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Investigating the Short-term and Long-term Impact of COVID-19 on Cardiovascular Health

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INVESTIGATING THE SHORT-TERM AND LONG-TERM IMPACT OF COVID-19 ON CARDIOVASCULAR HEALTH

by

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DISSERTATION

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ABSTRACT

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Recent data indicates that the risk of developing cardiovascular disease (CVD) is augmented following coronavirus disease 2019 (COVID-19). Moreover, emerging data indicates that almost 50% of individuals who had COVID-19 experience persisting symptoms which are often debilitating, beyond the acute phase of the illness. This condition that is given the diagnosis post-acute sequalae of COVID-19 (PASC), also appears to be associated with cardiovascular complications. However, it is unclear what factors contribute to CVD risk following COVID-19 and PASC. Accordingly, the aim of this dissertation was to investigate potential factors that may contribute to adverse cardiovascular outcomes following COVID-19 with a focus on several key determinants of cardiovascular health, namely peripheral and cerebral vascular function, arterial stiffness, and blood pressure (BP). Given that young adults between 18 – 29 years account for almost 25% of the COVID-19 cases in the United States, study 1 and 2 focused on this population. In study 1, we tested the hypothesis that young, otherwise healthy adults who are beyond 4 weeks from a COVID-19 diagnosis would exhibit blunted peripheral and cerebral vasodilator function and increased central arterial stiffness compared with those

who did not have COVID-19. The major novel findings from this study are that 1) peripheral macro- and microvascular vasodilator function are blunted in young adults still symptomatic from COVID-19, but not in those who were asymptomatic beyond the acute phase, and 2) cerebral vascular function and central arterial stiffness are unaffected beyond the acute phase irrespective of COVID-19 symptomology. Extending our findings, and those of others demonstrating detrimental effects of COVID-19 on vascular health in young adults, in study 2 we aimed to perform a comprehensive assessment of BP using ambulatory and laboratory techniques in this population. Findings from this study demonstrated that ambulatory and laboratory BP are higher in those closer to a COVID-19 diagnosis compared to those who are further out from diagnosis, suggesting a transient effect of COVID-19 to elevate BP in young adults. Finally, study 3 aimed to evaluate vascular health in PASC with a focus on investigating a potential link between cardiovascular health and symptomology. We show for the first time that patients with PASC have higher BP and central arterial stiffness whereas peripheral and cerebral vasodilator function appears to be unaffected. Moreover, although there were no associations between total symptom burden and measures of vascular function, we found that resting cerebral blood flow was inversely correlated with the severity of brain fog with those with the greatest severity of brain fog having the lowest cerebral blood flow. Collectively, the work described herein provides novel insight into the impact of COVID-19 on key determinants of cardiovascular health that may contribute to the greater CVD risk following COVID-19 in young adults and in PASC.

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List of Abbreviations

ACE	Angiotensin Converting Enzyme
ACE2	Angiotensin Converting Enzyme-2
AIx	Augmentation Index
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ARB	Angiotensin Receptor Blocker
ARV	Average Real Variability
ASYM	Asymptomatic
AT1R	Angiotensin Type 1 Receptor
AT2R	Angiotensin Type 2 Receptor
AUC	Area Under the Curve
BH_4	Tetrahydrobiopterin
BMI	Body Mass Index
BP	Blood Pressure
CA	Cerebral Autoregulation
CBF	Cerebral Blood Flow
CO_2	Carbon Dioxide
COVID-19	Coronavirus Disease 2019
CV	Coefficient of Variation
CVCi	Cerebrovascular Conductance Index
CVD	Cardiovascular Disease
CVMR	Cerebral Vasomotor Reactivity
DBP	Diastolic Blood Pressure
eNOS	Endothelial Nitric Oxide Synthase
ET-1	Endothelin -1
ET_A	Endothelin A
ET_B	Endothelin B
FMD	Flow-Mediated Dilation
GMP	Guanosine Monophosphate
GTP	Guanosine Triphosphate
HCO_3	Bicarbonate
HDL	High Density Lipoprotein
HR	Heart Rate
hsCRP	High Sensitivity C-Reactive Protein

IL	Interleukin
INF- γ	Interferon - gamma
LDL	Low Density Lipoprotein
LLFDI	Late Life Function and Disability Instrument
MAP	Mean Arterial Pressure
MCA	Middle Cerebral Artery
MERS	Middle East Respiratory Syndrome
MET	Metabolic Equivalent
MRI	Magnetic Resonance Imaging
NDRI	Norepinephrine–Dopamine Reuptake Inhibitor
NO	Nitric Oxide
O_2	Oxygen
OTC	Over the counter
PASC	Post-acute Sequalae of COVID-19
$P_{ET}CO_2$	Partial Pressure of End-tidal Carbon Dioxide
PWA	Pulse Wave Velocity
PWV	Pulse Wave Analysis
QOL	Quality of Life
RAS	Renin-Angiotensin System
RBD	Receptor Binding Domain
RH	Reactive Hyperemia
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-1	Severe Acute Respiratory Syndrome Coronavirus-1
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus-2
SBP	Systolic Blood Pressure
SD	Standard Deviation
SF-12	12 Item Short Form Survey
SNRI	Serotonin and Norepinephrine Reuptake Inhibitor
SNS	Sympathetic Nervous System
SSRI	Selective Serotonin Reuptake Inhibitor
SYM	Symptomatic
TCD	Transcranial Doppler
TMPRSS2	Transmembrane Serene Protease 2
TNF- α	Tumor Necrosis Factor - alpha

CHAPTER 1: GENERAL INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus -2 (SARS-CoV-2), was first reported among humans in December 2019 (1), and declared a global pandemic by March 2020 (2). As of July 2022, over 560 million cases and more than 6 million deaths from COVID-19 have been reported globally (3). Under the assumption that the pandemic would be considerably under control by fall 2021, the overall direct and indirect cost of COVID-19 in the United States was anticipated to be over $16 trillion, a number that is likely to have far exceeded today given the ongoing nature of the pandemic. Importantly, due to improved treatment strategies for the acute illness, vaccines that reduce the severity of illness, and emergence of less virulent variants, over 98% of those infected with COVID-19 survive the acute illness (4). However, recent data has shown that individuals who had COVID-19 and are beyond the first month after infection are at an elevated risk for development of cardiovascular disease (CVD) including heart disease, cerebrovascular diseases, and peripheral vascular diseases (5). Importantly, CVD is already the leading cause of morbidity and mortality globally (6), and in the United States (7). Thus, there is an urgent need to better understand the impact of COVID-19 on cardiovascular health and its potential to become a novel risk factor for CVD.

An important prerequisite to development of CVD is compromised vascular health (8–10). Notably, it has become increasingly apparent that SARS-CoV-2 can cause detrimental effects on the vasculature (11). Indeed, although first identified as an acute respiratory illness, early clinical and autopsy reports revealed that COVID-19 is a systemic illness which also affects the cardiovascular system (12–19). Cardiovascular complications such as thrombosis, myocardial infarction, and stroke were frequently observed during the acute phase of the illness even in young otherwise healthy individuals (12–19). However,

whether the potential detrimental effects of COVID-19 on the vascular system persists beyond the acute illness is not yet fully understood.

Accumulating data indicates that up to 50% of individuals, including those who had only mild-moderate acute illness, experience new, ongoing or returning symptoms from COVID-19 for months to years (20). While this condition still lacks a universal definition, it has been referred by several terms including “long COVID”, “post-COVID condition”, and a diagnosis of “post-acute sequalae of COVID-19” (PASC) (21). Many of these patients experience debilitating symptoms that affect their daily activities and quality of life (22–25). Notably, cardiovascular symptoms and complications are also reported among many PASC patients (24, 26). However, whether vascular health is compromised in PASC and the potential link between cardiovascular health and persistent symptomology is yet to be determined.

In summary, the burden of COVID-19 extends beyond the health effects of the acute illness. Almost half of those who had COVID-19 continue to experience ongoing health complications that impact their daily life, and moreover, those who had COVID-19 are at a higher risk for developing CVD. Given the substantial number of individuals who have had COVID-19 and the continuing endemic nature of the virus, it is important to begin to understand the possible factors that may contribute to the long-term health consequences of COVID-19, and factors that may increase the risk of CVD following COVID-19. Herein, the overall goal of this dissertation is to explore the potential persistent impact of COVID-19 on cardiovascular health. In order to achieve this, in chapter 2, several key determinants of cardiovascular health that are being explored in the subsequent studies in this dissertation [i.e., vascular function, arterial stiffness and blood pressure (BP)] are discussed. Factors that influence above determinants of cardiovascular health and methodologies used to evaluate them in the dissertation studies are discussed in detail. The

latter half of Chapter 2 will discuss the current literature on the impact of COVID-19 on cardiovascular health and these key determinants of cardiovascular health. The studies in this dissertation are focused on young healthy adults who had COVID-19 and patients with PASC. Notably, given that COVID-19 initially appeared to be affecting predominantly older individuals and those with comorbidities, early studies investigating the health effects of COVID-19 were primarily in this population. However, young adults between the ages of 18-29 years account for almost one-fourth of the COVID-19 cases in the United States (27). Thus, Chapter 3 and 4 are focused on this age group that has been understudied. Specifically, Chapter 3 investigates the persisting effects of COVID-19 on peripheral and cerebral vascular function and central arterial stiffness in young otherwise healthy adults. Given the importance of high BP as a risk factor for other CVDs, Chapter 4 will explore the influence of time since COVID-19 diagnosis on ambulatory and laboratory measured BP in young adults. Lastly, Chapter 5 explores the impact of PASC on cardiovascular health with an emphasis on the relationship between measures of vascular health and persistent symptomology. Collectively, findings from these studies will provide novel information that is pertinent to understanding the impact of COVID-19 and PASC on long-term cardiovascular health.

CHAPTER 2: REVIEW OF LITERATURE

CVD including coronary heart disease, heart failure, hypertension, and stroke affects almost 50% of the population over the age of 20 year, and is the number one cause of death globally as well as in the United States (7). Traditional risk factors for the development of CVD are numerous and are categorized as modifiable (e.g., smoking, physical activity) and non-modifiable (e.g., age, sex, family history) factors. Beyond traditional risk factors, accumulating research in the past few decades has identified that certain infections may lead to a greater risk of CVD (28). In this regards, recent data suggests that individuals who had the novel viral infection coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus – 2 (SARS-CoV-2), are at an elevated risk for development of CVD (5). This review will focus on discussing several key determinants of cardiovascular health that may contribute to augmenting the risk of CVD following COVID-19, and the current evidence on the impact of COVID-19 on cardiovascular health.

Determinants of Cardiovascular Health

Emerging evidence suggests that a potential first step in the development of CVDs through any risk factor is a pathological insult on the vasculature that causes alteration in normal vascular function (i.e., vascular dysfunction) and structure (8). The general constituents of a blood vessel are similar throughout the arterial tree although changes are observed in the relative amount and arrangement of these constituents based on the functional demand. Notably, each of the different cellular elements possess different

function. For example, the structural proteins collagen and elastin provide tensile strength and elasticity. Vascular smooth muscle cell contraction and relaxation cause vasoconstriction and dilation respectively, which maintains vascular tone. Connective tissue harbors the nerve endings and vasa vasorum for extrinsic control of blood vessels and nutrition, respectively. One of the most important component of the vascular wall is the endothelium, which is addition to its barrier function, is capable of responding to various physical and chemical signals and produce molecules that are vital for the regulation of vascular tone, inflammation, and also cause structural remodeling (29).

Vascular Function

Although blood vessels serve a multitude of functions, dysregulation in the intricate balance between vasodilators and vasoconstrictors that maintain vascular tone is often referred to as vascular dysfunction. Thus, for the purpose of this review, vascular function and dysfunction will refer to normal and altered vascular tone, respectively. Together with vascular smooth muscle cells, the vascular endothelium plays a crucial role in regulating vascular tone by responding to chemical and physical stimuli that activate cellular pathways that produce vasoactive molecules (29, 30). Under basal conditions, vascular tone maintains a slight vasodilatory state that ensures adequate tissue perfusion and maintains vascular resistance and systemic blood pressure (BP). Any adverse insult on the vasculature that causes a disruption in the cellular processes that produce or respond to vasoactive agents generally tips the balance in favor of vasoconstriction and thus could compromise vascular health and tissue perfusion. In general, vascular tone is regulated by similar pathways across all vascular beds, although there some specific differences exist depending on the function and metabolic needs of the tissues. (29, 31).

Peripheral Vascular Function

Modulators of Peripheral Vascular Function

One of the most potent regulators of vascular tone is nitric oxide (NO). NO is produced by endothelial cells from the amino acid L-arginine by the enzymatic action of endothelial nitric oxide synthase (eNOS) primarily in response to shear stress. NO is constitutively produced by the endothelium and freely diffuses to vascular smooth muscle cells. Within the vascular smooth muscles, NO activates guanylate cyclase to convert guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), which activates a cascade of phosphorylation reaction that ultimately causes smooth muscle relaxation and vasodilation. Under basal physiological conditions, NO is the primary vasoactive molecule that maintains vascular tone in favor of vasodilation, which means that when NO activity/bioavailability is reduced, it results in impaired vasodilation and/or unopposed vasoconstriction by other vasoactive molecules (30, 32). NO bioavailability can be reduced due to unavailability of substrate (L-arginine) or other co factors (e.g., BH_4) in the signaling pathway, reduced expression of eNOS, and/or increased inactivation of NO. Importantly, all of these alterations in NO pathway are known to be caused by inflammation and oxidative stress (10). It should also be noted that beyond its role in maintain vascular tone, NO possesses other vaso-protective properties such as inhibition of smooth muscle proliferation and leukocyte adhesion, and exerting anti- thrombotic, and anti-inflammatory effects (33).

While NO is one of the most important regulators of vascular tone, the endothelium produces other vasoactive molecules that help maintain the balance between vasoconstriction and dilation, among which endothelin 1 (ET-1), produced mainly by endothelial cells, is one of the most potent vasoconstrictors (34). The actions of ET-1 are transduced by 2 distinct receptor subtypes ET_A and ET_B which, depending on the location

can exert opposing effects. ET_A receptors are highly expressed in vascular smooth muscle cells whereas ET_B receptors are found on both vascular smooth muscle cells and endothelial cells, though the latter is the primary site. Activation of ET_A and ET_B receptors on the vascular smooth muscle cells, induce vasoconstriction whereas when ET_B receptors on endothelial cells are stimulated vasodilation results (35, 36). Thus, the net effect depends on the predominant action, which under basal conditions is a tendency towards vasoconstrictions which contributes to maintaining vascular tone together with the vasodilators. Upregulation of ET-1 expression and changes in sensitivity of different ET-1 receptors under pathological conditions leads to greater vasoconstriction and vascular dysfunction (37).

In addition to intrinsic mechanism, another important moderator of vascular function in health and disease is the renin-angiotensin-system (RAS). The classical RAS, as was discovered over 100 years ago consists of several steps starting with the enzyme renin that is secreted by the juxtaglomerular apparatus of the kidney primarily in response to reduced renal blood pressure and salt delivery to the macula densa (38). Renin converts angiotensinogen, a glycoprotein secreted by the liver, to the decapeptide angiotensin I (Ang I). Ang I is then converted to active form angiotensin II (Ang II) by angiotensin converting enzyme (ACE) found primarily but not exclusively in the pulmonary endothelial cells (39). Ang II is a vasoconstrictor that under basal conditions, exerts a modest effect (38). Notably, recent advances in research in this area has identified alternate pathways in the RAS as well as components of the RAS in different tissues (i.e., the "local" RAS) such as the heart, vessels, and brain with actions that extend beyond regulation of fluid and blood pressure (40). For example, in addition to causing vasoconstriction, Ang II, acting via angiotensin type 1 receptors (AT1R), have shown to increase oxidative stress, inflammation, fibrosis, and remodeling of the vasculature all outcomes that would lead to the development of

vascular dysfunction (40). In animal models, Ang II has also been shown to act via angiotensin type 2 receptors (AT2R) and oppose the deleterious actions of AT1R activation; the role of AT2R in humans is still unclear (39). Importantly, an alternate pathway that counter-regulates the actions of Ang II was also discovered recently, in which angiotensin converting enzyme 2 (ACE2), a receptor found on many cells including endothelial cells, plays a pivotal role (41, 42). ACE2 converts Ang II to Ang (1-7), a peptide molecule that is anti-inflammatory, antifibrotic, antiproliferative, antiangiogenic, and increases vasodilation, and therefore counteracts the effects of Ang II and acts to promote vascular health (43–45).

A second important extrinsic factor that influences vascular tone in health and disease is the sympathetic nervous system (SNS). The SNS is primarily involved in short-term regulation of vasomotor tone and thereby BP in the phase of various physiological or pathological conditions via reflex mechanisms, although increasing evidence indicates it is also involved in long-term BP regulation (46). The end result of sympathetic activation is norepinephrine-mediated vasoconstriction by activation of α_1 adrenergic receptors on vascular smooth muscle cells. Hence, heightened central sympathetic activity and/or heightened signal transduction at the level of the vasculature (i.e., sympathetic vascular transduction) leads to augmented vascular tone. Importantly, given the systemic nature of the response, augmented vascular tone due to elevated SNS activity results in not only local but also elevated systemic vascular resistance. Recent evidence indicates that the interaction between other vasoactive compounds and the SNS is highly complex and modulates vascular function via both central and peripheral mechanisms (47). For example, on one hand, NO has been shown to inhibit central sympathetic outflow (48) whereas on the other hand, circulating norepinephrine via endothelial adrenergic receptors can stimulate the release of NO. Experimental evidence also indicates that ET-1 can stimulated

SNS activity both centrally and peripherally, although studies in humans are limited (49). Furthermore, Ang II has also been shown to potentiate SNS activity both centrally and peripherally by direct and indirect mechanisms (50). Collectively, these findings provide clear evidence for the complex interactions between SNS activity and vascular function.

Notably, peripheral vascular dysfunction as evidenced by impaired NO-mediated vasodilation has been consistently detected in populations at risk of CVDs such as patients with hypercholesterolemia (51, 52), hypertension (53, 54), obesity and insulin resistance (55), in smokers (56), in ageing (57), and in those with a family history of premature CVD (53, 58, 59). Indeed, vascular dysfunction is considered to be the primary insult that leads to the development of atherosclerosis. Although NO pathway is the one that has been investigated more frequently, heightened vasoconstriction due to overactive ET-1 system has also been implicated in the peripheral vascular dysfunction observed with some CVD risk factors such as obesity, insulin resistance, hypertension, and aging (37). Moreover, inhibiting the actions of Ang II has been shown to improve endothelial function in hypertension and diabetes (60). Furthermore, studies have shown an inverse relationship between markers of SNS activity and vascular function in healthy adults (61, 62) and CVDs and CVD risk conditions that are characterized by peripheral vascular dysfunction (63) are also accompanied by heightened sympathetic activity (47). These findings collectively provide robust evidence for the role of peripheral vascular dysfunction in the development of CVD.

Assessment of Peripheral Vascular Function

Peripheral vascular function is assessed by invasive and non-invasive techniques that usually involve administration of vasoactive substances or inducing shear stress. One technique that is widely used in the research setting is flow-mediated dilation (FMD) test

using duplex Doppler ultrasound (64, 65). The FMD test involves measurement of brachial artery diameter before and after a shear stress stimulus on the artery induced by rapid inflation of a forearm cuff to a suprasystolic pressure for 5 min (64). Since the method was introduced in 1992 by Celermajer et al. (66), FMD technique has gained popularity due to its non-invasive nature and ability to carry out an *in vivo* assessment of macrovascular and microvascular function. In addition, and probably more importantly, brachial artery FMD% (i.e., the percent increase in brachial artery diameter following cuff release) has been repeatedly shown to be correlated with coronary artery endothelial function (67, 68) and independently predicts future cardiovascular events in those with and without preexisting CVD (69–74). Indeed, on a population basis, a 1% reduction in brachial artery FMD has been shown to increase the risk of cardiovascular events by 9 – 17% (71), highlighting the value of FMD in assessment of vascular health and CVD risk. Notably, it has been demonstrated that the dilation of the brachial artery following release of the cuff is at least in part mediated by NO, the contribution of which is greater with a forearm cuff occlusion compared to a upper arm occlusion though the CVD predictive value is greater with the test performed with an upper arm cuff (75).

In addition to providing the means for assessing macrovascular function, the FMD test can also be used to quantify microvascular function (76, 77). As detailed above, a transient period of ischemia is applied on the forearm during the test, which leads to metabolic vasodilation in the forearm microvasculature and generates a reactive hyperemia (RH) after the ischemia is released (76). With the availability of duplex Doppler ultrasound, this RH can be quantified when recording data post cuff release. To date, it is not clear what the best measure of RH is. Indeed, studies showing impaired microvascular function in clinical populations and/or with exposure to potential risk factors have expressed RH as both the blood flow response (78, 79) and blood velocity response (80–83). Notably

though, RH expressed as the blood velocity response appears to be the measure that is predictive of future cardiovascular disease risk (81–83).

Cerebral Vascular Function

Modulators of Cerebral Vascular Function

Cerebral blood flow (CBF) is tightly regulated by overlapping mechanisms to ensure optimal delivery of nutrients and oxygen and removal of waste products in a tight space within the cranial cavity (84, 85). An important mechanism by which CBF is regulated is via changing vasomotor tone. In this regard, arterial partial pressure of O_2 (PaO_2) and carbon dioxide ($PaCO_2$) are two primary regulators of cerebral vascular tone (86, 87). In fact, CO_2 is known to be the most potent vasodilators of the cerebral vasculature in humans. Elevated $PaCO_2$ (hypercapnia) increases cerebral blood flow by causing vasodilation while reduced $PaCO_2$ (hypocapnia) leads to vasoconstriction (86, 88). This effect is observed throughout the cerebral vasculature including both intracranial and extracranial vessels although with different levels of reactivity (88). Notably, the effect of $PaCO_2$ on cerebral blood flow is indirect in that CO_2 diffuses across the blood brain barrier, and then is converted to H^+ and HCO_3^- via the action of carbonic anhydrase (89). The resulting change in pH in the cerebral perivascular tissue is responsible for the changes in cerebral vascular tone (90–92).

The exact cellular mechanisms and signaling pathways that are involved in cerebral vasomotor reactivity (CVMR) to CO_2 are complex and appear to show redundancy(87), which is not unexpected given the importance of tight regulation of CBF. Although this complex interplay between different pathways haven't been fully elucidated yet, endothelial dependent mechanisms similar to those involved in regulation of peripheral vascular function have been found to be important in the cerebral vasculature (85, 87). For

example, studies have shown that administration of a NO donor (93) or L-arginine (94) improves cerebral vascular reactivity to CO_2 in humans, indicating a role of NO. Several other signaling pathways including adenosine, prostaglandins, and arachidonic acids, have been implicated to play a role in regulating cerebral vascular tone in studies that utilized primarily in-vitro models or animal models, However, their exact role in humans need to be fully elucidated. (87). In addition to metabolic vasodilation, the cerebral vasculature is capable of responding to changes in intravascular pressure, whereby increased pressure causes an intrinsic myogenic contraction while decreased pressure causes relaxation, thus leading to vasoconstriction and dilation respectively. This response is mediated via stretch sensitive calcium channels, that increase or decrease intracellular calcium concentrations in vascular smooth muscle cells (95). Myogenic regulation of vascular tone is fundamental in cerebral autoregulation, which is another important aspect of cerebral vascular function.

Importantly, cerebral vasodilator function has been demonstrated to be blunted in populations at risk of cerebrovascular diseases such as obesity (96), hypercholesterolemia (97), diabetes (98, 99), hypertension (99), aging (100), and dementia (101), although some inconsistencies in these finding have been reported especially with regards to aging and hypertension (87). However, despite some inconsistencies in findings in certain populations, it is apparent that cerebral vascular function is impaired in most preclinical populations at an elevated risk for adverse cerebrovascular outcomes. It is also well-established that reduced CVMR is predictive of future risk of stroke and mortality in patients with existing steno-occlusive disease (102, 103). Interestingly, to date, these findings have not been replicated in individuals without carotid stenosis (103), which stimulates some discussion regarding the value of assessing CVMR to determine future risk of cerebrovascular events in healthy populations. However, there is little debate over the fact that impaired cerebral vascular function is associated with CVD.

Assessment of Cerebral Vascular Function

While different methods exist to measure cerebral vascular function, given the profound impact of CO_2 on cerebral vasodilator function, CVMR to CO_2 measured as the magnitude of the change in cerebral blood flow or cerebrovascular conductance to a unit change in $PaCO_2$, is frequently used to quantify cerebral vasodilator function. The methods utilized to induce changes in $PaCO_2$ and imaging modalities used to measure cerebral blood flow are numerous (87, 104). For example, changes in $PaCO_2$ can be elicited by administration of acetazolamide (carbonic anhydrase inhibitor) (105–108), breath holding (109–111), administration of a known concentration of CO_2 in inspired air (103, 111, 112), and CO_2 rebreathing (96, 112–117). Given that the equipment required to perform rebreathing-induced hypercapnia are less expensive and readily available compared to some of the other techniques, this technique is quite frequently used to induce hypercapnia (87). In addition, due to the gradual increase in $PaCO_2$ that occurs with the participant rebreathing their expired air, when used in conjunction with a hyperventilation trial, this technique allows for the exploration of the dynamic relationship between change in CO_2 and cerebral blood flow across a wide range of $PaCO_2$.

Transcranial doppler (TCD) ultrasound is frequently utilized to measure the blood flow response to CVMR (118, 119). TCD allows the user to measure from large intracranial vessels such as the middle cerebral, anterior cerebral, basilar, and posterior cerebral arteries. One caveat with TCD ultrasound is that current systems allow for the measurement of only blood velocity and not vessel diameter in a B-mode image, and thus is unable to quantify the actual blood flow. Therefore, blood velocity is used as a surrogate for blood flow with the assumption that the diameter of the larger intracranial vessels does not change. While this assumption holds true during small changes in CO_2 (120–123), recent studies with high resolution MRI has shown that the diameter may change with larger

changes in CO_2 (120, 124, 125), and therefore it should be kept in mind that cerebral blood velocity measured during hyper- or hypocapnia using TCD may not be proportional to blood flow at certain magnitude changes of $P_{ET}CO_2$.

Arterial Stiffness

Modulators of Arterial Stiffness

Arterial stiffening can be defined as a reduced ability of the vessel to expand and contract in response to pressure changes, or in other words, reduced compliance. Stiffening of vessels occur as a result of complex interactions between structural and functional changes that occur in the vessel, which are influenced by intrinsic and extrinsic factors. (126). Collagen and elastin are the primary constituents that provide tensile strength and elasticity, respectively. By design, the larger central arteries that provide a cushioning effect on peripheral vessels by dampening the pressure oscillation of ventricular ejection (i.e., Windkessel effect) are rich in elastin, and thus have lower stiffness (i.e., greater compliance) compared to peripheral vessels that contain more collagen. However, an imbalance in the amount and functional capacity of these proteins will increase arterial stiffness (126).

In addition to structural changes, vascular function also contributes to arterial stiffness. Indeed, studies from animal models that utilized pharmacological methods to increase or decrease NO bioavailability suggest that NO is a determinant of arterial stiffness (127), although findings from human experiments have been equivocal. For example, administration of a NO donor has been shown to improves artery elasticity (128, 129), while inhibition of endogenous NO-production by an eNOS inhibitor has also been shown to decreased stiffness in the brachial artery (129), indicating a direct effect of NO on stiffness. However, others have shown that in the aorta, the increase in stiffness

observed following inhibition of eNOS was abolished when BP was controlled. (130). While these studies assessed changes in arterial stiffness acutely, given the role of NO in vascular remodeling, it is possible that chronic NO deficiency may also influence arterial stiffness by causing structural changes. Moreover, recent studies have identified a role of dysregulated vascular smooth muscle contraction in arterial stiffness (131). Collectively, these data suggest that arterial stiffness in influenced by several factors with a potentially variable outcome in different vascular beds.

Overwhelming evidence indicates that central arterial stiffness, is a strong independent predictor of future cardiovascular events and mortality in high-risk and low risk populations (132, 133). Interestingly though, the relationship between arterial stiffness and common risk factors for CVD such as obesity, hypercholesterolemia, diabetes, and smoking appears to be weak and less consistent, whereas, age and BP have been consistently shown to be strongly associated with stiffness (134). This suggests that the predictive value of arterial stiffness is likely due to factors related to arterial aging and hypertension more than other risk factors. Indeed, it is well-known that aging is the strongest risk factor for arterial stiffening. However, the cause-and-effect relationship between arterial stiffness and BP is still a topic of much debate. For example, acute increases in BP have been shown to increase arterial stiffness under experimental conditions (130) indicating that BP affects arterial stiffness. However, findings from longitudinal assessments on the temporal relationship between central arterial stiffness and incident hypertension indicates that arterial stiffness predates the development of hypertension (135–137). Overall, it is apparent that arterial stiffness and BP are tightly linked and interrelated, and thus both are important aspects of cardiovascular health.

Assessment of Arterial Stiffness

Among several methods used to assess arterial stiffness, two measures that are frequently used are pulse wave velocity (PWV), and pulse wave analysis (PWA). PWV is defined as the time taken for a pressure pulse to travel between two points, is a technique that is widely used both in the clinical setting as well as research setting (138–140). Higher PWV is indicative of greater stiffness. Notably, carotid-femoral PWV which is used as a surrogate for aortic PWV, has been shown to independently predict future risk of cardiovascular events, target organ damage, and mortality in those with and without preexisting risk factors across the lifespan (132, 137, 141). Carotid-femoral PWV is thus considered the gold standard for the assessment of arterial stiffness (138, 140). While the true aortic PWV can be measured using intra-arterial catheters or magnetic resonance imaging (140, 142), these techniques are invasive and/or expensive. However, carotid-femoral PWV can also be measured by other simple non-invasive techniques (139, 140, 143). Importantly, these methods show good reproducibility and have been validated against the direct measures (144–147).

Another parameter that is frequently used as an index of arterial stiffness is the augmentation index (AIx) through PWA. AIx is derived from the ascending aortic pressure waveform, and is defined as the difference between the second systolic peak (caused by the reflected wave) and first systolic peak (caused by the forward wave), expressed as a percentage of the pulse pressure (139). AIx is well known to be affected by heart rate, and thus should be corrected for differences in heart rate between participants (148, 149). Even though AIx is often reported as a measure of arterial stiffness the arterial waveform is affected by not only arterial factors but also cardiac factors (140, 150). Therefore it is recommended that AIx is used as an indirect measure of arterial stiffness (140).

Blood Pressure

Regulation of BP is likely one of the most complex physiological processes of the body and undoubtedly one of the most important. Indeed, in health, BP is tightly regulated to ensure appropriate perfusion of vital organs under different physiological and pathological conditions in the short-term and long-term. A multitude of mechanisms are involved in the regulation of BP, and therefore, there are many different factors that contribute to the pathophysiology of hypertension, all of which are still not well understood (151). However, BP is maintained via a balance between cardiac output and peripheral resistance, thus factors that affect these two components will alter BP. As briefly discussed in the preceding sections, there is a complex interaction between vascular function, arterial stiffness, and sympathetic nervous system activity, and dysregulation of each alone or collectively can lead to elevated vascular resistance, and/or changes in cardiac function. Thus all these derangements in the cardiovascular system have been implicated in the pathogenesis of hypertension (151–154). Indeed, in supporting this hypothesis, endothelial dysfunction (53, 59), arterial stiffness (135–137), and augmented sympathetic activity (155, 156) have been shown to precede hypertension in populations at risk of developing hypertension.

High BP is one of the strongest risk factors for the development of almost all other CVD (7). For example. data from a comprehensive meta-analysis on prospective observational studies of BP and mortality showed that risk of CVD increases in a log-linear fashion starting from BP values as low as 115 systolic BP and 75 diastolic BP in adults 40 – 89 years (157). From this study, it was also revealed that a 20 mmHg higher systolic BP or 10 mmHg higher diastolic BP was associated with at least a 2-fold higher risk of death from CVD in those between 40 – 69 years, and (157). Notably, similar findings have been observed in studies in young adults 18 years and above, in that systolic and diastolic BP

values above normal BP as per the guidelines (158) was found to be associated with a graded increase in risk of CV events (159). Importantly, although many factors can affect BP, if detected early, high BP is a modifiable risk factor. Indeed, a 10 mmHg reduction in systolic BP has been shown to reduce the risk of cardiovascular events by up to 20% and all-cause mortality by 13%, an association that was observed even in the presence of baseline BP below 130 mmHg (160). Collectively, these findings highlight the importance of early detection of high BP and the need to monitor BP over time in all age groups irrespective of the baseline BP.

Although it is usually measured intermittently, BP is a continuous variable that is regulated via complex interaction between internal cardiovascular regulatory mechanisms and external stimuli. Thus, fluctuations in BP, termed BP variability is a physiological phenomenon that maintains cardiovascular homeostasis and can be observed on a beat-to-beat basis as well as over the course of a day (i.e. day to night variability) (161, 162). Notably, beyond the risk of CVD associated with elevated means systolic and diastolic BP values, beat-to-beat and 24-hr BP variability has been shown to be independently predictive of cardiovascular outcome and target organ damage (163–165). Moreover, elevated BP variability has been demonstrated in normotensive healthy individuals at a greater risk for hypertension and CVD such as Black individuals (166, 167), indicating that augmented BP variability may precede the development of high BP. Thus, assessment of BP should include the measurement of mean systolic and diastolic BP as well as BP variability both in the clinical and research settings.

Assessment of Blood Pressure

Laboratory BP

Blood pressure measurement in the laboratory is performed using manual or automated devices and is frequently measured at the brachial artery. Participant should be seated or supine, and relaxed and rested before obtaining measurements. It is recommended that the first measurement is discarded due to possibility of obtaining an erroneous reading, and an average of ≥ 2 measurements taken 1 -2 min apart should be considered as the resting BP (168, 169). In addition to resting intermittent BP measurements, non-invasive beat-to-beat BP monitoring using finger photoplethysmography is a valuable tool that allows for the assessment of dynamic changes in BP at rest as well as during various perturbations used to assess cardiovascular and autonomic control mechanisms (170). However, one important consideration to be aware of is that the non-invasive beat-to-beat BP measurement devices that are widely available (e.g., Finometer) measure the BP at the finger and reconstructs a brachial pressure using a height-correction, the accuracy of which has been questioned (171). Thus, these measurements are more suited for quantifying changes in BP rather than absolute BP.

Ambulatory BP

Ambulatory BP measurement is usually carried out using automated portable BP monitors that are programmed to obtain BP measurements at a pre-defined interval throughout a continuous 24-hr period (172, 173). Measurement of BP over an extended period of time provides important information beyond that can be obtained by BP measurement during a single or multiple intermittent visits. For example, 24-hr ambulatory BP monitoring identifies phenomena such as “white coat hypertension” (i.e., elevated BP only in an office/clinic setting), “masked hypertension” (i.e., normal office/clinic BP

despite elevated ambulatory/home BP), nocturnal hypertension, and nocturnal dipping status, conditions which are associated with elevated CVD risk (172, 174, 175). In addition, elevated mean daytime and 24-hr ambulatory BP, and BP variability are also strongly predictive of cardiovascular and all-cause mortality independent of other risk factors (163, 176–178). Overall, 24-hr ambulatory BP monitoring provides the means for a more comprehensive assessment of BP and BP variability in clinical and research settings.

Cardiovascular Health and COVID-19

Coronaviruses are a family of large enveloped single stranded RNA viruses (179). Based on their structure, they are classified in to four genera: alpha, beta, delta, and gamma, and to date only the alpha and beta coronaviruses are known to infect humans (180, 181). Among seven different coronaviruses known to cause human disease, three have caused severe illness, namely Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) that caused the SARS epidemic in 2002, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) that caused the deadly MERS outbreak in 2012, and SARS-CoV-2, which is responsible for the current COVID-19 pandemic (182). Some similarities and differences exist between the three viruses. For example, SARS-CoV and MERS-CoV have higher fatality rates than SARS-CoV-2 whereas SARS-CoV and SARS-CoV-2 have high rates of transmission among humans compared to MERS-CoV. Overall, the high transmissibility, relatively lower fatality rate, and high reproductive rate of SARS-CoV-2 led to the unprecedented magnitude of the COVID-19 pandemic (183, 184). Indeed, the spectrum of clinical illness from SARS-CoV-2 infection ranges from asymptomatic to mild and moderate, to severe and critical (185). All these degrees of disease severity have been observed in all age groups and in individuals with and without preexisting comorbidities.

Pathogenesis of COVID-19

Coronaviruses are made up of 4 structural proteins, out of which the spike protein (S), which is made up of two subunits S1 and S2, is essential for the entry of the virus into host cells (181). The receptor binding domain (RBD) on the S1 subunit binds the virus to host cells and initiates infection while the S2 subunit is important in membrane fusion and entry of the virus into the host cells. Importantly, in the case of SARS-CoV-2 (and SARS-CoV-1), the ACE2 receptor in the RAS is the gateway to the cell (186). The virus binds to ACE2 on a target cell and activates a serene protease on the host cell (TMPRSS2) that cleaves and activates the S2 subunit to facilitate fusion of the viral envelope with the host cell membrane. This allows the viral genome to enter and replicate within the host cell (186). A second method of ACE2-mediated viral entry into the host cell involves endocytosis of the viral particle (187). The process of viral binding and entry into the host cells trigger a multitude of responses that leads to the pathogenesis and complications of COVID-19.

ACE2 is a membrane-bound peptidase receptor that is expressed in abundance in the respiratory epithelium, and as such results in a primary respiratory disease when infected by SARS-CoV-2. However, ACE2 has also been identified in many other tissues including the vascular endothelium (188). Therefore, direct infiltration of the virus in vascular endothelial cells and the ensuing local inflammation is one mechanisms that could alter vascular health following COVID-19 (189). Indeed, early post-mortem studies demonstrated the presence of endotheliitis (190, 191), although this has not been a consistent finding in later studies.

Binding of the virus to ACE2 has also been shown to downregulate the receptor (192) and/or release soluble ACE2 into the circulation (193), both of which leads to a reduction in ACE2 activity. As discussed in *"Modulators of Peripheral Vascular*

Function" reduced ACE2 activity reduces the production of Ang (1-7) and elevates the concentration of circulating Ang II (194). These changes would potentially shift the balance in the RAS towards greater Ang II activity leading to increased vascular inflammation, thrombosis, fibrosis, and vasoconstriction (194–196). In addition, severe and critical COVID-19 is associated with a cytokine storm which is also proposed to be a result of increased Ang II and reduced ACE2 activity (197). Notably, the cytokine storm in COVID-19 is characterized by a significant elevation in proinflammatory cytokines such as IL-6, IL-4, TNF-α, and INF-γ, which are well known to exert a detrimental effect on the cardiovascular system (198) as well as further downregulate ACE2 (199).

Cardiovascular Health During the Acute Phase of COVID-19

Given the pathways involved in the pathogenesis of COVID-19 that cause local and systemic inflammation and RAS imbalance, it is highly plausible to postulate that COVID-19 could exert detrimental effects on the cardiovascular system during acute illness. Indeed, cardiovascular and cerebrovascular complications such as myocardial infarctions, stroke, vasculitis, and venous thromboembolism have been observed during the acute phase of illness in those with mild or severe respiratory symptoms as well as in those with no respiratory symptoms. Further, cardiovascular complications were observed in young and old individuals with and without pre-existing comorbidities (12–19, 184). These clinical observations prompted many researchers to directly explore the impact of COVID-19 on vascular function during the acute phase of the illness (i.e., within 4 weeks from symptom onset/diagnosis) and in those who were hospitalized (200–206). Studies on hospitalized patients have consistently reported blunted peripheral macro (200, 204) and microvascular (205, 207) function. Notably, although some investigators revealed FMD to be predictive of COVID-19 outcome (204), others did not observe similar findings (208). While it might

be expected that patients with severe COVID-19 illness that warranted hospitalization exhibit vascular dysfunction due to a multitude of reasons, surprisingly, several studies have reported significant impairment in macro and micro vascular function in young previously healthy adults who had only mild to moderate symptoms from COVID-19 (202, 203, 206). For example, Trinity et al. (206) reported a 1.3% reduction in brachial artery FMD, reduction in forearm RH, and lower leg microvascular function from pre to post diagnosis in a case report of a 24-year old female while she was still asymptomatic from COVID-19. Similarly, Jud et al. (203) reported blunted brachial artery FMD in a 24-year-old previously healthy female with asymptomatic COVID-19. Findings from these case reports are further strengthened by a study by Ratchford et al. (202) that demonstrated significant impairment in brachial artery FMD and blood flow response to passive leg movement in young adults when tested within 3-4 weeks from mild to moderate COVID-19 diagnosis compared to historical controls. Collectivity, these findings provide strong evidence of impaired peripheral vascular function irrespective of disease severity, age, or previous health status.

Although there has been a substantial number of studies focused on evaluating peripheral vascular function, limited data is available on the effects of COVID-19 on cerebral vascular function. This is surprising given the unexpectedly high incidence of cerebral vascular events that have been reported during the acute illness in previously healthy adults, including young adults (18, 209–212). Furthermore, brain biopsies in patients who had cerebrovascular events showed endothelial injury (213), indicating the potential for SARS-CoV-2 to cause cerebral vascular dysfunction. Indeed, one study reported blunted reactivity to CO_2 following a breath hold test in hospitalized middle-aged patients with COVID-19 during acute illness (214). However, whether these findings extend to other age groups and to those with less severe disease is yet to be fully explored.

In addition to vascular dysfunction, another potential detrimental effect of COVID-19 on the vasculature is elevated arterial stiffness, a strong risk factor for CVD. Indeed, it is well known that chronic low-grade inflammation as well as acute inflammation contributes to arterial stiffness (215, 216). Although no studies to date have revealed a causative relationship between COVID-19 induced inflammation and arterial stiffness, several studies have reported elevated arterial stiffness in hospitalized older patients (217, 218) as well as young otherwise healthy adults with acute mild to moderate illness (202, 203, 219).

Cardiovascular Health Beyond the Acute Phase of COVID-19

Based on the previously discussed evidence of the negative impact of COVID-19 on vascular health during the acute phase, there has been a growing interest in investigating the persistent effects of COVID-19 on the cardiovascular system (200, 203, 220–227). Notably, majority of these studies have been performed in middle to older aged adults who were hospitalized and/or experienced severe or critical illness during the acute phase (200, 220, 221, 223–226). Findings from these studies in individuals with who had severe acute illness indicates persistent impairment in peripheral macro and microvascular function up to 1 year post diagnosis (200, 220, 223, 225, 226). These results are further supported by the finding of elevated cellular markers of endothelial activation and dysfunction several months post infection (228, 229). Notably, endothelial dysfunction was shown to be associated with elevated pro-inflammatory markers, suggesting a role of persistent inflammation in causing ongoing vascular dysfunction (228). Although not investigated in detail, cerebral vascular function has also been showed to be reduced several months post COVID in hospitalized patients (230, 231). Extending the findings during the acute phase

of the illness, few studies have reported persistently elevated arterial stiffness in patients who had severe acute COVID-19 (221–223).

Although these findings on patients with complicated acute COVID-19 are important, over 80% of individuals who are diagnosed with COVID-19 experience only mild to moderate symptoms, and therefore it is important to identify long-term effects of COVID-19 in these individuals. However, there is limited data on the persistent impact of COVID-19 on vascular health in this population (220, 223, 224, 226, 232), and findings from these studies are less consistent compared to data on those with severe disease. For example, one study reported no impairment in peripheral vascular function in young adults following mild acute illness (232), whereas another study reported persistently blunted FMD in older adults (223) compared to age-matched controls without COVID-19. Interestingly, when compared to those who had severe acute illness, Riou et al. (226) found that those who had mild acute illness had greater impairment in FMD while Santoro et al. (224) reported less impairment, and Gao et al. (220) reported no difference in the level of impairment in FMD between the different levels of disease severity.

Importantly, some investigators have found promising results of improvement in vascular health over the course of several months (221, 233, 234). However, others have reported vascular dysfunction at one year post diagnosis (220). Collectively, while there have been a substantial number of studies investigating the long-term impact of COVID-19 on cardiovascular health, findings are not unambiguous. Potential reasons for the equivocal findings between studies could be due to several factors including the differences in age of participants (young and old), time since COVID-19 diagnosis at the time of testing (few weeks to beyond a year), and presence or absence of other persistent complications from COVID-19. Thus, further studies are indicated to fully understand the long-term impact of COVID-19 on cardiovascular health.

Interestingly, despite the well-known associations between vascular dysfunction, arterial stiffness, high BP, and CVD risk (135, 152), limited studies have focused on exploring the acute and long-term effect of COVID-19 on BP. One study in middle and older-aged adults with no prior history of hypertension reported only 8% of individuals had elevated BP while hospitalized with COVID-19, while another study reported elevate BP in 12% of the studied group one month post hospital discharge following COVID-19 in middle-aged adults (235). Interestingly, Szeghy et al. (219) reported higher BP in young adults who had COVID-19 during the acute phase compared to those without a history of COVID-19, whereas others have reported no difference (219, 232). However, BP measurement has been performed in the laboratory/hospital setting with the exception of a case report that reported higher ambulatory BP in one patient who was previously normotensive (236), and no difference in ambulatory BP between those with and without fatigue following COVID-19 (237). As discussed under the section "*Blood Pressure"*, since measurement at a single time point does not capture the true BP or the fluctuation in BP in an individual, it is important to perform more comprehensive assessments of BP using both laboratory and ambulatory techniques to determine the extent of COVID-19 on BP.

Post-Acute Sequalae of COVID-19/ Post-Covid Condition/ Long COVID

While it is necessary to understand the long-term effects of COVID-19 in general, a significant proportion of individuals infected with SARS-CoV-2 experience a wide range of symptoms from COVID-19 that persists for weeks to months after initial diagnosis (20, 21). This condition has been referred to by several terms over the past 2.5 years including "COVID-long haulers," "long COVID," "post-acute sequalae of COVID-19" (PASC), or "post-COVID condition". Importantly, based on data from past coronavirus infections such

as SARS and MERS, persistence of symptoms or complications following severe acute COVID-19 illness with multiorgan involvement would not be unanticipated (238–241). However, debilitating persistent symptoms have been reported in a large number of previously healthy individuals of all age groups who had merely mild or moderate acute illness (22–25). To date, a universal definition for this condition is lacking. The Center for Disease Control currently defines PASC/post-COVID condition as *"a wide range of new, returning, or ongoing health problems experienced by patients at least 4 weeks after initially being infected by SARS-CoV-2"* (242), which would include those who have persistent symptoms that may or may not be affecting functionality, whereas the World Health Organization defines it as "*new onset, returning, or persistent symptoms, at 3 months from the onset of COVID-19 with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis, and impacts everyday functioning*" (243), which potentially excludes those within the first three months after diagnosis and those who do not have functional impairments. Given the discrepancies in the diagnostic criteria, follow up period, and selection biases such as inclusion of only hospitalized patients or only non-hospitalized patients in studies, the reported incidence of persistent symptoms from COVID-19 varies between 10 to ~50% at 6 months after diagnosis. (20, 22, 25, 244, 245). Female sex, older age, and preexisting comorbidities have been found to be potential risk factors (246, 247), although males and previously healthy individuals across all age groups have been found to present with persistent symptoms. Therefore, given the large number of individuals experiencing persistent symptoms, it is of the utmost importance to investigate the extent, potential contributing factors, and consequences of PASC to be able to identify potential targets for alleviating persistent symptoms and/or minimizing the occurrence of PASC following SARS-CoV-2 infection.

Cardiovascular Health in PASC

Similar to acute COVID-19, symptoms of post COVID condition appears to involve multiple organ systems. Over 50 post COVID sequalae have been reported, out of which fatigue, headache, cognitive problems and shortness of breath, are among the most frequently reported symptoms (20, 24, 245). The mechanisms contributing to this condition is still largely unknown and unexplored. However, based on what is known regarding other post viral conditions, pathogenesis of acute COVID-19, and common post COVID symptomology several theories involving cardiovascular system derangements have been postulated.

One pathophysiological process that has been proposed to contribute to PASC is an ongoing direct or indirect negative impact of COVID-19 on vascular health (248, 249). This theory is supported by several factors. Vascular dysfunction has been implicated to play a pathophysiological role in other post viral conditions such as chronic fatigue syndrome (250, 251), a diagnosis that has also been made in a subpopulation of patients with PASC (252). Furthermore, current evidence indicates persistent endothelial dysfunction beyond the acute phase of COVID-19 illness. However, the symptomology of the participants have been evaluated only in a handful of studies (200, 223, 227), and therefore it is still not fully understood whether PASC is associated with persistent vascular dysfunction. For example, one study reported that microvascular function assessed by peripheral arterial tonometry was impaired in only 30% of PASC patients at nine months since initial mild to moderate infection, compared to those without a history of COVID-19 (227), whereas, another study reported that brachial artery FMD was similar between those with and without a PASC diagnosis at 6 months after hospitalization but lower compare to controls with no history of COVID-19 (200). Improvement in brachial artery FMD was also observed in both groups (i.e., with and without PASC) over the 6 months period in

this study (200). The latter findings may indicate that there is a slow recovery from vascular dysfunction following COVID-19 regardless of symptomology. However, these findings do not provide robust evidence regarding whether peripheral vascular function is affected in PASC. Moreover, although many patients with PASC experience neurological symptoms such as impairments in memory, concentration, and articulation (i.e., brain fog), headaches, and fatigue, no studies have assessed cerebral vascular function in PASC. Interestingly in a recently reported case series (253), two patients with PASC who were treated with a stellate ganglion blockade, a procedure known to improve cerebral blood flow (254), showed improvement in PASC symptoms including brain fog. While these data provide preliminary evidence on potential dysregulation of cerebral blood flow in PASC, further studies are warranted to fully understand the impact of PASC on the cerebral vasculature and brain health.

Importantly, the actual symptoms, number of symptoms, and severity of each symptom varies considerably between PASC patients, as does the disabilities caused by these symptoms. These differences could potentially be leading to the discrepant findings between studies investigating cardiovascular health in PASC. Thus, better characterization of participants based on their symptomology, and evaluating whether there is an association between symptomology and cardiovascular health would be an important and essential consideration for future studies. Furthermore, although it is known that individuals who had COVID-19 are at an elevated the risk of future CVD (5), it remains to be determined if this is driven by PASC, and therefore, should undeniably be a focus of future studies.

Conclusion

Since the SARS-CoV-2 infection was first detected in humans in December 2019, millions of individuals have been affected and continue to be affected by COVID-19. In addition, the extend of PASC has also become an unforeseen challenge for the patients as well as the medical and scientific community. Moreover, potentially adding to the already existing health burden from COVID-19 and CVD, a recent study found that the risk of future CVD is elevated in individuals who had COVID-19. This includes those who had mild-moderate illness, who account for the majority of the survivors from COVID-19 (5). Therefore, it is imperative to identify effects of COVID-19 that may persist and augment CVD risk. Although there has been an immense response from the scientific community to fully understand the effects of COVID-19 and PASC on cardiovascular health, much is still unknown regarding the longer-term effects. The studies proposed in this dissertation explores the impact of COVID-19 on cardiovascular health in two specific populations; 1) an age group that has been greatly affected by COVID-19 (i.e., young adults 18 – 30 years), and 2) patients with PASC, a population that continues to suffer from long-term complications of COVID-19. The findings from the studies in this dissertation provides novel clinically relevant information that will significantly improve our understanding on short and long-term cardiovascular health after COVID-19. These findings will pave the way for future studies to identify potential targets aimed at reducing the long-term burden of COVID-19 on cardiovascular health and CVD risk.

CHAPTER 3: BLUNTED PERIPHERAL BUT NOT CEREBRAL VASODILATOR FUNCTION IN YOUNG OTHERWISE HEALTHY ADULTS WITH PERSISTENT SYMPTOMS FOLLOWING COVID-19

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Abstract

Recent findings suggest that COVID-19 causes vascular dysfunction during the acute phase of the illness in otherwise healthy young adults. To date, no studies have investigated the longer-term effects of COVID-19 on vascular function. Herein, we hypothesized that young, otherwise healthy adults who are past the acute phase of COVID-19 would exhibit blunted peripheral (brachial artery flow-mediated dilation (FMD) and reactive hyperemia) and cerebral vasodilator function (cerebral vasomotor reactivity to hypercapnia; CVMR) and increased central arterial stiffness. Sixteen young adults who were at least 4 weeks past a COVID-19 diagnosis and 12 controls who never had COVID-19 were studied. Eight COVID subjects were symptomatic (SYM) and 8 were asymptomatic (ASYM) at the time of testing. FMD and reactive hyperemia were not different between COVID and Control groups. However, FMD was lower in SYM (3.8 ± 0.6%) compared to ASYM (6.8 ± 0.9%; P = 0.007) and Control (6.8 ± 0.6%; P = 0.003) with no difference between ASYM and Control. Similarly, peak blood velocity following cuff release was lower in SYM (47 ± 8 cm/s) compared to ASYM (64 ± 19 cm/s; P = 0.025) and Control (61 ± 14 cm/s; P = 0.036). CVMR and arterial stiffness were not different between any groups. In summary, peripheral macro- and microvascular function, but not cerebral vascular function or central arterial stiffness were blunted in young adults symptomatic beyond the acute phase of COVID-19. In contrast, those who were asymptomatic had similar vascular function compared to controls who never had COVID.

New and Noteworthy

This study was the first to investigate the persistent effects of COVID-19 on vascular function in otherwise healthy young adults. We demonstrated that peripheral macro- and microvascular vasodilation was significantly blunted in young adults still symptomatic from COVID-19 beyond the acute phase (>4 weeks from diagnosis) while those who become asymptomatic have similar vascular function compared to controls who never had COVID-19. In contrast, cerebral vascular function and central arterial stiffness were unaffected irrespective of COVID-19 symptomology.

Introduction

The novel coronavirus disease – 2019 (COVID-19) has affected over 30 million individuals in the Unites States alone. To date, the prevalence of COVID-19 has been highest in young adults, with almost 1 in 4 cases being diagnosed in those between 18 – 29 years of age (255). Notably, early investigations revealed that SARS-CoV-2, the virus causing COVID-19, infects and damages vascular endothelial cells (190). However, limited studies have directly investigated the effects of COVID-19 on vascular function.

Notably, recent work reported that young adults who had COVID-19 and were within 4 weeks from diagnosis (i.e., within the acute phase of the illness (256)) at the time of testing exhibited attenuated peripheral vascular function (202) and elevated arterial stiffness (202, 219) compared to those who never had COVID-19. However, whether these detrimental effects on the vasculature persist or resolve beyond the acute phase of the illness remains unknown. This becomes quite important because recent evidence showed that up to 1 in 4 young adults, including those with mild symptoms during the acute phase, report persisting symptoms for up to 6 months from diagnosis (22). Also, individuals who have had COVID-19 frequently experience lingering headaches and cognitive deficits (22, 257) suggestive of cerebral vascular impairments. However, despite these observations, to our knowledge, no studies have investigated whether cerebral vascular function is altered following COVID-19.

With this background in mind, we sought to comprehensively assess the potential persisting effects of COVID-19 on vascular function in young adults. We hypothesized that young, otherwise healthy adults who are beyond 4 weeks from a COVID-19 diagnosis would exhibit blunted peripheral and cerebral vasodilator function and increased central arterial stiffness compared to those who did not have COVID-19.

Methods

Study Population

Sixteen young otherwise healthy adults who had a lab confirmed diagnosis of COVID-19 (COVID; SARS-CoV-2 RT-PCR or antigen test), were studied. All COVID subjects were a minimum of 4 weeks from their diagnosis and reported being symptomatic at the time of diagnosis. Severity of each symptom was ranked on a scale of 1 – 10 and quantified both for the time of COVID-19 diagnosis and on the date of the study visit. At the time of testing, eight were asymptomatic (ASYM; age: 22 ± 4 years; BMI: 22 ± 4 kg/m^2; 12 ± 5 weeks [range: 4 to 21 weeks] from diagnosis; Male/Female: 5/3) (mean ± SD), while 8 remained symptomatic (SYM; age: 24 ± 3 years BMI: 26 ± 3 kg/m^2; 14 ± 4 weeks [range: 7 to 20 weeks] from diagnosis; Male/Female: 1/7). Symptoms reported at the time of testing by SYM subjects were loss of smell and/or taste (n = 7), fatigue (n = 1), and severe muscle pain after exercise (n = 1), and the average symptom severity was 4 ± 1. All ASYM subjects reported that symptoms resolved within 1 month of their COVID-19 diagnosis. Twelve adults who did not have COVID-19 were also studied and served as Controls (age: 23 ± 3 years; BMI: 23 ± 3 kg/m^2; Male/Female: 6/6).

All subjects were non-smokers and were not on any prescription medication. Subjects reported being recreationally active (Control: 280 ± 230 exercise min/week, ASYM: 259 ± 193 min/week, SYM: 227 ± 98 min/week; no difference between groups), and free from any known cardiovascular, cerebrovascular, metabolic, or neurological diseases. All experiments were carried out following an overnight fast in a temperature-controlled (20 – 22 °C) dimly lit room. Subjects were instructed to abstain from caffeine and any over-the-counter medication for at least 12-hours and alcohol and exercise for at least 24-hours prior to the study. After receiving a detailed verbal and written explanation of the experimental protocol, subjects provided informed written consent. All experimental

procedures conformed to the Declaration of Helsinki and were approved by the Institutional Review Board at the University of Texas at Arlington (2021-0197).

Experimental Measurements

Subjects were instrumented with a standard lead II electrocardiogram (model Q710, Quinton, Bothell, WA) to continuously measure heart rate (HR) and a pneumobelt (Pneumotrace II 1132, UFI, Morro Bay, CA) to monitor respiration. Beat-to-beat arterial blood pressure (BP) was measured via finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, The Netherlands). Automated sphygmomanometer BPs (Welch Allyn, Skaneateles Falls, NY) were obtained for subject characterization and to confirm the Finometer blood pressures.

Experimental Protocol

Subjects rested supine for 20 minutes prior to data collection. For assessment of peripheral vascular function, brachial artery flow-mediated dilation (FMD) technique was performed using current guidelines and as previously described (64, 258). Briefly, the brachial artery was imaged using duplex Doppler ultrasound (GE Logiq P5, Milwaukee, WI) and an 11-MHz linear array transducer. Continuous blood velocity and diameter measures were simultaneously obtained with the sample volume encompassing the entire lumen but not extending beyond the arterial wall edges. Baseline data were recorded for 2 min, following which a rapidly inflating cuff (Hokanson, Bellevue, WA) placed ~2 cm distal to the antecubital fossa was inflated to a suprasystolic pressure (220 mmHg) for 5 min. Blood velocity and diameter data were recorded for 30 sec prior to and for 3 min following cuff release.

Following FMD, central arterial stiffness was assessed by carotid-femoral pulse wave velocity (PWV) and pulse wave analysis (PWA) (SphygmoCor, Atcor Medical, Sydney, Australia) as described previously (258). For PWV, a cuff was placed on the thigh, and carotid and femoral pulses were palpated at the strongest points. Measurements were made between 3 sites (carotid artery to sternal notch, sternal notch to thigh cuff, femoral artery to thigh cuff). An arterial BP waveform was detected using a handheld tonometer placed over the carotid artery while the thigh cuff was inflated. PWV was calculated (XCEL 1.3, Atcor Medical, Sydney, Australia) as the carotid-femoral artery distance divided by the pulse transit time. For PWA, a brachial cuff around the left arm measured peripheral pressure waveforms and generated a corresponding aortic waveform from which augmentation index normalized to a HR of 75 beats/min (AIx75) was derived. PWV and PWA measurements were not obtained in two control subjects.

Next, HR and automated brachial artery sphygmomanometer BPs were obtained during a 5 min quiet resting period. Subjects were then instrumented with a 2-MHz transcranial doppler (Multigon Industries Inc., Yonkers, NY) ultrasound probe placed over the left temporal window to obtain middle cerebral artery blood velocity (MCAv) measurements. The probe was held in place using a headband. A rebreathing protocol was carried out for assessment of cerebral vascular function (96). Briefly, subjects were fitted with a nose clip and breathed through a mouthpiece attached to a 3-way valve (Hans Rudolph Inc., Kansas City, KS,). Oxygen (O_2) was bled into the 5-L rebreathing bag throughout the protocol to ensure normal arterial O_2 saturation (SpO_2) based on the subject's basal metabolic rate as estimated using the Harris–Benedict formula (259). End-tidal carbon dioxide ($P_{ET}CO_2$) and SpO_2 were measured through a sampling line connected to the mouthpiece, and pulse oximeter on their index finger respectively, which were connected to a capnograph (Capnocheck Plus, Smiths Medical, Dublin, OH). Before the

start of the protocol, the rebreathing bag was filled with the subject's own air. A 3 min baseline was then performed while subjects breathed room air. The valve was then switched, and subjects rebreathed from the bag for 3 min or until they reached a $\Delta P_{ET}CO_2$ of at least 15 mmHg from baseline. The valve was then switched back to room air for 3 min of recovery. Two control and one COVID (ASYM) subject did not perform the rebreathing protocol.

Data Analysis

HR, BP, respiration, MCAv, $P_{ET}CO_2$ and SpO_2 were recorded continuously at 1,000Hz using PowerLab (ADInstruments, Bella Vista, Australia). For baseline subject characterization, HR was averaged over the 5 min resting period, and mean systolic (SBP) and diastolic BP (DBP) was calculated from 3 automated sphygmomanometer readings. A customized offline wall tracking and edge detection software (LabView, National Instruments, Austin, TX) was used to analyze brachial artery diameter and weighted mean blood velocity. For assessment of peripheral artery macrovascular function, brachial artery FMD was calculated as FMD% = (3-beat average peak diameter - baseline diameter)/baseline diameter × 100. Shear rate was calculated as 8 × mean blood velocity/diameter. The shear stimulus for FMD was calculated as the hyperemic shear rate area under the curve (AUC) via the sum of trapezoids method to peak brachial artery dilatation. Microvascular function was quantified as the 3-beat average peak blood velocity after cuff release. Central arterial stiffness was quantified using the average of two measures of PWV and AIx75. For quantification of cerebral vasomotor reactivity (CVMR), average values for $P_{ET}CO_2$, MCAv, MAP and cerebral vascular conductance index (CVCi = MCAv/MAP) were calculated over the last 1 min of baseline. During the rebreathe, 3-breath averages of MCAv and CVCi at $\Delta P_{ET}CO_2$ of 3, 6, 9, 12, and 15 mmHg were

calculated. CVMR was quantified as percent increase in MCAv (ΔMCAv%) and CVCi (ΔCVCi%) at each $\Delta P_{ET}CO_2$.

Statistical Analysis

Resting cardiorespiratory and hemodynamic parameters, FMD, reactive hyperemia, PWV, and AIx75 between Control and COVID group were analyzed using Student's t-test for independent samples (SPSS, version 25). Further comparisons between Control, ASYM and SYM were made using one-way ANOVA. When significant group differences were observed, pairwise comparisons were made using Fisher's least significant difference post hoc test. CVMR was analyzed using 2-way repeated-measures ANOVA for effects of group (Control vs. COVID and Control vs. ASYM vs. SYM) and time ($\Delta P_{ET}CO_2$ level). Due to the observation of differences in the shear stimulus for FMD between Control, ASYM and SYM, ANCOVA was performed to co-vary statistically for the impact of hyperemic shear rate on FMD values. All data are presented as mean ± SD, and the significance level was set at $\alpha < 0.05$.

Results

Control and COVID

Resting brachial artery diameter (Control: 0.34 ± 0.04 cm vs. COVID: 0.34 ± 0.06 cm) and mean blood velocity (Control: 7.3 ± 3.3 cm/s vs. COVID: 6.9 ± 2.3 cm/s) were similar between Control and COVID ($P > 0.05$ for both). FMD (Fig. 1A) and peak blood velocity following cuff release (Fig. 1B), were not different between groups. Similar results were observed for hyperemic blood velocity AUC to 30 s, 60 s, and 120 s after cuff release (all $P > 0.05$). Likewise, central arterial stiffness, assessed as PWV and AIx75 were also not different between groups (Fig. 1 C and D respectively). Resting MCAv (Control: 86 ±

5 cm/s vs. COVID: 76 ± 4 cm/s) and CVCi (Control: 1.04 ± 0.06 cm/s/mmHg vs. COVID: 0.92 ± 0.05 cm/s/mmHg) were similar between groups ($P > 0.05$ for both). For CVMR, there was no interaction between group and time ($P = 0.503$) or main effect of group ($P = 0.807$) for ΔMCAv% indicating no difference between groups at any level of $\Delta P_{ET}CO_2$. Similar results were observed for ΔCVCi% (interaction: $P = 0.505$, main effect of group: $P = 0.431$).

Control, ASYM, and SYM

Resting brachial artery diameter and mean blood velocity were not different between Control, ASYM, and SYM groups (Table 1). However, significant group differences for FMD were observed. Post hoc testing revealed that FMD was lower in SYM compared to both ASYM and Control groups with no difference between Control and ASYM (Fig. 2A). Shear rate AUC to peak diameter was lower in SYM (22501 ± 6633 a.u.) compared to ASYM (34956 ± 15589 a.u. $P = 0.034$) and Control (34230 ± 9799 a.u. $P = 0.029$); however, group differences in FMD remained ($P = 0.006$) after statistically co-varying for differences in shear stimulus (SYM: 4.3 ± 0.8%, ASYM: 6.6 ± 0.7%, Control: 6.6 ± 0.6%; $P < 0.05$ for both comparisons). Likewise, peak blood velocity after cuff release was lower in SYM compared to both ASYM and Control, with no differences between Control and ASYM (Fig. 2B). Similar results were obtained for hyperemic blood velocity AUC to 30 s, 60 s, and 120 s after cuff release. PWV and AIx75 were not different between any groups (Fig.2C and D). Resting MCAv was lower in ASYM compared to SYM and Control. CVCi was lower in ASYM compared to Control (Table 1). However, for CVMR, there was no significant interaction or main effect of group for ΔMCAv% or ΔCVCi% (Fig. 3). Resting cardiorespiratory and hemodynamic measures for all groups are presented in Table 1.

Discussion

This study was the first to investigate the persistent effects of COVID-19 on peripheral vascular function, central arterial stiffness, and cerebral vascular function in young otherwise healthy adults. The major novel findings are threefold. First, we demonstrate that in contrast to our hypothesis, vascular function was not different between young adults who are beyond 4 weeks from a COVID-19 diagnosis and controls. The second major finding is that peripheral vascular function assessed via brachial artery FMD (i.e., macrovascular function) and reactive hyperemia (i.e., microvascular function) were blunted in those who had COVID-19 and were still symptomatic beyond the acute phase compared to controls. Conversely, FMD and reactive hyperemia were not different in asymptomatic COVID-19 subjects compared to controls. Thirdly, in contrast to peripheral vascular function, we found no differences in markers of central arterial stiffness (i.e., carotid-femoral PWV and AIx75), and cerebral vascular function (i.e., CVMR) between any groups. Collectively, these findings indicate that peripheral vascular function, but not cerebral vascular function and central arterial stiffness is impaired in young adults who had mild COVID-19 illness but are still symptomatic past the acute phase. This impairment appears to be resolved in those that are no longer symptomatic.

A recent study by Ratchford et al. (202) reported that brachial artery FMD was blunted in young adults when tested during the acute phase of COVID-19 illness. The current study extends these findings by demonstrating that the impairment in brachial artery FMD persists in those who continue to be symptomatic beyond the acute phase. However, this impairment appears to be completely resolved in those who become asymptomatic. Notably, all individuals who had COVID-19 in the current study would be classified to have had mild-moderate illness (185), and were on average at 3 months from diagnosis. We also found that reactive hyperemia was blunted in symptomatic individuals.

Interestingly, Ratchford et al. (202) reported no impairment in reactive hyperemia during the acute phase suggesting that persistent, but not acute COVID-19 illness may be a primary mediator of microvascular dysfunction. In contrast, we found central arterial stiffness to be unaffected in those who had COVID-19 irrespective of their symptomology, whereas previous work reported that arterial stiffness was elevated in young adults during the acute phase of COVID-19 illness (202, 219). These findings may suggest that the detrimental impact of COVID-19 on central large arteries is an acute and transient phenomenon that resolves overtime. Nonetheless, additional studies are warranted.

Despite the consistent impairments in peripheral vasodilation observed, we found that cerebral vascular function was not impaired following COVID-19 in young adults even in those with persistent symptoms. However, it should be noted that only 2 individuals reported experiencing cognitive symptoms (i.e., 'brain fog') at the time of diagnosis and these symptoms resolved within 2 weeks. Thus, it would be important to perform additional studies to assess cerebral vascular function in those who report persistent cognitive impairments following COVID-19.

It is intriguing that those with persistent symptoms exhibited peripheral vascular dysfunction, whereas those who were asymptomatic at the time of testing had similar macro- and micro-vascular vasodilation to Controls. Moreover, the symptomatic group only reported 1 or 2 symptoms of mild to moderate severity. Nevertheless, while the impairments in peripheral vascular function in those with persisting symptoms were clear, the mechanism(s) responsible remain unclear. Although beyond the scope of this rapid report, some discussion is warranted. One proposed mechanism contributing to persistent COVID-19 symptoms is ongoing and/or a dysregulated immune response to the acute infection (256). In support of this hypothesis, de Melo et al. (260) demonstrated the presence of local inflammation in the olfactory epithelium in those with persistent loss of

smell following COVID-19. Moreover, despite the lack of direct evidence of a link between COVID-19 symptomology, vascular dysfunction, and inflammation, previous investigations have demonstrated a clear link between acute inflammation and impaired endothelial function (261, 262). Notably, improvement in endothelial function following acute viral infection has been shown to be accompanied by a reduction in blood inflammatory markers (261). Given these observations, it is plausible that ongoing inflammation contributes to both the persistent vascular dysfunction and lingering symptoms following COVID-19. Nonetheless, further studies are warranted to directly investigate the link between COVID-19 symptomology and vascular function.

Conclusion

The findings from the current study demonstrate that young otherwise healthy adults who continue to experience symptoms from COVID-19 beyond the acute phase of the illness exhibited peripheral vascular dysfunction. In contrast vascular function appears to be restored in those who are no longer symptomatic. Furthermore, central arterial stiffness and cerebral vascular function were unaffected in COVID-19 subjects beyond the acute phase irrespective of symptomology. Collectively, these findings highlight that the persistence of symptoms following COVID-19 is associated with peripheral vascular dysfunction in otherwise healthy young adults.

Tables and Figures

Table 1. Resting cardiorespiratory and hemodynamic measures

	Control (n = 12)	ASYM (n = 8)	SYM (n = 8)	P value
Heart rate (beats/min)	58 ± 8	61 ± 10	65 ± 10	0.259
Systolic blood pressure (mmHg)	112 ± 9	110 ± 9	111 ± 6	0.923
Diastolic blood pressure (mmHg)	66 ± 4	68 ± 5	70 ± 4	0.188
Brachial artery diameter (cm)	0.34 ± 0.04	0.34 ± 0.05	0.34 ± 0.07	0.971
Brachial artery mean blood velocity (cm/s)	7.4 ± 3.3	7.8 ± 2.7	6.0 ± 1.4	0.368
Middle cerebral artery blood velocity (cm/s)	86 ± 16	67 ± 17*†	84 ± 11	0.042
Cerebral vascular conductance index (cm/s/mmHg)	1.04 ± 0.20	0.82 ± 0.21*	1.01 ± 0.10	0.048
End-tidal carbon dioxide (mmHg)	47 ± 2	45 ± 7	44 ± 5	0.297
Respiratory rate (breaths/min)	14 ± 3	13 ± 3	15 ± 4	0.293

Values are mean ± SD. One-way ANOVA followed by post hoc testing was performed between Control, COVID asymptomatic (ASYM), and symptomatic (SYM) groups. Middle cerebral artery blood velocity, cerebral vascular conductance index, end-tidal carbon dioxide, and respiratory rate data are for n = 10 Controls and n = 7 ASYM. * $P < 0.05$ between ASYM and Control. † $P < 0.05$ between ASYM and SYM.

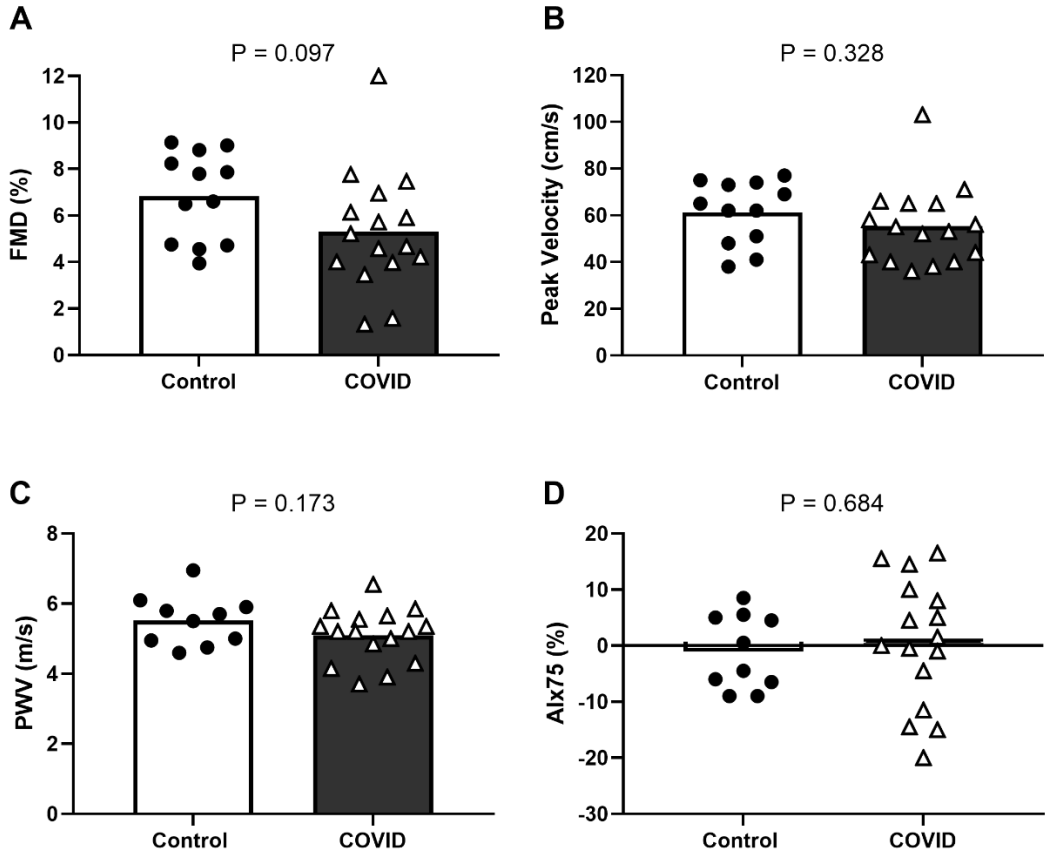

Figure 1. Group mean and individual data for flow-mediated dilation (FMD, A), peak blood velocity following cuff release (B) (n = 12 Control and n = 16 COVID for both), pulse wave velocity (PWV, C) and augmentation index normalized to heart rate of 75 beats/min (AIx75, D) (n = 10 Control and n = 16 COVID for both) between Control and COVID groups.

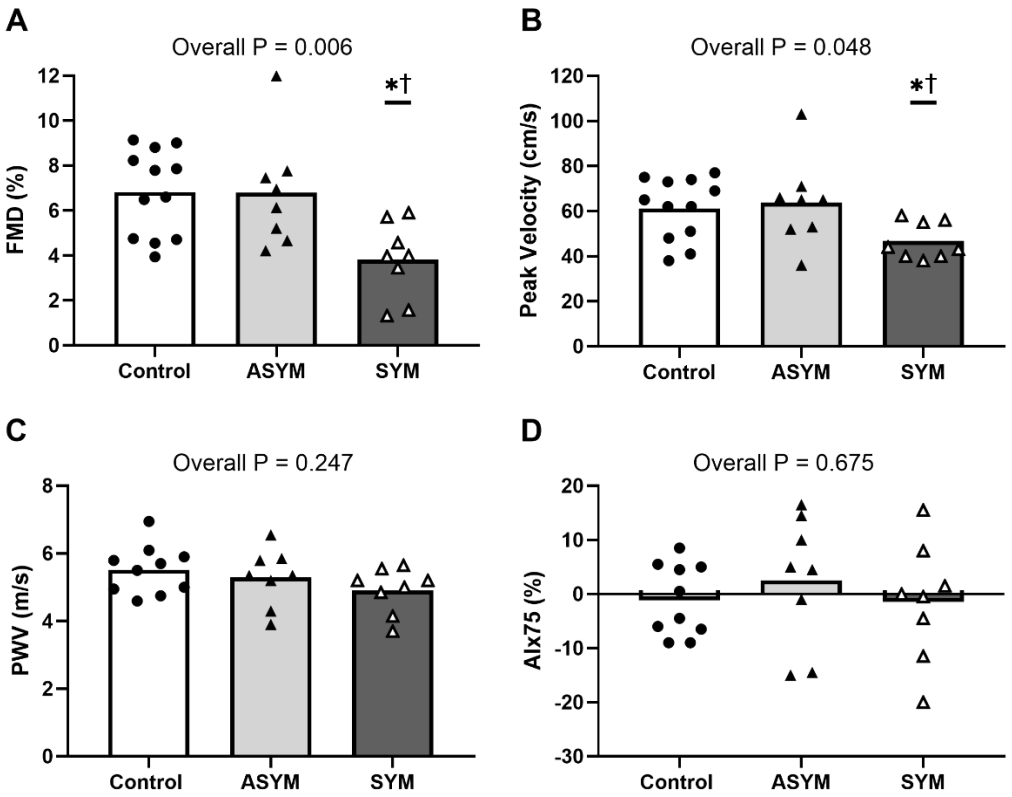

Figure 2. Group mean and individual data for flow-mediated dilation (FMD, A), peak blood velocity following cuff release (B) (n = 12 Control, n = 8 ASYM and n = 8 SYM), pulse wave velocity (PWV, C) and augmentation index normalized to heart rate of 75 beats/min (AIx75, D) (n = 10 Control, n = 8 ASYM and n = 8 SYM) between Control, Asymptomatic (ASYM) and Symptomatic (SYM) groups. * $P < 0.05$ between SYM and ASYM. † $P < 0.05$ between SYM and Control.

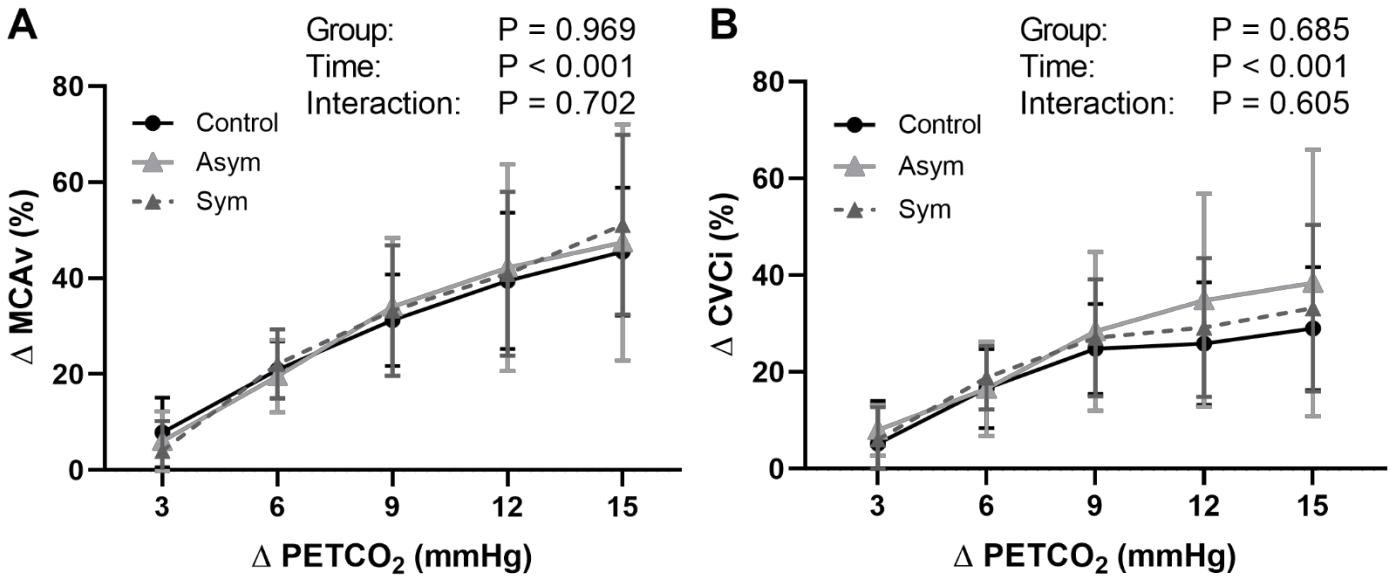

Figure 3. Group mean (± SD) data for percent change in middle cerebral artery blood velocity (ΔMCAv%, A) and cerebral vascular conductance index (ΔCVCi%, B) at increases in end-tidal carbon dioxide ($\Delta P_{ET}CO_2$) of 3, 6, 9, 12 and 15 mmHg from baseline between Control (n = 10), Asymptomatic (ASYM; n = 7) and Symptomatic (SYM; n = 8) groups.

CHAPTER 4: IMPACT OF COVID-19 ON AMBULATORY BLOOD PRESSURE IN YOUNG ADULTS: A CROSS-SECTIONAL ANALYSIS INVESTIGATING TIME SINCE DIAGNOSIS

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Abstract

Previous studies have reported detrimental effects of COVID-19 on the peripheral vasculature. However, reports on blood pressure (BP) are inconsistent and measurements made only in the laboratory setting. To date, no studies have measured ambulatory BP. Additionally, in previous studies, time since COVID-19 diagnosis among participants varied across a wide range, potentially contributing to the inconsistent BP results. Thus, we aimed to perform a comprehensive assessment of BP and BP variability using ambulatory and laboratory (brachial and central) measurements in young adults who had COVID-19. We hypothesized that ambulatory BP would be elevated post-COVID-19, and that measures of BP would be inversely related with time since diagnosis. Twenty-eight young adults who had COVID-19 (11 ± 6 [range 3 to 22] weeks since diagnosis) and 10 controls were studied. Ambulatory daytime, nighttime, and 24-hr systolic BP, diastolic BP, and mean BP were not different between control and COVID groups (e.g., daytime systolic BP: control,122 ± 12 mmHg; COVID,122 ± 10 mmHg; $P = 0.937$). Similar results were observed for laboratory BPs (all $P > 0.05$). However, ambulatory daytime, nighttime, and 24-hr BPs as well as laboratory brachial BPs were inversely correlated with time since COVID-19 diagnosis (e.g., daytime systolic BP: $r = -0.444$; $P = 0.044$, nighttime systolic BP: $r = -0.518$; $P = 0.016$). Ambulatory and laboratory measured BP variability were not different between groups nor correlated with time since diagnosis. Collectively, these data suggest that adverse effects of COVID-19 on BP in young adults are minimal and likely transient.

New and Noteworthy

We report for the first time that ambulatory daytime, nighttime, and 24-hr blood pressure (BP), as well as laboratory BP were not different between control and COVID participants. However, a significant inverse relationship with time since COVID-19 diagnosis was found (i.e., greater BP with more recent infection). Ambulatory and laboratory BP variability were unaffected and not related with diagnosis time. These findings suggest that COVID-19 may exert only short-lasting effects on BP in young adults.

Introduction

The number of COVID-19 cases reported to date globally has exceeded 400 million with over 6 million deaths since the first case was detected in December 2019 (255), and it is predicted that COVID-19 will remain an endemic disease for the foreseeable future (263). Notably, the incidence of future cardiovascular disease has been reported to be substantially higher in individuals who had COVID-19, including in those without pre-existing risk factors and those who had only mild-moderate acute illness (5). However, the underlying factors that contribute to a greater cardiovascular disease burden in COVID-19 are not fully understood. Although high blood pressure (BP) is a major risk factor for the development of cardiovascular disease, the short- and long-term effects of COVID-19 on BP remains unclear.

There is a growing interest in investigating the effects of COVID-19 on cardiovascular health in young adults given that this age group accounts for almost one-fourth of the reported cases in the United States (264). Studies have reported greater central arterial stiffness (202, 219), blunted peripheral vascular function (202, 203, 265), and elevated resting sympathetic nerve activity (266) in previously healthy young adults who had mild-to-moderate COVID-19 compared to those who never had COVID-19. In contrast to these findings of negative effects of COVID-19 on the vasculature in young adults, it is notable that reports on BP have been inconsistent. Indeed, while some studies report normal BP (202, 265) others have reported elevated BP (219) following COVID-19. Although the reason for this discrepancy is unclear, one important consideration is that all these studies assessed BP only in the laboratory environment.

Although laboratory BP measurements provide valuable information on cardiovascular health, it does not capture the circadian variation and short-term fluctuations in BP that occur throughout the day (161). Moreover, laboratory BP measurements may be

confounded by phenomena such as “white coat hypertension” (i.e., elevated BP only in an office/clinic setting) and “masked hypertension” (i.e., normal office/clinic BP despite elevated ambulatory/home BP) (174, 175). Thus, a more comprehensive assessment of BP using 24-hr ambulatory BP is warranted to fully capture the potential impact of COVID-19 on BP and BP variability.

Another consideration is that the duration since the onset of SARS-CoV-2 infection at the time participants were studied has been variable between the studies that reported BP. Indeed, some studied individuals within 4 weeks from diagnosis (202, 219), whereas others were beyond 4 weeks at the time of testing (265, 266) including up to 1 year post diagnosis (232). Notably, data from previous studies indicate that central arterial stiffness, which is well-known to effect BP (135), is elevated in young adults within 4 weeks from COVID-19 diagnosis (202, 219), but not when tested after 4 weeks (265). Whether time from COVID-19 diagnosis similarly impacts BP is yet to be determined.

Herein, we sought to perform a comprehensive assessment of BP and BP variability using ambulatory as well as laboratory measurements in young adults who had COVID-19. In this cross-sectional investigation, we hypothesized that ambulatory BP would be elevated in those who had COVID-19 compared to those with no prior history of COVID-19, and that measures of BP would be inversely correlated with time since COVID-19 diagnosis (i.e., greater BP in those closer to their diagnosis).

Methods

Study Population

Twenty-eight young otherwise healthy adults (11 males) who had a laboratory-confirmed diagnosis (SARS-CoV-2 RT-PCR or antigen test) of COVID-19 (COVID

group) and 10 adults (4 males) without a prior history of COVID-19 (control group) were recruited and studied between March 20th and November 1st, 2021. COVID participants were studied between 2 weeks to 6 months from their diagnosis. Sixteen of the COVID participants were unvaccinated against COVID-19 mainly due to unavailability at the time,10 participants were fully vaccinated, and 2 others had received their 1st dose. All except 1 of our control participants were vaccinated at the time of assessment. All participants were nonsmokers, were not on any prescription medications, and were free from any known cardiovascular, cerebrovascular, metabolic, or neurological diseases based on a health history questionnaire. After receiving a detailed verbal and written explanation of the experimental protocol, participants provided informed written consent. All experimental procedures conformed to the Declaration of Helsinki and were approved by the Institutional Review Board at the University of Texas at Arlington (#2021-0197). Participants were instructed to abstain from caffeine and any over-the-counter medication for at least 12-hrs and alcohol and exercise for at least 24-hrs prior to the study visit. All participants completed the long form of the self-administered International Physical Activity Questionnaire to estimate average physical activity (267). Laboratory assessments were carried out following an overnight fast in a temperature-controlled (20°C – 22°C) dimly lit room.

Experimental Protocols

Laboratory BP

Participants were instrumented with a standard lead II electrocardiogram (model Q710, Quinton, Bothell, WA) to continuously measure heart rate (HR). Resting brachial artery BP was obtained using an automated sphygmomanometer (Welch Allyn, Skaneateles Falls, NY). Beat-to-beat arterial BP was measured via finger

photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, The Netherlands). Respiratory movements were monitored using a strain-gauge pneumobelt (Pneumotrace II 1132, UFI, Morro Bay, CA) around the abdomen to monitor for respiratory driven fluctuations in BP and HR (e.g., deep breath, sigh etc.). After instrumentation, participants rested supine for at least 20 min prior to data collection. Then, HR, beat-to-beat BP, and respiration were recorded continuously during a 5-min resting baseline. Automated brachial artery BPs were obtained every minute. Participants were instructed to remain quiet and awake during this period.

Central BP and arterial stiffness

For assessment of central BP, a brachial BP measurement was obtained using the Sphygmocor device and software (XCEL 1.3, Atcor Medical, Sydney, Australia), which analyzes the brachial waveform and provides an estimate of central BP (268). In addition, we also measured central arterial stiffness as carotid-femoral pulse wave velocity (PWV) as previously described (265). Briefly, a cuff was placed on the thigh, and carotid and femoral pulses were palpated at the strongest points. Measurements were made between three sites (carotid artery to sternal notch, sternal notch to thigh cuff, and femoral artery to thigh cuff). An arterial BP waveform was detected using a handheld tonometer placed over the carotid artery while the thigh cuff was inflated. PWV was calculated (XCEL 1.3, Atcor Medical, Sydney, Australia) as the carotid-femoral artery distance divided by the pulse transit time. Some of the PWV data used for this study have been previously published (265); however, the hypothesis tested and relationships examined are novel and independent from the previous study.

Ambulatory BP

Following laboratory measurements, participants were fitted with an appropriately sized brachial cuff and Oscar 2 oscillometric ambulatory BP monitor (model 250, Sun Tech Medical, Morrisville, USA) to wear for a continuous 24-hr period. Participants were instructed to perform normal daily activities but not to perform any moderate-vigorous physical activity during the 24-hr period. Measurements were obtained every 20 min during daytime and every 30 min during nighttime. Day and night periods were pre-programmed based on each individual's expected sleep time and wakeup time for the day of the assessment (172, 173, 269). Sleep and wakeup times were also confirmed post-assessment and adjusted if needed via the software, prior to downloading the report. Ambulatory BP was measured in all controls and 24 COVID participants.

Data analysis

All continuous data were recorded at 1,000Hz using PowerLab (ADInstruments, Bella Vista, Australia) and stored offline for later analysis. HR was averaged over the 5-min resting period. Brachial artery BP in the laboratory setting was measured and reported as the average of 3 readings. The first measurement during the 5 min baseline period was discarded to avoid potential erroneous readings at the start of data collection. Central BP was calculated as the average of 2 readings. Central arterial stiffness was quantified using the average of two measures of PWV that were within 0.5 m/s of each other (140). For ambulatory BP, following criteria were used to identify a satisfactory assessment per published guidelines: 1) 24-hr recording with ≥70% of expected measurements, 2) ≥ 20 valid awake readings, and 3) ≥ 7 valid asleep readings (172, 269). For quantification of daytime BP, 2 hours immediately after waking and immediately before bedtime were discarded from the analysis to avoid measurement artifacts during the transition period.

Likewise, for quantification of nighttime BP, 1 hour immediately after bedtime and immediately before waking up was removed (172, 269). Adequate number of readings for daytime and nighttime were obtained in all controls and 21 COVID participants. Average systolic BP, diastolic BP, and mean BP were quantified separately for daytime, nighttime, and 24 hrs. Nocturnal dip was calculated as percentage difference between average daytime and night-time systolic BP (270). Ambulatory BP variability was quantified as standard deviation of daytime BP (SD_{day}), nighttime BP (SD_{night}), 24-hr SD weighted for daytime and nighttime BP variability ($SD_{dn} = \frac{(day\ SD \times day\ hours)+(night\ SD \times night\ hours)}{day\ hours+night\ hours}$) (271), and average real variability (ARV = $\frac{1}{N-1}\sum_{k=1}^{N-1}|BP_{k+1} - BP_k|$, where N denotes the number of BPs and k denotes the chronological order of the measurements) (272). Beat-to-beat BP variability was also quantified from the 5-min Finometer derived BP measures which were calibrated to the average of 3 automated sphygmomanometer readings for systolic BP, diastolic BP, and mean BP to ensure absolute values were matched. Following parameters of BP variability were then calculated: SD, coefficient of variation (CV% = $[SD/mean] \times 100$), and ARV (272).

Statistical analysis

Normality was assessed using the Shapiro–Wilk test. All comparisons between control and COVID were made using Student's t-test for independent samples or Mann-Whitney *U* test when data was not normally distributed (SPSS, version 25). To examine the relationship between time since diagnosis and the outcome variables, we performed curve-fitting analysis to determine the nature of the relationship between the independent and dependent variables and observed that the data fit both a linear model and an exponential model. However, the difference between these models were not significant and did not change the interpretation of the data. Therefore, we performed a linear regression

analysis to determine the relationship between time since COVID-19 diagnosis and measures of BP, BP variability, and PWV. All data are presented as mean ± SD, and the significance level was set *a priori* at $\alpha < 0.05$.

Results

Participant characteristics

The control and COVID groups were matched for age (control, 23 ± 3 yrs; COVID, 23 ± 4 yrs; P = 0.757), body mass index (control, 23.0 ± 2.6 kg/m^2; COVID, 24.5 ± 3.1 kg/m^2; P = 0.172), and physical activity levels (control, 4177 ± 3624 METmin/week; COVID, 5080 ± 4912 METmin/week; P = 0.909). Resting HR was also not different between the groups (control, 58 ± 8 beats/min; COVID, 62 ± 11 beats/min; P = 0.244). For the COVID group, the mean time since diagnosis was 11 ± 6 (range: 3 to 22) weeks. All COVID participants had mild illness (185) and none had required hospitalization. Twelve participants reported having 1 – 3 persistent symptoms (loss of smell and/or taste, fatigue, and muscle pain after exertion) while 16 reported having no symptoms at the time of testing. However, there were no differences in any of the reported experimental measures between those with and without symptoms (data not shown, P> 0.05 for all measures).

Ambulatory and laboratory BP and arterial stiffness

There was no difference in ambulatory daytime or nighttime systolic BP, diastolic BP, or mean BP between control and COVID groups (Figure 1). Similar results were obtained for the overall 24-hr period (P > 0.05 for all). There was also no difference in nocturnal dipping between the two groups (control, 14 ± 5%; COVID, 14 ± 4%; P = 0.844). Similarly, there were no differences in laboratory measured brachial systolic BP, diastolic

BP, or mean BP between control and COVID groups (Table 1). Lastly, central systolic BP (control, 100 ± 7 mmHg; COVID, 103 ± 9 mmHg; P = 0.334), diastolic BP (control, 68 ± 6 mmHg; COVID, 72 ± 7 mmHg; P = 0.168), mean BP (control, 79 ± 6 mmHg; COVID, 82 ± 8 mmHg; P = 0.207), and PWV (control, 5.5 ± 0.7 m/s; COVID, 5.4 ± 0.9 m/s; P = 0.552) were not different between groups.

Relationships with time since diagnosis

Ambulatory daytime and nighttime systolic BP, diastolic BP and mean BP were inversely related with time since COVID-19 diagnosis (Figure 2). Similar results were observed for 24-hr systolic BP (r = -0.516; P = 0.017), diastolic BP (r = -0.574; P = 0.006), and mean BP (r = -0.592; P = 0.005). Likewise, laboratory brachial systolic BP (r = -0.474; P = 0.011), diastolic BP (r = -0.449; P = 0.017), and mean BP (r = -0.462; P = 0.013), were inversely correlated with time since diagnosis, whereas no relationships were found for central systolic, diastolic, or mean BP (all P > 0.05). PWV was also inversely related with time since diagnosis (Figure 3). This relationship remained after accounting for the potential influence of laboratory measured BP on PWV (r = 0.699, P < 0.001). Both time since diagnosis (P = 0.016) and mean BP (P = 0.020) were significant determinants of PWV.

Ambulatory and beat-to-beat BP variability

There was no difference in ambulatory BP variability for systolic BP, diastolic BP, or mean BP (i.e., SD_{day}, SD_{night}, SD_{dn}, and ARV) between control and COVID groups (Table 2). Likewise, beat-to-beat BP variability measurements were not different between control and COVID groups (Table 1). There were also no significant relationships between

time since COVID-19 diagnosis and ambulatory or beat-to-beat BP variability with the exception of CV% for beat-to-beat diastolic BP variability (Table 3).

Discussion

To our knowledge, this was the first study to comprehensively investigate the effects of COVID-19 on ambulatory BP and BP variability in young adults. Contrary to our hypothesis, ambulatory BP was not different between young adults who had COVID-19 and controls who never had COVID-19. However, we found that ambulatory daytime, nighttime, and 24-hr BP as well as laboratory brachial BP was inversely correlated with time since COVID-19 diagnosis, with higher BP presenting closer to the onset of infection. Interestingly, a similar inverse relationship was observed between time since diagnosis and central arterial stiffness. In addition, we show that COVID-19 does not adversely impact ambulatory or laboratory BP variability in young adults and no relationship to time since COVID-19 diagnosis was found when studying individuals within 6 months from diagnosis. Collectively, these data suggest that COVID-19 does not have major effects on BP in young adults; however, transient effects of COVID-19 to increase BP and central arterial stiffness closer to diagnosis may be present.

We (265) and others (202, 232) have previously reported that brachial artery BP measured in the laboratory setting is not different between young adults who had COVID-19 and those who never had COVID-19. While measuring BP in the laboratory setting is conventional and provides important information, it does not allow for the measurement of BP over an extended time period during regular daily activities, nor does it consider nocturnal dipping, the presence of whitecoat hypertension, or masked hypertension. Indeed, elevated daytime, nighttime, and 24-hr ambulatory BP as well as having either

reduced or exaggerated nocturnal dipping are strong independent predictors of adverse cardiovascular outcomes (177, 178). In the current study, we found that ambulatory daytime, nighttime, 24-hr BP, and nocturnal dipping were not different between young adults who are within 6 months from diagnosis and those without a history of COVID-19. Ambulatory and beat-to-beat BP variability, which are known to offer independent prognostic information on cardiovascular outcomes, (164, 272) were also unaffected by COVID-19. For our study, we recruited control participants during the pandemic since that would ensure better matching of participants with regards to any lifestyle changes that may have been unavoidable during this period. One caveat to this is that it is impossible to know whether some of the control participants may have had asymptomatic infection. Nevertheless, given the growing research indicating an elevated cardiovascular disease risk associated with COVID-19 (5, 249), these negative findings are promising in that we found no major effects on BP or BP variability in young adults who had COVID-19. Nevertheless, comprehensive studies of BP in those who are older, have more severe acute illness, and those with persistent sequalae from COVID-19 are warranted.

Our findings also indicated that central BP was not elevated following COVID-19. This is in contrast to findings by Szeghy et al. (219) who reported that central systolic BP and mean BP were elevated in young adults who had COVID-19 compared to controls. Notably, a key difference is that all participants (n = 15) in the study by Szeghy et al. (219) were within 4 weeks from diagnosis, whereas only 4 participants in the current study were within that time frame at the time of testing. Hence, the difference in the timing since infection may have contributed to the divergent findings between studies. Indeed, in line with this hypothesis, in the current study we found that ambulatory as well as laboratory brachial BP was inversely correlated with time since diagnosis, with individuals who were closer to COVID-19 diagnosis presenting with greater BP values compared to those further

away from diagnosis up to 6 months. Likewise, arterial stiffness was also shown to be inversely related to time since diagnosis. These findings are in agreement with the findings of a recent follow up study by Szeghy et al. (233) which showed improvement in carotid-femoral PWV over the first 6 month from diagnosis in young otherwise healthy adults who had COVID-19. Although no significant relationship with time since diagnosis was found for central BP in the current study, Szeghy et al. (233) reported an improvement in central systolic BP and mean BP at 6 months compared to one month after diagnosis. Collectively, these findings provide evidence for a potential transient impact of COVID-19 on indices of cardiovascular health in young adults.

Data from previous studies lend some insight into factors that may contribute to a transient elevation in BP. For example, studies have demonstrated that during the early phase of the illness, young adults who had COVID-19 exhibit impaired vasodilation (202) and the potential for increased vasoconstriction with elevated sympathetic nervous system activity (266). Central artery stiffness and central BP has also been shown to be higher within 3-4 weeks from COVID-19 diagnosis compared to controls (202). Findings from studies that included individuals further out from diagnosis suggests no elevation in arterial stiffness (265), and no impairment in vascular function (232), or impairment in only those with persistent symptoms (265). While the temporal relationship between vascular dysfunction, elevated arterial stiffness, and elevated BP is a topic of some debate, it has been suggested that increased peripheral vascular resistance due to alterations in smaller arteries (i.e., impaired vasodilation and increased vasoconstriction) leads to elevated brachial BP, which causes greater large artery stiffness (131, 273), followed by a rise in central BP. Increased central BP is then thought to contribute to structural changes in the smaller arteries, which again leads to a rise in brachial BP (131, 273). Although previous studies suggest that central BP is elevated within 4 weeks from COVID-19 diagnosis (219),

our data indicates that the effects of COVID-19 on brachial BP and arterial stiffness in young adults is likely not sufficient to cause a significant impact on central BP, nor cause permanent structural alterations on the vasculature.

Perspectives and Significance

Despite previous studies reporting negative effects of COVID-19 on the peripheral vasculature and autonomic function in young adults, it is encouraging to see that the impact of COVID-19 on BP, a primary risk factor for development of cardiovascular disease, is likely minimal and not persistent in this age group. Indeed, this is important because to date, nearly 15 million young adults (i.e., ~ 30% of those between 18 – 29 years) have been affected by COVID-19 in the United States alone (27). Given that SARS-CoV-2 virus is continuously changing and evolving into new variants with varying virulence, investigating the long-term effects of COVID-19 becomes complex and challenging. However, longitudinal studies are necessary to fully understand the risk of future cardiovascular disease with COVID-19. In contrast to our findings in young adults, several studies have reported new onset hypertension post COVID-19 in older adults and those with preexisting comorbidities (235, 236, 274). Whether this is a permanent outcome of COVID-19 in this population is still unknown and warrants further investigation. Moreover, the exact mechanisms that trigger negative vascular alterations following COVID-19 are still unclear. However, two plausible mechanisms are direct vascular inflammation (190, 191) and imbalance in the renin-angiotensin-aldosterone system as a result of viral binding and downregulation of angiotensin converting enzyme-2 receptors (275). Additional studies investigating the role of these potential mechanisms in causing

adverse cardiovascular outcomes are needed to better understand the short and long-term influence of COVID-19 on overall cardiovascular health.

Tables and Figures

Table 1. Laboratory brachial blood pressure and blood pressure variability

Parameter		Control	COVID	P value
Systolic (mmHg)	BP	110 ± 7	111 ± 8	0.722
	SD	5.0 ± 1.0	4.4 ± 0.9	0.134
	CV%	4.5 ± 1.1	4.0 ± 0.8	0.116
	ARV	2.1 ± 0.6	2.3 ± 0.7	0.471
Diastolic (mmHg)	BP	66 ± 4	69 ± 6	0.135
	SD	3.5 ± 0.8	3.5 ± 0.8	0.900
	CV%	5.2 ± 1.2	5.1 ± 1.2	0.683
	ARV[a]	1.8 ± 0.9	1.8 ± 0.7	0.590
Mean (mmHg)	BP	81 ± 5	83 ± 6	0.334
	SD[a]	3.7 ± 0.6	3.7 ± 0.9	0.732
	CV%	4.6 ± 0.8	4.5 ± 1.1	0.809
	ARV	1.3 ± 0.5	1.3 ± 0.3	0.977

Values are means ± SD. ARV, average real variability; BP, blood pressure; CV, coefficient of variation; SD, standard deviation. Independent sample t-tests were used to compare between control (n = 10; 4 males) and COVID (n = 28; 11 males) groups.
[a]Non-normalized data was analyzed using Mann-Whitney U test.

Table 2. Ambulatory blood pressure variability

Parameter		Control	COVID	P value
Systolic BP (mmHg)	SD_{day}	9.3 ± 2.5	10.4 ± 2.0	0.175
	SD_{night}	8.3 ± 2.9	8.8 ± 3.0	0.615
	SD_{dn}	9.0 ± 2.1	9.8 ± 1.7	0.228
	ARV[a]	9.0 ± 1.6	9.6 ± 1.6	0.201
Diastolic BP (mmHg)	SD_{day}[a]	8.1 ± 1.5	9.0 ± 2.5	0.441
	SD_{night}	6.7 ± 1.9	6.6 ± 2.2	0.905
	SD_{dn}	7.6 ± 1.2	8.2 ± 2.0	0.414
	ARV	7.4 ± 1.0	7.8 ± 1.5	0.428
Mean BP (mmHg)	SD_{day}	7.7 ± 1.8	8.7 ± 2.3	0.244
	SD_{night}	6.8 ± 1.9	6.6 ± 2.4	0.803
	SD_{dn}	7.4 ± 1.4	7.9 ± 1.9	0.456
	ARV	7.2 ± 0.9	7.6 ± 1.4	0.363

Values are means ± SD. ARV, average real variability; BP, blood pressure; SD_{day}, daytime standard deviation; SD_{night}, nighttime standard deviation; SD_{dn}, 24-hr standard deviation weighted for daytime and nighttime variability. Independent sample t-tests were used to compare between control (n = 10; 4 males), and COVID (n = 21; 8 males) groups.
[a]Non-normalized data was analyzed using Mann-Whitney U test.

Table 3. Relationship between time since COVID-19 diagnosis and blood pressure variability

Parameter	Ambulatory BP variability			Beat-to-beat BP variability		
		r	P value		r	P value
Systolic BP (mmHg)	SD_{day}	-0.212	0.357	SD	0.025	0.901
	SD_{night}	-0.143	0.537	CV%	0.205	0.295
	SD_{dn}	-0.240	0.295	ARV	0.058	0.771
	ARV	-0.109	0.639			
Diastolic BP (mmHg)	SD_{day}	0.020	0.932	SD	0.298	0.124
	SD_{night}	-0.265	0.246	CV%	0.460	0.014
	SD_{dn}	-0.095	0.681	ARV	0.343	0.074
	ARV	0.050	0.828			
Mean BP (mmHg)	SD_{day}	0.020	0.931	SD	0.119	0.548
	SD_{night}	-0.181	0.433	CV%	0.262	0.178
	SD_{dn}	-0.094	0.686	ARV	0.095	0.632
	ARV	0.004	0.986			

n = 21 (8 males) for ambulatory BP variability and n = 28 (11 males) for beat-to-beat BP variability. ARV, average real variability; BP, blood pressure; SD, standard deviation; CV, coefficient of variation; SD_{day}, daytime standard deviation; SD_{night}, nighttime standard deviation; SD_{dn}, 24-hr standard deviation weighted for daytime and nighttime variability; r, correlation coefficient.

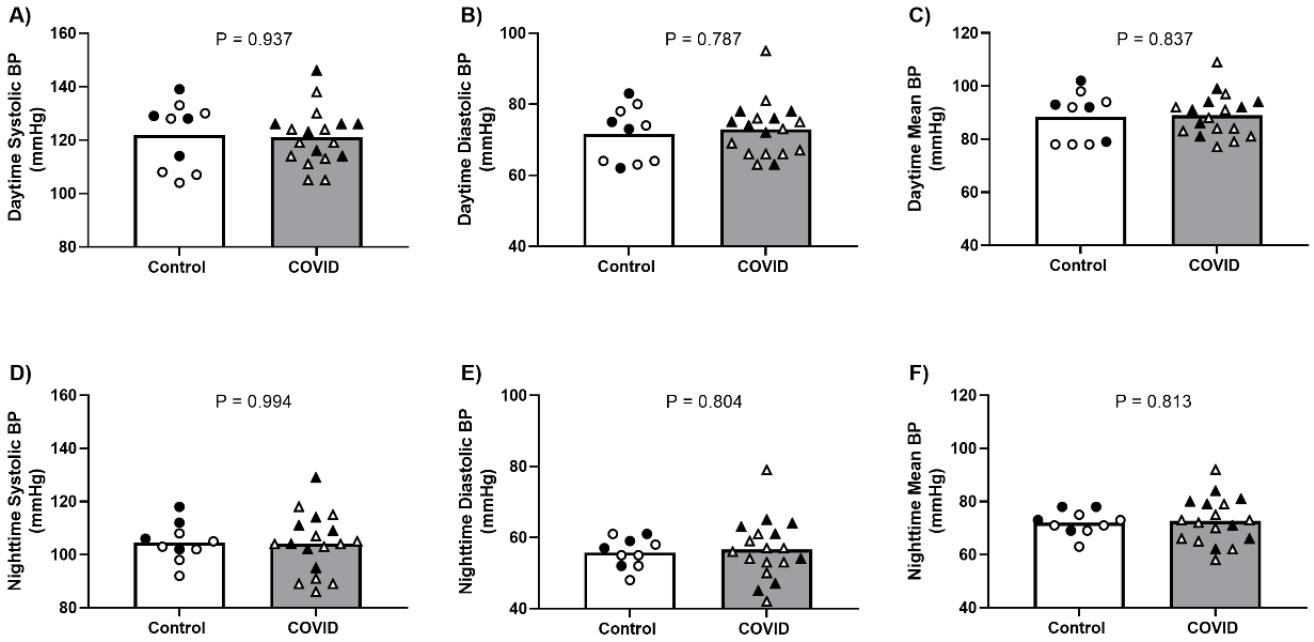

Figure 1. Group and individual data for ambulatory daytime (A, B, and C) and nighttime (D, E, and F) systolic blood pressure (BP), diastolic BP, and mean BP between control (white bars and circles; n = 10; 4 males) and COVID (grey bars and triangles; n = 21; 8M males). Black symbols represent males and white symbols represent females. Comparisons between groups were made using Student's t-test for independent samples.

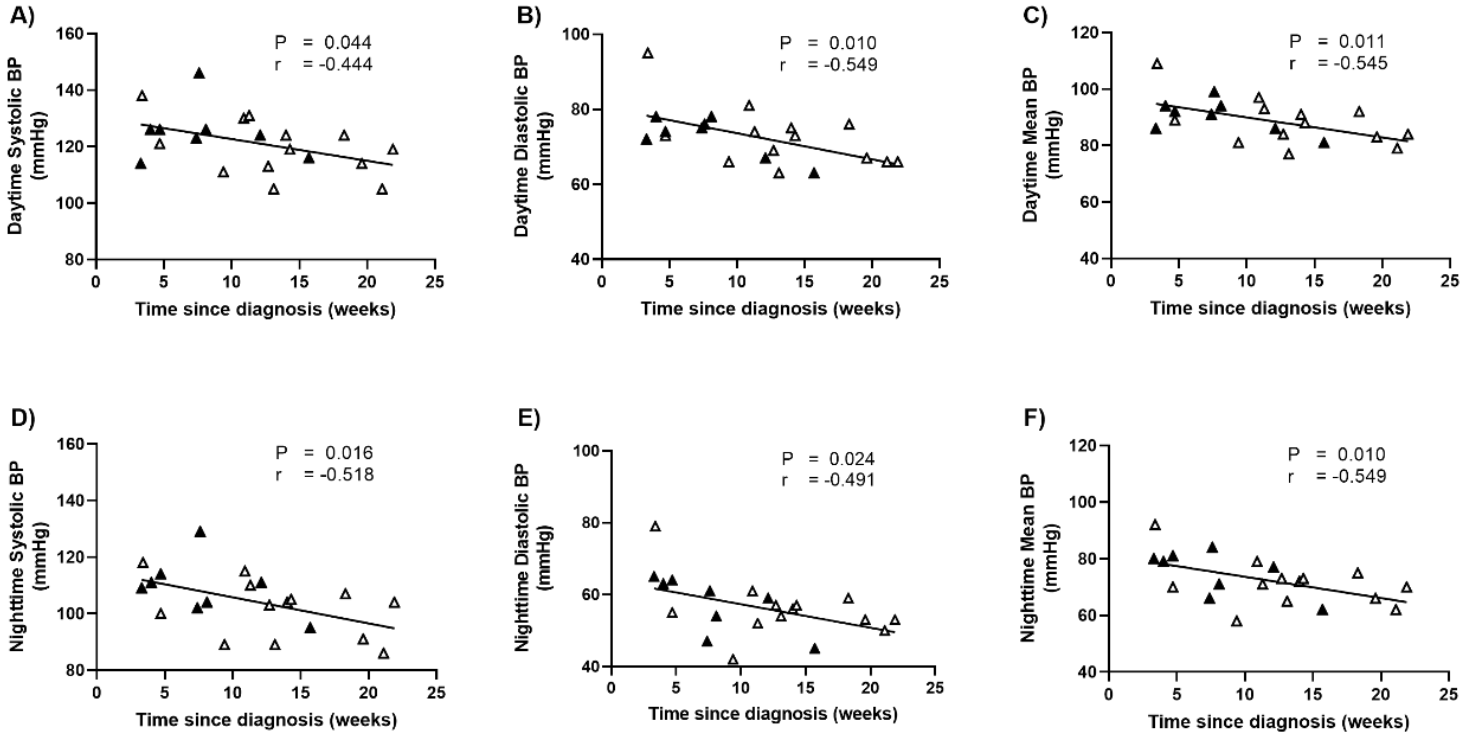

Figure 2. Relationship between time since diagnosis and ambulatory daytime (A, B, and C) and nighttime (D, E, and F) systolic blood pressure (BP), diastolic BP, and mean BP in the COVID group (n = 21; 8 males). Black symbols represent males and white symbols represent females. r = correlation coefficient.

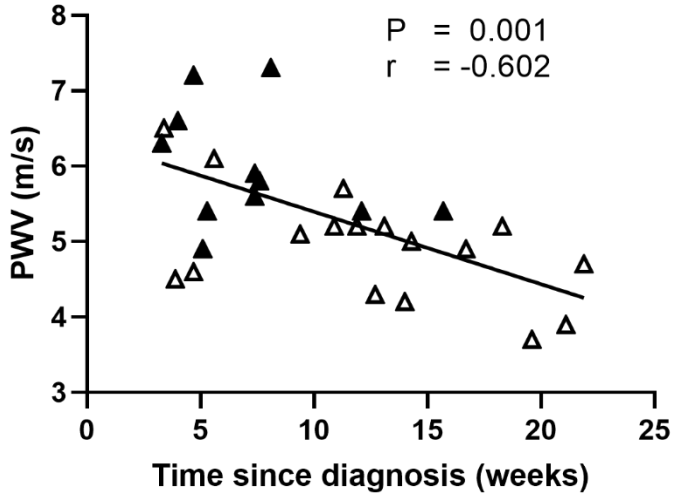

Figure 3. Relationship between time since diagnosis and carotid-femoral pulse wave velocity (PWV) in the COVID group (n = 28; 11 males). Black symbols represent males and white symbols represent females. r = correlation coefficient.

CHAPTER 5: CARDIOVASCULAR HEALTH IN POST-ACUTE SEQUALAE OF COVID-19 (PASC) AND THE INFLUENCE OF SYMPTOM BURDEN

Manuscript in preparation

Abstract

Many individuals who had COVID-19 experience debilitating persistent symptoms [i.e., post-acute sequalae of COVID-19 (PASC)] including adverse cardiovascular outcomes. Although studies show detrimental effects of COVID-19 on vascular health, findings in PASC are limited and equivocal. Whether this is due to the heterogenicity of symptomology of PASC patients is unknown and warrants investigation. We hypothesized that peripheral and cerebral vascular function would be blunted, and central arterial stiffness would be elevated in PASC patients compared to age-matched controls, and that impairments in vascular health would be greater in PASC patients with greater symptom burden. Brachial artery flow-mediated dilation (FMD) and reactive hyperemia, cerebral vasomotor reactivity to hypercapnia (CVMR), carotid femoral pulse wave velocity, (PWV), and resting brachial and central blood pressure (BP) were measured in 10 females with diagnosed PASC and 10 age-matched controls. FMD and reactive hyperemia were not different between groups ($P > 0.05$ for both). Brachial BP (e.g., systolic BP: 128 ± 20 vs. 109 ± 8 mmHg; $P = 0.013$) central BP (e.g., systolic BP: $P = 0.002$), and PWV (7.2 ± 1.3 vs. 6.1 ± 0.8 m/s; $P = 0.032$) were higher in PASC compared to controls. CVMR was not different between groups. Severity of brain fog was inversely correlated with resting middle cerebral artery blood velocity ($r = -0.675$; $P = 0.046$) and cerebrovascular conductance index ($r = -0.743$; $P = 0.022$). Total symptom burden was not correlated with measures of vascular health. Collectively, these findings indicate that BP and arterial stiffness are elevated in PASC patients, whereas peripheral and cerebral vascular function are unaffected. Further, those with greater severity of brain fog appear to have lower resting cerebral blood flow.

New and Noteworthy

We demonstrate for the first time that blood pressure and central arterial stiffness are elevated in PASC patients. In contrast, peripheral and cerebral vascular function appear to be unaffected. In addition, while there was no relationship between total PASC symptom burden and measures of vascular health, severity of brain fog was inversely correlated with resting cerebral blood flow. These findings provide novel insight into the cardiovascular consequences of PASC and the influence of symptomology.

Introduction

Recent data indicates that between 40 - 50% of individuals who had COVID-19, the illness caused by SARS-CoV-2 experience new, returning, or ongoing symptoms or health complications that persist beyond the acute illness (20, 245). This condition, referred to as "long COVID" and a diagnosis of "post-acute sequalae of COVID-19 (PASC), is more prevalent among females (23, 276), results in debilitating symptoms and is observed among not only those who had severe acute COVID-19 but also in those who had mild acute infection (24, 276) and even those with breakthrough infections (277). Thus, given the large number of individuals who have been and continue to be diagnosed with COVID-19, PASC is becoming a concerning health burden of substantial magnitude. Emerging data has demonstrated more than 50 long-term effects of COVID-19 involving multiple organ systems (24), including many cardiovascular complications (26). However, to date, it is not well understood what drives these post-acute cardiovascular sequalae of COVID-19.

A growing number of studies investigating the long-term impact of COVID-19 on cardiovascular health suggest that detrimental effects of COVID-19 on the vasculature may gradually improve with time (221, 233, 234, 278). Moreover, data from our lab (265) and others (223) indicate that vascular impairments may be related to COVID-19 symptomology, and symptom resolution appears to be associated with improvement in vascular health (234, 265). Although these studies provide preliminary insight into the potential association between COVID-19 symptomology and vascular alterations, limited studies have investigated vascular health in those with a diagnosis of PASC (200, 227). Notably, findings from these studies are equivocal with reports of impaired forearm microvascular function in some, but not all patients with PASC (227), or similar impairment in brachial artery flow-mediated dilation (FMD) in those with and without a PASC diagnosis (200). Neither study related vascular function measures to symptomology

of PASC patients and the latter study included only those who required hospitalization during acute SARS-CoV-2 infection, thus limiting the interpretation regarding peripheral vascular function in PASC. In addition, among PASC patients, neurological symptoms such as impairment in memory and concentration (i.e., brain fog), dizziness, and recurrent headaches are frequently reported (24). To date, one study has reported a lower cerebral vasoreactivity to a breath hold test assessed using transcranial doppler ultrasound in those with persistent neurological symptoms following COVID-19 (231). However, participants in this study were within 2 months from COVID-19 diagnosis and it was not reported whether they had a PASC diagnosis. To our knowledge, no studies have investigated cerebral vascular function, and the potential link between cerebral vascular function and symptomology in patients with PASC.

Importantly, the symptomology of PASC patients varies greatly in terms of the particular symptoms they experience (22, 24) and the perceived severity of each symptom (279). Therefore, it is possible that the inconsistency in measures of cardiovascular health between studies is due to the heterogenicity of symptomology of the participants; however, to date, this has not been investigated. Herein, we sought to assess cardiovascular function in patients with PASC with a focus on examining a potential link to symptomology. We hypothesized that patients with PASC will have blunted peripheral and cerebral vascular function, and elevated arterial stiffness compared to age-matched controls. We further hypothesized that impairments in cardiovascular outcome measures would be greatest in those with the highest symptom burden.

Methods

Study Population

Ten females who had a previously documented COVID-19 diagnosis (positive antigen or PCR test), persistent COVID-19 related symptoms beyond 4 weeks after COVID-19 diagnosis, and a physician diagnosis of PASC were recruited from the University of Texas Southwestern Medical Center, Dallas, Texas, COVID-recover clinic. Ten age-matched females without PASC were recruited from the Dallas-Fort Worth community and studied as the control group. All participants were non-smokers and were not pregnant or breast-feeding. After verbal explanation of the experimental protocol, participants provided informed written consent. All experimental procedures conformed to the Declaration of Helsinki and were approved by the Institutional Review Board at the University of Texas at Arlington.

Participants were instructed to abstain from food, caffeine, and any medication for at least 12-hours and alcohol and exercise for at least 24-hours prior to the study. After consenting, all participants completed a detailed questionnaire on their medical history. Recent physical activity was quantified using the International Physical Activity Questionnaire (IPAQ) (280). In addition, PASC participants completed a questionnaire regarding their COVID-19 related persistent symptoms. Current severity of each symptom was ranked on a scale of 1 – 10 with a higher score indicating greater symptom severity.

Experimental Protocol

Experiments were performed in a temperature-controlled (20 – 22 °C) dimly lit room. Participants were instrumented with standard lead II electrocardiogram (model Q710, Quinton, Bothell, WA) to continuously measure heart rate (HR) and respiratory excursions were monitored using a pneumobelt (Pneumotrace II 1132, UFI, Morro Bay,

CA). Subjects rested supine for 20 minutes prior to data collection. Peripheral vascular function was assessed using the brachial artery FMD technique according to current guidelines and as previously reported (64, 265). Briefly, brachial artery diameter and blood velocity were obtained using duplex Doppler ultrasonography (GE Logiq P9, Milwaukee, WI) and an 11-MHz linear array transducer. Baseline data were obtained for 5 min, after which a rapidly inflating cuff (Hokanson, Bellevue, WA) placed 2 cm distal to the antecubital fossa was inflated to a suprasystolic pressure (220 mmHg) for 5 min. Brachial artery blood velocity and diameter were continuously recorded for 30 seconds before and 3 min after the cuff was released. FMD data is for 10 PASC and 9 control participants since data from one control participant could not be used due to a movement artifact.

Following FMD, central arterial stiffness was measured as carotid-femoral pulse wave velocity as previously described (PWV) (265). Carotid and femoral pulses were palpated at the strongest points and a cuff was placed on the thigh. Distance measurements were made between the carotid artery and sternal notch, sternal notch and thigh cuff, and femoral artery and thigh cuff. While the thigh cuff was inflated, a handheld tonometer placed over the carotid artery was used to detect the arterial blood pressure (BP) waveform (SphygmoCor, Atcor Medical device and XCEL 1.3 software, Sydney, Australia). PWV was calculated as the carotid-femoral artery distance divided by the pulse transit time. In addition, an estimate of central BP was acquired via pulse wave analysis of the brachial waveform obtained from a brachial BP measurement (SphygmoCor, Atcor XCEL 1.3 software, Sydney, Australia). Following this, a venous blood sample was obtained and sent to a commercial blood processing laboratory (Labcorp) for measurement of complete metabolic panel, lipid panel, and high sensitivity C-reactive protein (hsCRP).

Next, participants were instrumented to measure beat-to-beat arterial BP using finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam,

The Netherlands) from the left hand, and automated sphygmomanometer BPs (Welch Allyn, Skaneateles Falls, NY) from the right arm during 5-mins of quiet rest. HR and beat-to-beat BP were continuously recorded while automated BPs were obtained every min for baseline participant characterization. A pneumobelt was placed around the abdomen (Pneumotrace II 1132, UFI, Morro Bay, CA) to monitor for respiratory excursions. Following this, a 2-MHz transcranial doppler (Multigon Industries Inc., Yonkers, NY) ultrasound probe was held in place over the left temporal window using a headband to obtain middle cerebral artery blood velocity (MCAv) measurements. A rebreathing protocol was performed for the assessment of cerebral vasomotor reactivity (CVMR) as described previously (265). Briefly, participants were fitted with a nose clip and breathed through a mouthpiece attached to a 3-way valve (Hans Rudolph Inc., Kansas City, KS,). A 5-L rebreathing bag was connected to one end of the valve and filled with the participants expired air. Following a 3-min baseline period where participants breathed room air, the valve was switched so that the participant breathed from the rebreathing bag until they reached an increase in end tidal carbon dioxide concentration ($\Delta P_{ET}CO_2$) of at least 15 mmHg from baseline, indicated to stop, or diastolic BP (DBP) increased above 100 mmHg. The valve was then switched back to room air for 3 min of recovery. Oxygen (O_2) was introduced into the rebreathing bag throughout the protocol to maintain normal arterial O_2 saturation (SpO_2) (259). $P_{ET}CO_2$ and SpO_2 were measured through a sampling line connected to the mouthpiece and pulse oximeter on their index finger respectively, which were connected to a capnograph (Capnocheck Plus, Smiths Medical, Dublin, OH). One participant from each group opted not to perform this test, and the protocol was stopped early in two control and one PASC participant due to the rise in DBP > 100 mmHg, or participant request. Therefore, CVMR data are reported for eight control and nine PASC participants who reached at least a $\Delta PETCO_2$ level of 12 mmHg.

Following a minimum interval of 1 hour, for assessment of physical function, all PASC participants completed a 6-min walk test. Participants were instructed to walk as far as possible in 6 mins at a comfortable pace on a flat surface. The 6-min walk test took place in a 30-m corridor with two turning points and markers every 5 meters. In addition, they completed a 32-item questionnaire on physical functioning (adapted from the late life function and disability instrument [LLFDI](281)) with a 5 point scale for each question giving a total score ranging from 32 to 160. Quality of life (QOL) was assessed using the SF-12 health survey (SF12v2 Standard, US Version 2.0) (282).

Data Analysis

Brachial artery diameter and mean blood velocity were analyzed using a customized offline wall tracking and edge detection software (LabView, National Instruments, Austin, TX). Macrovascular function was quantified as FMD% = (3-beat average peak diameter - baseline diameter)/baseline diameter × 100. Shear rate was calculated as 8 × mean blood velocity/diameter. The shear stimulus for brachial artery dilation was calculated as the hyperemic shear rate area under the curve (AUC) to peak brachial artery dilatation using the sum of trapezoids method. Microvascular function was quantified as the 3-beat average peak blood velocity. For PWV, PWA, and central BP, the average of 2 consistent measurements were used according to recommended guidelines (140).

HR, beat-to-beat BP, and respiration were recorded continuously at 1,000Hz using PowerLab (ADInstruments, Bella Vista, Australia). For baseline subject characterization, HR was averaged over the 5 min resting period and BP was calculated from 4 automated sphygmomanometer readings obtained during the 5 min resting period. The first measurement was discarded to avoid including potential erroneous readings at the start of

data collection. For quantification of CVMR, Finometer derived mean arterial pressure (MAP) values were square wave calibrated to the average of two automated BPs obtained during the baseline of the rebreathing protocol. Values for $P_{ET}CO_2$, MCAv, corrected MAP, and cerebral vascular conductance index (CVCi = MCAv/corrected MAP) were averaged over the last 1 min of baseline. During the rebreathe, 3-breath averages of MCAv and CVCi were calculated. CVMR was quantified as percent increase in MCAv (ΔMCAv%) and CVCi (ΔCVCi%) at Δ 12 mmHg $P_{ET}CO_2$ and the linear regression slope of ΔMCAv% and ΔCVCi% vs. $\Delta P_{ET}CO_2$. Only slopes with a correlation coefficient above 0.5 were included in the analysis.

Total symptom burden for each participant was calculated as the sum of severity of all reported symptoms where a higher score represents greater symptom burden. For physical function, 6-min walk distance and total score from the physical function questionnaire were calculated separately. A higher 6-min walk distance and lower physical function score indicated better function. For quantification of QOL, the total score for physical health domain and mental health domain were combined to obtain an overall QOL score. A higher score denoted better quality of life (283).

Statistical Analysis

Normality of the data were assessed using the Shapiro–Wilk test. All comparisons between control and PASC group, were made using Student's t-test for independent samples or Mann-Whitney *U* test when data were not normally distributed (SPSS, version 25). FMD was corrected for differences in shear stress using analysis of covariance. The relationship between symptom severity, physical function, and QOL, and cardiovascular outcomes were determined using Pearson's correlation coefficient (Graphpad prism,

v9.4.0). All data are presented as mean ± SD, and the significance level was set *a priori* at $\alpha < 0.05$.

Results

Participant characteristics

The PASC and control groups were matched for age (48 ± 10 yrs vs. 52 ± 12 yrs; P = 0.482) and body mass index (29.2 ±3.6 kg/m^2 vs. 26.1 ± 5.8 kg/m^2; P = 0.165). Resting HR (Table 1) and recent physical activity assessed using the IPAQ (PASC, 3708 ± 4150 METmin/week vs. control, 3994 ± 3081 METmin/week; P = 0.696) was not different between groups. However, resting SBP, DBP, and MAP were higher in PASC compared to controls (Table 1). There was no difference in hsCRP (PASC, 3.8 ± 3.5 mg/L vs. control, 2.1 ± 2.6 mg/L; P = 0.071), fasting blood sugar or LDL-cholesterol between the groups (Table 1). In contrast, HDL-cholesterol was lower, and triglycerides were higher in PASC compared to controls (Table 1). The number of participants with diagnosed medical conditions and on prescription medication are reported in Table 2.

Median time since COVID-19 diagnosis for the PASC groups was 531 days (range 82 to 691). Four PASC patients had been hospitalized during the acute illness (1 day, 2 days, 13 days, and 17 days each), and two patients required supplemental O_2 for 10 -12 weeks. A total of 18 persistent symptoms were reported by PASC patients (Table 2), of which fatigue (n = 10), shortness of breath (n = 9), and brain fog (n = 9) were the 3 commonest symptoms. Two of the control participants had a past diagnosis of COVID-19 (48 and 91 days since diagnosis at the time of testing); however, both had mild acute illness and neither had persistent symptoms beyond 4 weeks from diagnosis.

Peripheral vascular function and central arterial stiffness

Brachial artery baseline diameter (PASC, 0.321 ± 0.028 cm; control, 0.299 ± 0.037 cm; P = 0.165) and blood velocity (PASC, 6.7 ± 2.4 cm/s; control, 8.5 ± 4.0 cm/s; P = 0.254) were not different between PASC and control groups. FMD% was also not different between groups (Figure 1A). Likewise, there was no difference between the groups for absolute brachial artery dilation (PASC, 0.013 ± 0.006 cm vs. control, 0.019 ± 0.011 cm; P = 0.178) or FMD% when corrected for shear AUC to peak diameter (PASC, 4.49% ± 2.78%; control, 6.02 ± 2.79%; P = 0.258). Reactive hyperemia assessed as peak blood velocity following cuff release (Figure 1B) or hyperemic blood velocity AUC to 30s, 60s or 120s was not different between the two groups (P > 0.05 for all).

PWV was greater in PASC compared to controls (Figure 2A). Likewise, central SBP, DBP (Figure 2B and 2C respectively), and MAP (PASC, 96 ± 11 mmHg; control, 82 ± 5 mmHg; P = 0.003), and pulse pressure (PASC, 41 ± 9 mmHg; control, 33 ± 3 mmHg; P = 0.014) were higher in PASC compared with controls.

Cerebral vascular function

There was no difference in baseline MCAv (72 ± 8 cm/s vs. 69 ± 9 cm/s; P = 0.503) and CVCi (0.77 ± 0.16 cm/s/mmHg vs. 0.84 ± 0.12 cm/s/mmHg) between PASC and control. CVMR to hypercapnia was not different between groups when assessed as percent change in MCAv and CVCi at $\Delta P_{ET}CO_2$ of 12 mmHg or the respective slopes (Figure 3). The mean correlation coefficients (r) for the slopes were not different between the groups (ΔMCAv% slope r: PASC, 0.94 ± 0.04 vs. control, 0.91 ± 0.10; P = 0.424; ΔCVCi% slope r: PASC, 0.83 ± 0.10 vs, control, 0.78 ± 0.17; P = 0.464).

Relationship between symptom burden, and measures of cardiovascular health, physical functioning, and quality of life

There were no correlations between total symptom burden and measures of peripheral vascular function, central arterial stiffness, cerebral vascular function, or blood pressure (Table 3). However, total symptom burden was directly correlated with the physical function questionnaire score, and inversely correlated with 6-min walk distance and overall QOL score (Table 3). Baseline MCAv ($r = -0.67$; $P = 0.046$) and CVCi ($r = -0.74$; $P = 0.022$) were inversely correlated (Figure 4), and the CVMR slope for ΔCVCi% was positively correlated ($r = 0.683$; $P = 0.043$) with the severity of brain fog. However, the slope for ΔMCAv% did not show a correlation with the severity of brain fog ($r = 0.287$; $P = 0.454$).

Discussion

This study was the first to investigate peripheral and cerebral indices of cardiovascular health in PASC and explore the relationship to symptom burden. A major novel finding of this study is that patients with PASC exhibit higher resting BP and central arterial stiffness compared to controls. In contrast, peripheral vascular function and cerebral vascular function were not different between PASC and controls. In addition, we found that there was no relationship between total symptom burden and measures of cardiovascular health in PASC patients with the exception that severity of brain fog was inversely correlated with resting cerebral blood velocity and cerebral vascular conductance index. Although indices of cardiovascular health were not correlated with total symptom burden, physical function and QOL were inversely related with symptom burden indicating that those with greater symptom burden experience poor physical function and QOL.

Collectively, these data indicate that PASC patients are likely at risk of developing high BP regardless of symptomology, while lower resting cerebral blood flow may contribute to brain fog and total symptom burden to physical function and QOL.

Previous studies have reported higher BP in a subset of individuals following COVID-19 (235, 236). However, these studies did not assess persistent symptomology, and to our knowledge the current study is the first to report elevated BP in PASC patients. Indeed, two PASC patients in the current study had received a new diagnosis of hypertension while two others had resting BP level classifiable as stage II hypertension, one with stage I hypertension, and two with elevated BP based on the current guidelines (158). PASC patients also exhibited higher central arterial stiffness. Notably, while elevated arterial stiffness has been previously reported in individuals who had COVID-19 (221–223, 235, 236), findings from some studies suggest an improvement BP and arterial stiffness over the course of 6 months (221, 278). However, these studies were not in PASC patients. Therefore, in contrast to the potential transient impact of COVID-19 on BP and arterial stiffness, findings from the current study suggests that high BP and arterial stiffness are likely long-term cardiovascular outcomes of PASC.

There is robust evidence from previous studies showing blunted peripheral vascular function following COVID-19, an impairment that appears to persist well beyond the acute phase of the illness (200, 207, 220, 223, 225, 226, 265). Limited studies have reported that peripheral macro and micro vascular function are blunted in PASC (200, 227). However, in contrast to these findings, our data demonstrates that peripheral macro- and microvascular function are not blunted in PASC patients. Several differences between the previous studies that included patients with a PASC diagnosis, and the current study may explain these equivocal findings. In the study by Oikonomou and colleagues (200), brachial artery FMD was reported to be blunted at 6 months following SARS-CoV-2 infection in

those with and without a long COVID diagnosis who had required hospitalization for a median duration of 14 days during the acute illness. In contrast to the participants in the aforementioned study, the median time since COVID-19 diagnosis of the PASC patients in the current study was 17.5 months, and only 2 patients had required hospitalization for an extended period. Moreover, Oikonomou and colleagues (200) showed improvement in brachial artery FMD from the acute stage of the illness to up to 6 months, in patients with and without Long COVID. In the study by Haffke et al. (227), it was reported that forearm microvascular function is blunted only in 30% of PASC patients at 9 months since initial SARS-CoV-2 infection. Collectively, these findings would suggest that the impairment in peripheral vascular function observed following COVID-19 is likely a transient detrimental consequence of COVID-19 that is unrelated to PASC. Data from the current study strengthens these findings.

Similar to peripheral vascular function, we found no impairment in cerebral vasodilator function in PASC patients compared to controls. A previous study by Marcic et al. found that cerebrovascular reactivity to hypercapnia induced by a breath hold test was blunted in those who had non-specific neurological symptoms 30 - 60 days post COVID-19 diagnosis compared to controls without a history of COVID-19 (231). However, this study did not include a group without neurological symptoms, and therefore, it is not possible to infer whether the impairment in cerebrovascular reactivity was related to symptomology and the timing of when participants were studies prevents the ability to make any inference on long-term effects. Our data suggest that similar to peripheral vascular dysfunction, cerebral vascular dysfunction may also be a consequence of COVID-19 but not PASC.

Interestingly, we found that resting MCAv and CVCi were inversely correlated with the severity of brain fog. Although TCD does not provide a measure of vessel

diameter, this may suggest that lower resting cerebral blood flow and greater resting cerebral vascular tone is associated with greater impairment in cognitive function. These findings are in agreement with studies that have assessed cerebral blood flow in other conditions that are associated with brain fog such as chronic fatigue syndrome (284). Interestingly, CVMR to hypercapnia assessed as the slope of ΔCVCi% vs. $\Delta P_{ET}CO_2$ was positively correlated with the severity of brain fog although the slope of ΔMCAv% vs. $\Delta P_{ET}CO_2$ was not correlated. These data may indicate that PASC patients with greater severity of brain fog had a greater cerebrovascular conductance reserve due to the low baseline conductance as has been documented with aging by some researchers (116). However further studied are warranted to better understand the potential role of cerebral blood flow and its regulation in PASC-related cognitive dysfunction.

Beyond the association between cerebral blood flow and brain fog, we did not find any relationships between measures of peripheral vascular function, arterial stiffness or BP and symptom burden. The heterogenous nature of the symptomology of PASC patients could be contributing to these non-associations. Indeed, a total of 18 symptoms were reported among all PASC patients with varying degrees of severity. However, we observed that physical function and QOL were inversely correlated with total symptom burden, thus providing objective evidence that symptomology does play a role in overall health.

To date, the mechanisms contributing to persistent symptoms of PASC are not well understood. However, it is postulated that several mechanisms may be involved (247, 285). Notably, a preliminary study involving two case studies has shown improvement in long COVID symptoms including brain fog following stellate ganglion blockade, a procedure that blocks the sympathetic outflow to the head and neck region. While these findings suggest that sympathetic overactivity may contribute to PASC symptomology, others have reported autonomic dysregulation in individuals with persistent COVID-19 related

symptoms (286–289). Our findings of elevated BP in PASC could be at least in part caused by elevated sympathetic activity though future studies are needed to identify the potential contribution of autonomic dysfunction in causing adverse effects of PASC.

Conclusion

In conclusion, we demonstrate that patients with a diagnosis of PASC exhibit elevated resting BP and central arterial stiffness. In contrast, peripheral and cerebral vascular function appears to be unaffected in PASC. Furthermore, peripheral vascular function, arterial stiffness, and BP were not associated with symptom burden whereas resting cerebral blood flow was inversely correlated to severity of brain fog and total symptom burden was inversely correlated to measures of physical function and QOL. Collectively, these findings suggest that although peripheral and cerebral vascular function appear to be unaffected, brain fog appears to be greater in those with lower resting cerebral blood flow and elevated BP and central arterial stiffness are adverse cardiovascular outcomes of PASC.

Tables and Figures

Table 1. Resting hemodynamics and metabolic parameters

	Control (n = 10)	PASC (n = 10)	P value
Heart rate, beats per min	59 ± 4	62 ± 9	0.434
Brachial SBP, mmHg	109 ± 8	128 ± 20	0.013
Brachial DBP, mmHg	68 ± 4	78 ± 8	0.003
Brachial MAP, mmHg	82 ± 5	95 ± 11	0.005
Fasting blood glucose, mg/dl	94 ± 8	101 ± 33	0.539
LDL-cholesterol, mg/dl	104 ± 18	125 ± 42	0.153
HDL-cholesterol, mg/dl	68 ± 19	54 ± 7	0.045
Triglycerides, mg/dl	70 ± 22	144 ± 78	0.010

Data are mean ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure, MAP, mean arterial pressure; LDL, low density lipoprotein; HDL, high density lipoprotein. Independent sample t-test between control and PASC.

Table 2. Medical conditions, medication, and persistent COVID-19 related symptoms

	Control (n = 10)	PASC (n = 10)
Diagnosed medical conditions[a]		
Hypertension	1	3 (2)
Diabetes	0	2 (1)
Dyslipidemia	1	2 (1)
Depression	2	4 (1)
Anxiety	2	5 (2)
Hypothyroidism	2	2 (0)
Lupus	0	1 (1)
Medication		
β-blockers	0	2
Ca-channel blockers	0	1
ACE inhibitors/ARB	0	2
Diuretics	1	1
Statins	1	2
Aspirin	1	2
SSRI/SNRI/NDRI	2	4
Over the counter vitamins/supplements	9	8
Persistent COVID-19 related symptoms		
Fatigue	-	10
Brain fog	-	9
Shortness of breath	-	9
Muscle pain	-	7
Joint pain	-	6
Palpitations	-	6
Chest pain	-	5
Sleep disturbances	-	4
Tingling/numbness in extremities	-	3
Altered smell/taste	-	2
Cough	-	2
Swelling in extremities	-	2
Muscle weakness	-	1
Hair loss	-	1
Fainting episodes	-	1
Diarrhea	-	1
Recurrent headaches	-	1
Extremities changing color	-	1

n, number of participants. PASC, Post-acute sequalae of COVID-19; OTC, over the counter; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blockers; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin and norepinephrine reuptake inhibitor; NDRI, norepinephrine and dopamine reuptake inhibitors.

[a]values within parentheses for medical conditions are the number of participants who were diagnosed after their COVID-19 diagnosis.

Table 3. Relationship between measures of vascular health and total symptom burden in PASC patients

	r	P value
Brachial SBP, mmHg	0.279	0.453
Brachial DBP, mmHg	0.232	0.518
Brachial MAP, mmHg	0.270	0.451
FMD, %	0.451	0.191
Peak blood velocity, cm/s	-0.020	0.956
PWV, m/s	-0.188	0.603
Central SBP, mmHg	0.154	0.671
Central DBP, mmHg	-0.096	0.793
Central MAP, mmHg	0.016	0.965
ΔMCAv slope, %/mmHg	0.184	0.636
ΔCVCi slope, %/mmHg	0.295	0.441
Physical function score	0.694	0.026
6-min walk distance	-0.864	0.001
QOL score	-0.795	0.006

PASC, Post-acute sequalae of COVID-19; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; FMD, flow-mediated dilation; PWV, pulse wave velocity; MCAv, middle cerebral artery blood velocity; CVCi, cerebrovascular conductance index; QOL, quality of life; r, Pearson's correlation coefficient.

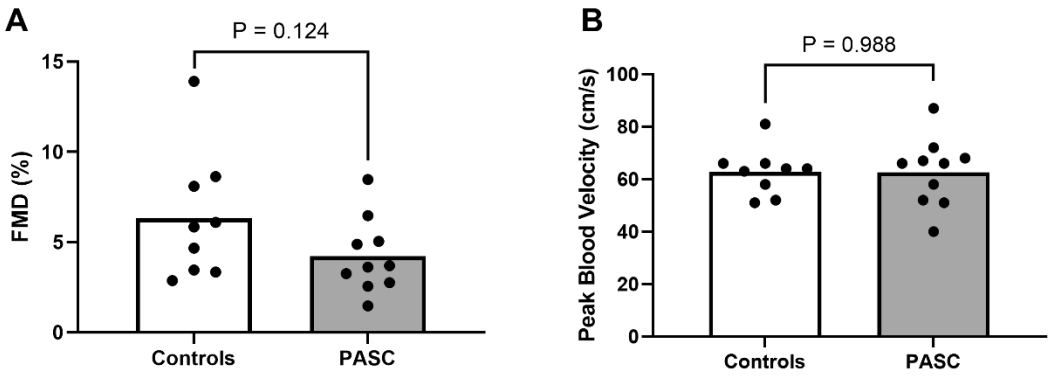

Figure 1. Group means and individual data for flow-mediated dilation (FMD, A) and peak blood velocity following cuff release (B) between controls (n = 9) and patients with post-acute sequalae of COVID-19 (PASC, n = 10). Group comparisons were made using two-tailed independent samples t-test.

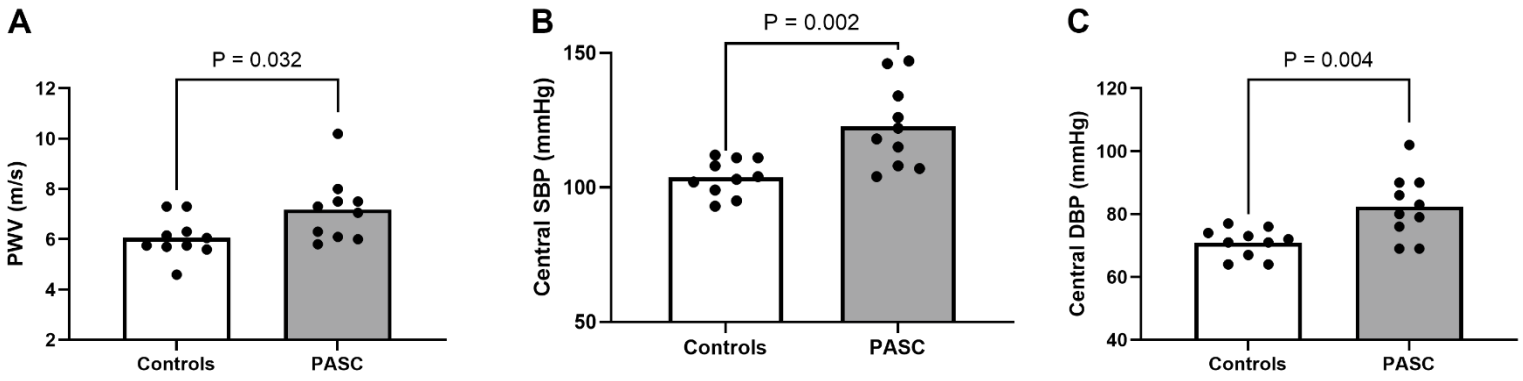

Figure 2. Group means and individual data for carotid-femoral pulse wave velocity (PWV, A), central systolic blood pressure (SBP, B). and diastolic BP (DBP, C) between controls (n = 10) and patients with post-acute sequalae of COVID-19 (PASC, n = 10). Group comparisons were made using two-tailed independent samples t-test.

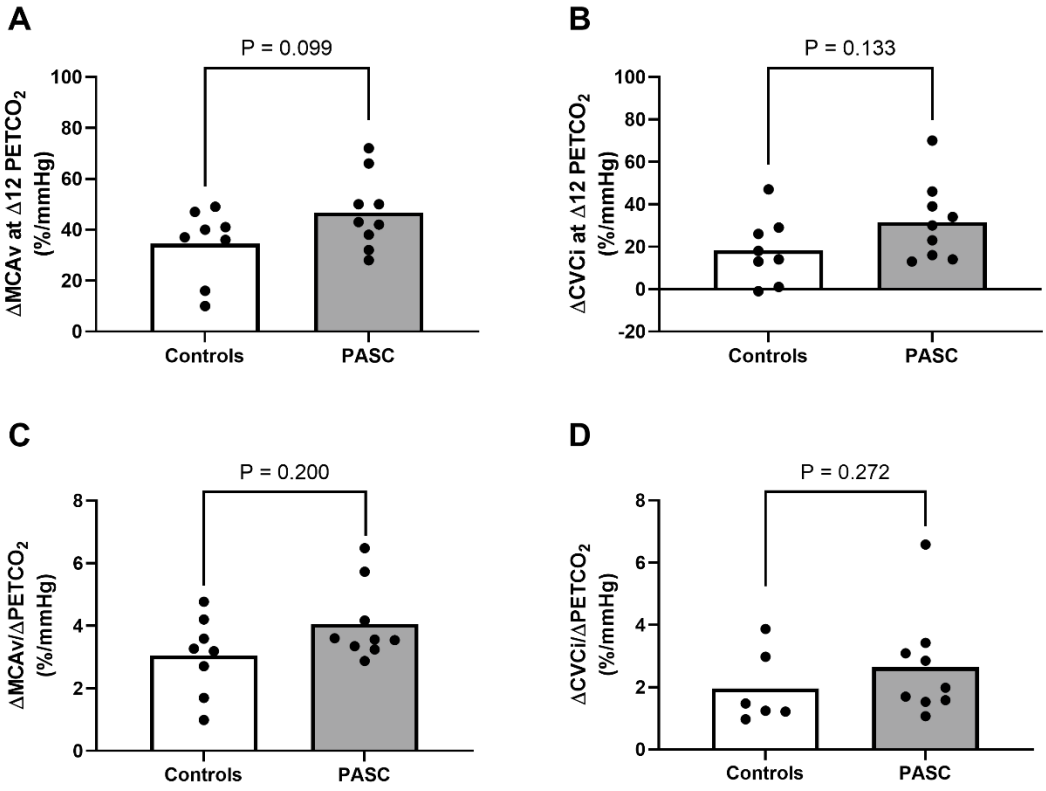

Figure 3. Group means and individual data for percent change in middle cerebral artery blood velocity (ΔMCAv%) and cerebral vascular conductance index (ΔCVCi%) at an increase in end-tidal carbon dioxide ($\Delta PETCO_2$) of 12 mmHg (A and B respectively) and the slope of ΔMCAv% vs. $\Delta PETCO_2$) and ΔCVCi% vs. $\Delta PETCO_2$ (C and D respectively) between controls (n = 8) and patients with post-acute sequalae of COVID-19 (PASC, n = 9). Group comparisons were made using two-tailed independent samples t-test.

[a]n = 6 controls for ΔCVCi% vs. $\Delta PETCO_2$ slope due to correlation coefficient being below the threshold in 2 controls.

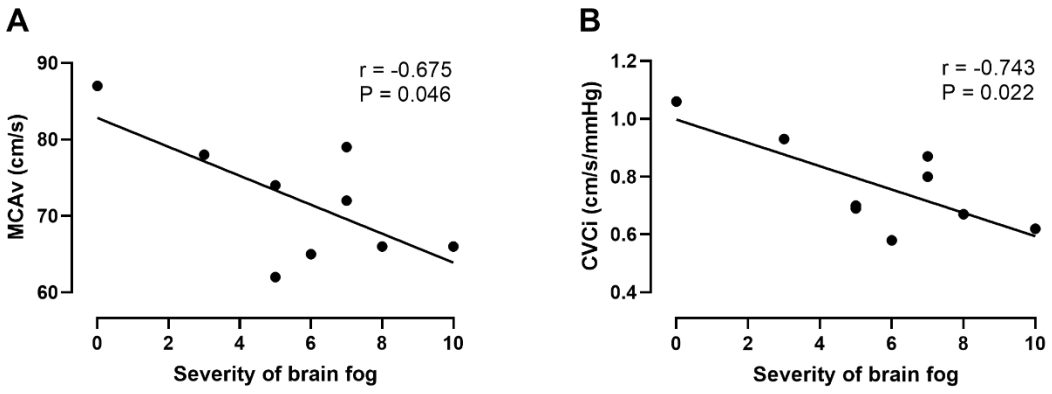

Figure 4. Relationship between severity of brain fog and resting middle cerebral artery blood velocity (MCAv, A) and cerebral vascular conductance index (CVCi, B) in patients with post-acute sequalae of COVID-19. r = correlation coefficient.

CHAPTER 6: CONCLUSION AND FUTURE DIRECTIONS

Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus -2 (SARS-CoV-2) is a continuing health burden with adverse effects that extend well beyond the acute illness. One such long-term consequence of COVID-19 is its potential to increase the future risk of developing cardiovascular diseases (CVD) (5). Given that millions of individuals have been, and continue to become infected by SARS-CoV-2, the already existing enormous burden of CVD (6, 7) could be substantially increased in the future. Therefore, it is important to identify factors that may lead to this augmented CVD risk following COVID-19 in order to identify potential targets to direct preventive and/or therapeutic strategies. The studies described in this dissertation aimed to provide novel fundamental information regarding the impact of COVID-19 on key components of cardiovascular health that are known to contribute to the development of CVD.

Vascular dysfunction, as evidenced by blunted vasodilator function is a known risk factor for CVD (8–10). In chapter 3 of this dissertation, we show that young adults who continue to experience symptoms beyond the acute phase of COVID-19 exhibit blunted peripheral macro-and microvascular function. Peripheral vascular function was not impaired in those who had recovered from symptoms. These results, for the first time suggest a potential association between COVID-19 symptomology and peripheral vascular function. In this study, we also found that cerebral vascular function and central arterial stiffness is unaffected by COVID-19 beyond the acute phase of the illness. While these latter findings are encouraging, it is important to realize that this study was in young otherwise healthy adults with mild acute illness. Indeed, since this original work, others have reported blunted peripheral vascular function (200, 220, 222–227), cerebral vascular

function (230, 231), and elevated arterial stiffness (221, 223) beyond the acute illness in older age groups with varying COVID-19 disease severity. Thus, collectively, it is evident that COVID-19 exert detrimental effects on the vasculature that appear to extend beyond the acute illness.

Extending our findings from chapter 3 and work of others suggesting persistent adverse effects of COVID-19 on vascular function and arterial stiffness, in young adults, in chapter 4 we aimed to comprehensively assess the influence of COVID-19 on blood pressure (BP) in this population given that vascular homeostasis is vital for regulation of BP. In this study, we additionally aimed to investigate the influence of time since COVID-19 diagnosis on BP using both ambulatory and laboratory BP measurement techniques. We demonstrate that the impact of COVID-19 on BP in young adults is likely transient, with BP being higher in individuals closer to COVID-19 diagnosis compared to those further out, up to 6 months from infection. A similar inverse relationship was observed between central arterial stiffness and time since COVID-19 diagnosis. While these findings indicate that COVID-19 likely does not exert permanent damage to the vasculature in young adults who had mild acute illness, others have also shown similar promising results of improvement in measures of vascular health over time in older individuals who had COVID-19 (200, 234).

Overall, our data and work from others indicate that the detrimental effects of COVID-19 on cardiovascular health may extend beyond the acute illness but recover over time. However, post-acute sequalae of COVID-19 (PASC) is a condition that arises from SARS-CoV-2 infection which is characterized by debilitating symptoms and health effect that persist, recur, or are newly developed following COVID-19, indicating the presence of more long-lasting adverse consequences of COVID-19. Findings from chapter 5 of this dissertation demonstrates that PASC patients exhibit high BP and central arterial stiffness

but not impairments in peripheral or cerebral vascular function. We also show an inverse association between the severity of brain fog in PASC patients and resting cerebral blood flow which points to a potential link between PASC symptoms and alterations in cerebral blood flow regulation.

Collectively, these data provide direct evidence of the impact of COVID-19 on long-term cardiovascular health in two extremes of COVID-19: young previously healthy adults who had mild acute illness and patients with PASC, a significant proportion of who also were previously healthy. Importantly, some research (233, 234), including findings from chapter 4 of this dissertation suggests that some adverse effects of COVID-19 on cardiovascular health may not be permanent, but merely takes time to resolve. While this is encouraging, it is unknown whether there is complete resolution of detrimental effects of COVID-19 on cardiovascular health. SARS-CoV-2 continues to evolve into different variants with varying degrees of infectiousness and disease severity. Notably, we recently showed that cardiovascular health appears to be unaffected in young adults who contracted the Omicron variant (290). The differing pathogenicity of the virus and the introduction of vaccines against COVID-19 makes investigation of the long-term impact of COVID-19 challenging and interpretations complex. Nonetheless, large scale follow-up studies, and future studies investigating the impact of new SARS-COV-2 variants would provide important information on the long-term impact of COVID-19 on CVD risk and overall health.

Importantly, it should be recognized that PASC is a condition in which those affected experience ongoing debilitating symptoms without any signs of recovery even after one year since initial SARS-CoV-2 infection. This prompts the question whether the elevated CVD risk following COVID-19 could in fact be due to PASC. Indeed, our data suggests that elevated BP may be a complication of PASC. Given that hypertension is a

primary risk factor for other CVD, understanding the mechanisms leading to elevated BP in PASC is important. In this regard, recent studies have reported persistence of viral particle for several months post SARS-CoV-2 infection (291, 292), including preliminary studies showing the presence of the SARS-CoV-2 spike protein in patients with PASC (293). Persistence of viral particles indicate the possibility of an ongoing inflammatory response. Indeed, a recent study reported the presence of an ongoing, sustained inflammatory response in PASC patients with high levels of circulating pro-inflammatory mediators (294). It is well known that inflammation and oxidative stress can trigger and mediate the progression of hypertension and other CVD (295, 296). Whether persistent inflammation contributes to adverse cardiovascular outcomes of PASC is still unknown. Indeed, although it is plausible that inflammation may lead to elevated arterial stiffness and high BP in PASC, it could be expected that inflammation would cause vascular dysfunction as well. However, we did not observe vascular dysfunction in PASC patients, and therefore, further studies are warranted to determine the potential contribution of inflammation to CVD risk in PASC. In addition, several clinical reports have indicated that a significant proportion of individuals who experience persistent COVID-19 related symptoms show clinical features suggestive of autonomic dysregulation (286, 297–299). Given that the sympathetic nervous system plays a vital role in the regulation of BP, it is reasonable to postulate that autonomic dysregulation in the form of augmented sympathetic activity may cause high BP in this population. In fact, a recent study involving stellate ganglion block, which blocks the sympathetic outflow to the head and neck region and upper limbs, was shown to improve PASC symptoms in two patients (253). While these findings provide preliminary evidence of involvement of sympathetic overactivity in PASC, further studies are needed to confirm these findings.

Collectively, the work presented in this dissertation provides novel information regarding the impact of COVID-19 and PASC on long-term cardiovascular health and CVD risk. Findings from these studies pave the way for future studies to explore potential therapeutic options that could reverse and/or mitigate the elevated CVD risk following COVID-19 and PASC.

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