Microvascular function variation in a healthy population:

Insights from Oxygenation-Sensitive CMR

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1. Abbreviation Glossary

CAD: Coronary Artery disease
CMD: Coronary microvascular disease
CMR: Cardiovascular Magnetic Resonance Imaging
OS-CMR: Oxygenation-Sensitive Cardiovascular Magnetic Resonance Imaging
BOLD: Blood Oxygen Level Dependent
MORE: Myocardial oxygenation reserve
B-MORE: Breathing Induced Myocardial Oxygenation Reserve
CBF: Coronary blood flow
VSMCs: Vascular smooth muscle cells
CAD: Coronary artery disease
ROS: Reactive oxygen species
CVD: Cardiovascular disease
TTDE: Transthoracic doppler echocardiography
CT: Computed tomography
MBF: Myocardial blood flow
PET: Positron emission tomography
MRI: Magnetic resonance imaging
RF: Radiofrequency
MPRI: Myocardial perfusion reserve index
SSFP: Steady-state free precession
SPECT: single-photon emission computerized tomography
FFR: Fractional Flow Reserve
INOCA: Ischemia without obstructive coronary artery disease
CO_2: Carbon dioxide:
T: Tesla

2. Abstract- English

Microvascular dysfunction has significant impacts on patient prognosis, even in absence of obstructive coronary artery disease, and may predict future cardiovascular events. Factors like age, sex, and body composition contribute to vascular dysfunction over the lifetime. In otherwise healthy individuals, the impact of these factors is unclear.

Oxygenation-sensitive cardiovascular magnetic resonance imaging (OS-CMR) is a validated methodology to examine vascular function which exploits the blood oxygen level dependent effect, in which deoxygenated hemoglobin acts as an innate contrast agent in a magnetic field. Through varied OS-CMR signal intensities, regional tissue oxygenation changes during vasodilation are detected. Previous research utilizing OS-CMR observed blunted vascular function in many cardiovascular pathologies. Vasoactive breathing maneuvers, a period of paced hyperventilation followed by a voluntary maximal breath hold, are an endogenous alternative to pharmaceutical vasodilation in OS-CMR. Carbon dioxide variations, inducing vasoconstriction during hyperventilation and vasodilation during the breath hold, act on endothelial cells to modulate vascular tone. The breathing-induced myocardial oxygenation reserve (B-MORE) describes the myocardial oxygenation response during breathing maneuvers. This endothelial-dependent mechanism may provide a valuable assessment of microvascular function, based on meaningful biomarkers instead of secondary surrogate markers such as blood flow or tracer uptake. Previous work demonstrated a transmural gradient of vascular function during endothelial-independent adenosine vasodilation in subjects with risk factors but no cardiovascular disease. In a similar cohort, breathing maneuvers may provide valuable insight into microvascular dysfunction before the onset of cardiovascular disease.

The thesis aims to investigate tissue oxygenation variation as a marker of microvascular function in healthy subjects, providing a better understanding of microvascular dysfunction pathophysiology in such populations. The included study retrospectively assessed the impact of demographic factors on global and regional B-MORE in healthy adults from 4 prospective OS-CMR studies in Montreal, Canada. We hypothesize that demographic factors will impart slight changes to microvascular function across the lifetime, differentially impacting B-MORE.

This thesis includes the first study investigating factors impacting B-MORE in healthy subjects. Several factors differentially impacted microvascular function within the cohort. This indicates that in a healthy population, sex and body composition, including height and body size analysis, are associated with microvascular function and thus lead to baseline value variations. Female sex-specific risk factors may be associated with reduced function (lower B-MORE values). Further research is needed to fully understand the impact of healthy aging on the microvasculature over the lifetime, as it may be impacted by factors not assessed in this study.

Future work should investigate the prognostic value and utility of B-MORE as a marker for microvascular function. Blunted B-MORE may indicate an increased risk for cardiovascular events. As breathing maneuvers employ endothelial-dependent vasodilation it is possible that slight B-MORE variations seen in healthy subjects reflect the biological spectrum of microvascular (endothelial) function. If validated by the invasive reference standards of microvascular function, this methodology could provide a non-invasive and informative test of microvascular function and tissue status. Given the relationship of B-MORE to factors affecting the vasculature in healthy individuals, it appears to be a sensitive

marker to detect microvascular dysfunction at an early, subclinical stage. A methodology of this nature may be instrumental in diagnosing early microvascular disease, improving the prognosis of those at risk of ischemic heart disease.

3. Abstract- Français

Le dysfonctionnement microvasculaire est important pour le pronostic des patients, même en l'absence de maladie coronarienne obstructive, et peut prédire les événements cardiovasculaires. L'âge, le sexe, et la composition corporelle contribuent au dysfonctionnement vasculaire. Chez les individus par ailleurs en bonne santé, l'impact de ces facteurs n'est pas clair.

L'imagerie par résonance magnétique cardiovasculaire sensible à l'oxygénation (OS-CMR) est une méthode validée pour voir la fonction vasculaire, dans lequel l'hémoglobine désoxygénée agit comme agent de contraste dans un champ magnétique. Les variations du signal OS-CMR représentes les changements d'oxygénation des tissus pendant la vasodilatation. Des études utilisant l'OS-CMR ont observé une fonction vasculaire réduite dans plusieurs pathologies cardiovasculaires. Les manœuvres de respiration vasoactive, composées d'une période d'hyperventilation suivie d'une retenue du souffle, sont une alternative endogène à la vasodilatation pharmaceutique dans l'OS-CMR. Le dioxyde de carbone, induisant une vasoconstriction pendant l'hyperventilation et une vasodilatation pendant l'apnée, agissent sur les cellules microvasculaires endothéliales pour moduler le tonus vasculaire. La réserve d'oxygénation du myocarde induite par la respiration (B-MORE) décrit l'oxygénation du myocarde au cours des manœuvres respiratoires. Ce mécanisme endothélial est peut-être une évaluation directe de la fonction microvasculaire. Des travaux antérieurs ont observé un gradient transmural de fonction vasculaire réduite chez des sujets

avec des facteurs de risque mais non la maladie cardiovasculaire, lors d'une vasodilatation à l'adénosine. L'application de manœuvres respiratoires à une cohorte similaire pourrait fournir des informations précieuses sur le dysfonctionnement microvasculaire avant l'apparition de la maladie cardiovasculaire.

L'objectif de cette thèse est d'étudier l'oxygénation des tissus chez des sujets sains comme marqueur de la fonction microvasculaire, permettant de mieux comprendre la pathophysiologie de la dysfonction microvasculaire. L'étude incluse dans cette thèse a évalué rétrospectivement l'impact des facteurs démographiques sur la B-MORE globale et régionale chez des adultes en santé provenant de 4 études prospectives OS-CMR, à Montréal, Canada. Nous émettons l'hypothèse que des facteurs démographiques entraîneront de légers changements dans la fonction microvasculaire au cours de la vie, ce qui aura un impact différentiel sur la B-MORE.

Cette thèse comprend la première étude étudiant les variables impactant le B-MORE au sujets sains. Plusieurs facteurs ont eu un impact différentiel sur la fonction microvasculaire dans la population saine, comme le sexe et la composition corporelle, y compris l'analyse de la taille et de la corpulence. Ces résultats pourraient améliorer la compréhension de la physiologie microvasculaire. Les risques spécifiques au sexe féminin peuvent être associés à une réduction du B-MORE. Des recherches supplémentaires sont nécessaires pour mieux comprendre l'impact de vieillissement sur la microvasculature, car elle peut être influencée par des facteurs non évalués dans cette étude.

La pronostique de le B-MORE doivent être étudier. Un B-MORE réduit indique peut-être un risque d'événements cardiovasculaires. Comme la méthodologie utilise un

vasodilatation endothéliale-dépendante, les variations en B-MORE reflètent peut-être des anomalies endothéliales. Si c'est valider contre les références invasives, cette méthodologie pourrait devenir un test non invasif et informatif de la fonction microvasculaire. Comme c'est sensible aux facteurs affectant le système vasculaire chez les individus sains, cette méthodologie est probablement sensible au dysfonctionnement précoce. Une méthodologie comme ça pourrait être utile pour diagnostiquer la maladie microvasculaire précoce, améliorant ainsi le pronostic des personnes à risque de cardiopathie ischémique.

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5. Thesis Overview

This body of work is divided into 3 chapters. The main objective of this work is to improve the understanding of the pathophysiology of microvascular dysfunction over the lifetime, by examining tissue oxygenation variation in healthy subjects as a marker of microvascular function. Chapter 1 summarizes the physiology of microvascular function and coronary microvascular disease, reviews the methodology behind cardiac magnetic resonance imaging, and summarizes existing research and limitations of oxygenation-sensitive cardiac magnetic resonance. Chapter 2 is an original research manuscript investigating the variation in vascular function seen in healthy individuals, through the breathing induced myocardial oxygenation reserve viewed with oxygenation-sensitive cardiac magnetic resonance. Chapter 3 will summarize the previous chapters, contextualizing their implications in the context of clinical cardiovascular research.

6. Contribution of Authors

Chapter 1:

Kate Lindsay (KL) was the primary writer and editor of the chapter. Dr. Elizabeth Hillier (EH) and Dr. Matthias G. Friedrich (MGF) contributed by editing the text after the first draft was completed by KL.

Chapter 2:

This original research manuscript is being prepared for future submission and publication with KL as the first author. KL was the primary writer and editor of the chapter. KL was involved in writing the study protocols for ethics submission, study design, data organization and statistical analysis, interpretation of data, figure and table creation, as well as development, writing, and editing of the manuscript. Elisavet Konidis contributed by revising study protocols for ethics submission after their first draft was completed by KL. EH contributed by analysing the CMR images retrospectively accessed for this work, conceptual design of the study, and editing of the manuscript after the first draft was completed by KL. MGF was involved in conceptual design of the study and editing of the manuscript after the first draft was completed by KL. Dr. Judy Luu (JL) contributed by editing the manuscript after the first draft was completed by KL.

Chapter 3:

KL was the primary writer and editor of the chapter. JL and MGF contributed by editing the text after the first draft was completed by KL.

7. Chapter 1: Introduction and OS-CMR Literature Review

7. a. Summary

The microvasculature is an essential part of the cardiovascular system, adapting to stressors and the functional demands of the heart. Over time, cardiovascular risk factors impart structural, molecular, and functional alterations to the microvasculature, progressively inducing dysfunction. Microvascular dysfunction has significant impacts on patient symptoms, prognosis, and cardiovascular event risk. Factors such as age, sex, and body composition impart subclinical deficits to microvascular function over the lifetime, though their impact in otherwise healthy individuals is unclear. Oxygenation-sensitive cardiovascular magnetic resonance imaging (OS-CMR) is a validated non-invasive methodology to examine vascular function. Previous research utilizing OS-CMR has observed a blunted microvascular oxygenation reserve during pharmaceutical vasodilation in several cardiovascular pathologies, including those with cardiovascular risk factors but no overt cardiovascular disease. Vasoactive breathing maneuvers, composed of hyperventilation followed by a voluntary maximal breath hold, offer an endogenous alternative to pharmaceutical vasodilation due to the vasodilatory properties of carbon dioxide. Unlike adenosine, breathing maneuvers induce endothelial-dependent vasodilation, and thus, in conjunction with OS-CMR, offer a true assessment of microvascular physiology. Though novel, and not without limitations, OS-CMR in conjunction with breathing maneuvers is a safe and efficient methodology to assess vascular function and tissue oxygenation status. Further validation could establish this non-invasive methodology as an informative assessment of microvascular function.

7. b. Introduction

Diagnostic testing is key in the prevention of ischemic heart disease, the leading cause of mortality and morbidity worldwide (1). However, a significant proportion of patients with suspected coronary artery disease (CAD) show normal or non-obstructive coronary arteries on coronary angiography (2). Nonetheless, this population has significant symptoms and increased cardiovascular risk, for which microvascular abnormalities are likely responsible (3,4). It is now known that microvascular dysfunction, independent of obstructive CAD, is an important component of ischemic heart disease, modulator of disease burden, and predictor of patient outcomes (5). As such, diagnostic testing capable of visualizing deficits in microvascular function is important in preventing all manifestations of ischemic heart disease.

Coronary microvascular disease (CMD) is an overarching term which encompasses all pathologies affecting the coronary microvasculature (5). Inflammatory, endocrine, and epigenetic pathways can encourage CMD over the lifetime, driving dysfunction of endothelial and vascular smooth muscle cells (6). Cardiovascular risk factors and underlying cardiomyopathies may contribute to the adverse vascular remodeling observed in CMD (6). The structural and functional alterations seen in CMD may be initial warnings of the burden of cardiovascular risk factors on the microvasculature, visible before epicardial disease (7). CMD has significant impacts on patient symptoms, prognosis, and cardiovascular event risk. Women with CMD are at a greater risk of symptoms, comorbidities, and poor prognosis, despite being less likely than men to present with obstructive CAD, possibly due to female-sex specific risk factors acting in conjunction with traditional cardiovascular risk factors (5,8,9). These findings highlight the need for a deeper understanding of the impact of cardiovascular risk factors on microvascular function.

The microvasculature directly maintains organ function through the shuttling of oxygen and essential nutrients to tissue, interacting with nearly every living cell in the body (10,11). CMD manifests through metabolic, inflammatory, and structural changes that impair the vascular response to stressors (5). This dysfunction appears to precede overt injury in vascular pathologies like cardiovascular disease (CVD) (5,12). Microvascular dysfunction may act as a key early marker of future vascular disease, due to cardiometabolic risk factors such as hypertension, obesity, and diabetes mellitus (5,12). As such, the understanding of microvascular function, and early detection of its dysfunction, is important in preventing adverse vascular outcomes across the lifetime.

Cardiovascular magnetic resonance imaging (CMR) is a non-invasive imaging modality useful in assessing vascular function. It has high spatial resolution, and can measure several parameters, such as tissue characterization, within one exam (13–15). First-pass perfusion CMR offers a semi-quantitative assessment of vascular function through measures of myocardial blood flow at rest and during pharmaceutical stress (4,16,17). However, contraindications to gadolinium and pharmaceutical stress agents can limit its use in some patient populations (18).

A novel non-invasive method to assess vascular function without exogeneous contrast agents is oxygenation-sensitive cardiovascular magnetic resonance imaging (OS-CMR). The paramagnetic properties of deoxygenated hemoglobin in blood allow it to act as an endogenous contrast agent, creating inhomogeneities in the local magnetic field and thus altering signal intensity when its concentration is varied (19,20). Known as the blood oxygen level dependent (BOLD) effect, this property can be exploited to detect regional tissue

oxygenation changes visible on OS-CMR images. In the heart, signal intensity changes reflect the oxygen supply and demand balance of the myocardium, represented by the myocardial oxygenation reserve (MORE) (20). MORE, the ratio of myocardial oxygenation during stress and at baseline, is reduced in several cardiovascular pathologies, indicative of vascular dysfunction. Notably, MORE deficits during adenosine stress have been observed in subjects with cardiovascular risk factors but no clinical disease, demonstrating MORE's sensitivity to the early impacts of risk factors on the vasculature (21).

Recent work has identified standardized vasoactive breathing maneuvers, hyperventilation followed by a voluntary maximal breath hold, as a suitable alternative to pharmaceutical vasodilation in OS-CMR due to the vasodilatory properties of carbon dioxide (22,23). This methodology shows a greater sensitivity to myocardial oxygenation changes than adenosine, while reducing adverse effects (24). Carbon dioxide is an endothelial-dependent vasodilator, unlike adenosine (25,26). Acting on the endothelial cells which line the microvasculature, it may provide direct insights into microvascular physiology and function, visualized with OS-CMR. Breathing induced MORE (B-MORE), describing the tissue oxygenation response to breathing maneuvers, is sensitive to inducible ischemia, and has been observed to be blunted in several patient populations (27–31), and animal models of CAD (32). Notably, women with ischemia without obstructive CAD show a heterogeneous response to breathing maneuvers during OS-CMR, likely reflecting underlying microvascular dysfunction (29). While novel, OS-CMR with breathing maneuvers is a safe and informative test of vascular function, validated in detecting inducible ischemia, that shows great promise in assessing microvascular dysfunction.

7. c. Physiology and Function of the Microvasculature

The microvasculature, representing the smallest vessels of the circulatory system, directly supports organ function through the transfer of oxygen and nutrients to tissues, making it an essential component of the cardiovascular system (11). The coronary microvasculature adapts to the functional demands of the heart to ensure adequate perfusion both, during resting conditions and during periods of higher demand (5,33). Malfunction of the microvascular system may result in a disbalance caused by an insufficient adjustment of coronary blood flow (CBF) during stress or, even under resting conditions, resulting in a cellular lack of oxygen, i.e. myocardial ischemia (5,33).

Microvascular dysfunction, though often excluded in historical definitions of heart disease which focused on epicardial atherosclerosis, plays a significant role in modulating cardiovascular event risk, prognosis, and symptoms (5). This is likely due to the microvasculature's essential role in modulating CBF through the capillaries to meet the functional needs of the myocardium. Coronary microvascular disease (CMD) refers to pathologies specifically affecting the microvasculature (5). It has been suggested that CMD may act as a window into the risk of future cardiovascular events (34) and may be the first indicator of CVD risk, well before the manifestation of detectable coronary artery stenosis (7).

CMD manifests through structural changes, known as microvascular remodeling, and a spectrum of functional abnormalities (5). Structural changes, typically narrowing of microvascular vessels, have been linked to cardiovascular risk factors such as hypertension and diabetes (1,5,35). Functional abnormalities, on the other hand, mainly manifest through dynamic endothelial dysfunction, impairing smooth muscle function in the presence of

physiological or pharmacological stress (5). In healthy microvasculature, the endothelium modulates smooth muscle function through release of vasodilatory compounds, primarily nitric oxide, which increase CBF and thereby myocardial perfusion during stress (5). In CMD, endothelial dysfunction attenuates this response, resulting in blunted CBF augmentation, or even vasoconstriction, during stress (36). Vascular smooth muscle cells (VSMCs), which regulate arterial tone, can also show functional abnormalities in CMD, independent of the endothelium. Such changes have been shown to lead to a blunted vasodilation during pharmaceutical stress targeting VSMCs, notably in populations at risk of CVD such as metabolic syndrome (37) and hypertension (38). Other abnormalities include acetylcholine-induced spasms of the epicardium or microvasculature, in roughly half of patients with angina but no CAD, which may be related to endothelial dysfunction and atherosclerosis (39,40).

The origins of CMD are still under investigation, though molecular, functional, and structural abnormalities all play a role (6). Inflammation with molecular oxidative stress is likely a key driver of CMD pathogenesis, resulting from reactive oxygen species (ROS) (41). Increased intracellular ROS inhibits the endothelial production of vasodilatory substances, promoting vasoconstriction (42). On a functional level, impaired dilation and increased constriction caused by endothelium-dependent or independent mechanisms are likely responsible for CMD (6,33,43). Endothelial-independent mechanisms, however, are less well understood, but likely also involve impaired VSMC relaxation. This deficit may be due to increased vasoconstrictors, overreactions to normal stimuli, or abnormal sympathetic activity (6). On a structural level, alterations due to underlying cardiomyopathies, or the inflammatory impact of metabolic disease, may be responsible. Adverse remodeling, through pathways such as smooth muscle hypertrophy and increased collagen deposition, alters CBF as microvascular

lumina narrow and fibrosis develops (44,45). These abnormalities are well-documented in cases of hypertensive heart disease and hypertrophic cardiomyopathy (44,45).

CMD and associated abnormalities do not exist in a vacuum. The pathophysiology of the microvasculature is intrinsically linked to macrovascular, i.e. epicardial, disease (5). In fact, patients may concurrently have macrovascular and CMD, complicating its recognition and diagnosis (5,46). If CMD is undiagnosed, it can lead to inappropriate clinical decisions such as with-holding medication or unnecessary revascularization procedures; this may worsen patient prognosis. Indeed, recent research acknowledges that CMD is an important predictor of cardiovascular outcomes, independent of epicardial disease, and should not be ignored (5,34,47–49). These adverse cardiovascular outcomes are observed in both men and women with CMD. However, women are at a greater risk of symptoms, comorbidities, and poor prognosis, possibly due to specific female risk factors in conjunction with traditional CVD risk factors, despite data showing that they are less likely than men to have obstructive CAD (5,9). As such, the abnormalities that characterize CMD may have a stronger prognostic value in women, especially in those with vascular risk factors (5). These findings highlight the need for a deeper understanding of microvascular function variations with vascular risk factors across the lifespan. However, a major impediment in the assessment of microvascular function are difficulties of its verification or exclusion in clinical settings. Traditional methods to assess epicardial disease in-vivo cannot visualize the microvasculature (13). Therefore, to diagnose CMD, a direct interrogation of microvascular function is required (5). To meet this need, several imaging modalities to assess microvascular function have been proposed and validated in recent years.

7. d. Assessment of Vascular Function

Though formerly exclusive to invasive, catheterization-based methods, microvascular function can now be assessed non-invasively. Unlike in epicardial disease, the small vessels of the microvasculature cannot be imaged in vivo with coronary angiography or intracoronary imaging due to limits of reach and spatial resolution (13). To clinically assess CMD, indirect measurements of CBF are used. To do so, the microvascular response to vasodilatory stimuli is assessed, measuring CBF at baseline and during maximal vasodilation. The vasodilatory stimulus, usually pharmaceutical, is selected to target either VSMCs, in endothelium-independent vasodilation, or endothelial cells themselves, in endothelium-dependent vasodilation (13).

Non-invasive methods include transthoracic doppler echocardiography (TTDE), myocardial contrast echocardiography, dynamic myocardial perfusion computed tomography (CT), positron emission tomography (PET), and CMR (13). TTDE measures CBF velocity as an indirect measure of CBF, at baseline and during vasodilator stimuli, by pulsed wave doppler echocardiography. TTDE is a cost-effective method that can be used at the patient bedside (13). However, evidence so far is limited to its use in the left anterior descending artery, the method is limited in patients with a limited acoustic window or patients with obesity, and is highly dependent on operator experience (5,13). Myocardial contrast echocardiography utilizes the properties of microbubbles with air administered intravenously as a contrast agent, to assess myocardial blood flow (MBF). Though it is cost-effective, widespread use is limited. In addition to being operator-dependent, major adverse events due to microbubbles occur in some patients, it is difficult to maintain the image plane during microbubble replenishment, and image quality is often compromised due to several microbubble variables (13,50,51). PET is a technique which acquires images using specific radionuclide tracers to

reveal tissue function, and it shows exceptional sensitivity and specificity to study the microcirculation. Its greatest advantage is that it obtains a quantitative MBF measurement, before and during stress, and it can assess global and regional microvascular function (13). However, PET is expensive, time-consuming, has a poor spatial resolution, and relies on the use of radioactive material with associated risks for patients (13,52). Dynamic CT acquires multiple images as iodinated contrast moves through the myocardium, estimating myocardial blood flow and providing a functional assessment of the coronary arteries and myocardium (5). Limitations include its low temporal resolution and exposure of both patients and physicians to ionizing radiation. Lastly, CMR is a promising methodology to assess vascular function. It shows a high spatial resolution, is non-invasive, and allows measures of several parameters, such as tissue morphology, in the same exam (14,15). Its methodology in assessing vascular function will be further described in Section 7.f. Notably, the innate principles of magnetic resonance imaging (MRI) allow CMR to acquire images without ionizing radiation, further differentiating this modality from PET and CT. As such, CMR has the potential to assess CMD non-invasively, while overcoming the common limitations of other imaging modalities.

7. e. Principles of MRI

MRI is based on the principles of nuclear magnetic resonance technology and the innate properties of hydrogen nuclei, which act like small magnets with positive and negative poles. Hydrogen nuclei, throughout the human body, are aligned randomly in free conditions. However, when placed into magnetic fields, like that of an MRI scanner, these nuclei align in the direction of that magnetic field. Put simply, magnetic resonance signals are collected from hydrogen in body tissues and transformed into visible images (53). This is advantageous as it avoids the use of ionizing radiation, known to have deleterious effects on the heart (54).

The magnetic field of the MRI scanner is used to align the hydrogen nuclei in the body. High energy radiofrequency (RF) pulses are used in bursts to push the nuclei out of their alignment, and into an excited state of resonance (53). After the RF pulse is turned off, the nuclei realign in their non-excited, equilibrium state, while emitting an electromagnetic signal. MRI scanner hardware receives this signal and utilizes spatial encoding gradients to store signal data in what's known as a k-space matrix (55). Fourier transformations are then used to mathematically process the k-space matrix and produce an interpretable MRI image (55). In the resulting image, the distribution of known signal intensities within anatomical structures allows clinicians to identify their components, including fat, pathologic abnormalities, edema, or fibrosis (15,56,57).

The realignment of the nuclei to their equilibrium, once the RF pulse is turned off, is characterized by two parameters: T1 (longitudinal) and T2 (transverse) relaxation. These parameters describe tissue presence and type, providing MRI with very good soft-tissue contrast (58). As different tissues have different relaxation times, they can be quantified accurately using T1 or T2 imaging. T1 quantifies the longitudinal component's recovery, in the direction of the magnetic field, to equilibrium (58). T1 values are increased in several pathologies primarily due to increase of interstitial space, as in fibrosis or amyloid deposition (57–59). T2 relaxation describes the loss of coherence in the spins of the nuclei, following their alignment due to the RF pulse. When tissue water content increases, T2 is more substantially increased than T1 (15). As such, T2 values are sensitive to myocardial edema in several pathologies, such as acute infarction and myocarditis (60,61). Tissue characterization with T1 and T2 values is a unique aspect of CMR which distinguishes it from other imaging modalities and contributes to its utility in multiple clinical populations (14).

7. f. Utility of Cardiovascular MRI

CMR is not only used for tissue characterization but is also the current reference standard in quantitative imaging of cardiovascular function and morphology, with a role for the non-invasive assessment of vascular function (62). Techniques used are case-specific, selected based on suspected clinical conditions to make up the CMR acquisition protocol (14).
In cases of suspected CMD, CMR first pass stress perfusion can be used to obtain diagnostic and prognostic information on vascular function (16,63). This technique uses pharmaceutical vasodilator stress and gadolinium-based contrast agents to evaluate myocardial ischemia, by quantifying MBF at-rest and during stress-induced maximal hyperemia. The images collected, known as first-pass perfusion images, are evaluated semi-quantitatively to calculate stress-induced myocardial perfusion reserve index (MPRI) (17).

CMR has been used to assess CMD in a wide variety of clinical populations, including those with CVD risk factors and CAD patients (64,65). However, it is nonetheless limited by reliance on gadolinium-based contrast agents, contraindicated in some patient populations (18). Promisingly, recent developments suggest that exogeneous contrast may not be necessary to assess vascular function, effectively advancing CMR as a risk-free assessment of CMD, through techniques that exploit the endogenous contrast of hemoglobin in blood.

7. g. Oxygenation-sensitive Cardiovascular MRI (OS-CMR)

In 1936, Linus Pauling first discovered that the magnetic properties of blood are not homogeneous (66). He found that arterial and venous blood differ in their magnetic properties due to the greater concentration of the deoxygenated form of hemoglobin in venous blood. This discovery would later form the basis of the BOLD effect and its utility in MRI.

In a magnetic field as in that of an MRI scanner, deoxygenated hemoglobin in blood acts as a paramagnetic material, slightly attracted to the magnetic field. This property causes spin-spin interaction in the nearby magnetic field, altering signal intensity when the concentration of deoxygenated hemoglobin varies, and accelerating the decay of transverse (T2) relaxation (20). T2*, a subset signal of T2, refers to the loss of the transverse signal, which deoxygenated hemoglobin accelerates. In short, its paramagnetic properties allow the de-oxygenation of hemoglobin to act as an innate contrast agent, visualized on magnetic resonance images (20). This is known as the BOLD effect. Imaging can exploit this effect and use it to detect regional changes in tissue oxygenation, termed oxygenation-sensitive imaging.

Landmark work by Ogawa (1992) showed that external stimuli cause minute changes to blood flow in the brain. The results of these changes were detectable with oxygenation-sensitive imaging (67). This work kickstarted the field of functional brain MRI, utilizing color maps to view dynamic oxygenation changes in the brain (67). The utility of oxygenation-sensitive imaging to investigate tissue oxygenation was later transitioned to the myocardium, denoted as OS-CMR (19,68–70).

In the myocardium, signal intensity changes viewed by OS-CMR reflect the oxygenation of blood in capillary beds, which in turn represent the oxygen supply and demand balance of the myocardium (20). These changes are quantitatively measured through the MORE, the ratio of myocardial oxygenation during vasodilatory stress to the myocardial oxygenation at baseline (20,68). As such, MORE viewed by OS-CMR is a direct marker of tissue oxygenation which can identify vascular dysfunction or inducible ischemia (71–73). Early OS-CMR studies in

animal models and healthy volunteers validated MORE as a marker of tissue oxygenation before its use could be established in patient populations.

7. h. OS-CMR in Healthy Volunteers

Healthy volunteers show a characteristic response to vasoactive stimuli which is visible on OS-CMR images. OS-CMR is capable of visualizing myocardial oxygenation changes due to the properties of deoxygenated hemoglobin, as in cerebral studies. The concentration of deoxyhemoglobin will be diluted if the supply of oxygenated blood increases without a matched increase in myocardial oxygen demand (20). Therefore, if a vasodilatory stimulus is applied, the concentration of deoxyhemoglobin should decrease in healthy vasculature, leading to an increase in T2*, and therefore increased signal intensity on OS-CMR images (20).

Several early OS-CMR studies validated this principle. Pre-clinical studies utilized ex-vivo models (70) to demonstrate the association of signal intensity to blood oxygenation, and animal models (74) demonstrated hypoxia-induced changes in myocardium due to the BOLD effect. Niemi (1996) and Wacker (1999) respectively observed that dipyridamole-induced increases in myocardial blood flow correlated to increased myocardial signal intensity in healthy volunteers (19,75). Li et al. (1996) determined that signal intensity changes in healthy volunteers largely reflected changes in myocardial oxygenation, not blood flow (69). During a simultaneous increase of blood flow and oxygen demand with dobutamine, there was no significant change in signal intensity. However, an increase in blood flow without increased oxygen consumption, using dipyridamole, did result in an increase in myocardial signal intensity, showing that signal intensity changes likely reflect oxygenation changes rather than blood flow (20,69). Additionally, myocardial signal intensity changes in canine myocardium

were validated against microsphere studies (76). By successfully demonstrating the BOLD effect in the myocardium, these pre-clinical studies allowed for further investigations of signal intensity differences between healthy volunteers and patients with known or suspected CAD and, later, diverse cardiovascular pathologies.

7. i. OS-CMR in Cardiovascular Pathologies

Unlike in healthy individuals, dysfunctional vasculature cannot effectively respond to vasoactive stimuli, and an inflow of oxygenated blood will not occur to match increased oxygen demand. In cases of ischemia, or myocardial deoxygenation, there is in fact a greater concentration of deoxyhemoglobin in capillary blood, which leads to T2* shortening and therefore decreased signal intensity on OS-CMR images (20).

A blunted MORE as an indicator of vascular dysfunction has been validated in several pathologies. Early OS-CMR studies demonstrated that BOLD signal intensity at rest and during stress could identify stenotic coronary arteries without exogeneous contrast agent use (77,78). Later work explored the potential of a steady-state free precession (SSFP) sequence to identify stenotic vessels in animal models (79,80). The first clinical OS-CMR study using the SSFP sequence in human models was led by Karamitsos (2010) (72). They observed that in CAD patients, reduced perfusion does not always coincide with reduced oxygenation, suggesting that oxygenation measured by OS-CMR may be a better marker for coronary artery stenosis than PET-derived perfusion. Several studies assessed CAD in animal models (81,82) and human subjects, observing a decrease in signal intensity in stenosed territories during dipyridamole or adenosine stress compared to non-stenosed territories, distinguishing CAD patients from healthy subjects (71,73,83–86). Several of the above-mentioned studies found that BOLD performed favorably to other techniques such as thallium single-photon

emission computerized tomography (SPECT) (78), PET (72), and fractional flow reserve (FFR) (85,86).

Other pathologies studied with OS-CMR include cardiomyopathies (87,88), chronic kidney disease (89,90), subjects with cardiovascular risk factors such as diabetes (21,91), pulmonary arterial hypertension (92), ST segment elevation myocardial infarction (93), heart failure (94), and myocardial scar (95). All observed varying degrees of decreased signal intensity during stress, thus blunted MORE. However, a study of Syndrome X, now known as ischemia without obstructive coronary artery disease (INOCA), found no global perfusion or oxygenation deficits compared to healthy volunteers, suggesting that microvascular dysfunction in this population may occur regionally rather than through a homogeneous global deficit (96). Following OS-CMR's validation in animal models, healthy volunteers, and several cardiovascular pathologies, recent work revealed a novel method of assessing vascular function with OS-CMR without the need for pharmaceutical vasodilators, reducing the methodology's limitations without compromising its utility.

7. j. Breathing Maneuvers

Endothelium-independent pharmaceutical agents, such as adenosine, have historically been used to assess perfusion or oxygenation. Adenosine has been used in OS-CMR to reveal regional MORE deficits in several patient populations as reviewed in Section 7.i. Adenosine acts on VSMCs to augment synthesis of nitric oxide, a vasodilator (97,98). However, contraindications in some patient groups, and significant adverse effects in over half of patients, limit its universal adoption (99,100).

Carbon dioxide (CO_2) is a strong direct vasodilator and thus can be used as an endogenous alternative to pharmaceutical vasodilation. CO_2 is a powerful vasodilator which acts upon the endothelial cells of the microvasculature as an endothelial-dependent stimuli (25,26). As with other vasoactive stimuli, endothelial dysfunction results in an attenuated vasodilation and vasoconstriction response to varying CO_2 concentrations (101,102). Though some studies utilized inhaled CO_2 in lieu of pharmaceutical vasodilators, limitations such as limited patient compliance, adverse effects, and the need for specialized equipment have prevented the widespread adoption of this method (103,104). More recently, vasoactive breathing maneuvers consisting of a paced hyperventilation followed by a voluntary maximal breath hold have been validated as a suitable alternative to inhaled CO_2, and used in several patient populations (27–32).

Early work by Guensch and colleagues (2013, 2014) confirmed previously published experimental studies from the 1960's that CO_2 concentration regulates myocardial blood flow and thus imparts signal intensity variations on OS-CMR images (23,23). Breathing maneuvers in healthy subjects induce myocardial oxygenation changes monitored in vivo by OS-CMR (23) or in first-pass perfusion imaging (105). In hypocapnia caused by hyperventilation, the decreased concentration of CO_2 induces vasoconstriction and increased vascular resistance (22,23). With the oxygen consumption by the myocardium remaining constant, these conditions result in increased oxygen extraction and a relative increase in deoxyhemoglobin concentration, therefore decreasing OS-CMR signal intensity (22,23). The subsequent breath hold-induced hypercapnia induces vasodilation and decreases vascular resistance. This results in luxury perfusion in post-capillary venules, a relative decrease in deoxyhemoglobin, and an increased OS-CMR signal intensity (22). The parameter describing the tissue oxygenation response is denoted as B-MORE.

Fischer et al. (2015) showed that breathing maneuvers can be used safely and may show greater sensitivity to myocardial oxygenation changes than adenosine, while increasing subject comfort and reducing adverse effects (24). Later work observed that B-MORE sensitively detects areas inducible ischemia due to stenoses in animal models (32) and multi-vessel CAD patients (27), in addition to observing blunted microvascular function in patients with obstructive sleep apnea (28), heart failure (30,106), and after heart transplantation (31). Recently, a heterogeneous B-MORE response, without globally reduced oxygenation, has been observed in women with INOCA, likely reflecting underlying CMD (29). These results, considering previous work which found no global deficits in oxygenation during adenosine stress in INOCA patients (96) suggest that microvascular dysfunction manifests through regional, heterogeneous patterns over time rather than homogeneous deficit across the whole myocardium. While novel, OS-CMR using breathing maneuvers is a methodology that shows great promise in investigating microvascular dysfunction and inducible ischemia through B-MORE. However, as a novel methodology, it is not without limitations.

7. k. Limitations of OS-CMR

Despite its promise in assessing tissue oxygenation in several pathologies, several limitations to OS-CMR exist that limit its current implementations and must be addressed in future work.

Firstly, field strength must be considered. Implementation of OS-CMR at 1.5 Tesla (T) is limited due to the slight differences in signal that distinguish normal from deoxygenated myocardium (20). At 3T, the T2 signal is more sensitive to oxygenation, and the contrast between normal and deoxygenated myocardium is increased due to a higher signal to noise ratio at higher field strengths (68,80). However, the advantages due to higher field strength come at the cost of artifacts due to magnetic field inhomogeneities, requiring precise shimming

by the scanner operator (20). Other artifacts in OS-CMR may occur, such as in the inferolateral wall due to the great cardiac vein and the lung-heart interface, or due to stents in certain patients (20).

Physiological variables may also affect B-MORE. Hematocrit level has also been seen to significantly affect OS-CMR, both at baseline and during breathing maneuvers (107). As such, factors which are associated to varied hematocrit, such as subject hydration status (107) or exposure to high altitudes (108,109), may then impact the OS-CMR signal intensity and therefore B-MORE.

There are multiple variables which may affect microvascular function even in individuals considered healthy, which have yet to be assessed by OS-CMR. Sex, age, and body composition impart subclinical variations to vascular function which may contribute to CMD before the onset of clinically observable CAD (110–112). Understanding whether and if, how strongly these factors impact microvascular function in healthy individuals would allow to interpret blunted B-MORE with greater accuracy. Recent research has found that blunted signal intensity on OS-CMR images, during adenosine vasodilation, may act as an early tissue marker in individuals at risk of developing CAD, even without overt disease (21). A transmural gradient of vascular function was observed in individuals with hypertension, smoking history, dyslipidemia, diabetes mellitus, or a family history of CAD. The range of MORE results observed in the at-risk population was between the healthy population and those with diagnosed CAD (21). This supports the hypothesis that CMD is an early marker of cardiovascular risk and validates OS-CMR's sensitivity in detecting slight vascular function deficits. Though this study used adenosine vasodilation, past work has shown that breathing maneuvers are a validated alternative to pharmaceutical vasodilators with fewer adverse

effects, suggesting that a future study utilizing breathing maneuvers would show similar results (24). The impact of aging on B-MORE has also not yet been clearly defined. Preliminary work by Hillier (2019) found reduced B-MORE values in healthy aging (113). Aging is known to have a significant impact on cardiovascular health (114,115). The limited understanding of the effect of aging on B-MORE limits its use across different age groups.

Lastly, there are no standard reference values to denote B-MORE ranges in healthy volunteers or for any cardiovascular pathologies. Multi-site OS-CMR studies have yet to be performed. As such, B-MORE values can only be compared within the site they've been acquired from. It is yet unknown if site-specific scan procedures, or external factors impacting the population at the site, impact B-MORE. Reader bias may also affect calculations of B-MORE, as current publications include only manual segmentation and analysis of OS-CMR images.

7.1. Conclusions

Microvascular dysfunction has a significant impact on symptoms and prognosis of patients with cardiovascular disease, even in the absence of obstructive CAD. CMD may be indicative of the burden of vascular health risk factors, perceivable before the onset of macrovascular pathologies. OS-CMR provides a validated assessment of coronary vascular function, observing deficits in myocardial oxygenation in several cardiovascular pathologies, and in subjects with cardiovascular risk factors without CAD. In conjunction with standardized breathing maneuvers, OS-CMR is a sensitive, safe, and efficient measure of microvascular function. With no reference values established, further research is needed to understand B-MORE variations, due to environment, physiological parameters, or subject characteristics, and thus allow for a deeper understanding of how these factors contribute to CMD pathogenesis and progression.

7. m. Chapter 1 References

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8. Chapter 2: Original Research Manuscript

8. a. Foreword

OS-CMR is a validated non-invasive methodology to investigate tissue oxygenation status in several cardiovascular pathologies, such as coronary artery disease. In conjunction with vasoactive breathing maneuvers, this methodology offers a non-invasive assessment of vascular function without gadolinium-based contrast agents or pharmaceutical vasodilators. A previous study utilizing OS-CMR identified a transmural gradient of blunted vascular function during adenosine infusion in subjects with cardiometabolic risk factors without overt coronary artery disease, underscoring the notion that vascular dysfunction may accompany risk factors even before the development of coronary artery stenosis. Though this study utilized pharmaceutical vasodilation, a novel study utilizing OS-CMR with vasoactive breathing maneuvers may likely reveal similar deficits in vascular function, due to the endothelial-dependent mechanism of breathing maneuvers. Modifiable and non-modifiable risk factors, such as age, sex, and body composition, impact vascular health and can progressively induce endothelial dysfunction. However, the relationship of such factors with vascular function in otherwise healthy individuals (without risk factors) remains unclear. The following chapter aims to shed light on that relationship, applying breathing-enhanced OS-CMR in a cohort of individuals considered healthy. The results of this study could provide a deeper understanding of vascular function in the general healthy population and its potential as an early marker for cardiovascular risk.

8. b. Original Research Manuscript

Breathing Induced Myocardial Oxygenation REserve and vascular function in the general HEALTHY population (BMORE-HEALTHY)

Abstract

Introduction: Non-modifiable factors like age and sex, along with factors like body composition, impact vascular health. In otherwise healthy individuals, the impact of these factors on the microvasculature is unclear. Oxygenation-Sensitive Cardiac Magnetic Resonance (OS-CMR) is a validated methodology to examine vascular function using the quantitative marker breathing-induced myocardial oxygenation reserve (B-MORE). Previous research has observed a transmural gradient with a blunted B-MORE, reflecting vascular dysfunction, in subjects with vascular risk factors but no overt cardiovascular disease (CVD). The aim of this study was to assess the impact of demographic factors on vascular function as defined by the response of myocardial oxygenation to a standardized breathing maneuver. We hypothesized that demographic factors have a demonstrable relationship with microvascular function as assessed by B-MORE.

Methods: We retrospectively assessed B-MORE in 80 healthy adults from 4 prospective OS-CMR studies, performed in Montreal, Canada. Independent T-tests were used to assess for statistically significant differences in microvascular function between the sexes. B-MORE was analyzed in the entire myocardium as well as regionally, in the subendocardial and subepicardial myocardial layers and myocardial coronary territories. ANOVA was used to assess for differences in B-MORE between age and CVD risk groups, and between myocardial layers. Linear regression was used to view the relationship of individual factors with B-MORE. A principal component regression analysis was used to assess the differential

impact of all factors (age, sex, height, weight, heart rate, blood pressure, BMI, BSA, Framingham's risk score) on B-MORE.

Results: Globally, male subjects showed a greater mean B-MORE (7.8 (CI 6.2-9.3)) compared to female subjects (6.0 (CI 4.9-7.2)), though this difference was statistically not significant. Global B-MORE did also not differ significantly between age groups or CVD risk groups. Subject height significantly impacted B-MORE analysis (R^2=0.09, p=0.009). Age, height, BSA, sex, and CVD risk each had an independent correlation with B-MORE (p=0.008, 0.001, 0.025, 0.006, 0.035, respectively), explaining 14% of its variation (R^2=0.14). B-MORE was homogeneous across the myocardial layers. Regionally, several factors impacted B-MORE in the sub-endocardium and sub-epicardium, with height significantly impacting B-MORE in univariable analysis, and age and height significantly impacting B-MORE in multivariable analysis, among others which varied by myocardial layer. In all coronary territories, male subjects showed a significantly higher mean B-MORE compared to female subjects (p=0.008, p=0.02, p=0.008), and in the LAD territory, B-MORE significantly decreased in older age groups (p=0.04). Sex and height were significantly correlated with B-MORE in all territories, amongst others which varied by territory. Furthermore, age, height, and BSA showed significant correlations with B-MORE in all coronary territories, while others which varied by territory.

Conclusions: In a healthy population, sex, age, and body composition, are related with vasculature function. Female sex may be associated to a lower B-MORE. Further research is needed to clarify the impact of confounders not captured in this study.

Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide (1). Recognizing of cardiovascular risk is key in preventing the development of CVD and in reducing disease burden (2,3). Non-modifiable factors like age and sex, and difficult-to-modify factors like body composition, impact vascular health. Alongside environmental, socioeconomic, and genetic risk factors, these characteristics are used to predict the risk of overt CVD and future cardiovascular events, often through clinical risk calculators, such as the Framingham risk score (4–6). These characteristics may also impact the vasculature sub-clinically, before the onset of overt clinical cardiac disease (7–9).

In otherwise healthy individuals, the relative contribution of these factors in driving vascular dysfunction is unclear. Aging progressively induces vascular dysfunction. Over the lifetime, vascular remodeling results in increased vessel stiffness, inflammation, calcification, and increased likelihood of ischemia (10,11). Vascular differences also manifest between sexes through female sex-specific factors, such as menopause, resulting in a distinct pathophysiology for microvascular disorders in women (8,12–16). In both sexes, body composition impacts the vasculature due to excess adipose tissue. Functioning as an endocrine organ, excess adipose tissue promotes vascular dysfunction through production of pro-inflammatory compounds acting on the endothelium, and secretion of hormones contributing to nutrition, insulin processing, and inflammatory pathways (17,18).

Oxygenation-sensitive cardiac magnetic resonance imaging (OS-CMR) has recently emerged as a fast, non-invasive technique to assess vascular function, which avoids the use of intravenous exogeneous contrast and pharmaceutical vasodilatory agents (19). OS-CMR utilizes the blood oxygenation level dependent (BOLD) effect to directly assess myocardial

oxygenation changes (20). Recent work has shown that vascular function assessed with OS-CMR is blunted in subjects with coronary artery disease, as well as those with vascular risk factors (21). This illustrates the significant effects of risk factors on vascular function, even in subclinical disease states. Guensch and colleagues (2013, 2014) showed that OS-CMR can visualize the known impact of carbon dioxide (CO_2) on myocardial blood flow (22–24). Specifically, the drop of blood CO_2 during hyperventilation leads to increased vascular resistance and induces vasoconstriction. A subsequent breath hold induces an increase of CO_2, lowering vascular resistance, i.e., leading to vasodilation. Vasoactive breathing maneuvers consisting of paced hyperventilation followed by a voluntary maximal breath hold have been validated as a suitable alternative to pharmaceutical vasodilation in a number of patient populations, resulting in myocardial oxygenation changes visible with OS-CMR (20,25–29).

Age, sex, and body composition may result in subclinical alterations to vascular function, even in individuals with no history of cardiac disease, attenuating the myocardial oxygenation response to breathing maneuvers visible with OS-CMR. The aim of this study is to investigate the relationship of risk factors with vascular function as defined by the response of myocardial oxygenation to breathing maneuvers.

Methods

Study Design

This was a retrospective, cross-sectional cohort study.

OS-CMR parameters were retrospectively obtained from study data at the McGill University Health Centre (MUHC) in Montreal, Canada. Study data came from prospective studies

approved by the appropriate local ethics boards: B-MORE (2016-2428, 2019-4137), B-CORE (2017-3002), HAPI-FIT (MP-21-2018-1760), SYNDX (2019-4635).

Participants

Healthy volunteer inclusion criteria for the studies included subject age above 18, informed consent, no history of cardiac or pulmonary conditions, no tobacco consumption, body mass index (BMI) below 30, and no consumption of medications affecting the cardiac or pulmonary system. Exclusion criteria were known contraindications to CMR, such as implantable electronic devices and metallic foreign bodies (30). Data was accessed retrospectively from subjects with acceptable image quality recruited as healthy volunteers in their respective studies, with whom informed consent for secondary use of their data was given.

Clinical Variables

Data accessed included global and segmental signal intensity values during breath hold, as well as demographic characteristics from study records (BMI, body size analysis (BSA), weight, height, heart rate, blood pressure, age, and sex), recorded at the time of the OS-CMR scan.

CMR Protocol

MRI scans were performed on a clinical 3 Tesla MAGNETOM Skyra (Siemens Healthineers, Erlangen, Germany). Subjects were instructed to fast the morning of the scan and avoid caffeine for 12 hours before the scan. Subjects practiced the breathing maneuvers before the scan and performed the maneuvers according to recorded audio instructions.

OS-CMR images were acquired during the breathing maneuvers in one basal and one mid-ventricular short-axis slice. The breathing maneuver protocol consisted of a baseline cine acquisition, a 60-second period of metronome-paced hyperventilation, and a voluntary maximal breath-hold throughout which OS-CMR images were continuously acquired. The OS-CMR sequence and parameters have been previously described in detail (24,28,31).

CMR Image Blinding & Analysis

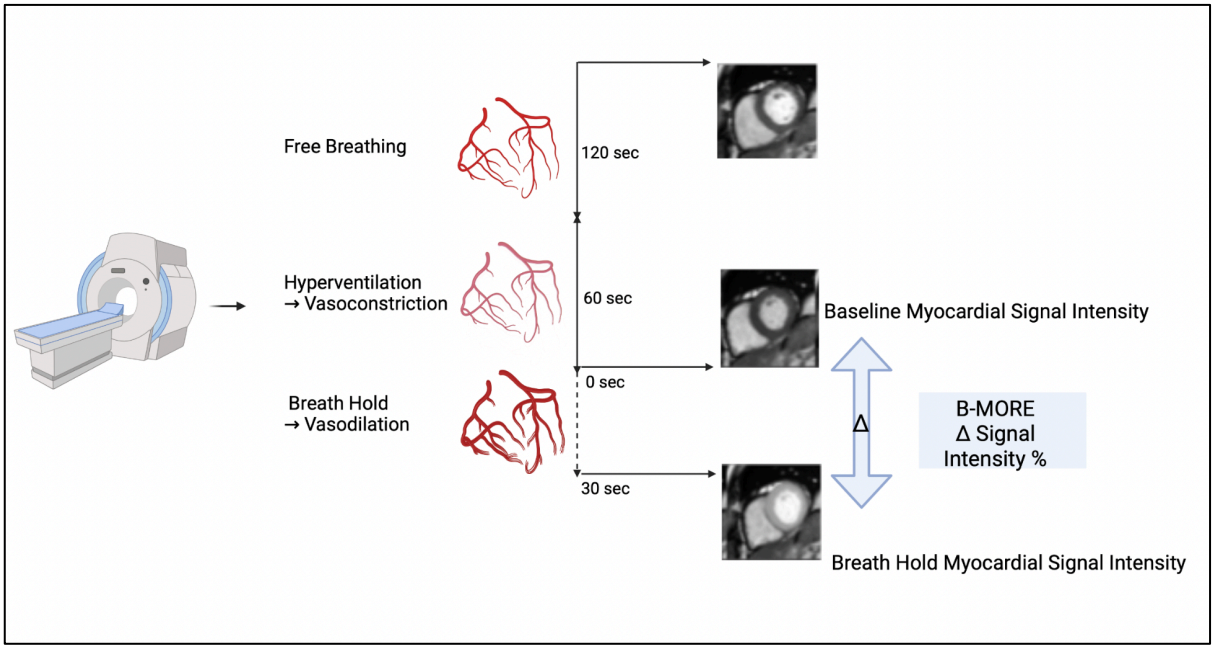


Figure 1: To view dynamic myocardial oxygenation changes, change in Breathing Induced Myocardial-Oxygenation Reserve (B-MORE) was calculated as the percentage difference (Δ) in signal intensity (B-MORE ΔSI%) between the signal intensity of the first post-hyperventilation end-systolic image (at end vasoconstriction due to hypocapnia), and the signal intensity of the end-systolic image closest to 30 seconds into the breath hold, (during vasodilation due to hypercapnia). Figure created by Katherine Lindsay with Biorender.com (32)

CMR images were anonymized using a primary code, then given a secondary code to blind the participant's identity. An experienced CMR reader, EH, then quantitatively analyzed all images using cvi42™ (Circle Cardiovascular Imaging, Calgary, Canada), manually contouring the epicardial and endocardial borders as per clinical standards (28). OS-CMR data were reported as global and segmental values in accordance with the American Heart Association's standardized myocardial segmentation model (33). Assessments of global and

regional breathing induced myocardial oxygenation reserve (B-MORE) were determined from the first post-hyperventilation end-systolic image, and the end-systolic image closest to 30 seconds of the breath-hold were used for statistical analysis (Figure 1). Changes in B-MORE were therefore expressed as the percentage difference in signal intensity (B-MORE ΔSI%) between the first post-hyperventilation end-systolic image, and the end-systolic image closest to 30 seconds into the breath hold (Figure 1).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). BMI and BSA, using the Mosteller formula, were calculated from patient height and weight (34). Full 30-year CVD-risk was calculated for each participant using the Framingham Risk Score (6). B-MORE ΔSI% values for each participant were grouped by sex, age (>36, 36-55, 55<), and CVD-risk (>10, 10-20, 21-31, 31< %). One-way analysis of variance (ANOVA) tests were performed to compare mean B-MORE ΔSI% between the age and CVD-risk groups, respectively. A t-test was performed to compare mean B-MORE ΔSI% between male and female participants. Unadjusted analysis was performed using univariable linear regression analysis for all variables (age, sex, CVD-risk, height, weight, BMI, BSA, heart rate, and blood pressure). Adjusted, multivariable analysis was performed using principal component analysis (PCA) and principal component regression (PCR), including all demographic variables as independent variables. B-MORE ΔSI% was the dependent variable for the regression analyses. The same statistical tests were performed on segmental and global B-MORE ΔSI% values. To determine intraobserver variability, intraclass correlation coefficient (ICC) analysis was performed in Excel (version 16.5, 2021) on a subgroup of 21 subject images analyzed twice by EH, an experienced CMR reader. To determine interobserver

variability, intraclass correlation coefficient (ICC) analysis was performed in Excel (version 16.5, 2021) on a subgroup of 16 subject analyzed images analyzed both by EH and a second experienced CMR reader.

Results

Study population

The baseline characteristics of all analyzed subjects are summarized in Table 1. Data from 80 subjects were included. All subjects completed the study protocol and tolerated the breathing maneuver well. The study population had a mean age of 40.6 (95% confidence interval (CI) 37.1, 44.12). The population was largely female (61%) with a mean CVD 30-year risk score (%) of 18.3% (CI 14.8-21.7) and mean BMI of 24.8 (CI 24.1-25.4).

Intra and Interobserver variability

The intraobserver variability of B-MORE (ΔSI%) measurements was small. Assessed on a subgroup of 21 subject images read twice by EH, the ICC was 0.96, indicating excellent intra-observer reliability (35). The interobserver variability was also small, as assessed on a subgroup of 16 subject images read by two readers. For all slices, ICC results were above 0.8, and for global values, the ICC was above 0.9, indicating good and excellent reliability between readers, respectively (35).

A. Global Myocardial Oxygenation Changes

The overall mean ΔSI% over the breath hold (B-MORE) was 6.7 (CI 5.8-7.6). Mean B-MORE did not differ significantly between CVD risk groups. Though not statistically significant in ANOVA analysis, a decrease in mean B-MORE was observed across age groups (Figure 2, Table 2). Male subjects showed a greater mean B-MORE (7.8 (CI 6.2-9.3) compared to females (6.0 (CI 4.9-7.2)) though this was not statistically significant in a t-test

(p>0.05). Height significantly impacted B-MORE in unadjusted analysis (R^2=0.09, p=0.009) (Figure 3). For each centimeter of height, B-MORE ΔSI% increased by 0.12 in this univariable model (Figure 3, Table 3). Age, sex, BSA, height, and CVD risk significantly impacted B-MORE ΔSI% (Table 3). This multivariable model explained 14% of variance in B-MORE ΔSI% (R2=0.14). For each year of age, the model showed B-MORE ΔSI% decreased by 0.03. Female sex decreased B-MORE ΔSI% by 0.77 in this model. For each unit of BSA, the model showed B-MORE ΔSI% increased by 1.35. For each cm of height, the model showed B-MORE ΔSI% increased by 0.04. For each percentage increase in CVD risk, the model showed B-MORE decreased by 0.02 (Table 3).

B. Regional Myocardial Oxygenation Changes

i. Myocardial oxygenation changes in the myocardial layers

B-MORE was homogeneous across the myocardial layers, with no significant difference in mean B-MORE in the sub-endocardium compared to the sub-epicardium. Mean B-MORE did not differ significantly between age groups, CVD risk groups, or between the sexes, in either the sub-endocardium or the sub-epicardium. In unadjusted analysis, height significantly impacted B-MORE in both layers, with B-MORE ΔSI% increasing by 0.12 for each centimeter increase of height in the epicardium (p=0.02, R^2=0.07), and 0.13 in the endocardium (p=0.02, R^2=0.08) (Table 4).

In the sub-endocardium, sex, age, and height were significantly distinct (p<0.05), with the model explaining 7.6% of variation of B-MORE ΔSI% (R^2= 0.076). In the sub-epicardial layer, age, height, and CVD risk were significant (p<0.05) with the model explaining 8% of variation of B-MORE ΔSI% (R2=0.08) (Table 4). The endocardial multivariable model

showed that female sex decreased B-MORE ΔSI% by 0.68, each year of age decreased B-MORE ΔSI% by 0.03, and each centimeter of height increased B-MORE ΔSI% by 0.04 (Table 4). In the epicardial layer, each year of age was associated with a drop of B-MORE ΔSI% by 0.03, while each centimeter of height increased B-MORE ΔSI% by 0.04. Moreover, one percent increase of CVD risk decreased B-MORE ΔSI% by 0.02 (Table 4).

ii. Myocardial oxygenation changes in coronary territories

Several demographic variables impacted B-MORE ΔSI% in specific coronary territories. Mean B-MORE ΔSI% differed significantly ($p<0.05$) between age groups in the LAD territory, decreased with increasing age ($p=0.04$), and was significantly greater in male subjects compared to female subjects in the RCA, LAD, and LCX, respectively ($p=0.008$, $p=0.02$, $p=0.008$) (Figure 5, Figure 6). In unadjusted analyses, sex and height significantly impacted B-MORE ΔSI% in all territories, amongst others which varied by territory (Table 5). In unadjusted analyses of the RCA, B-MORE ΔSI was 3.62% lower in women, each centimeter of height increased B-MORE ΔSI% by 0.25, and each unit of BSA increased B-MORE ΔSI% by 7.91, respectively (R^2=0.09, 0.18, 0.07). In the LAD territory, B-MORE ΔSI was 2.63% lower in women, each year of age decreased B-MORE ΔSI% by 0.08, each centimeter of height increased B-MORE ΔSI% by 0.13, and each percentage increase of CVD Risk decreased B-MORE ΔSI% by 0.08, respectively (R2=0.07, 0.07, 0.08, 0.06). In the LCX, B-MORE ΔSI was 2.43% lower in women, each centimeter of height increased B-MORE ΔSI% by 0.15, and each unit of BSA increased B-MORE ΔSI% by 4.98, respectively (R2=0.09, 0.15, 0.06).

Age, height, and BSA significantly impacted B-MORE ΔSI% in all territories, amongst others which varied by territory (Table 5). In the RCA, the multivariable model explained 22% of variation in BMORE ΔSI% (R^2=0.22), In the RCA model, BMORE ΔSI% was 1.61% lower in women, each year in age decreased BMORE ΔSI% by 0.06, each centimeter in height increased BMORE ΔSI% by 0.08, each percentage increase of CVD risk decreased BMORE ΔSI% by 0.03, each beat per minute increase of heart rate increased BMORE ΔSI% by 0.07, each unit of BMI decreased BMORE ΔSI % by 0.15, and each unit of BSA increased BMORE ΔSI% by 2.09. (Table 5)

In the LCX, the multivariable model explained 15% of variation in BMORE ΔSI% (R^2=0.15). In this model, BMORE ΔSI% was 0.89% lower in women, each year in age decreased BMORE ΔSI% by 0.03, each centimeter in height increased BMORE ΔSI% by 0.04, and each unit of BSA increased BMORE ΔSI% by 1.2. (Table 5)

In the LAD, the multivariable model explained 15% of variation in BMORE ΔSI% (R^2= 0.15). In this multivariable model, each year in age decreased BMORE ΔSI% by 0.04, each centimeter in height increased BMORE ΔSI% by 0.05, each unit of BSA increased BMORE ΔSI% by 1.66, and each percentage increase of CVD risk decreased BMORE ΔSI% by 0.03. (Table 5)

Discussion

Our results from four prospective studies show numerous correlations of known modifiers of cardiovascular risk such age, sex, and body composition with vascular function as measured with breathing-enhanced OS-CMR. This approach may therefore allow to identify an increased risk in apparently healthy subjects.

To our knowledge, this was the first study of its kind. While previous animal and clinical studies have used OS-CMR to observe blunted vascular function in subjects with coronary artery disease and cardiovascular risk factors, and recent work has suggested that a blunted B-MORE is associated to microvascular pathologies, this was the first OS-CMR study with vasoactive breathing maneuvers to investigate variation in vascular function within a healthy cohort.

Impact of age on myocardial oxygenation response

In adjusted, multivariable analysis, age significantly reduced global and regional B-MORE ΔSI%. These findings agree with previous work observing decreased mean B-MORE across healthy aging (36). Aging induces structural modifications in the heart, manifesting through cardiomyocyte apoptosis, myocardial and vascular stiffening, and hypertrophy (4). Coronary microvascular function is also known to decline with aging. Research suggests that oxidative stress, increased over the lifetime, induces changes to arterioles which impacts vasoactive function of coronary vessels (37). Our findings are consistent with these reports by showing an age dependence of B-MORE as a marker for vascular function. Overall, this also indicates the need for age-specific B-MORE reference values acquired from a truly healthy cohort.

Impact of sex on myocardial oxygenation response

Male subjects demonstrated a higher mean B-MORE ΔSI% compared to female subjects, globally and in regional subsets. While the mean age of study subjects was 40.6, the majority of female subjects were aged 50 years or older, suggesting some female subjects may have been post-menopausal, as the Canadian median age at natural menopause is estimated at 51 (38), while most male subjects were below 50 years of age. Further, the results of the adjusted multivariable analyses, globally, in the sub-endocardial layer, and the RCA and LCX coronary territories, respectively, revealed that sex impacted B-MORE independent of other factors. In post-menopausal women, current literature suggests a significant association between estrogen and microvascular pathologies like coronary microvascular dysfunction (CMD) (39). As such, the vascular function deficit we observed in females may have occurred due to the unique effects of post-menopausal estrogen circulation. Our findings suggest that sex remains significant in modulating vascular function, even in those considered healthy, and that sex-specific reference values are necessary in future studies of B-MORE, notably if the cohort studied includes post-menopausal subjects.

Impact of body composition on myocardial oxygenation response

BSA significantly impacted myocardial oxygenation, increasing myocardial oxygenation response with increasing body composition values. Excess body fat is a known CVD risk factor which may affect cardiac structure and function (5). Namely, excess adipocytes contribute to vascular dysfunction pathogenesis through endocrine and pro-inflammatory pathways (17,18). All included subjects had a BMI < 30, so it is possible that their body fat did not have a significant impact on their vascular function. Notably, BMI and BSA are limited in defining body fat distribution, such as excess visceral adipose tissue, a known marker for adverse cardiometabolic outcomes and CVD (40,41). Some literature suggests that waist circumference or waist-to-height ratio should be used in conjunction with, or instead of

BMI or BSA (42,43). Waist circumference was not assessed in this study. A further study of microvascular function comparing multiple metrics of body composition is suggested, to further investigate its impact in healthy individuals, and confirm if B-MORE should be indexed to body composition.

Impact of height on myocardial oxygenation response

Increasing height was associated to increasing B-MORE, across all analyses. These findings support previous research suggesting that a taller stature reduces cardiovascular risk. A recent meta-analysis by Yano found that short stature is associated with a risk of CV morbidity and mortality 1.5 times greater than taller individuals (44).

Related work found that short stature is also associated with a blunted vasodilatory capacity, possibly due to decreased endothelial repair potential in the vasculature (45,46). Short stature can be interpreted as a representation of multiple factors acting on the cardiovascular system, including as poor nutrition, environmental factors, and genetics (47,48). As such, it is likely our findings simply reflect the high sensitivity of B-MORE to identify even mild vascular dysfunction resulting from a variety of factors, even in subjects considered healthy, and may not be an effect due to subject height itself. To determine if indexing B-MORE to height is necessary, future work should investigate if the impact of height on B-MORE is independent of determinants of height, which affect the cardiovascular system.

Myocardial oxygenation in myocardial layers

The sub-endocardium and sub-epicardium were homogeneous and did not show significantly different B-MORE responses. This agrees with previous observations of a homogenous myocardial oxygenation response to pharmacologic vasodilation across the myocardial layers in healthy subjects, unlike subjects with cardiovascular risk factors who showed a transmural

gradient (21). Further research should investigate if the transmural gradient previously observed in those with cardiovascular risk factors would be replicated using endothelial-dependent breathing maneuvers rather than endothelial-independent adenosine. Across the coronary territories, however, not all results were homogeneous. Notably, in an independent ANOVA analysis, only the LAD territory showed a significantly lower B-MORE in older age groups, despite all territories being equally exposed to age. These results suggest that microvascular function in the LAD territory may be the first indicator of the impact of healthy aging, before a significant effect is observed globally or in other territories. However, further research with a larger cohort is needed to establish this.

Limitations

This study has a number of limitations. Risk factors were self-reported, so unknown risk factors amongst the cohorts may be possible. Further, not all possible risk factors were included in this analysis, which may have contributed to the remaining BMORE ΔSI% variation unexplained by factors in this study. A number of additional factors may have been impacting the vasculature in the study, including environmental factors such as pollution, or socioeconomic factors such as income and education level, or physiological factors modulating the B-MORE response, like hematocrit level and baseline coronary blood flow (31,49–53). Future studies should include a wider range of factors to assess their potential contributions to vascular and microvascular function in the general healthy population.

Conclusions and Future Directions

Our results indicate that age, sex, and body composition affect coronary vascular function in healthy individuals. Though several questions remain unanswered and not all results were in line with these observations, our study suggests a strong potential of the breathing-induced

myocardial oxygenation reserve (B-MORE) as measured by breathing-enhanced OS-CMR as an early risk marker. Height, BSA, sex, CVD risk, and age were independently predictive of B-MORE, in addition to BMI and heart rate in subsets of our population. This preliminary research aids in the understanding of the underlying subclinical variations in vascular function which occur even in individuals considered healthy.

Future studies should incorporate a wider range of factors with a known impact on coronary microvascular function across the lifespan, including socioeconomic, environmental, and sex-specific factors, to better understand variations of vascular function in the diverse general population. B-MORE's utility as a marker for vascular dysfunction, and the potential prognostic value it may hold, must be further explored in various clinical settings but also as a screening marker.

Tables and Figures:

Figure 1:

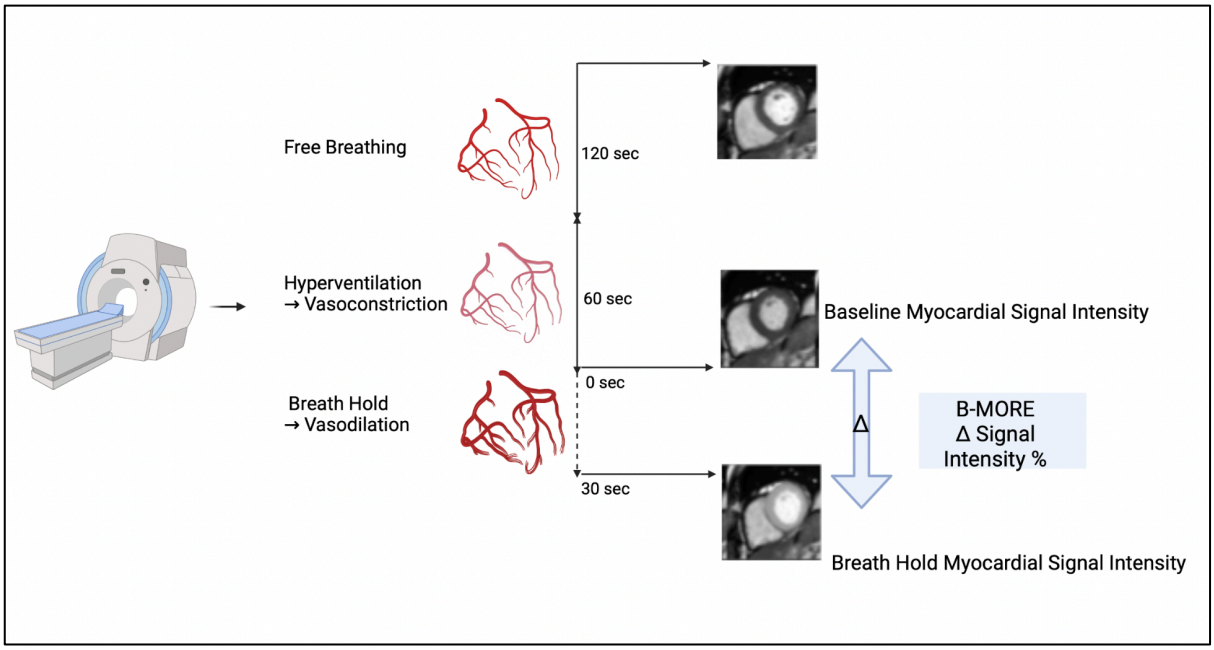


Figure 1: The change of myocardial oxygenation in response to a combined breathing maneuver (Breathing- induced Myocardial-Oxygenation REserve (B-MORE) was calculated as the percentage difference in signal intensity (ΔSI%) between the end-systolic myocardial signal intensity of the first post-hyperventilation (vasoconstriction due to an decrease of CO_2), and at 30 seconds into the breath hold, (vasodilation due to an increase of CO_2).

Figure created by Katherine Lindsay with Biorender.com (32)

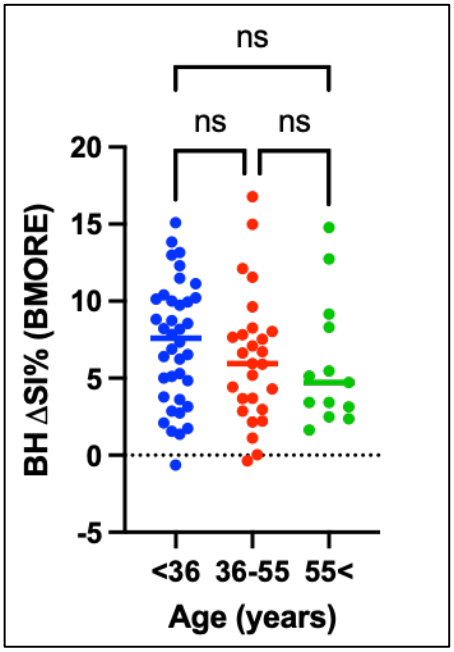
Table 1: Subject demographics

		Sample Size	Mean ± SD	95% CI of mean
Age (years)	Total	80	40.64 ± 15.81	[37.12, 44.15]
	Under 35	39	25.26 ± 4.44	[23.82, 26.70]
	35-55	27	52.37 ± 1.78	[51.67, 53.07]
	56-70	14	60.86 ± 4.26	[58.4, 63.32]
Cardiovascular Risk (%)	Total	80	18.28 ± 15.51	[14.82, 21.73]
	0-9%	32	3.88 ± 1.98	[3.161, 4.589]
	10-20%	12	14.5 ± 3.94	[11.99, 17.01]
	21-31%	20	24.35 ± 2.46	[23.20, 25.50]
	32+%	16	42.31 ± 10.99	[36.36, 48.17]
Gender	Female	49 (61%)	-	-
BMI (kg/m²)		80	24.78 ± 2.96	[24.12, 25.44]
BSA(m²)		80	1.79 ± 0.20	[1.749, 1.839]
Height (cm)		80	167.29 ± 10.34	[165, 169.6]
Weight (kg)		80	69.64 ± 12.16	[66.93, 72.35]
Heart Rate (bpm)		80	71.4 ± 11.27	[68.81, 73.99]
Systolic Blood Pressure (mmHg)		80	118.38 ± 12.62	[115.6, 121.2]
Diastolic Blood Pressure (mmHg)		80	74.14 ± 10.41	[71.82, 76.45]

Table 2: Mean B-MORE ΔSI% by age group

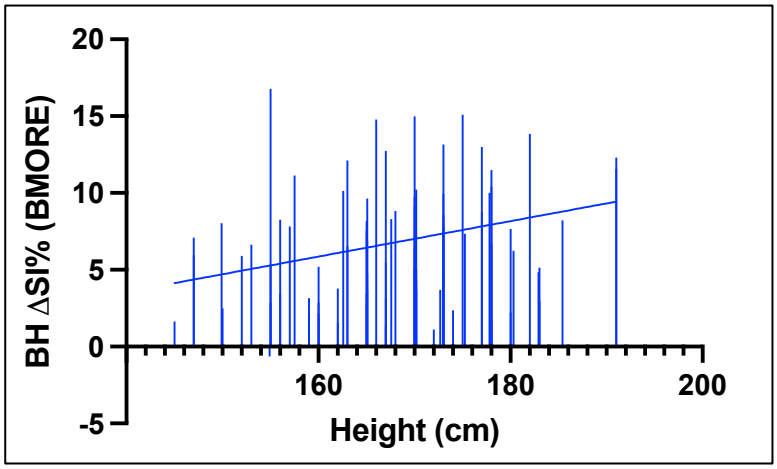
Age Group	B-MORE (Mean ± SD)	95% CI of mean
Total	6.7 ± 4.06	[5.78, 7.62]
Below 36	7.29 ± 3.95	[5.99, 8.58]
36-55	6.26 ± 4.21	[4.59, 7.92]
56+	5.91 ± 4.15	[3.40, 8.41]

Figure 2



Mean B-MORE ΔSI% by age group: There is a trend to lower mean B-MORE ΔSI% values with increasing age, although these were not statistically significant.

Figure 3

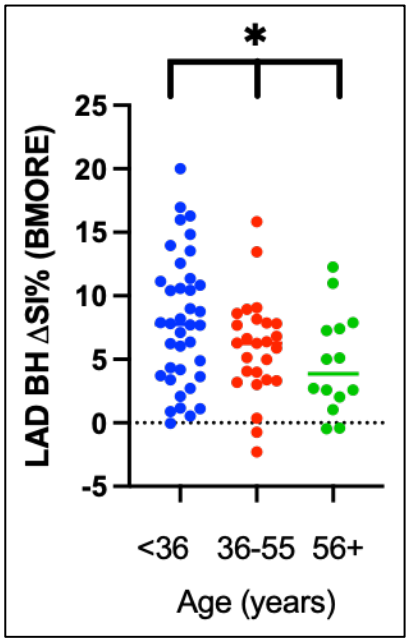


Height significantly impacts B-MORE: In univariate linear regression, height had a significant (R^2=0.12; p=0.0085) positive impact on B-MORE ΔSI%.

Table 3: Unadjusted and adjusted analysis of demographic factors impacting global B-MORE ΔSI%. *Significant (p<0.05) results are highlighted in red.*

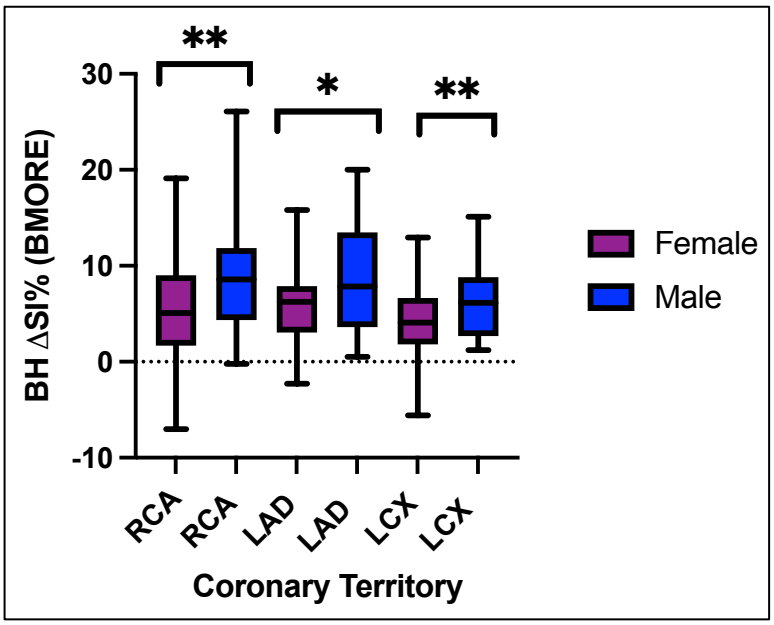
Variable	Unadjusted model		Adjusted model	
	p-value	Coefficient	p-value	Coefficient
Age	0.25	-0.03	0.008	-0.03
BMI	0.39	-0.14	0.30	-0.05
BSA	0.09	3.91	0.025	1.35
Heart Rate	0.76	-0.01	0.226	0.02
Systolic Blood Pressure	0.96	0.002	0.527	0.008
Diastolic Blood Pressure	0.84	-0.01	0.867	-0.003
Height	0.0085	0.12	0.001	0.04
Weight	0.23	0.047	0.107	0.016
CVD Risk	0.29	-0.032	0.035	-0.02
Sex	0.07	-1.73	0.006	-0.77

Figure 4



Age significantly impacts B-MORE ΔSI% in the LAD territory. Mean B-MORE ΔSI% decreased sequentially in each age group and was significant in ANOVA analysis (p=0.04)

Figure 5



Sex significantly impacts B-MORE ΔSI% in all coronary territories: In independent t-tests, male subjects showed significantly (p<0.05) higher mean B-MORE ΔSI% compared to female subjects in each the RCA (p=0.008), LAD (p=0.02), and LCX (p=0.008), in individual t-tests.

Table 4: Significant factors impacting B-MORE ΔSI% in the sub-epicardial and sub-endocardial myocardial layers, identified in unadjusted and adjusted analysis

	Sub-epicardium				**Sub-endocardium**			
Variable	Unadjusted model		Adjusted model		Unadjusted model		Adjusted model	
	p-value	**Coefficient**	**p-value**	**Coefficient**	**p-value**	**Coefficient**	**p-value**	**Coefficient**
Sex	-	-	-	-	-	-	0.047	-0.68
Age	**-**	**-**	0.023	-0.03	-	-	0.036	-0.03
Height	0.02	0.12	0.027	0.037	0.01	0.13	0.024	0.037
CVD Risk	**-**	-	0.038	-0.025	-	-	-	-

Table 5: Significant factors impacting B-MORE ΔSI% in each coronary territory, in unadjusted (univariable) and adjusted (multivariable) analysis

	RCA				LAD				LCX			
Variable	**Unadjusted model**		**Adjusted model**		**Unadjusted model**		**Adjusted model**		**Unadjusted model**		**Adjusted model**	
	p-value	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value	Coefficient
Sex	0.008	-3.62	<0.0001	-1.61	0.015	-2.63	-	-	0.0075	-2.43	0.001	-0.89
Age	-	-	0.0005	-0.06	0.0199	-0.078	0.009	-0.037	-	-	0.012	-0.028
Height	<0.0001	0.247	<0.0001	0.08	0.0122	0.127	0.004	0.047	0.0004	0.148	0.001	0.0424
CVD Risk	-	-	0.0115	-0.034	0.0257	-0.076	0.0099	-0.03	-	-	-	-
HR	-	-	0.0156	0.07	-	-	-	-	-	-	-	-
BMI	-	-	0.0452	-0.15	-	-	-	-	-	-	-	-
BSA	0.02	7.91	0.016	2.09	-	-	0.024	1.66	0.025	4.98	0.041	1.195

8. c. Chapter 2 References

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9. CHAPTER 3: DISCUSSION AND CONCLUSIONS

9. a. Summary

In addition to summarizing the information presented in Chapter 1, this concluding chapter will review the findings of the original research manuscript, its limitations, and implications for our understanding of coronary microvascular function. Cardiometabolic risk factors are associated with structural, molecular, and functional alterations to the vasculature, and ultimately dysfunction. Even in absence of obstructive epicardial disease, underlying factors can result in significant deficits of microvascular function over the lifespan. It is known that age, sex, and body composition alter vascular function, though the relationship of these factors with vascular function in healthy individuals is unclear.

OS-CMR is a safe, efficient, patient-friendly, and informative non-invasive approach to assess myocardial tissue oxygenation. When used with vasoactive breathing maneuvers, this method offers a direct assessment of endothelial function, which is pertinent to microvascular disease. The study presented in this thesis utilized OS-CMR in conjunction with vasoactive breathing maneuvers to evaluate vascular function in healthy subjects. It showed that the B-MORE response is not homogeneous across all healthy subjects, but differs between the sexes, and is related to age, sex, height, and body composition.

This work contributes to the growing body of evidence demonstrating the utility of OS-CMR with vasoactive breathing maneuvers in assessing vascular function, and the possibility that this approach may identify early microvascular dysfunction.

9. b. Introduction

CMD, though undetectable by coronary angiography, is a form of ischemic heart disease which modulates disease burden and patient outcomes with or without obstructive coronary artery disease (1–3). Structural and functional alterations, likely due to the burden of

cardiometabolic risk factors, induce microvascular dysfunction before overt injury occurs (3,4). Diagnostic testing capable of visualizing microvascular function is important in detecting CMD and preventing adverse outcomes. If undetected, unnecessary revascularization and worse prognosis may result (2,3). Many diagnostic imaging techniques are limited in the reach and resolution needed to assess the microvascular system and its function in-vivo, contributing to its poor characterization and adverse outcomes (5).

OS-CMR is a non-invasive imaging modality for assessing coronary vascular function by detecting regional tissue oxygenation changes during vasodilation (6). The non-invasive assessment of vascular function without injections or contrast agents is unique (6). Blunted myocardial oxygenation changes during adenosine vasodilation, indicative of vascular dysfunction, has been observed in several cardiovascular pathologies, including CAD (7), heart failure (8), and individuals with cardiovascular risk factors but no coronary artery stenosis (9). Vasoactive breathing maneuvers, composed of hyperventilation followed by a voluntary maximal breath hold, have recently been validated as an alternative to pharmaceutical vasodilation in OS-CMR, with B-MORE as a marker for vascular function (10,11). Vasoactive breathing maneuvers increase patient comfort and may show a greater sensitivity to myocardial oxygenation changes than adenosine (12). Unlike adenosine, breathing maneuvers modulate vascular tone through an endothelial-dependent pathway (11,13,14). Carbon dioxide, a powerful vasodilator, varies during hyperventilation and apnea, modulating vascular tone by acting on the endothelial cells, which line the microvasculature (14). This endothelial-dependent mechanism gives this approach the potential to provide a direct assessment of microvascular function. OS-CMR with breathing maneuvers is non-invasive, fast, and clinically feasible, having been validated in CAD, obstructive sleep apnea, heart transplant, and heart failure cohorts (15–19). Notably, women with INOCA showed a

heterogeneous B-MORE, further suggesting that OS-CMR with breathing maneuvers is a modality which can detect underlying microvascular dysfunction (20). As recently shown, breathing-enhanced OS-CMR is a preferred test among both referring physicians and patients (21).

This thesis presents and discusses the results of a study of demographic factors in a general healthy population that may affect B-MORE as the quantitative OS-CMR-derived marker for coronary vascular function, and as such, sheds light on the variation of coronary vascular function in this population. In Chapter 2, several factors differentially affected B-MORE. This work not only contributes to the existing body of literature supporting OS-CMR with vasoactive breathing maneuvers as a non-invasive, feasible, and informative assessment of vascular function, but also expands on the current understanding of microvascular dysfunction. Chapter 2 highlighted that the B-MORE response was not fully homogeneous among subjects, despite all being healthy volunteers. Instead, the myocardial oxygenation response to breathing maneuvers was dependent on age, sex, and metrics of body composition, such as height and body size analysis. As B-MORE is a validated marker of coronary vascular function, our study provides strong evidence for a direct effect of age, sex, and metrics of body composition including height and body size on vascular function, detectable in individuals considered healthy, with no prior history of cardiovascular disease. Additionally, this suggests that individuals with vascular risk factors may show a spectrum of blunted B-MORE as their risk of cardiovascular disease increases.

9. c. Contributions to understanding of microvascular function

The findings of Chapter 2 support the thesis hypothesis that demographic factors impart slight changes to microvascular function over the lifespan. These differential results likely

represent structural or functional alterations resulting from a multifactorial impact on the subject's vasculature. Though invasive measurements of microvascular function were not assessed in this thesis, it can be inferred that these results specifically reflect microvascular function and thereby blunted endothelial signalling pathways. This is supported by the known endothelial-dependent vasodilation of breathing maneuvers, in which carbon dioxide modulates vascular tone by acting on endothelial cells lining the microvasculature (14). As such, it is likely that the results shown in Chapter 2 do indeed reflect true variations in microvascular function.

Cardiometabolic risk factors are considered to first affect the microvasculature on a subclinical level, before damage to larger vessels occurs (3,4,22,23). With or without concurrent atherosclerosis, these factors drive CMD pathogenesis through structural and functional alterations (3). Notably, in Chapter 2, B-MORE variations due to certain factors mirror known microvascular abnormalities identified in previous studies. Namely, as described in Chapter 2, we observed that female subjects, who were largely above the mean Canadian post-menopausal age, showed lower global and regional B-MORE values compared to their male counterparts (24). Past research has observed that post-menopausal women undergo alterations to microvascular function, likely due to modified estrogen signalling pathways (25). Microvascular abnormalities seen in CMD are thought to be more prognostically useful in women, as woman with CMD are more likely to experience symptoms, comorbidities, and worse prognosis, despite data showing they're less likely than men to have obstructive CAD (3). As such, the observed B-MORE variations may be of particular prognostic importance in women, namely those with cardiometabolic risk factors, though further research is needed to confirm this. Subjects of shorter stature showed reduced global and regional B-MORE compared to taller subjects. Previous work examining

microvascular signalling supports these results, having shown that shorter stature is linked to a less strong vasodilation through decreased endothelial repair in the microvasculature (26–28). Lastly, though B-MORE did not always differ significantly between age groups in univariable analysis, aging may nonetheless contribute to slight alterations to B-MORE over the lifespan, as the multivariable analyses suggest. These results indicate that B-MORE reflects a complex interplay of multiple factors which include age, sex, height, and body size analysis. This supports previous hypotheses that microvascular dysfunction progresses due to the impact of several abnormalities, not one sole factor, including aging-induced modifications, female-sex specific risk modifiers, and traditional cardiometabolic risk factors (3).

Characterization of microvascular dysfunction in vivo remains challenging, as the limits of reach and spatial resolution restrict the use of traditional methods to assess vascular disease (5). Sensitive diagnostic tools are nonetheless necessary to (early) diagnose CMD, avoid unnecessary revascularization, and inform preventive and therapeutic management. The current reference standard in the field is invasive interrogation of microvascular function (3,5). A non-invasive yet sensitive and informative methodology capable of directly assessing microvascular function is an unmet need in the field. This thesis supports the promising capability of OS-CMR with vasoactive breathing maneuvers to fill this need, as a non-invasive, feasible, and efficient methodology, with B-MORE as a direct marker of microvascular function. Future studies should compare B-MORE to the invasive reference standards of microvascular function to validate that OS-CMR with breathing maneuvers is an equally informative yet non-invasive method to assess the microvasculature.

B-MORE's value as a prognostic marker for microvascular function has not yet been established. Longitudinal studies examining its variation over the lifespan have yet to be performed. Presumably, B-MORE would decrease as subjects age, following the results observed in Chapter 2 and the known impacts of age on the vasculature, but that has not yet been studied. Longitudinal studies should also investigate if B-MORE acts as a marker for future cardiovascular events. Blunted B-MORE may signify the early impact of cardiometabolic risk on the microvasculature, acting as a primordial predictor of cardiovascular disease or CMD. If validated in future studies, B-MORE could represent a snapshot of microvascular function over the lifetime, encompassing modifiable and non-modifiable risk factors. Future work should study the potential impact of other cardiometabolic risk factors, including environmental and socioeconomic variables. Importantly, this research has also formed the premise for future, large scale population-based studies to establish normal reference values for B-MORE.

9. d. Limitations

This study observed that varied demographic risk factors impart changes in microvascular function, modulating the vascular response to vasoactive breathing maneuvers. However, this work has limitations. The original research manuscript included in this thesis is a cross-sectional retrospective study with a sample size of less than 100 subjects. Studies with larger sample sizes are necessary to attain sufficient statistical power for further multivariable analyses of B-MORE across various populations, and to provide robust reference ranges for age- and sex-specific B-MORE values.

The B-MORE values obtained in the study varied, despite all individuals fitting criteria for healthy volunteers, and their variation is not fully explained by the factors included in the

statistical analysis. It is possible that subjects had yet unknown risk factors or other factors such as lifestyle or female sex-specific risk modifiers. It is unknown if site environment or site-specific scan procedures impact B-MORE. Multi-site and international studies are necessary to compare B-MORE across multiple sites. Interpretation of B-MORE values is currently restricted to comparison within the site where they've been acquired, and solely on Siemens MRI scanners. Physiological variables may have also affected the results, such as subject hematocrit. Hematocrit level, which varies with subject hydration, has been previously shown affect myocardial signal intensity (29). However, all subjects followed the same instructions regarding hydration which likely limited this variation.

No invasive measurements of microvascular function were performed, and subjects were not examined to rule out unknown epicardial disease. To date, there have been no studies correlating breathing-enhanced OS-CMR to invasive measures of microvascular function, although plans for such studies are underway. Future work should compare B-MORE obtained with breathing maneuvers to the invasive reference standards, to validate this methodology as a non-invasive measure of microvascular function.

It is not yet possible evaluate the potential prognostic value of a blunted myocardial oxygenation response to breathing maneuvers. To evaluate this, longitudinal studies repeatedly performing OS-CMR with vasoactive breathing maneuvers would be necessary, observing lifetime changes to B-MORE and their correlation with cardiovascular events. Similarly, future studies comparing B-MORE's variation over the lifetime in healthy individuals, at-risk cohorts, and cardiovascular disease patients may be useful.

9. e. Conclusion

Coronary microvascular dysfunction is a contributor to ischemic heart disease, the leading cause of death worldwide, that worsens patient symptoms and prognosis. However, it remains poorly understood and challenging to diagnose, unobservable through traditional methods of diagnosing obstructive coronary artery disease. A rapid, non-invasive, and efficient methodology, capable of visualizing early microvascular dysfunction imparted by vascular risk factors, would be extremely valuable in preventing its pathogenesis. The preliminary research encompassed by this thesis poses that OS-CMR with vasoactive breathing maneuvers may be the methodology to fill this need, with B-MORE as a sensitive marker of microvascular function. Through differential myocardial oxygenation responses to vasoactive breathing maneuvers, it was observed that demographic factors, including age, sex, and height, significantly impact the vasculature in healthy individuals. The endothelial-dependent mechanism of vasoactive breathing maneuvers suggests that our results reflect variations in microvascular function across the lifetime even in individuals considered healthy. These findings support B-MORE's sensitivity as a marker of minute underlying microvascular abnormalities. Future research will continue to investigate B-MORE's variation due to physiologic variables and vascular risk factors, aim to validate this methodology as a true assessment of microvascular function, and determine the potential prognostic value it may hold.

9. f. Chapter 3 References

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11. Declarations

Conflicts of Interest:

KL reports no conflicts of interests. MGF is an external consultant to Circle Cardiovascular Imaging Inc and co-founder of Area19 Medical Inc. MGF is listed as a holder of: United States Patent No. 14/419,877: Inducing and measuring myocardial oxygenation changes as a marker for heart disease; United States Patent No. 15/483,712: Measuring oxygenation changes in tissue as a marker for vascular function; United States Patent No 10,653,394: Measuring oxygenation changes in tissue as a marker for vascular function - continuation; Canadian Patent CA2020/051776: Method and apparatus for determining biomarkers of vascular function utilizing bold CMR images. MGF is shareholder and advisor of Circle Cardiovascular Imaging Inc. and Area19 Medical Inc.

EH is listed as a holder of Canadian Patent CA2020/051776: Method and apparatus for determining biomarkers of vascular function utilizing bold CMR images.