, Hwang D, et al. Coronary flow reserve and microcirculatory resistance in patients with intermediate coronary stenosis. J. Am. Coll. Cardiol. 2016;67:1158–1169.

190. Ahn SG, Hung OY, Lee J-W, et al. Combination of the Thermodilution-Derived Index of Microcirculatory Resistance and Coronary Flow Reserve Is Highly Predictive of Microvascular Obstruction on Cardiac Magnetic Resonance Imaging After ST-Segment Elevation Myocardial Infarction. JACC Cardiovasc. Interv. 2016;9:793–801.

191. Sax FL, Cannon ROI, Hanson C, Epstein SE. Impaired forearm vasodilator reserve in patients with microvascular angina. N. Engl. J. Med. 1987;317:1366–1370.

192. Anderson TJ, Uehata A, Gerhard MD, et al. Close relation of endothelial function in the human coronary and peripheral circulations. J. Am. Coll. Cardiol. 1995;26:1235–1241.

193. Hozumi T, Yoshida K, Akasaka T, et al. Noninvasive assessment of coronary flow velocity and coronary flow velocity reserve in the left anterior descending coronary artery by Doppler echocardiography: Comparison with invasive technique. J. Am. Coll. Cardiol. 1998;32:1251–1259.

194. Gullu H, Erdogan D, Caliskan M, et al. Interrelationship Between Noninvasive Predictors of Atherosclerosis: Transthoracic Coronary Flow Reserve, Flow-Mediated Dilation, Carotid Intima-Media Thickness, Aortic Stiffness, Aortic Distensibility, Elastic Modulus, and Brachial Artery Diameter. Echocardiography 2006;23:835–842.

195. Park C-S, Youn H-J, Kim J-H, et al. Relation between peripheral vascular endothelial function and coronary flow reserve in patients with chest pain and normal coronary angiogram. Int. J. Cardiol. 2006;113:118–120.

196. Raft KF, Frestad D, Michelsen MM, et al. Peripheral endothelial function and coronary flow velocity reserve are not associated in women with angina and no obstructive coronary artery disease: The iPOWER Study. J. Vasc. Res. 2017;54:309–319.

197. Bonetti PO, Pumper GM, Higano ST, Holmes DR, Kuvin JT, Lerman A. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. J. Am. Coll. Cardiol. 2004;44:2137–2141.

198. Matsuzawa Y, Sugiyama S, Sugamura K, et al. Digital assessment of endothelial function and ischemic heart disease in women. J. Am. Coll. Cardiol. 2010;55:1688–1696.

199. Michelsen MM, Mygind ND, Pena A, et al. Peripheral reactive hyperemia index and coronary microvascular function in women with no obstructive CAD: The iPOWER Study. JACC Cardiovasc. Imaging 2016;9:411–417.

200. Ng MKC, Yeung AC, Fearon WF. Invasive assessment of the coronary microcirculation: Superior reproducibility and less hemodynamic dependence of index of microcirculatory resistance compared with coronary flow reserve. Circulation 2006;113:2054–2061.

201. Lanza GA, Giordano A, Pristipino C, et al. Abnormal cardiac adrenergic nerve function in patients with syndrome X detected by [123I]Metaiodobenzylguanidine myocardial scintigraphy. Circulation 1997;96:821–826.

202. Vatner SF, McRitchie RJ, Braunwald E. Effects of dobutamine on left ventricular performance, coronary dynamics, and distribution of cardiac output in conscious dogs. J. Clin. Invest. 1974;53:1265–1273.

203. Willerson JT, Hutton I, Watson JT, Platt MR, Templeton GH. Influence of dobutamine on regional myocardial blood flow and ventricular performance during acute and chronic myocardial ischemia in dogs. Circulation 1976;53:828–833.

204. Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. J. Physiol. 2000;525:263–270.

205. Pijls NHJ, Bruyne BD, Smith L, et al. Coronary thermodilution to assess flow reserve: Validation in humans. Circulation 2002;105:2482–2486.

206. Kobayashi Y, Fearon WF, Honda Y, et al. Effect of sex differences on invasive measures of coronary microvascular dysfunction in patients with angina in the absence of obstructive coronary artery disease. JACC Cardiovasc. Interv. 2015;8:1433–1441.

207. Zou KH, O'Malley AJ, Mauri L. Receiver-operating characteristic analysis for evaluating diagnostic tests and predictive models. Circulation 2007;115:654–657.

208. Al-Badri A, Kim JH, Liu C, Mehta PK, Quyyumi AA. Peripheral microvascular function reflects coronary vascular function. Arterioscler. Thromb. Vasc. Biol. 2019.

209. Ludmer PL, Selwyn AP, Shook TL, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N. Engl. J. Med. 1986;315:1046–1051.

210. Quyyumi AA, Dakak N, Andrews NP, et al. Nitric oxide activity in the human coronary circulation. Impact of risk factors for coronary atherosclerosis. J. Clin. Invest. 1995;95:1747–1755.

211. Faizi AK, Kornmo DW, Agewall S. Evaluation of endothelial function using finger plethysmography. Clin. Physiol. Funct. Imaging 2009;29:372–375.

212. Broxterman RM, Witman MA, Trinity JD, et al. Strong relationship between vascular function in the coronary and brachial arteries. Hypertens. Dallas Tex 1979 2019.

213. Monahan KD, Feehan RP, Sinoway LI, Gao Z. Contribution of sympathetic activation to coronary vasodilatation during the cold pressor test in healthy men: effect of ageing. J. Physiol. 2013;591:2937–2947.

214. Zeiher AM, Drexler H, Wollschlaeger H, Saurbier B, Just H. Coronary vasomotion in response to sympathetic stimulation in humans: Importance of the functional integrity of the endothelium. J. Am. Coll. Cardiol. 1989;14:1181–1190.

215. Barbato E, Bartunek J, Wyffels E, Wijns W, Heyndrickx GR, De Bruyne B. Effects of intravenous dobutamine on coronary vasomotion in humans. J. Am. Coll. Cardiol. 2003;42:1596–1601.

216. Sverrisdóttir YB, Jansson LM, Hägg U, Gan L-M. Muscle sympathetic nerve activity is related to a surrogate marker of endothelial function in healthy individuals. PLOS ONE 2010;5:e9257.

217. Hijmering ML, Stroes ESG, Olijhoek J, Hutten BA, Blankestijn PJ, Rabelink TJ. Sympathetic activation markedly reduces endothelium-dependent, flow-mediated vasodilation. J. Am. Coll. Cardiol. 2002;39:683–688.

218. Thijssen DHJ, Atkinson CL, Ono K, et al. Sympathetic nervous system activation, arterial shear rate, and flow-mediated dilation. J. Appl. Physiol. 2014;116:1300–1307.

219. Kaplon RE, Walker AE, Seals DR. Plasma norepinephrine is an independent predictor of vascular endothelial function with aging in healthy women. J. Appl. Physiol. 2011;111:1416–1421.

220. Padilla J, Young CN, Simmons GH, et al. Increased muscle sympathetic nerve activity acutely alters conduit artery shear rate patterns. Am. J. Physiol. - Heart Circ. Physiol. 2010;298:H1128–H1135.

221. Parent R, al-Obaidi M, Lavallée M. Nitric oxide formation contributes to beta-adrenergic dilation of resistance coronary vessels in conscious dogs. Circ. Res. 1993;73:241–251.

222. Dawes M, Chowienczyk PJ, Ritter JM. Effects of inhibition of the L-arginine/nitric oxide pathway on vasodilation caused by beta-adrenergic agonists in human forearm. Circulation 1997;95:2293–2297.

223. Gobel F L, Norstrom L A, Nelson R R, Jorgensen C R, Wang Y. The rate-pressure product as an index of myocardial oxygen consumption during exercise in patients with angina pectoris. Circulation 1978;57:549–556.

224. Dawson EA, Green DJ, Cable NT, Thijssen DHJ. Effects of acute exercise on flow-mediated dilatation in healthy humans. J. Appl. Physiol. 2013;115:1589–1598.

225. Cassar A, Chareonthaitawee P, Rihal CS, et al. Lack of Correlation Between Noninvasive Stress Tests and Invasive Coronary Vasomotor Dysfunction in Patients With Nonobstructive Coronary Artery Disease. Circ. Cardiovasc. Interv. 2009;2:237–244.

226. Kaski JC, Collins P, Nihoyannopoulos P, Maseri A, Poole-Wilson PA, Rosano GMC. Cardiac syndrome X: Clinical characteristics and left ventricular function: Long-term follow-up study. J. Am. Coll. Cardiol. 1995;25:807–814.

227. Maseri A, Crea F, Kaski JC, Crake T. Mechanisms of angina pectoris in syndrome X. J. Am. Coll. Cardiol. 1991;17:499–506.

228. Nihoyannopoulos P, Carlos Kaski J, Crake T, Maseri A. Absence of myocardial dysfunction during stress in patients with syndrome X. J. Am. Coll. Cardiol. 1991;18:1463–1470.

229. Panza JA, Laurienzo JM, Curiel RV, et al. Investigation of the Mechanism of Chest Pain in Patients With Angiographically Normal Coronary Arteries Using Transesophageal Dobutamine Stress Echocardiography. J. Am. Coll. Cardiol. 1997;29:293–301.

230. Pathak A, Velez-Roa S, Xhaët O, Najem B, Borne PVD. Dose-dependent effect of dobutamine on chemoreflex activity in healthy volunteers. Br. J. Clin. Pharmacol. 2006;62:272–279.

231. Al-Hesayen A, Azevedo ER, Newton GE, Parker JD. The effects of dobutamine on cardiac sympathetic activity in patients with congestive heart failure. J. Am. Coll. Cardiol. 2002;39:1269–1274.

232. Biaggioni I, Killian TJ, Mosqueda-Garcia R, Robertson RM, Robertson D. Adenosine increases sympathetic nerve traffic in humans. Circulation 1991;83:1668–1675.

233. Costa F, Diedrich A, Johnson B, Sulur P, Farley G, Biaggioni I. Adenosine, a Metabolic Trigger of the Exercise Pressor Reflex in Humans. Hypertension 2001;37:917–922.

234. Thames MD, Kinugawa T, Dibner-Dunlap ME. Reflex sympathoexcitation by cardiac sympathetic afferents during myocardial ischemia. Role of adenosine. Circulation 1993;87:1698–1704.

235. Gnecchi-Ruscone T, Montano N, Contini M, Guazzi M, Lombardi F, Malliani A. Adenosine activates cardiac sympathetic afferent fibers and potentiates the excitation induced by coronary occlusion. J. Auton. Nerv. Syst. 1995;53:175–184.

236. Gulati M, Cooper-DeHoff RM, McClure C, et al. Adverse cardiovascular outcomes in women with nonobstructive coronary artery disease: A report from the Women's Ischemia Syndrome Evaluation Study and the St James Women Take Heart Project. Arch. Intern. Med. 2009;169:843–850.

237. Camici PG, Crea F. Coronary Microvascular Dysfunction. N. Engl. J. Med. 2007;356:830–840.

238. Pepine CJ. Multiple causes for ischemia without obstructive coronary artery disease: Not a short list. Circulation 2015;131:1044–1046.

239. Celermajer DS. Reliable Endothelial Function Testing. Circulation 2008;117:2428–2430.

240. Hamburg NM, Benjamin EJ. Assessment of endothelial function using digital pulse amplitude tonometry. Trends Cardiovasc. Med. 2009;19:6–11.

241. Mark DB, Shaw L, Harrell FE, et al. Prognostic value of a treadmill exercise score in outpatients with suspected coronary artery disease. N. Engl. J. Med. 1991;325:849–853.

242. Silvestro A, Scopacasa F, Oliva G, de Cristofaro T, Iuliano L, Brevetti G. Vitamin C prevents endothelial dysfunction induced by acute exercise in patients with intermittent claudication. Atherosclerosis 2002;165:277–283.

243. Hwang I-C, Kim K-H, Choi W-S, et al. Impact of acute exercise on brachial artery flow-mediated dilatation in young healthy people. Cardiovasc. Ultrasound 2012;10:39.

244. Finaud J, Lac G, Filaire E. Oxidative Stress: Relationship with Exercise and Training. Sports Med. 2006;36:327–358.

245. Ji LL. Exercise-induced Modulation of Antioxidant Defense. Ann. N. Y. Acad. Sci. 2002;959:82–92.

246. Harris RA, Padilla J, Hanlon KP, Rink LD, Wallace JP. The flow-mediated dilation response to acute exercise in overweight active and inactive men. Obesity 2008;16:578–584.

247. Johnson BD, Padilla J, Wallace JP. The exercise dose affects oxidative stress and brachial artery flow-mediated dilation in trained men. Eur. J. Appl. Physiol. 2012;112:33–42.

248. Sakr SA, Abbas TM, Amer MZ, et al. Microvascular angina. The possible role of inflammation, uric acid, and endothelial dysfunction. Int. Heart. J. 2009;50:407–419.

249. Brant LCC, Barreto SM, Passos VMA, Ribeiro ALP. Reproducibility of peripheral arterial tonometry for the assessment of endothelial function in adults. J. Hypertens. 2013;31:1984–1990.

250. Liu J, Wang J, Jin Y, Roethig HJ, Unverdorben M. Variability of peripheral arterial tonometry in the measurement of endothelial function in healthy men. Clin. Cardiol. 2009;32:700–704.

251. Di Carli MF, Charytan D, McMahon GT, Ganz P, Dorbala S, Schelbert HR. Coronary circulatory function in patients with the metabolic syndrome. J. Nucl. Med. 2011;52:1369–1377.

252. Nahser PJ, Brown RE, Oskarsson H, Winniford MD, Rossen JD. Maximal coronary flow reserve and metabolic coronary vasodilation in patients with diabetes mellitus. Circulation 1995;91:635–640.

APPENDIX A: ARTERIAL STIFFNESS AND CORONARY FUNCTION

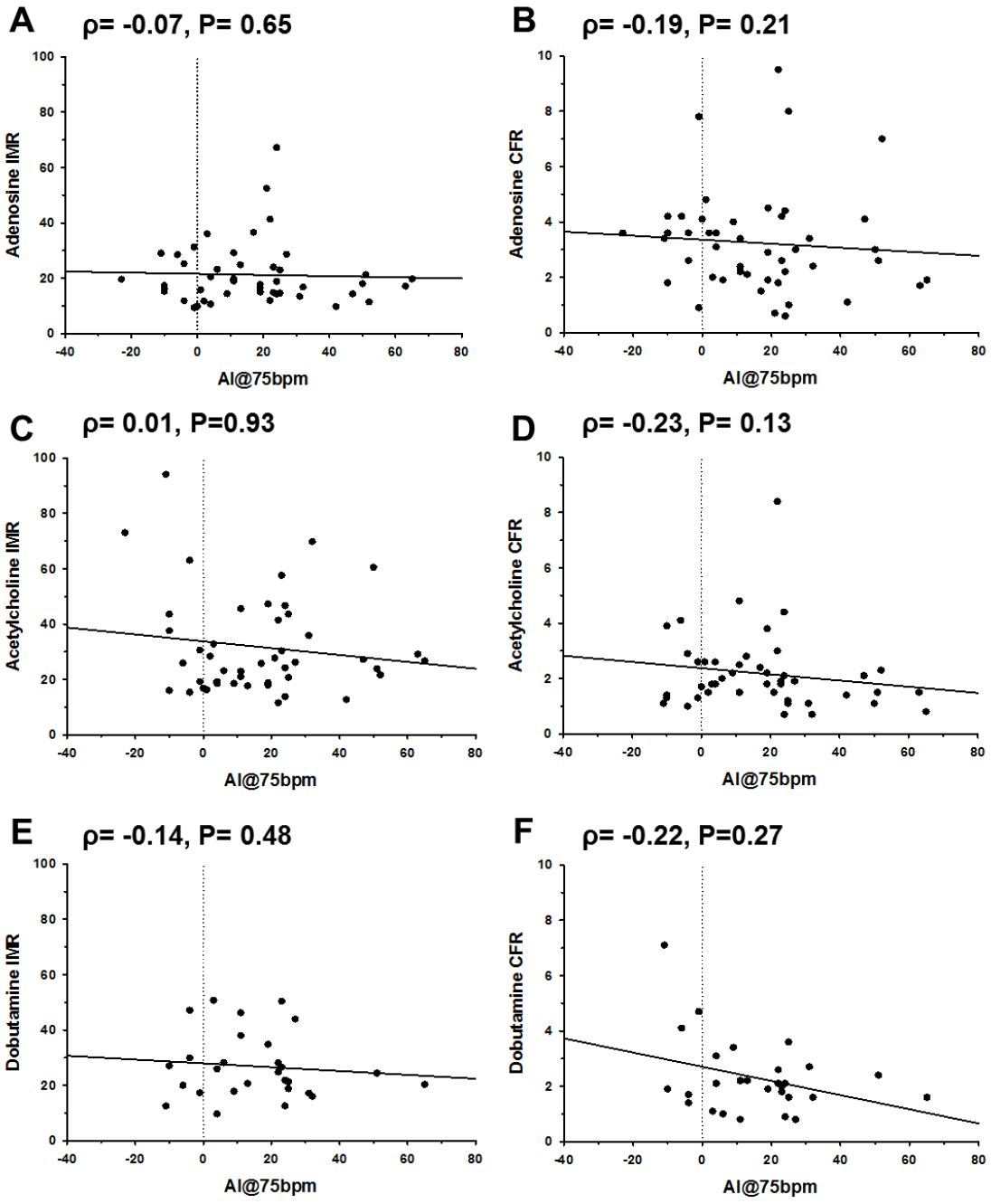


Figure 1: Correlations between the augmentation index at a controlled heart rate of 75bpm (AI@75bpm) and the index of microvascular resistance (IMR; left panels) and the coronary flow reserve (CFR; right panels) to adenosine (A and B; n=46), acetylcholine (C and D; n=46), and dobutamine (E and F; n=28). ρ: Spearman's Rho.

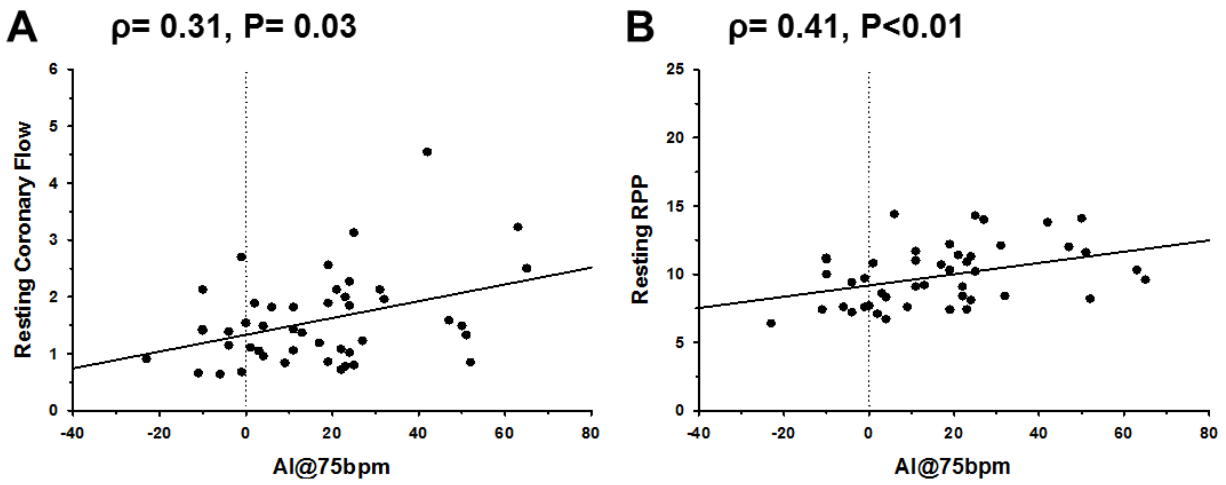


Figure 2: Correlations between the augmentation index at a controlled heart rate of 75bpm (AI@75bpm) and resting coronary flow (A; n=46) and the resting rate pressure product (RPP; B; n=46). ρ: Spearman's Rho.

APPENDIX B

Influence of standard exercise stress testing on peripheral microvascular function in patients with coronary microvascular dysfunction.

Massimo Nardone[1], Steven Miner[1,2], Mary McCarthy[2], Heather Edgell[1,2]

[1] School of Kinesiology and Health Science, York University, Toronto, Ontario, Canada.

[2] Southlake Regional Health Center, Newmarket, Ontario, Canada.

Running Title: Microvascular function following acute exercise

Word Count of Manuscript: 2915

Word Count of Abstract: 242

Number of figures: 4

To be submitted to the Canadian Journal of Cardiology

Abstract

The effect of exercise on the microvasculature of patients with suspected coronary microvascular disease (CMD), assessed by reactive hyperemia peripheral arterial tonometry (RHPAT), is unknown. Further, RHPAT assessments may be particularly useful for screening patients with suspected CMD, though exercise stress testing is also commonly conducted in clinical practice. Therefore, the present study aimed to determine if standard clinical exercise stress testing (Bruce treadmill protocol; GXT) affected peripheral microvascular function, as determined by reactive hyperemia index (RHI and LnRHI), in patients with suspected CMD. Patients (n=68) were grouped based on whether the GXT was performed; 1) prior to (exercisers; n=26), or 2) after the vascular assessment (non-exercisers; n=42). Patients with abnormal coronary flow or resistance responses to adenosine- and/or acetylcholine-induced hyperemia were confirmed to have CMD (n=41). LnRHI was lower in patients with CMD compared to patients with normal coronary arteries, while LnRHI was also lower in exercisers compared to non-exercisers (LnRHI: CMD Non-Exercisers: 0.65±0.23; CMD Exercisers: 0.50±0.23; No CMD Non-Exercisers; 0.85±0.23; No CMD Exercisers: 0.65±0.25; Condition and Exercise Main Effects: Both $P<0.01$). In all exercisers, there was a significant association ($r=0.54$; $P<0.01$) comparing the time between the GXT and RHPAT tests and LnRHI, and the correlations did not differ between conditions (CMD: $r=0.57$ vs No CMD: $r=0.55$; $P=0.95$). Our results demonstrate that patients with CMD display impaired microvascular function, and suggests that exercise impairs microvascular function in all patients. We recommend that exercise should be avoided prior to RHPAT determination in clinical settings.

Key words: peripheral arterial tonometry, EndoPAT, acute exercise, augmentation index.

Summary

Reactive hyperemia peripheral arterial tonometry (RHPAT) is increasingly recognized as a potential screening measure for patients with suspected coronary microvascular dysfunction (CMD), though the effects of exercise are unknown. The current study highlights that patients with confirmed CMD have impaired peripheral microvascular function, and that exercise stress testing attenuates microvascular function in all patients with suspected CMD. We recommend that exercise should be avoided prior to RHPAT determination in clinical settings.

Introduction

Coronary microvascular dysfunction (CMD), defined by abnormal coronary microvascular vasomotion, is increasingly recognized as an important contributor to adverse cardiac outcomes (6, 7, 236). Although unclear and multifactorial, endothelial dysfunction likely plays an important role in facilitating the observed abnormalities in CMD (155, 237, 238). Given that endothelial dysfunction is thought to systemically affect the vasculature (191, 195), measuring endothelial function from the peripheral circulation may be an effective time and cost-efficient analysis for identifying patients with CMD. In particular, reactive hyperemia peripheral arterial tonometry (RHPAT), as measured by EndoPAT, is emerging as a novel measure of microvascular function, and is obtained by quantifying the hyperemic response within the finger microvasculature following brief ischemia (239, 240). Poor peripheral microvascular function, using RHPAT, has been shown to identify patients with both non-obstructive coronary artery disease(198), and early coronary atherosclerosis (197), supporting it's utility within clinical settings.

The impact of acute exercise on RHPAT in patients with CMD is currently unknown, however, conclusions from studies conducted in the conduit arteries of healthy participants have highlighted that acute exercise briefly attenuates endothelial function (dependent on the duration, intensity, and type of exercise, as reviewed by Dawson et al.(224)). It is currently unclear whether patients with cardiovascular disease, in particular CMD, experience an exaggerated reduction of endothelial function following exercise, given that baseline impairments in endothelial function are thought to impair the post-exercise vascular response (224). Therefore, the present study aimed to determine; 1) if patients with confirmed CMD exhibit impaired peripheral microvascular function, and 2) if standard clinical exercise stress testing, commonly conducted within clinical practice, acutely affects peripheral microvascular function in patients with suspected CMD. We hypothesized that 1) patients with CMD will have significantly lower peripheral microvascular function, 2) exercise will impair peripheral microvascular function in all patients with a greater effect in those with confirmed CMD.

Methods

Patient selection

Sixty eight patients with suspected coronary microvascular disease (CMD) who attended the Cardiovascular Integrative Physiology Clinic at Southlake Regional Health Centre were recruited for this study. Subjects were included in this study if they underwent reactive hyperemia peripheral arterial tonometry (RHPAT) assessments and coronary reactivity testing, to functionally assess peripheral and coronary microvascular function, respectively. Coronary reactivity testing was conducted if participants were experiencing typical or atypical chest pain, and had angiographic evidence of normal coronary arteries. Participants did not undergo coronary reactivity testing if the cardiologist determined that there was evidence of coronary obstruction based on prior angiography, or recent treatment (<1 years) of a percutaneous coronary intervention. All procedures were approved by the Research Ethics Boards of Southlake Regional Healthcare Centre and York University.

Experimental Protocol

In a cross-sectional design, patients were grouped based on whether an exercise stress test was performed 1) prior to, or 2) following the vascular assessment. The exercise assessment consisted of a symptoms-limited maximal exercise stress test (GXT) using the Bruce protocol with concurrent 12-lead electrocardiography. The Duke Treadmill Score (DTS) was subsequently calculated, using the formula: DTS= Exercise time – (5 x Max exercise ST) – (4 x Angina Index), as previously ascribed for risk classification (241). Maximum exercise ST depression was the maximal depression present during exercise, where a maximal ST segment depression of ≥0.1 mV was considered relevant myocardial ischemia. The angina index was ranked 0-2; 0 defined as no chest pain, 1 defined as non-limiting chest pain, and 2 defined as limiting chest pain. The time duration between the GXT and the vascular assessment was documented.

Prior to the vascular assessment, blood pressure was manually obtained, and height and weight were recorded. Non-invasive assessment of peripheral microvascular function was conducted using reactive hyperemia peripheral arterial tonometry (RHPAT) (EndoPat, Itamar Medical, Israel). Briefly, an applanation tonometry cuff was placed on the index finger of both hands. A standard blood pressure cuff was then positioned on the right arm, immediately distal to

the elbow joint. Five minutes of baseline recordings preceded 5 minutes of forearm ischemia, which involved supra-systolic cuff inflation to either 200 mmHg or 50 mmHg above systolic blood pressure. Subsequently, the blood pressure cuff was quickly deflated to allow reperfusion of the distal limb for an additional 5 minutes. The pulse amplitude recordings were stored, digitized and analyzed by an automated algorithm, which quantified the hyperemic response as the reactive hyperemic index (RHI) and the natural logarithm of the RHI (LnRHI) (to account for the non-normal distribution of RHI) (102). The finger-based peripheral artery tonometry device concurrently quantified arterial stiffness by measuring the augmentation index (AI) of the arterial waveforms in the right finger during baseline, averaged over more than 100 cardiac cycles. Further, due to the temporal relationship between heart rate and augmentation index (204), AI was also calculated at a controlled heart rate of 75 beats per minute (AI@75bpm).

Approximately 5 months later, patients underwent coronary reactivity testing using the Doppler guidewire method, as previously described (200, 206). In brief, a guiding catheter was advanced into the left anterior descending coronary artery (LAD), followed by the insertion of a 0.014-in pressure-temperature sensor-tipped Doppler guidewire as distal as possible into the coronary vessel. Simultaneously, the catheter and the Doppler guidewire measured coronary pressure at a proximal arterial segment (Pa) and distal arterial segment (Pd), respectively. Coronary blood flow was measured using the thermodilation method, where a 3 mL room temperature saline bolus was infused from the catheter to the Doppler guidewire. The time taken for the saline to arrive at the Doppler guidewire (transit time) was used to determine coronary flow (205). This was repeated in triplicate and averaged to obtain the mean transit time (Tmn). The fractional flow reserve (FFR) was collected to confirm patients did not have significant coronary epicardial stenosis. An FFR<0.80 was considered to be hemodynamically significant coronary epicardial stenosis, and if present, patients were excluded.

Measures of coronary pressure (i.e. Pa, Pd) and flow (i.e. Tmn) were collected during baseline and pharmacological-induced hyperemia. Two pharmacological stimuli were infused into the LAD in the following order; intravenous adenosine (140mcg/kg/min), and intracoronary acetylcholine (20μg followed by a 100μg slow injection over 90 seconds). Prior to infusion of subsequent pharmacological stimuli, baseline measures of pressure and flow were again collected to ensure hemodynamics returned to baseline, including resolution of any chest pain.

Two measures were calculated during infusion to diagnostically determine coronary microvascular function; 1) coronary flow reserve (CFR), and 2) index of microvascular resistance (IMR). CFR was calculated as baseline Tmn divided by the hyperemic Tmn. IMR was calculated as the product of the hyperemic Pd and the hyperemic Tmn (206). Patients were considered to have CMD if any of the following criteria were observed (151, 175, 181): 1) Adenosine IMR>25, 2) Adenosine CFR<2.0, 3) Acetylcholine IMR>30, 4) Acetylcholine CFR<1.5.

Data and Statistical Analysis

Anthropometric data, GXT data, coronary reactivity testing data, and RHPAT data were compared using a two by two factorial ANOVA. Levene's test of equality of variances was conducted prior to all ANOVA analyses. If a significant interaction was observed from the ANOVA, post hoc testing was conducted with the Bonferroni adjustment. The time between the GXT and the vascular assessment was compared between groups using an independent samples t-test. Medication regimen was grouped based on if subjects were taking; 1) any anti-hypertensive medications (i.e. one or more of the following: beta-adrenergic antagonist, angiotensin converting enzyme (ACE) inhibitor, angiotensin receptor blocker (ARB), diuretics, or a calcium channel blocker), and 2) any anti-cholesterol medications (i.e. one or more of the following: statin or cholesterol absorption inhibitors). Medication usages were compared between groups using Chi squared testing. Further, use of either medication (i.e. anti-hypertensive and anti-cholesterol medications) and fasted state were utilized as covariates for ANCOVA analysis.

Lastly, to investigate the effect of time between the GXT and the vascular assessment, Pearson correlations between the LnRHI and the time between tests in exercisers with or without CMD were conducted. The correlations between the LnRHI and the time between tests in both conditions were calculated and compared using a Fisher r-z transformation, calculated using an online calculator (VassarStats). All other statistical analyses were performed using IBM SPSS Statistics 23 (Armonk, NY). Parametric data is presented as Mean ± SD, while non-parametric data is presented as Count (%). Significance was defined as $P<0.05$.

Results

Between May 2017 and April 2019, 134 patients with suspected coronary microvascular dysfunction (CMD) attended the Cardiovascular Integrated Physiology Clinic at Southlake Regional Healthcare Centre for reactive hyperemia peripheral arterial tonometry (RHPAT) assessments. After exclusion of patients who experienced technical errors during RHPAT assessments (n=9), patients who did not receive a referral for coronary reactivity testing (n=20), patients who did not complete coronary reactivity testing (n=31), and patients who had hemodynamically significant coronary epicardial stenosis (n=6), the sample consisted of 27 patients with normal coronary microvascular function and 41 patients with CMD (Figure 1).

In patients with CMD, 26 patients did not exercise prior to assessment, and 15 patients exercised prior to assessment. In those without CMD, 16 patients did not exercise prior to assessment, and 11 patients exercised prior to assessment. There were no differences in anthropometrics, resting blood pressure, or fasted state between all groups ($P>0.05$; Table 1). Resting heart rate was elevated in exercisers compared to non-exercisers ($P=0.02$; Table 1). Prior to coronary reactivity testing, a greater proportion of patients with confirmed CMD were taking anti-hypertensive and anti-cholesterol medications compared to patients without CMD ($P<0.05$; Table 1). Patients with confirmed CMD had greater index of microvascular resistance (IMR) responses to both adenosine and acetylcholine ($P<0.001$; Table 2), and lower coronary flow reserve (CFR) responses to both adenosine and acetylcholine ($P<0.05$; Table 2) compared to patients without CMD. There was no difference in coronary microvascular function between non-exercisers and exercisers in both conditions ($P>0.05$; Table 2).

Five patients in both non-exerciser groups did not complete the graded exercise stress test at any time as a part of their normal clinical care (GXT; Table 3). In patients who were able to exercise, there were no differences in symptoms of exertional chest pain, exercise-induced myocardial ischemia or the Duke Treadmill Score between groups ($P>0.05$; Table 3). Although there was an interaction between groups and exercise duration (Interaction: $P=0.048$; Table 3), post hoc testing indicated that there were no significant differences in exercise duration between groups ($P>0.05$). In exercisers, the time between the GXT and the vascular assessment was similar between patients with and without CMD ($P>0.05$; Table 3).

Prior to the adjustment for pharmaceutical use and fasting, the RHI and LnRHI were lower in patients with confirmed CMD compared to patients without CMD ($P<0.01$), and

exercisers demonstrated an attenuated RHI and LnRHI compared to non-exercisers ($P<0.01$; Table 4). Augmentation indices (AI and AI@75bpm) were similar between patients with and without CMD ($P>0.05$), and exercisers demonstrated an attenuated AI compared to non-exercisers ($P<0.01$), while the AI@75bpm were similar between exercisers and non-exercisers ($P>0.05$, Table 4). After adjusting for pharmaceutical use and fasting, the RHI and LnRHI were still lower in patients with confirmed CMD compared to patients without CMD ($P<0.01$; Figure 2). Further, RHI and LnRHI were still lower in exercisers compared to non-exercisers ($P<0.01$; Figure 2). There were no exercise or condition effects in the adjusted AI and AI@75bpm ($P>0.05$; Figure 3).

Correlations of the time between tests and the LnRHI were conducted for exercisers with CMD, exercisers without CMD, and for all exercisers combined. In exercisers with confirmed CMD (n=15), there was a positive correlation ($r=0.57$, $P<0.05$), and in exercisers without CMD (n=11) a trend towards a positive correlation ($r=0.55$, $P=0.08$) was observed. Since there was no difference when comparing the correlation coefficients between patients with and without CMD ($P=0.95$), all exercisers were grouped and a positive correlation was observed ($r=0.54$, $P<0.01$; Figure 4).

Covariate analysis of the adjusted model (data not shown) found that there were no effects of anti-hypertensive medication use, anti-cholesterol medication use, or fasted state on RHI, LnRHI, or AI@75bpm ($P>0.05$). Further, anti-hypertensive medication use and anti-cholesterol medication use had no effect on AI ($P>0.05$), however, fasted state increased AI ($P=0.046$).

Discussion

The effect of acute exercise on peripheral microvascular function in patients with coronary microvascular dysfunction (CMD) was previously unknown. First, patients with CMD demonstrated impaired coronary and peripheral microvascular function. Second, consistent with studies in conduit arteries and healthy participants (224), acute exercise impaired microvascular function in patients with and without CMD. Third, contrary to our hypothesis, acute maximal exercise impaired peripheral microvascular function equally in patients with and without CMD.

Several studies have investigated the relationship between coronary microvascular function (using measurements of coronary flow) and RHPAT in cardiac patients. For example,

patients with abnormal coronary blood flow responses to acetylcholine and patients with non-obstructive coronary artery disease have lower RHI (197, 198). However, most recently, RHI was not associated with coronary flow reserve (CFR) in women with chest pain and non-obstructive coronary arteries (199). When additionally considering coronary resistance (i.e. IMR), a stronger predictor of adverse cardiac events (189) and a more hemodynamically stable measure of coronary microvascular function (200), our results suggest that patients with abnormal coronary function demonstrate an impaired RHI.

In the current study, all suspected CMD patients who exercised prior to RHPAT testing exhibited an attenuation of RHI and LnRHI, supporting previous work that peripheral endothelial function is attenuated following acute exercise (224, 242, 243). This was further supported by our observation that LnRHI was correlated with the time between tests in all patients. During high intensity exercise, oxidative stress is increased (244, 245), facilitating the reduction in endothelial function following acute exercise in health (246, 247) and disease (242). Since oxidative stress has been shown to be elevated in patients with CMD (248), we had hypothesized that baseline impairments in peripheral endothelial function in CMD patients would exacerbate post-exercise microvascular function compared to the non-CMD group, yet this was not observed. We suggest that measurements of oxidative stress should be added to future longitudinal investigations of the effect of exercise in CMD.

The use of RHPAT is a clinically appealing method of measuring peripheral microvascular function, given that RHPAT assessments are relatively cost efficient, operator independent, possess objective and automated analyses (240), and are highly reproducible (249, 250). However, the standardizations for RHPAT assessments have not been determined in health or disease. Our data shows that RHI and LnRHI were not influenced by pharmacological use or fasting. In particular, the observation that fasting did not demonstrate a significant influence is important for CMD patients since fasting prior to exercise could lead to hypoglycemia during their clinical visit and/or exercise stress test, given the high prevalence of diabetes mellitus (251, 252). Further, our data suggests that RHI and LnRHI are reduced by standard exercise stress testing, reducing the accuracy of RHPAT assessments in patients with suspected CMD. Notably, patients without CMD that exercised appeared to have similar peripheral microvascular function as patients with CMD who did not exercise. Therefore, our data suggests that when RHPAT

testing is performed in a clinical setting, exercise stress testing should not be performed beforehand.

Contrary to previous reports (171, 174), the present study observed that measures of arterial stiffness were similar in patients with and without CMD. Several studies observed that both the aortic augmentation index (174) and the carotid artery augmentation index (171) were associated with the CFR response to intravenous adenosine in patients with CMD, implying that arterial stiffness in the large, mainly elastic arteries, is elevated in patients with CMD. In the current study, augmentation index was obtained from the finger microvasculature using the EndoPAT device, suggesting that arterial stiffness in the peripheral vasculature is similar in both conditions. Further, the validity of the augmentation indices obtained from the EndoPAT device have yet to be compared to a gold-standard measurement such as applanation tonometry of conduit vessels.

We acknowledge several important considerations. First, this study was a cross-sectional study design. To fully elucidate the relationship between RHI and acute exercise, a repeated measures study design should be completed. However, to date, no study has investigated the relationship between RHI and exercise in patients with CMD, and these results provide strong evidence to continue this work. Second, since the time delay between the RHPAT assessment and the coronary reactivity testing was ~5 months, we cannot rule out that peripheral vascular function could have been altered within this time period due to changes in lifestyle or severity of condition, despite medication use being unchanged. Thirdly, although patients with and without CMD had similar exercise tolerances during the GXT (suggesting equal levels of cardiorespiratory fitness), this study did not objectively assess physical activity or oxygen consumption via a full cardiopulmonary exercise test. Lower physical activity has been linked with impaired endothelial function following acute exercise (243) potentially influencing our results. Therefore, future studies should obtain accurate measurements of fitness using accelerometry and oxygen consumption.

In conclusion, these data support our previous work that patients with confirmed CMD have impaired peripheral microvascular function compared to patients without CMD, and are the first data to suggest that exercise stress testing impairs peripheral microvascular function in all patients with suspected CMD equally. Further, in exercisers with and without CMD, there was a positive relationship between peripheral microvascular function and the time between exercise

testing and vascular assessment. Lastly, while longitudinal studies are recommended in the future to confirm our findings, we recommend that RHPAT measurements in patients with suspected CMD should not be conducted after exercise has been performed.

Grants

This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), Canadian Foundation for Innovation (CFI) and the Ontario Research Fund (ORF). M.N. was supported by a CIHR Fredrick Banting and Charles Best Canada Graduate Scholarship.

Disclosures

The authors declare no conflicts of interest relevant to the content of this study.

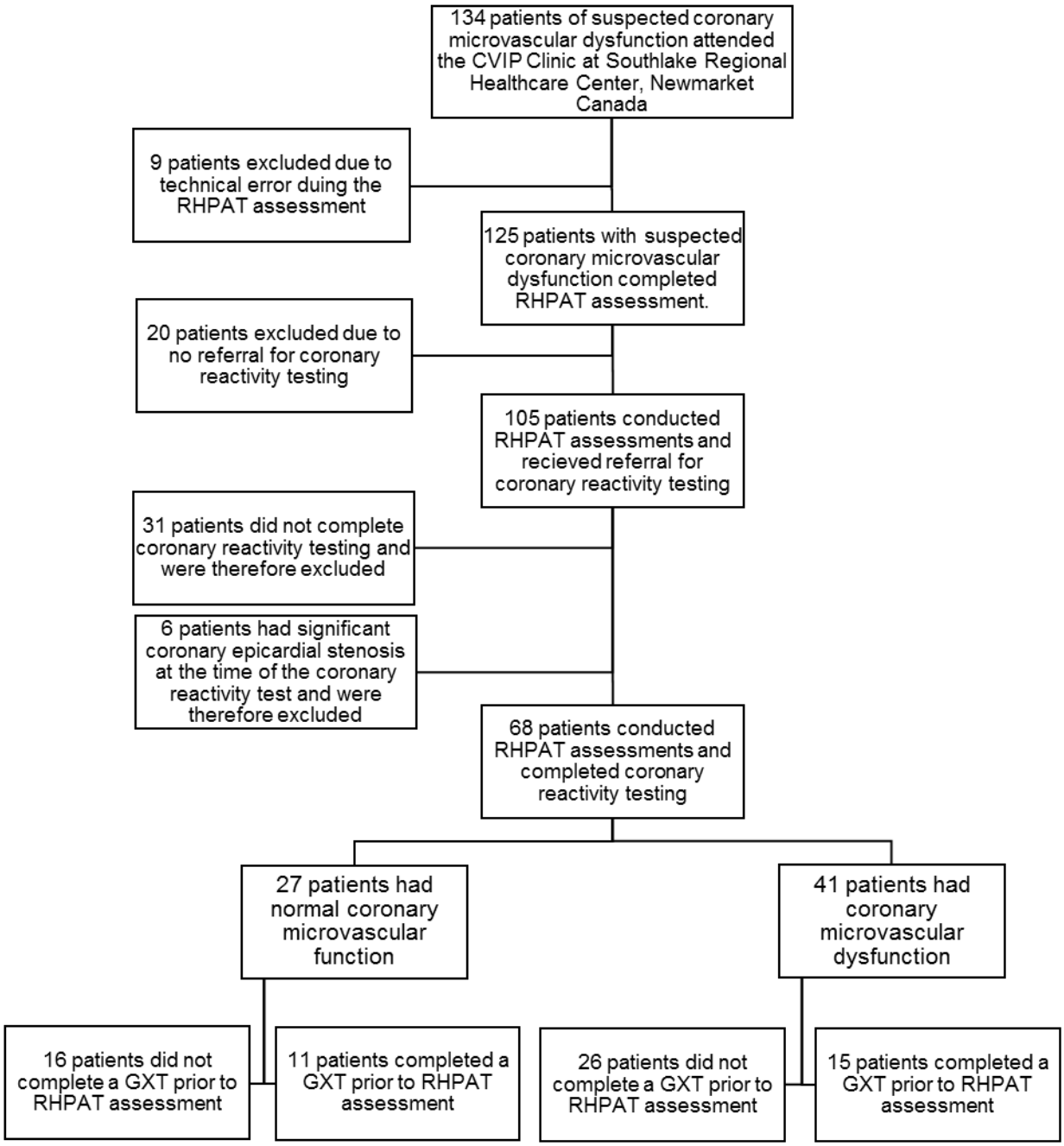
Figure Legends

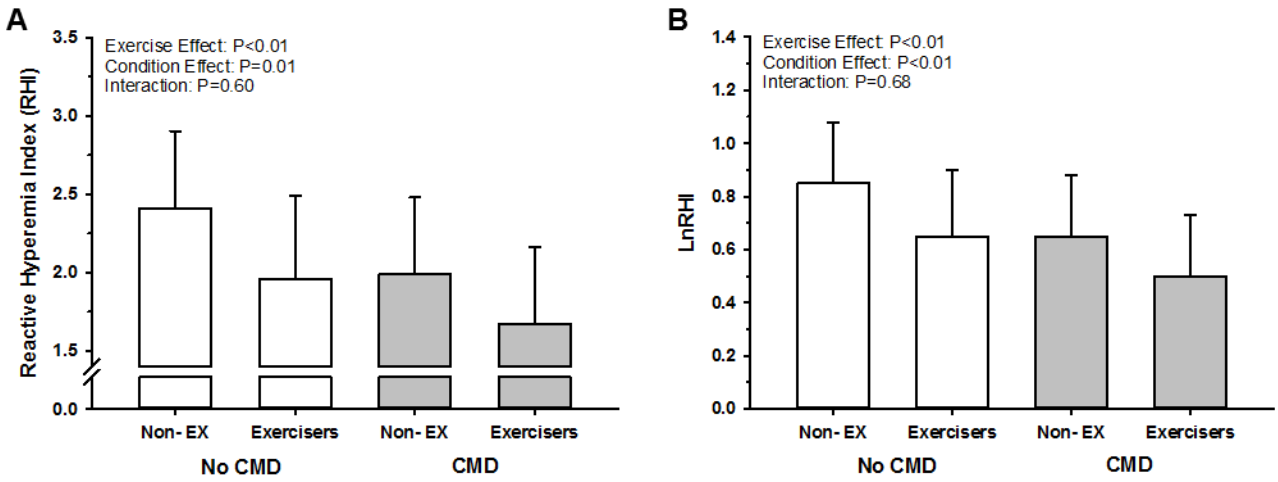
Figure 1: Schematic of patients recruited from the Cardiovascular Integrative Physiology Clinic (CVIP) at Southlake Regional Health Centre. RHPAT: Reactive Hyperemia Peripheral Arterial Tonometry; GXT Graded Exercise Test.

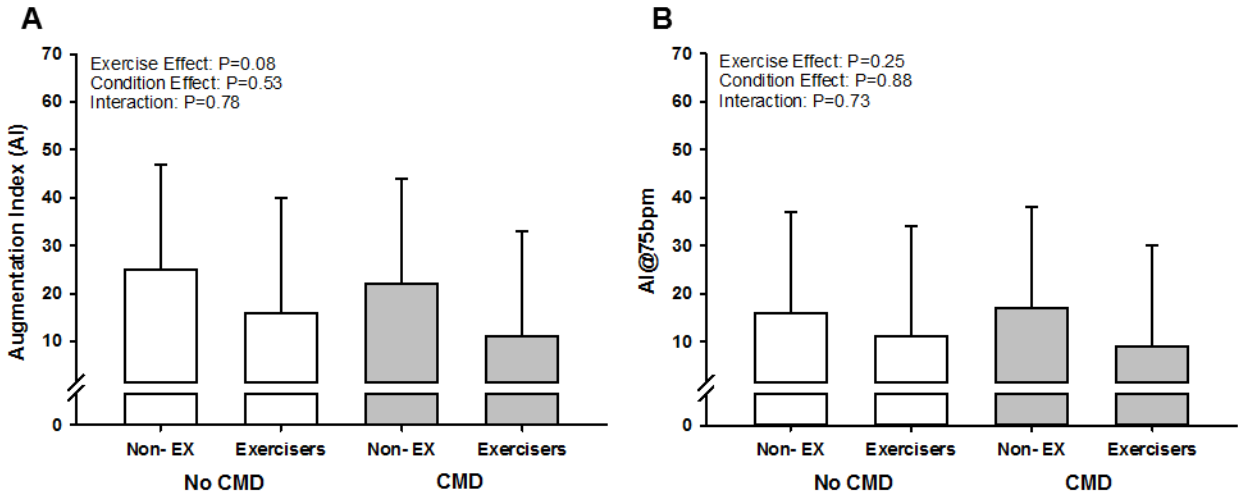
Figure 2: Adjusted reactive hyperemia index (RHI; A) and natural logarithm of the RHI (LnRHI; B) in patients with or without coronary microvascular dysfunction (CMD). Patients were separated into groups who did and did not exercise prior to vascular assessment. White bars represent patients without CMD. Grey bars represent patients with CMD. Non-EX indicates patients who did not exercise prior to assessment. Mean ± SD.

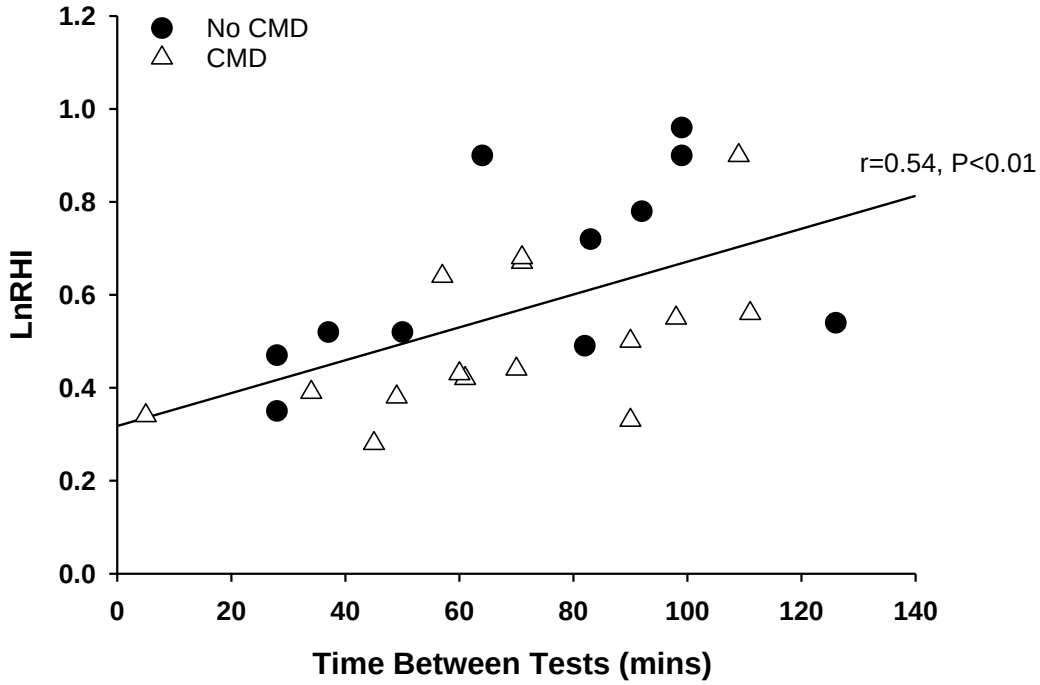
Figure 3: Adjusted augmentation index (AI; A) and augmentation index at 75 bpm (AI@75bpm; B) in patients with or without coronary microvascular dysfunction (CMD). Patients were separated into groups who did and did not exercise prior to vascular assessment. White bars represent patients without CMD. Grey bars represent patients with CMD. Non-EX indicates patients who did not exercise prior to assessment. Mean ± SD.

Figure 4: Correlation between the natural logarithm of the reactive hyperemia index (LnRHI) and the time between the graded exercise stress test and the vascular assessment in patients who exercised prior to the vascular assessment. Black circles represent patients without coronary microvascular dysfunction (CMD). White triangles represent patients with CMD.


134 patients of suspected coronary microvascular dysfunction attended the CVIP Clinic at Southlake Regional Healthcare Center, Newmarket Canada
9 patients excluded due to technical error duing the RHPAT assessment
125 patients with suspected coronary microvascular dysfunction completed RHPAT assessment.
20 patients excluded due to no referral for coronary reactivity testing
105 patients conducted RHPAT assessments and recieved referral for coronary reactivity testing
31 patients did not complete coronary reactivity testing and were therefore excluded
6 patients had significant coronary epicardial stenosis at the time of the coronary reactivity test and were therefore excluded
68 patients conducted RHPAT assessments and completed coronary reactivity testing
27 patients had normal coronary microvascular function
41 patients had coronary microvascular dysfunction
16 patients did not complete a GXT prior to RHPAT assessment
11 patients completed a GXT prior to RHPAT assessment
26 patients did not complete a GXT prior to RHPAT assessment
15 patients completed a GXT prior to RHPAT assessment


A
Exercise Effect: P<0.01
Condition Effect: P=0.01
Interaction: P=0.60
Reactive Hyperemia Index (RHI)
3.5
3.0
2.5
2.0
1.5
0.0
Non- EX
Exercisers
Non- EX
Exercisers
No CMD
CMD
B
Exercise Effect: P<0.01
Condition Effect: P<0.01
Interaction: P=0.68
LnRHI
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Non- EX
Exercisers
Non- EX
Exercisers
No CMD
CMD


A
Exercise Effect: P=0.08
Condition Effect: P=0.53
Interaction: P=0.78
Augmentation Index (AI)
70
60
50
40
30
20
10
0
Non- EX
Exercisers
Non- EX
Exercisers
No CMD
CMD
B
Exercise Effect: P=0.25
Condition Effect: P=0.88
Interaction: P=0.73
AI@75bpm
70
60
50
40
30
20
10
0
Non- EX
Exercisers
Non- EX
Exercisers
No CMD
CMD


No CMD
CMD
r=0.54, P<0.01
LnRHI
Time Between Tests (mins)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
140

Tables

Table 1: Anthropometrics, hemodynamics, medication use, and fasted state in patients with or without coronary microvascular dysfunction (CMD).

	No CMD		CMD	
	Non-EX	Exercisers	Non-EX	Exercisers
n	16	11	26	15
Sex, male, n (%)	5 (31)	4 (36)	10 (38)	7 (47)
Age (years)	54 ± 12	55 ± 17	62 ± 13	57 ± 8
Height (m)	1.66 ± 0.11	1.71 ± 0.07	1.67 ± 0.08	1.69 ± 0.14
Weight (kg)	77.5 ± 20.7	83.7 ± 20.4	78.4 ± 13.8	80.1 ± 16.3
BMI (kg/m^2)	28 ± 6	29 ± 7	28 ± 4	28 ± 3
SBP (mmHg)	119 ± 15	121 ± 12	126 ± 15	121 ± 9
DBP (mmHg)	75 ± 9	75 ± 9	77 ± 9	75 ± 7
Resting HR (bpm)	62 ± 9	71 ± 12*	65 ± 13	71 ±14*
Medication use, n (%)				
Anti-Hypertensive†	9 (56)	6 (55)	22 (85)	12 (80)
Anti-Cholesterol†	7 (44)	2 (18)	17 (65)	10 (67)
Fasted, n (%)	3 (19)	0 (0)	7 (27)	3 (20)

Non-EX: Non-Exercisers; CMD: Coronary Microvascular Dysfunction; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; HR: Heart Rate; BMI: Body Mass Index. * indicates a significant exercise effect. † indicates a significant effect of CMD. Mean ± SD.

Table 2: Coronary flow and resistance responses to coronary infusions of adenosine and acetylcholine in patients with or without coronary microvascular dysfunction (CMD) undergoing coronary reactivity testing.

	No CMD		CMD	
	Non-EX	Exercisers	Non-EX	Exercisers
Adenosine				
IMR	14.1 ± 3.6	17.4 ± 4.8	23.7 ± 12.5	29.0 ± 14.1
CFR	4.4 ± 2.0	3.1 ± 1.0	2.8 ± 1.9	3.0 ± 1.2
Acetylcholine				
IMR	19.9 ± 5.2	17 ± 4.6	41.2 ± 19.7	45.8 ± 23.4
CFR	2.9 ± 1.8	3.2 ± 0.9	1.5 ± 0.7	2.2 ± 1.8

Non-EX: Non-Exercisers; CMD: Coronary Microvascular Dysfunction; CFR: Coronary Flow Reserve; IMR: Index of Microvascular Resistance. Mean ± SD.

Table 3: Symptoms-limited maximal exercise testing data in patients with or without coronary microvascular dysfunction (CMD).

	No CMD		CMD	
	Non-EX	Exercisers	Non-EX	Exercisers
n	11	11	21	15
Time between test (mins)		72 ± 33		68 ± 29
Exercise duration (mins)	8.91 ± 2.06	6.92 ± 2.68	7.11 ± 3.15	8.13 ± 2.57
Chest pain symptoms (%)	2 (18)	6 (55)	10 (48)	6 (40)
ST Depression (%)	7 (64)	7 (64)	11 (52)	7 (47)
DTS	3 ± 5	1 ± 5	1 ± 7	2 ± 5

Non-EX: Non-Exercisers; CMD: Coronary Microvascular Dysfunction; DTS: Duke Treadmill Score. No significant effect of exercise or CMD for all variables (All $P>0.05$). Mean ± SD. Count (%).

Table 4: Unadjusted reactive hyperemia index (RHI), natural logarithm of the RHI (LnRHI), augmentation index (AI), and augmentation index at 75 bpm (AI@75bpm) in patients with or without coronary microvascular dysfunction (CMD). Patients were separated into groups who did and did not exercise prior to vascular assessment. Mean ± SD.

	No CMD		CMD	
	Non-EX	Exercisers	Non-EX	Exercisers
RHI†	2.43 ± 0.55	1.95 ± 0.41*	1.96 ± 0.55	1.67 ± 0.30*
LnRHI†	0.86 ± 0.23	0.65 ± 0.21*	0.64 ± 0.26	0.50 ± 0.17*
AI	25 ± 21	13 ± 19*	23 ± 25	11 ± 18*
AI@75bpm	17 ± 19	11 ± 15	17 ± 25	8 ± 16

Non-EX: Non-Exercisers; CMD: Coronary Microvascular Dysfunction; RHI: Reactive Hyperemia Index; LnRHI: Natural logarithm of the RHI; AI: Augmentation Index; AI@75bpm: Augmentation Index at 75 bpm. * indicates a significant exercise effect. † indicates a significant effect of CMD. Mean ± SD.