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Cardiovascular Disease Risk in Young Adults Following Covid-19

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Abstract

Introduction: SARS-CoV-2, the virus responsible for COVID-19, may cause a dysregulated systemic inflammatory response that could lead to cardiovascular damage and cause individuals recovering from COVID-19 to be at an increased risk for future cardiovascular disease (CVD). Physical activity (PA) is inversely associated with systemic inflammation and CVD risk, which may make it a useful cardioprotective lifestyle factor for individuals recovering from COVID-19. **Purpose:** 1) Compare arterial stiffness and systemic inflammatory levels between individuals recovering from COVID-19 and uninfected controls, 2) explore systemic inflammation as a predictor of arterial stiffness, and 3) explore PA as a mediator for the relationship for COVID-19 history with arterial stiffness and systemic inflammation. **Methods:** Cross-sectional analysis was performed on 23 SARS-CoV-2 participants (8M/15F, 25.0±8.9 years, 24.1±3.5 kg/m^2) and 32 uninfected controls (14M/18F, 24.4±6.5 years, 25.1±3.5 kg/m^2). Arterial stiffness, as a proxy for CVD risk, was estimated as pulse wave velocity (PWV) during 24-hour ambulatory blood pressure monitoring using an oscillometric blood pressure device. Systemic inflammation was assessed as salivary cytokine and C-reactive protein (CRP) levels collected using the passive drool method. PA was objectively measured via accelerometry and assessed as moderate-to-vigorous physical activity (MVPA). An independent samples *t*-test was used to compare measures of arterial stiffness and systemic inflammation between the SARS-CoV-2 and control groups. Simultaneous multiple regression was used to assess how well proinflammatory cytokine and CRP levels predicted arterial stiffness. Mediational analysis was used to determine whether there is a significant indirect effect of COVID-19 history through MVPA on arterial stiffness and CRP levels. **Results:** Participants recovering from COVID-19 were studied, on average, 111.6±118.3 days after testing positive, experienced 5.2±3.8 symptoms, and had mild COVID-

19 disease severity. The results from independent samples *t*-test showed no significant differences (all $p>0.05$) between the SARS-CoV-2 and control group in PWV (5.0±0.5 m/s vs 5.1±0.5 m/s) , IL-8 (821.1±772.6 pg/mL vs 843.8±958.4 pg/mL), IL-1β (126.3±102.2 pg/mL vs 143.6±157.9 pg/mL), IL-6 (11.7±25.4 pg/mL vs 5.9±7.9 pg/mL), TNF-α (4.8±3.9 pg/mL vs 5.1±5.9 pg/mL), or CRP (765.4±672.9 pg/mL vs 526.3±674.8 pg/mL). Additionally, the combination of IL-8, IL-1β, IL-6, TNF-α, and CRP were not found to significantly predict PWV, with no individual measure of systemic inflammation significantly contributing to the regression equation (all $p>0.05$). Finally, mediational analysis did not find a significant indirect effect of COVID-19 history through MVPA on PWV (estimate = 0.0220, 95% CI = -0.0488 – 0.2427) or of COVID-19 history through MVPA on CRP levels (estimate = 0.0254, 95% CI = -0.0675 – 0.1646). **Conclusion:** This investigation found no differences in arterial stiffness and systemic inflammation between young adults recovering from COVID-19 and uninfected controls. Additionally, systemic inflammation was not found to be a significant predictor of arterial stiffness. Finally, MVPA was not found to significantly mediate the relationship for COVID-19 history with arterial stiffness and systemic inflammation.

CARDIOVASCULAR DISEASE RISK IN YOUNG ADULTS FOLLOWING COVID-19

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Glossary of terms and abbreviations

Abbreviation	Term
ACE2	Angiotensin converting enzyme receptor 2
Anti-S-IgG	Anti-S protein-neutralizing antibody
ARDS	Acute respiratory distress syndrome
CFR	Case fatality rate
COVID-19	Coronavirus disease of 2019
CRP	C-reactive protein
CVD	Cardiovascular disease
HCoV	Human coronavirus
hsTnT	High-sensitivity troponin T
IL-6	Interleukin-6
MERS-2012	Middle East respiratory syndrome of 2012
MERS-CoV	Middle East respiratory syndrome coronavirus
MVPA	moderate-to-vigorous physical activity
PWV	Pulse wave velocity
SARS-2003	Severe acute respiratory syndrome of 2003
SARS-CoV	Severe acute respiratory syndrome coronavirus
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
TNF-α	Tumor necrosis factor-alpha

Chapter 1: Introduction

On March 11, 2020, the World Health Organization declared the coronavirus disease of 2019 (COVID-19) a global pandemic [1]. As of August 25, 2021, there have been more than 213 million confirmed cases of COVID-19 worldwide [2]. The United States has been the country hardest hit by the COVID-19 pandemic, accounting approximately 38 million of those confirmed cases [2]. COVID-19 is caused by a virus (SARS-CoV-2) that comes from the same coronavirus family that is responsible for the previous SARS-2003 and MERS-2012 epidemics [3]. While COVID-19 shares many similarities to SARS-2003 and MERS-2012, it is dissimilar to those coronaviruses in two significant ways. First, the infectivity index, a measure of the degree of contagiousness of a virus, is notably higher for COVID-19 compared to SARS-2003 and MERS-2012 [3] and is likely responsible for the marked increase in infected persons. Second, the case fatality rate of COVID-19 is much lower than SARS-2003 and MERS-2012 [4]. Taken together, the increased infectivity and decreased case fatality rate of COVID-19 suggests that there will be a greater number of individuals recovering from coronavirus than ever before. As such, it is of increasing importance to understand how COVID-19 may affect the long-term health of those recovering from it.

With that in mind, individuals recovering from COVID-19 may be at an increased risk for future cardiovascular disease (CVD). The defense of the body against COVID-19 is characterized by an inflammatory response that may be detrimental to the vasculature and lead to an increased risk for future CVD events. SARS-CoV-2 enters the body by binding to an angiotensin converting enzyme 2 (ACE2) host receptor, leading to the destruction and downregulation of ACE2 [5-7]. In response to the downregulation of ACE2, there may be a dysregulated systemic inflammatory response where proinflammatory cytokines are severely

upregulated to create a “cytokine storm” [5-7]. The “cytokine storm” induced by SARS-CoV-2 may then have negative consequences for vascular health. Proinflammatory cytokines lead to the local production of C-reactive protein (CRP) in vascular smooth muscle cells [8]. In turn, CRP promotes vascular inflammation [8]. Increased vascular inflammation causes increases in vascular fibrosis, smooth muscle cell proliferation and endothelial dysfunction; all of which lead to an increase in arterial stiffness [8]. Previous studies have supported this pathway by showing that acute systemic inflammation leads to a transient increase in arterial stiffness [9]. Arterial stiffness is an independent risk factor for CVD and is a strong predictor for future CVD events and all-cause mortality [10].

This inflammatory pathway may be responsible for acute cardiovascular damage during COVID-19 illness that could put those recovering from the disease at an increased risk for future CVD. A common clinical characteristic of COVID-19 patients is elevated cardiac troponin levels [11-14], an indicator of myocardial damage [14]. Cardiac troponin levels in COVID-19 patients have significant positive linear correlations with C-reactive protein levels [12, 15], suggesting that myocardial injury and systemic inflammation are closely related. Cardiac troponin levels have also been shown to be associated with arterial stiffness in clinical and community-dwelling populations [16, 17], providing a link between myocardial and vascular damage that may potentially have harmful long-term implications for cardiovascular health. Finally, there is emerging evidence of damage to the vasculature during COVID-19 illness, with findings indicating that arterial stiffness is increased in COVID-19 patients and predictive of length of hospital stay and all-cause mortality in these patients [18, 19].

Furthermore, there is emerging evidence to suggest that the heightened systemic inflammatory response, and subsequent increase in CVD risk, from COVID-19 may persist even

after recovery from the virus [20]. Continued cardiac involvement and myocardial inflammation was observed in middle-aged adults whose COVID-19 cases ranged from asymptomatic to severe [21]. Additionally, reduced endothelial function has been noted in middle-to-older aged adults who experienced severe-to-critical COVID-19 illness three months after viral recovery [22]. These findings are supported by initial investigations noting vascular alterations in young adults, with reduced endothelial function and increased aortic stiffness compared to healthy controls 3 to 4 weeks after testing positive for SARS-CoV-2 infection [23, 24]. These findings stress the need for a better understanding of the cardiovascular health of the individuals recovering from COVID-19. Knowledge regarding the short and long-term cardiovascular implications of coronavirus infections is limited and whether COVID-19 increases the risk for future CVD events is unknown.

Physical activity (PA) may be a method for improving regulation of the systemic inflammatory response and helping to reduce the risk for future CVD events in individuals recovering from COVID-19. PA is associated with reduced systemic inflammation through inverse associations with serum cytokines and CRP levels [25, 26]. Additionally, many studies have noted inverse associations for all levels of PA with arterial stiffness [27-29]. These findings suggest that PA may be potentially important in protecting against future CVD events in individuals recovering from COVID-19.

Therefore, the purposes of this study are to 1) examine whether individuals recovering from COVID-19 have an increased CVD risk by comparing arterial stiffness measures, as a proxy for CVD risk, between individuals recovering from COVID-19 and controls without a history of COVID-19; 2) (a) examine whether individuals recovering from COVID-19 have increased systemic inflammation by comparing markers of salivary systemic inflammation,

notably salivary proinflammatory cytokines and CRP, between individuals recovering from COVID-19 and uninfected controls, and (b) explore how well the combination of those salivary systemic inflammatory markers (proinflammatory cytokines and CRP) predicts arterial stiffness; and 3) explore PA as a possible protector against increased CVD risk by determining whether PA mediates the relationship between COVID-19 history and arterial stiffness and COVID-19 history and salivary systemic inflammation. It is hypothesized that individuals recovering from COVID-19 will have increased arterial stiffness compared to controls (Figure 1), that (a) cytokines and CRP will be higher in individuals recovering from COVID-19 than controls and (b) the combination of cytokines and CRP will be associated with arterial stiffness, and that physical activity will mediate the relationship between COVID-19 history and arterial stiffness (Figure 2), as well as the relationship between COVID-19 history and CRP levels (Figure 3). If these hypotheses are confirmed, it could help guide healthcare professionals in their treatment of patients with a history of COVID-19, with physical activity being a potential intervention for individuals recovering from COVID-19 in managing the systemic inflammatory response and reducing CVD risk. It would also stress the need for clinical trials examining anti-inflammatory drugs as a potential pharmaceutical intervention for improving the cardiovascular health profile of individuals recovering from COVID-19.

Chapter 2: Literature Review

Introduction

COVID-19 illness may increase the risk for future cardiovascular disease. Due to SARS-CoV-2 viral infection and the subsequent exacerbated systemic inflammatory response, individuals recovering from COVID-19 may be at an increased risk for a future adverse cardiovascular event. This review will focus on the pathophysiology of COVID-19, the pathogenesis of the "cytokine storm," myocardial injury during COVID-19 illness, how systemic inflammation may lead to a stiffening of the arteries, and the role arterial stiffness plays in adverse CVD events. Additionally, the relationship between physical activity with systemic inflammation and arterial stiffness will be explored. Finally, this review will briefly discuss methods for adapting human vascular research to a global pandemic.

COVID-19

Coronaviruses are large, enveloped, single-stranded RNA viruses belonging to the *Coronaviridae* family [5, 30]. They are divided into four genera based on their structure: alpha, beta, gamma and delta; with only the alpha and beta coronaviruses being found to infect humans (HCoVs) [31]. HCoVs are further classified as low and highly pathogenic coronaviruses [5]. Low pathogenic coronaviruses infect the upper airways and are responsible for illnesses like the common cold [30, 32]. These types of coronaviruses are usually alpha coronaviruses [31]. On the other hand, highly pathogenic coronaviruses, such as beta coronaviruses [31], infect the lower respiratory tract and can lead to pneumonia and acute respiratory distress syndrome (ARDS) [30]. Highly pathogenic beta coronaviruses have been previously responsible for two major public health crises, with the SARS-CoV and MERS-CoV being responsible for the SARS-2003

and MERS-2012 epidemics, respectively [33, 34]. The novel coronavirus SARS-CoV-2, which causes the coronavirus disease of 2019 (COVID-19), is now the third highly pathogenic beta coronavirus to cause severe disease in humans in the past two decades [5, 30-32]. SARS-CoV-2 likely originated in bats and was passed to humans through an intermediate mammalian host, such as the pangolin [30]. It was originally discovered in December of 2019 and was linked to a seafood and wet animal market in Wuhan, Hubei Province, China [7, 32]. As of August 25, 2021, there have been over 213 million global cases of COVID-19, with approximately 38 million of those cases occurring in the United States [35].

The coronavirus genome encodes for four structural proteins: spike (S), membrane (M), envelope (E) and nucleocapsid (N) [32]. The spike protein gives a coronavirus its "crown" shaped appearance and is responsible for receptor binding [32]. The functional receptor for SARS-CoV-2 is the Angiotensin Converting Enzyme Receptor 2 (ACE2), which is highly expressed in the lungs, heart and blood vessels [7]. SARS-CoV-2 is unique in that its infectivity index, a measure for the contagiousness of a virus, is much higher compared to other highly pathogenic beta coronaviruses, such as SARS-CoV and MERS-CoV [3]. The increased infectivity index in SARS-CoV-2 is likely due to structural differences in the spike protein. It has been noted that the SARS-CoV-2 receptor binding domain has a more compact conformation and an increased number of binding hotspots compared to SARS-CoV; thereby, allowing for greater affinity to the ACE2 host receptor [7, 36, 37]. This causes the increased infectivity index of SARS-CoV-2 and is likely responsible for the large number of COVID-19 cases when compared to SARS-2003 and MERS-2012.

Despite having a higher infectivity index than other highly pathogenic beta coronaviruses, the case fatality rate (CFR) of COVID-19 appears to be lower than the CFR for

both SARS-2003 and MERS-2012. The CFR for COVID-19 has been estimated to be around 5% [3, 4], while SARS-2003 and MERS-2012 were reported to be around 10% and 35%, respectively [32, 38, 39]. Moreover, the CFR of COVID-19 may be overestimated given that asymptomatic and mild cases are likely unaccounted for when calculating the CFR. In Singapore, where intensive contact tracing and viral testing measures have been implemented to account for asymptomatic and mild cases, the CFR was just 0.3% [40]. Additionally, on the Diamond Princess cruise ship, an isolated scenario where passengers were quarantined for over a month, the CFR was just 0.99% [40, 41]. This is notable, as isolation on the cruise ship removed any potential cofounders, such as healthcare inequities and screening capabilities [40]. Taken together, these data suggest that the CFR for COVID-19 is much lower than both SARS-2003 and MERS-2012. This lower CFR, combined with an increased infectivity index, indicates that there will be more individuals recovering from COVID-19 than any highly pathogenic coronavirus to come before. As such, it is of the utmost importance to understand how COVID-19 may impact the long-term health of those recovering from it.

Pathogenesis of the "Cytokine Storm"

With that in mind, individuals recovering from COVID-19 may be at an increased risk for future cardiovascular disease (CVD) due to an exacerbated systemic inflammatory response during illness. Once SARS-CoV-2 has entered the host cell, the body defends itself through an inflammatory response where proinflammatory cytokines are produced to up-regulate inflammatory reactions [42, 43]. However, in many cases of COVID-19, the viral infection induces an excessive systemic inflammatory response, as proinflammatory cytokines are overproduced to create an uninhibited positive feedback loop that leads to an exponential growth in inflammation [42, 43]. This is known as the "cytokine storm" [43]. Essentially, this "cytokine

storm" results in an extreme activation of the immune system that attacks the body and can lead to ARDS and multiple organ failure [42]. More research needs to be done on the mechanism via which SARS-CoV-2 induces the "cytokine storm;" however, previous research on the SARS-CoV infection – which shares a 79% similarity [44] and also uses the ACE2 receptor [45] – has suggested three likely mechanisms [6].

ACE2 Downregulation

As previously mentioned, the functional receptor for SARS-CoV-2 is the ACE2 receptor [7]. When SARS-CoV-2 binds to an ACE2 receptor, the spike protein is cleaved to trigger the process of endocytosis for cell entry [30, 42]. During the process of endocytosis, both the virus and the ACE2 membrane receptor are fused into the host cell [30, 42]. This process externally removes the ACE2 receptor from the membrane surface and causes the down-regulation of the ACE2 receptors [42]. This is important, as the ACE2 receptor plays a crucial role in the regulation of angiotensin II [46]. Angiotensin II has been shown to mediate a number of adverse reactions, such as myocardial dysfunction, endothelial dysfunction and enhanced inflammation [46]. Moreover, angiotensin II may interfere with the immune response, as it plays a role in activating macrophages that lead to an increase in proinflammatory cytokines [47-50], potentially causing the "cytokine storm".

Angiotensin II is up-regulated by the *ACE* → *Angiotensin II* → *AT1* receptor axis [46]. In contrast, ACE2 receptors reduce angiotensin II levels by degrading angiotensin II and through the counter-regulatory *ACE2* → *Angiotensin*$_{1-7}$ → *Mas receptor* axis [46]. When SARS-CoV-2 enters the cell and down-regulates ACE2 receptors, this deactivates the *ACE2* → *Angiotensin*$_{1-7}$ → *Mas receptor* axis and augments the *ACE* → *Angiotensin II* → *AT1* receptor axis [46]. Essentially, downregulation of the ACE2 receptor causes the production of angiotensin II to be

un-checked. This leads to angiotensin II interfering with the immune response by activating macrophages that will markedly increase the production of proinflammatory cytokines, such as IL-6 and TNF-α [47-50]. Thus, SARS-CoV-2 mediated downregulation of ACE2 may lead to the excessive activation of angiotensin II to subsequently induce the "cytokine storm."

Rapid Viral Replication and Cellular Death

Another potential mechanism for the "cytokine storm" is through the early onset of rapid viral replication and cellular death [6]. The ACE2 receptor is expressed in the vascular endothelium and respiratory epithelium, suggesting that these cells are likely to be infected by SARS-CoV-2 and undergo apoptosis, or cellular death [51]. As the cells lining the epithelium and endothelium are virally infected and go through the cellular death process, this leads to substantial vascular leakage and a subsequent release of proinflammatory cytokines that may contribute to the "cytokine storm" [6]. Additionally, ACE2 receptors are expressed in macrophages where viral entry and replication will lead to cellular death [51]. Previous studies on SARS-CoV have shown that macrophages can be directly infected by the virus [52], which suggests the same is likely for SARS-CoV-2. Unlike epithelial and endothelial cells that will undergo apoptosis, macrophages undergo a type cellular death called pyroptosis [53]. Pyroptosis is an inflammatory cell death process that is dependent upon caspase 1, which acts to rupture the plasma membrane to initiate cell death, release intracellular proinflammatory contents, and activate proinflammatory cytokines [53]. Taken together, rapid viral replication and subsequent cellular death of cells in the vascular epithelium, respiratory endothelium and macrophages may lead to a substantial increase in proinflammatory cytokines that could be the cause for "cytokine storm" [6, 51].

Anti-Spike IgG

Antiviral neutralizing antibodies play an important role in the body's defense against viral disease; however, they may also play a part in altering the inflammatory response [6]. While research on SARS-CoV-2 is limited, previous research on SARS-CoV provides insight into the role that antiviral neutralizing antibodies may play in the pathogenesis of the "cytokine storm," particularly the anti-S protein-neutralizing antibody (anti-S-IgG). Anti-S-IgG plays an important role in reducing viral replication, but also can alter the inflammatory response to cause severe damage to the lungs. Previous research on SARS-CoV in macaques showed that increases in anti-S-IgG led to greater lung injury [54], while serum samples from persons during infection with SARS-CoV showed that the presence of anti-S-IgG led to greater a buildup of inflammatory macrophages that would activate proinflammatory cytokines [55, 56]. Given the similarities between SARS-CoV and SARS-CoV-2, this suggests that the anti-S-IgG may induce a robust inflammatory response that could play a role in the "cytokine storm" [6].

Cytokines Involved in the "Cytokine Storm"

Whether through ACE2 downregulation, viral replication and cellular death, the anti-S-IgG, or any combination thereof; it is the excessive systemic inflammatory response, or "cytokine storm," induced by SARS-CoV-2 that could be detrimental to the cardiovascular system and cause individuals recovering from COVID-19 to be at an increased risk for future CVD events. More research needs to be done on the cytokines involved in the "cytokine storm" induced by SARS-CoV-2, but clinical characteristics of elevated cytokine levels in patients hospitalized with COVID-19 may elucidate which cytokines are involved in the pathogenesis of the "cytokine storm" [11, 57-65] (see Table 1). In particular, IL-6 levels have been shown to be predictive of COVID-19 disease severity [57], suggesting that it likely plays a role in COVID-19 illness and "cytokine storm" progression.

Myocardial Injury during COVID-19

The “cytokine storm” caused by infection with SARS-CoV-2 may play a role in damage to the cardiovascular system during COVID-19 illness and subsequently increase future risk for CVD. Myocardial injury is of particular concern for patients with COVID-19, as a common clinical characteristic for patients who are hospitalized with COVID-19 is elevated cardiac troponin levels, an indicator of myocardial damage [11, 13, 15, 66-68]. Initial reports from China estimate that one-fourth to one-third of patients exhibited myocardial injury [12]. A summary of these reports is presented in Table 2 [11, 13, 15, 66-68].

Myocardial injury during illness with COVID-19 is likely due to the progression of the systemic inflammatory response and the pathogenesis of the “cytokine storm” [12]. Plasma troponin levels and C-reactive protein levels were found to exhibit significant positive linear correlations [15], suggesting that myocardial injury is closely related to systemic inflammation. Additional mechanisms for myocardial injury during COVID-19 illness have been proposed. The up-regulation of proinflammatory cytokines promotes atherosclerosis and plaque rupture of the coronary arteries through local inflammation, which could lead to increased blood clotting and ischemia [68]. Additionally, the ACE2 receptor is highly expressed in the heart, suggesting that SARS-CoV-2 could play a direct role in damaging the myocardium [68].

Vascular Damage during COVID-19

The excessive systemic inflammatory response from COVID-19 may also play a role in causing vascular damage that could increase the risk for future CVD. Increased angiotensin II activity is associated with increases in arterial stiffness [69], providing a direct mechanistic link between SARS-CoV-2 infection and stiffening of the arteries. As previously mentioned, SARS-

CoV-2 infection may induce the “cytokine storm” through downregulation of the ACE2 receptor, causing an increasing in angiotensin II activity [6, 46]. This leads to an excessive systemic inflammatory response through the activation of proinflammatory cytokines [47-50], which can detrimentally affect the vasculature. Proinflammatory cytokines promote the production of C-reactive protein (CRP) in vascular smooth muscle cells [8]. In turn, CRP promotes vascular inflammation [70-72], which can lead to increases in vascular fibrosis, smooth muscle cell proliferation and endothelial dysfunction – all of which lead to a stiffening of the arteries [70, 73]. An outline of this process is provided in Figure 1.

Initial autopsy and histological reports in patients with COVID-19 suggest that endothelial dysfunction and endotheliitis may play a part in the pathogenesis of COVID-19. In a review of 23 autopsy cases of patients with COVID-19, a common finding among these cases was endothelial injury [74]. Indeed, there was even evidence of direct infection of SARS-CoV-2 into endothelial cells, as viral particles were identified as having infected endothelial cells in the kidney, liver and spleen [74]. These observations make sense, as the ACE2 receptor is expressed in the endothelium [7], providing a mechanism for direct infection of SARS-CoV-2 into endothelial cells. Findings from those autopsy reports are supported by findings of endothelial dysfunction and endotheliitis in histological reports of patients with COVID-19 [75]. Histological analyses found evidence towards direct infection of SARS-CoV-2 into endothelial cells and showed widespread endotheliitis in the lung, heart, kidneys and liver [75].

Endothelial dysfunction in COVID-19 patients, as seen through autopsy and histological reports, could cause arterial stiffening and an increased risk for future CVD. The endothelium lines the internal surface of the blood vessel and responds to hemodynamic changes by releasing substances that maintain vascular homeostasis through vasoconstriction or relaxation [76].

However, endothelial dysfunction shifts this vascular homeostasis towards a vasoconstrictive state. A hallmark characteristic of a damaged endothelium is impaired nitric oxide release, a substance important for counteracting the vasoconstrictive effects of angiotensin II [77, 78] and promoting vasorelaxation [79]. This impaired nitric oxide release leads to a sustained increase in vascular tone that, ultimately, causes arterial stiffening [76].

Endothelial dysfunction and arterial stiffening have been linked in numerous studies [80-84]. In patients with established CVD, endothelial dysfunction was associated with increases in large artery stiffness [81-84]. Additionally, in healthy subjects, worsened global endothelial function was independently associated with increases in large artery stiffness, wave reflections and central pulse pressure [80]. These findings suggest that endothelial dysfunction is linked with arterial stiffness and that COVID-19 induced endothelial dysfunction may put patients at a higher risk for developing arterial stiffness and CVD. Indeed, in patients hospitalized with COVID-19, arterial stiffness has been shown to be elevated during acute COVID-19 illness, with arterial stiffness also being predictive of length of hospital stay and all-cause mortality in COVID-19 patients [18, 19].

Arterial Stiffness and CVD risk

To understand how a stiffening of the large arteries during COVID-19 illness may lead to an increased risk for adverse CVD events, it is necessary to examine the structure and function of the large arteries. Large elastic arteries, such as the aorta, are composed of three layers: the tunica intima, tunica media and tunica adventitia [85]. The tunica intima is the innermost layer and contains a single layer of endothelial cells [85]. The tunica media, the middle layer, is composed mostly of elastin, a rubber-like material that accounts for most of the stretch of a

blood vessel [85]. The tunica adventitia is the outermost layer and is composed primarily of collagen, a fibrous material with a low distensibility [85].

In large arteries (i.e. the aorta) there is a sizeable tunica media and, thus, a large degree of elastin [85]. This gives the large arteries great elastic properties, which is important for increasing windkessel function and reducing wave reflections [85-87]. The windkessel function of the large arteries means that these arteries act as an elastic buffer after a heartbeat [87]. During systole, the large arteries will expand to store some of the stroke volume, and during diastole, the elastic forces of the vessel wall will propel the buffered stroke volume into the peripheral circulation [87]. This allows for healthy, continuous blood flow to target organs [87]. Additionally, compliant (less stiff) arteries lead to a decrease in wave reflections [86]. When the heart beats it sends a forward pressure wave that precedes blood flow [86]. When this forward pressure wave encounters a point of impedance mismatch (i.e. a bifurcation) some of the forward wave is reflected, creating a wave reflection [86]. This wave reflection will combine with the new, forward pressure wave created by the next heartbeat to increase the overall pressure that is resistant to flow and, ultimately, increase the burden on the heart [86]. In compliant arteries, there is a smaller reflected wave and reduced afterload that the heart must overcome to expel blood [86].

However, when large arteries stiffen, there is a reduced windkessel function and increased wave reflections [85-87]. As previously mentioned, excessive systemic inflammation can lead to a stiffening of the arteries [70]. This occurs through a remodeling process where the blood vessel wall thickens as a result of fractured elastin fibers and increases in collagen deposition [88]. Consequently, the artery has reduced distensibility and a worsened windkessel function [86, 87]. The artery is less capable of expanding to buffer stroke volume, leading to an

increase in pulsatile flow and, ultimately, target organ damage [87]. Additionally, stiffening of the arteries leads to an increase in wave reflections, as the stiffer artery leads to a greater reflected wave and creates an increased pressure for the heart to pump blood against [86]. Taken together, reduced functioning of the arteries due to increased stiffness causes a higher risk for adverse CVD events, such as myocardial infarction, heart failure and stroke [89-94].

Evidence associating arterial stiffness and risk for CVD is widespread. Cross-sectional studies have established an association between arterial stiffness and CVD risk [92-94], while longitudinal studies have pinpointed arterial stiffness as an independent predictor for CVD risk and all-cause mortality (Table 3) [89-91]. In fact, in a systematic review and meta-analysis of 17 longitudinal studies with a mean follow up of 7.7 years, Vlachopoulos et al. found that aortic pulse wave velocity (PWV), the gold standard measure for arterial stiffness, is a strong, independent predictor for future cardiovascular events and all-cause mortality [10].

Evidence of Long-Term Cardiovascular Consequences from COVID-19 Illness

Due to the novel nature of COVID-19, exploration of the long-term impact of SARS-CoV-2 infection on the cardiovascular system is limited. A study by Puntmann et al., found that high-sensitivity troponin T (hsTnT) was detectable in 71 out of 100 individuals recovering from COVID-19, while 5% had significantly elevated levels of hsTnT [21]. Additionally, 78 patients had ongoing myocardial inflammation detected via cardiac magnetic resonance imaging, of which 60 patients had no preexisting conditions [21]. These findings suggest that cardiac issues may continue even after recovery from viral infection, and more research needs to examine the cardiac consequences of COVID-19 illness.

Additionally, there is an emerging literature showing that vascular dysfunction continues after COVID-19 illness. Reduced endothelial function has been noted in middle-to-older aged adults who experienced severe-to-critical COVID-19 illness three months after viral recovery [22]. Additionally, in middle-to-older aged adults with cases ranging from mild to severe COVID-19 illness, endothelial and vascular dysfunction persisted four months into recovery [95]. These findings may also extend to younger adults with reduced COVID-19 disease severities, as initial investigations have noted increased resting sympathetic tone, reduced brachial artery endothelial, and increased carotid artery and aortic stiffness compared to controls, in young adults 3 to 4 weeks after testing positive for SARS-CoV-2 infection [23, 24, 96]. Young adults with persistent COVID-19 symptoms also had reduced peripheral vasodilation compared to individuals recovering from COVID-19 who were now asymptomatic, and healthy controls, four weeks into recovery [97]. Finally, COVID-19 illness may contribute to reduced exercise capacity in young adults, as brachial artery blood flow was lower in young adults who experienced mild COVID-19 illness compared to healthy controls [98]. Taken together, these findings suggest continued vascular involvement after COVID-19 illness and stresses the need for further research on the vascular consequences of COVID-19.

With COVID-19 potentially causing both cardiac and vascular damage, it is important to note the relationship between myocardial and vascular injury to understand how these factors may interplay to increase future CVD risk. Cardiac troponin levels are independently associated with aortic PWV in a clinical population of patients who had suffered an acute ST-elevated myocardial infarction [17]. This suggests that overt myocardial injury is linked with vascular damage. Additionally, minimally elevated cardiac troponin levels are associated with carotid-

femoral PWV in a community-dwelling population [16], suggesting that even subclinical myocardial injury is linked to vascular damage.

Furthermore, given the similarities between SARS-CoV and SARS-CoV-2 infection, additional insight into the long-term consequences of COVID-19 may be found through the lens of the SARS-2003 epidemic. For example, previous research on individuals recovering from the SARS-2003 epidemic suggests that the heightened systemic inflammatory response induced by viral infection, and subsequent increase in CVD risk, may persist long after recovery from the virus [20]. In a follow-up study of 25 individuals recovering from SARS-2003, Wu et al. found that these individuals had altered lipid metabolism 12-years after recovery from viral infection [99]. Systemic inflammation has been shown to adversely affect lipid metabolism, while also playing a significant role in mediating the pathogenesis to dyslipidemia [100, 101]. This suggests that heightened systemic inflammation may have persisted to alter lipid metabolism in individuals recovering from SARS-CoV and increase their risk for dyslipidemia. Dyslipidemia has been linked with increased arterial stiffness [102] and is a significant risk factor for CVD, such as atherosclerosis, stroke and myocardial infarction [103-105].

The harmful effects of a continued heightened systemic inflammatory response on the cardiovascular system is further supported by evidence linking systemic inflammation to arterial stiffness. Links between proinflammatory cytokines and arterial stiffness have been established in certain clinical populations [106-111], while there are also a substantial number of studies that have associated CRP with arterial stiffness in both healthy and clinical populations [106-112]. These studies are summarized in Table 4. Additional research needs to be conducted to determine if there is a specific link between inflammation and arterial stiffness in individuals recovering from COVID-19.

In addition, follow-up studies on respiratory recovery in individuals recovering from SARS-2003 have shown long-term implications for health status and exercise capacity [113-115]. In a 1-year follow up, 37% of individuals recovering from SARS-2003 reported reductions in physical health [115]; while health status at 6 months and 2 years was shown to be worse compared to normative controls [113, 114]. Most notably, performance on the 6-minute walk test, a submaximal exercise test, was lower for those recovering from SARS-2003 at 6 months, 1 year and 2 years [113-115]; suggesting that viral infection may have long-term consequences with regard to exercise capacity. Performance on the 6-minute walk test is independently associated with adverse cardiovascular events and all-cause mortality in community-dwelling adults and clinical populations [116-118], indicating that individuals recovering from SARS-2003 are likely at an increased CVD risk. Given the similarities between SARS-2003 and COVID-19, it is likely that these results will also apply to individuals recovering from COVID-19, but more research needs to be done to determine this.

Insight into future CVD risk in individuals recovering from COVID-19 may also be found through examination of common complications associated with COVID-19, most notably pneumonia. COVID-19 pneumonia is of significant concern to those infected with SARS-CoV-2 [119], and those infected whose disease conditions developed to pneumonia may have an increased risk for future CVD. In a study of 591 community-dwelling adults with no prior history of CVD, it was found that the risk for CVD was increased 4-fold in the 30 days after having pneumonia and remained elevated (1.5-fold increase) in the 10 years afterwards [120]. It was concluded that pneumonia is a significant risk factor for CVD and that the magnitude for CVD risk was equal to or higher than traditional risk factors of CVD, such as obesity, diabetes and

hypertension [120]. Given that many COVID-19 patients are hospitalized with COVID-19 pneumonia, it is likely that these patients have an increased risk for future CVD.

Finally, it should be noted that there is an overlap between risk factors for COVID-19 disease severity and arterial stiffness, which suggests that these groups are at an increased risk for COVID-19 related long-term cardiovascular consequences. Populations at the greatest risk for increased COVID-19 disease severity include those who are older [11], diabetics [121, 122], obese, hypertensive, have a history of CVD [11], or are African American [123]. These groups are also at a heightened risk for developing arterial stiffness [124, 125]. Furthermore, myocardial injury and endothelial dysfunction are more likely to occur in COVID-19 patients who are older, diabetic, hypertensive, or have a history of CVD [74, 75]. Myocardial damage and endothelial function are both linked to arterial stiffness in these groups [126-130], suggesting that they may be at an increased risk for developing arterial stiffness after COVID-19 illness. Thus, these groups are likely to be vulnerable to vascular damage induced by COVID-19 and research should focus on these groups when determining the consequential effects of COVID-19 illness to the cardiovascular system.

Physical Activity, Inflammation and Arterial Stiffness

Physical activity is defined as any movement produced by skeletal muscles that requires the body to expend energy [29]. It is a modifiable lifestyle factor that could provide many benefits towards improving cardiovascular health in individuals recovering from COVID-19 by contributing to reductions in inflammation and arterial stiffness [26]. More specifically, regular involvement in moderate-to-vigorous physical activity (MVPA) has been shown to lower CVD risk, as regular MVPA reduces the risk for myocardial infarction, stroke and coronary heart disease [131-133]. Despite this, social distancing guidelines during the COVID-19 pandemic

have led to decreased MVPA and increased sedentary time [134, 135]. Additionally, individuals recovering from COVID-19 may have reduced physical function that may cause lesser engagement in PA [136]. Given the potential effects of social distancing guidelines and SARS-CoV-2 infection on physical activity, it is necessary to understand how PA engagement may contribute to improved cardiovascular health in individuals recovering from COVID-19.

Physical activity may act as a protective lifestyle factor against any persistent heightened systemic inflammation that individuals recovering from COVID-19 could experience after recovery from the viral infection [26]. Inverse associations for physical activity with CRP have been consistently observed, suggesting it could be beneficial in managing inflammatory levels. The association between physical activity and proinflammatory cytokines is less clear [25, 137-141]. The association between physical activity and measures of inflammation is presented in Table 5.

Whether improvements in inflammatory markers are due to physical activity or changes in body composition is still to be determined. Body fat seems to play an important role in inflammation, while obesity is characterized by chronic low-grade inflammation [26, 142]. Many studies that examine the relationship between physical activity and levels of inflammation also report improvements in body composition; thus, it is difficult to determine whether the changes in inflammation are directly due to increased physical activity or improvements in body composition [26]. Nevertheless, increasing physical activity levels remains a potentially important lifestyle modification for reducing inflammation in individuals recovering from COVID-19 that could be useful for bettering their CVD outlook.

In addition to inflammation, all levels of physical activity have been associated with improvements in arterial stiffness. Although MVPA has been shown to have the most substantial

benefits towards reducing arterial stiffness [27, 143-146], even light physical activity has shown efficacy in improving arterial stiffness [28]. Cross-sectional studies examining these relationships have consistently demonstrated that physically active individuals have reduced arterial stiffness compared to those who are sedentary [27, 28, 146], while longitudinal studies have revealed that physical activity effectively slows the progression of arterial stiffness (see Table 6) [143-145]. In conjunction with improvements in inflammation, this evidence suggests that increasing physical activity levels may be a cardioprotective lifestyle modification that could have important benefits for managing future CVD risk in individuals recovering from COVID-19.

Adapting Human Vascular Research to a Global Pandemic

In attempting to prevent the spread of COVID-19, face-to-face laboratory research has been discontinued, severely impacting human research. Established vascular research methods, such as applanation tonometry for the assessment of aortic PWV, flow-mediated dilation for the examination of endothelial function and serum biomarkers for assessing systemic inflammation, have been halted to comply with social distancing guidelines and to prevent COVID-19 transmission. As such, novel methods for assessing vascular function need to be utilized to continue conducting important research during this global pandemic. Our study is proposing a novel approach for assessing cardiovascular function using ambulatory (24-hour) conditions and oscillometric methods, as well as salivary biomarkers for examining assessing systemic inflammation.

The accuracy of oscillometric devices, such as the Mobil-O-Graph, in estimating aortic PWV has been validated against intra-aortic catheter and non-invasive applanation tonometry measurements in healthy and clinical populations [147-149]. Additionally, these oscillometric

devices have proven to be highly feasible and reproducible in estimating aortic PWV [150]. Thus, based on their accuracy and reproducibility, oscillometric devices may be a valid and reliable method for assessing arterial stiffness.

Additionally, 24-hour PWV may add prognostic value to assessing CVD risk. A systematic review and meta-analysis of studies utilizing 24-hour PWV determined that 24-hour PWV independently predicted cardiovascular morbidity and mortality and was associated with different indices of arterial damage [151]. Those conclusions were supported by studies examining the use of 24-hour PWV in clinical populations. In a study examining the usefulness of 24-hour PWV in patients with hypertension, it was found that 24-hour PWV, augmentation index and central blood pressure were all higher in hypertensive than in normotensive subjects [152]. This suggests that ambulatory PWV may be useful for detecting differences in arterial function and vascular impairment in hypertension [152]. Additionally, in hemodialysis patients, 24-hour PWV was an independent predictor of all-cause mortality during a 36-month follow-up period [153].

Furthermore, salivary collection methods may provide a unique, non-invasive, and stress-free way to assess systemic inflammatory levels during a global pandemic [154]. Salivary collection methods, such as the passive drool method for whole saliva samples, are easily accessible and can be completed at home for ease of participants [154]. Salivary biomarkers of inflammation, especially CRP, have shown moderate to strong correlations with serum biomarkers of inflammation, suggesting their utility for assessing systemic inflammation [154-156]. That being said, a major challenge that may limit the utility of saliva samples for measuring systemic inflammation is the influence of local oral cavity inflammation [155]. In studies that have not found significant correlations between salivary and serum inflammatory

markers, major limitations included not controlling for oral health [155]. Thus, it is important that studies utilizing salivary methods include an oral health assessment to help account of local oral cavity inflammation [155].

In addition, salivary biomarkers of inflammation may help to assess CVD risk. In a study examining women exposed to intimate partner violence, salivary CRP differentiated between high and low levels of plasma CRP, suggesting it may be useful for assessing low-grade inflammation and potentially being used to screen for CVD risk [157]. Furthermore, salivary biomarkers of inflammation have reflected serum biomarkers of inflammation for individuals with hypertension, myocardial infarction, myocardial necrosis, and heart failure, suggesting that salivary biomarkers of inflammation could be a useful tool for assessing CVDs [156]. Finally, salivary inflammatory mediators are positively associated with arterial stiffness, subclinical atherosclerosis, and mean arterial pressure, suggesting that they may be useful in the assessment of CVD risk [158].

Based on these findings, 24-hour PWV using oscillometric devices may be a viable method for assessing cardiovascular function, while salivary biomarkers may be a useful assessment of systemic inflammation, in the midst of a global pandemic. 24-hour PWV measurements have been proven to be accurate and reproducible [150], while also adding prognostic value in assessing the risk for CVD and mortality in both healthy and clinical populations [151-153]. Additionally, salivary biomarkers of inflammation have moderate to strong correlations with serum biomarkers of inflammation and may provide insight into CVD risk [156-158]. As such, with the COVID-19 pandemic halting face-to-face laboratory research, measurements using 24-hour PWV via oscillometric devices and salivary biomarkers of

inflammation may be a solution for continuing human vascular research during these uncertain times.

Proposed Study

The above literature review informed the following study proposal and associated hypotheses. COVID-19 illness has been shown to elicit an exacerbated systemic inflammatory response [11, 57-65]. The marked increase in proinflammatory cytokines, or "cytokine storm," could lead to a rise in CRP levels; thereby, increasing vascular inflammation [8]. In turn, the increase in vascular inflammation causes vascular fibrosis, smooth muscle cell proliferation and endothelial dysfunction; all of which, ultimately, lead to the development of arterial stiffness [70]. On the flip side, physical activity may be a useful lifestyle modification for controlling inflammatory levels [26], improving arterial stiffness [27, 28, 143-146] and reducing CVD risk [131-133]. Therefore, the purposes of this study are to 1) examine whether individuals recovering from COVID-19 have an increased CVD risk by comparing arterial stiffness measures, as a proxy for CVD risk, between individuals recovering from COVID-19 and controls without a history of COVID-19; 2) (a) examine whether individuals recovering from COVID-19 have increased systemic inflammation by comparing markers of systemic inflammation, notably proinflammatory cytokines and CRP, between individuals recovering from COVID-19 and uninfected controls, and (b) explore how well the combination of those systemic inflammatory markers predicts arterial stiffness; and 3) explore PA as a possible protector against increased CVD risk by determining whether PA mediates the relationship between COVID-19 history and arterial stiffness. It is expected that individuals recovering from COVID-19 will have increased arterial stiffness compared to uninfected controls, that (a) cytokines and CRP will be higher in individuals recovering from COVID-19 than uninfected

controls and (b) the combination of cytokines and CRP will be significantly predictive of arterial stiffness, and that physical activity will mediator for the relationship between COVID-19 history and arterial stiffness.

Chapter 3: Methodology

Participants: Sixty-two participants, 26 adults who tested positive for SARS-CoV-2 infection (confirmed through documentation of either a positive viral or antibody test for SARS-CoV-2 infection) and 36 adults without a history of SARS-CoV-2 infection were recruited and enrolled for this study. Participants without a history of COVID-19 tested negatively on a SARS-CoV-2 antibody IgG/IgM fingerstick test. This study was designed to limit face-to-face interactions in an attempt to prevent potential exposure to COVID-19. As such, participants were excluded from the study if they were unable or unwilling to engage in Zoom video calls with the researchers. Zoom video calls have been shown to be a viable method for data collection and may be more suited to data collection compared to other videoconferencing services [159]. In addition, participants were excluded from the study if they were actively COVID-19 positive during the study period and were required to provide documentation of a negative viral test in the two weeks prior to study participation. Participants were also screened for COVID-19 symptomology and out-of-state travel prior to study participation, with participants being excluded if they had experienced any COVID-19 symptoms or had traveled outside the state of New York in the two weeks prior to study participation. Finally, participants were excluded from analysis if they did not complete blood pressure measurements or if salivary measures were outside of the assay ranges. In total, four participants did not complete blood pressure measurements (1 SARS-CoV-2 and 3 controls) and three participants exhibited salivary measures outside of assay ranges (2 SARS-CoV-2 and 1 control), leaving 55 participants (23 SARS-CoV-2 and 32 controls) as the final sample size for this study (Figure 4). All participants provided oral informed consent prior over a Zoom meeting prior to study initiation (Appendix 2) and all study procedures were approved by the Syracuse University Institutional Review Board (Appendix 3).

Study Design: Participants were asked to avoid food, sugary drinks, alcohol and caffeine for 12 hours, as well as exercise for 24 hours, prior to undergoing study measures. Study measurements were initially completed during a data collection Zoom meeting to maintain social distancing guidelines. Study equipment was dropped off at the participant's place of residence and all study procedures, as well as blood pressure monitor fitting, were completed during the data collection Zoom meeting. Participants performed one manual blood pressure measurement to ensure that the blood pressure cuff was appropriately sized and connected properly to the ambulatory blood pressure device. However, when in-person human research was allowed to return, these procedures were performed during a 15-minute in-person visit to the Human Performance Lab on the Syracuse University campus. After data collection, participants were sent a RedCap link with a series of questionnaires to complete while undergoing ABPM.

Study Measurements

Anthropometrics and Pulse Oximetry: Participants self-reported height, a measure that has been validated in young and middle-aged adults [160]. In a subsample of our participants who completed in-person data collection (n = 18), height was also measured using an automatic stadiometer for comparison of self-reported height to stadiometer measured height. Weight and body composition were assessed using a digital scale with bioelectrical impedance analysis. Body mass index (BMI) was calculated as kg/m^2. Arterial oxygen saturation was assessed at rest using a fingertip pulse oximeter placed on the index finger.

Cardiovascular Disease Risk: CVD risk was operationally defined as arterial stiffness measured as pulse wave velocity (PWV) using ambulatory blood pressure monitoring (ABPM) with simultaneous ambulatory pulse wave analysis (PWA). ABPM with simultaneous ambulatory PWA was performed using the oscillometric Mobil-O-Graph device. The Mobil-O-Graph (IEM,

Stolberg, Germany) device has been validated against gold standard invasive (intra-aortic catheter) and non-invasive (applanation tonometry) methods in a variety of populations [139-141]. Participants wore an appropriately sized upper arm blood pressure (BP) cuff and BP readings were taken every 20 minutes during the day (07:00 – 22:00) and every 30 minutes during the night (22:00 – 07:00). The device initially inflates and deflates to perform a brachial oscillometric BP measurement, then reinflates again for approximately 10 seconds at the level of diastole to record brachial pulse waveforms. Participants were instructed to avoid exercise during ABPM and to keep their arm still and relaxed during measurements. ≥ 70% of the measurements had to be valid for the data to be considered good quality. All ABPM and PWA measures were calculated as averages over the 24-hour period.

Mean arterial pressure (MAP) was calculated from the brachial systolic (SBP) and diastolic (DBP) blood pressures as MAP = DBP + 0.4(SBP – DBP). Central aortic pulse waves were calculated from brachial pulse waveforms using an applied transfer function derived from the ARCSolver algorithm [150]. The C2 method, which uses oscillometric MAP and DBP measurements, was used to calibrate central aortic waveforms. The C2 calibration method has shown greater accuracy than the traditional C1 method for estimating PWV when compared to gold standard invasive and non-invasive methods [161]. Pulse wave velocity (PWV), our surrogate measure of arterial stiffness, was estimated based on the central aortic waveform reconstructions [150].

Systemic Inflammation: Salivary cytokine (IL-1β, IL-6, IL-8 & TNF-α) and C-reactive protein (CRP) levels, measured as pg/mL, were used to assess systemic inflammation. The aforementioned cytokines have all been shown to be elevated in patients with COVID-19 and likely play a role in the "cytokine storm" [11, 57, 58, 65]. Salivary levels of inflammatory

mediators are associated with arterial stiffness and subclinical atherosclerosis and may be a viable method for evaluating CVD risk [158]. Participants were asked to avoid eating for one hour and rinse their mouth out with water 10 minutes prior to performing the passive drool test. Participants then provided a 1.6 mL saliva sample using the passive drool method by tilting their head forward and guiding their saliva into a cryovial. Whole saliva samples were collected with SalivaBio's 2 mL cryovials and the Saliva Collection Aid (Salimetrics, Carlsbad, CA), a collection device specifically designed to improve volume collection and increase participant compliance, and validated for use with salivary cytokine and C-reactive protein. Saliva samples were stored at -80 degrees Celsius prior to shipment on dry ice via overnight shipping to Salimetrics, Carlsbad, CA for analysis. Saliva samples were assayed for IL-1β, IL-6, IL-8, TNF-α and CRP using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit and samples were analyzed in duplicate. The assay ranges were 0.05 – 2256 pg/mL, 0.06 – 3068 pg/mL, 0.07 – 2336 pg/mL, 0.04 – 1360 pg/mL and 25 – 1600 pg/mL for IL-1β, IL-6, IL-8, TNF-α and CRP, respectively. Participants with salivary values outside of these assay ranges were excluded from analyses.

Physical Activity: Physical activity was assessed via accelerometry. Participants were asked to continuously wear an accelerometer for 24 hours per day for nine consecutive days, except for during water-based activities (e.g. swimming and bathing). The accelerometer was attached to an adjustable elastic belt and was worn around the waist and above the right hip. Accelerometer data was collected using a triaxial accelerometer: the ActiGraph wGT3X-BT (ActiGraph LLC, Pensacola, FL, USA). The device measures acceleration in three perpendicular planes (vertical, antero-posterior, and medio-lateral) and provides activity counts based on the composite vector magnitude of all three axes [162]. Data was initialized and downloaded using the wGT3X-BT,

ActiLife software (ActiGraph LLC, Pensacola, FL, USA). Data was collected at 80 Hz and processed in 60-s epochs. Sleep and wake wear times were classified using a previously published and validated algorithm (SAS syntax is available at https://www.pbrc.edu/pdf/PBRCSleepEpisodeTimeMacroCode.pdf) using SAS version 9.4 [163]. Accelerometer data was considered valid if the participant had at least 4 days with 10+ hours of wake/wear time, including one weekend day. Non-wear time was considered as 60+ consecutive minutes with zero activity counts, with the exception of up to two minutes of activity counts less than 100 [164]. Physical activity levels were then assessed as moderate-to-vigorous physical activity (MVPA). MVPA was defined as greater than 2020 activity counts per minute [164]. MVPA was averaged across all valid days and reported as mean duration (minutes) per day.

Questionnaires: Potential covariates of CV function, systemic inflammation, and PA levels were assessed via questionnaires. Participants self-reported their health history and socioeconomic characteristics. To account for local inflammation within the oral cavity that may affect salivary measures of systemic inflammation, oral health history was assessed using five questions on periodontal health. Sleep quality was determined using the Pittsburgh Sleep Quality Index (PSQI). Anxiety and depressive symptomology were appraised using the Generalized Anxiety Disorder 7-item scale (GAD-7) and Center for Epidemiological Studies Depression scale (CES-D), respectively. Perception of stress was assessed using the 10-item Perceived Stress scale (PSS-10). Post-traumatic stress disorder (PTSD) symptoms since the beginning of the COVID-19 pandemic were assessed using the PTSD Checklist for the DSM-5 (PCL-5). Finally, participants who tested positive for SARS-CoV-2 infection self-reported their COVID-19 history and COVID-19 symptomology. COVID-19 disease severity was determined using the World

Health Organization's guidelines for COVID-19 disease severity based on self-reported symptomology [165].

Statistical Analysis: Descriptive statistics were reported as mean ± SD for continuous variables and percentages for categorical variables. An independent samples *t*-test for continuous variables and a chi square test for categorical variables were used to compare descriptive statistics between the SARS-CoV-2 and control groups. All data were checked for normality using box plots, histograms, Q-Q plots, and skewness and kurtosis values. Salivary markers of systemic inflammation were highly skewed and $\log_{10}$-transformed for subsequent analyses. In our subsample of participants with stadiometer measured height, the criterion-related validity of self-reported height was assessed by comparing measured (criterion) and self-reported height using the Pearson correlation coefficient, One-samples *t*-test, Bland-Altman analysis, and linear regression.

An independent samples *t*-test was conducted to compare arterial stiffness, systemic inflammation, and physical activity measures between the SARS-CoV-2 and control groups. Simultaneous multiple regression using the enter method was used to examine a combination salivary markers of systemic inflammation as predictors of PWV. Variables entered into the model as predictors of PWV included salivary IL-8, IL-1β, IL-6, TNF-α, and CRP. Assumptions of linear relationships, independence of residuals, normal distributions, and homoscedasticity were checked and met. Multicollinearity was also checked; however, IL-8 and TNF-α were strongly correlated ($r = 0.851$), which may reduce the statistical power of our regression model. To further examine potential interactions between COVID-19 history, systemic inflammation, and arterial stiffness, we utilized multiple linear regression with an interaction variable of COVID-19 history and CRP levels to determine whether the new interaction variable was a

significant predictor of PWV. Finally, we used the SPSS PROCESS v3.5 by Andrew F. Hayes to explore possible mediator effects of MVPA on the relationship between COVID-19 history and 24-hour PWV, as well as COVID-19 history and CRP levels. To test for mediation, COVID-19 history was entered as the 'x' variable, PWV and CRP were entered as the 'y' variable, and MVPA was selected as the mediator variable. The model number 4 was selected and set to 5,000 bootstrap samples to minimize sampling error. Statistical significance for a mediator effect was set at a 95% confidence interval that does not contain 0. For additional exploratory analysis, Pearson correlations were used to explore the relationships for arterial stiffness and systemic inflammation with COVID-19 disease severity, COVID-19 symptomology, and days since testing positive for SARS-CoV-2 infection. Statistical significance was set *a priori* at $p < 0.05$. All statistical analyses were performed using IBM SPSS Statistics version 27 (SPSS Inc., Chicago, IL).

Chapter 4: Results

Participant Characteristics

Fifty-five participants were included in the final sample size and used for data analysis (23 SARS-CoV-2 and 32 controls). Participant characteristics are presented in Table 7. Participants were predominantly young adults who were normotensive and of normal weight status. Age, sex, race and BMI were well matched between the SARS-CoV-2 and control groups ($p > 0.05$). Body composition, blood oxygen levels, sleep quality, percentage of participants meeting recommended PA guidelines, and oral health did not significantly differ between groups. In considering mental health symptomology, anxiety, depressive, stress and PTSD symptoms did not significantly differ between groups. Participants in the SARS-CoV-2 group were studied, on average, 111 days after testing positive for SARS-CoV-2 infection, reported approximately 5 COVID-19 symptoms, and experienced mild COVID-19 disease. None of the participants in the SARS-CoV-2 group were hospitalized for COVID-19.

Validity of Self-Reported Height

The criterion-related validity of self-reported height was explored in a subsample of participants ($n = 18$). The results of Pearson correlation coefficients showed a strong and significant association between measured (criterion) and self-reported height ($r = 0.993$, $p < 0.001$). One samples *t*-test showed no significant difference between the mean difference of measured and self-reported height compared to zero, $t(17) = -1.15$, $p = 0.226$. Bland-Altman analysis was used to assess the agreement between measured and self-reported height. The assumption of normality for the distribution of differences was checked and met. Bland-Altman plots showed strong agreement between measured and self-reported height (Figure 5) and linear regression showed

no proportional bias (t = -0.489, $p = 0.632$). Taken together, these results suggest that there is high criterion-related validity for self-reported height in our sample of adults.

Arterial Stiffness, Systemic Inflammation and Physical Activity Levels Between SARS-CoV-2 and Control Groups

The results from the independent samples *t*-test examining differences in arterial stiffness, systemic inflammation and physical activity levels between the SARS-CoV-2 and control groups are presented in Table 8. PWV did not significantly differ between the SARS-CoV-2 and the control group (all $p > 0.05$). For ease of interpretation, raw values for saliva inflammatory markers are presented alongside *p*-values after log-transformation. For salivary cytokine measures, IL-8, IL-1β, IL-6 and TNF-α did not significantly differ between the SARS-CoV-2 group and the control group (all $p > 0.05$). CRP also did not significantly differ between the SARS-CoV-2 and control groups ($p < 0.05$), but did trend towards significance. Finally, accelerometer measured MVPA did not significantly differ between the two groups ($p > 0.05$).

Systemic Inflammatory Markers as Predictors of Arterial Stiffness

As can be seen from Table 9, results from simultaneous multiple regression analysis show that the combination of IL-8, IL-1β, IL-6, TNF-α, and CRP does not significantly predict PWV ($p = 0.699$). The multiple correlation coefficient for the model (R) is 0.243 when using all five of the predictors simultaneously. The adjusted R^2 value is -0.039, indicating that the combination of IL-8, IL-1β, IL-6, TNF-α, and CRP predicts 3.9% of the variance in PWV; however, as the R^2 value is a negative value, this suggests that the model is a very poor predictor of PWV [166]. Table 10 shows the variables that significantly contribute to the overall multiple regression equation for predicting PWV. None of the systemic inflammatory markers – IL-8, IL-1β, IL-6, TNF-α, and

CRP – are significantly contributing to the overall multiple regression equation for predicting PWV (all $p > 0.05$).

We also created an interaction term for COVID-19 history and CRP levels to determine whether the relationship between COVID-19 history and arterial stiffness is significantly influenced by CRP levels. Overall, the model predicted 1.6% ($R^2 = 0.016$) of the variance, and was not a significant predictor ($p = 0.670$), of PWV. Additionally, multiple linear regression showed no significant impact of our interaction term (*CovidCRP*, $p = 0.997$), suggesting that CRP levels do not make a significant difference in the interaction between COVID-19 history and PWV.

Physical Activity as a Mediator for COVID-19 History with Arterial Stiffness and Systemic Inflammation

The findings from mediational analysis are presented in Table 11 and Table 12. Mediational analysis did not show a significant indirect effect of COVID-19 history through MVPA on PWV (estimate = 0.0220, 95% CI = -0.0488 – 0.2427). These results suggest that MVPA does not statistically mediate the relationship between COVID-19 history and PWV. Furthermore, mediational analysis did not show a significant indirect effect of COVID-19 history through MVPA on CRP levels (estimate = 0.0254, 95% CI = -0.0675 – 0.1646), suggesting that MVPA does not statistically mediate the relationship between COVID-19 history and CRP levels.

Associations for Arterial Stiffness, Systemic Inflammation and Physical Activity with COVID-19 History

The results for the associations of arterial stiffness, systemic inflammation and physical activity levels with COVID-19 history are presented in Table 12. PWV was not significantly correlated

with COVID-19 disease severity, the number of COVID-19 symptoms, or days since testing positive for SARS-CoV-2 ($p > 0.05$. Additionally, no markers of systemic inflammation – IL-8, IL-1β, IL-6, TNF-α, and CRP – were significantly associated with COVID-19 disease severity, number of COVID-19 symptoms, or days since testing positive for SARS-CoV-2 (all $p > 0.05$). Finally, MVPA showed weak and non-significant associations with COVID-19 disease severity, number of COVID-19 symptoms, and days since testing positive for SARS-CoV-2 ($p > 0.05$).

Chapter 5: Discussion

This study compared an ambulatory measure of arterial stiffness, as a proxy of CVD risk, and markers of systemic inflammation between individuals recovering from COVID-19 to controls without a history of COVID-19. This study also explored how well a combination of systemic salivary inflammatory markers, proinflammatory cytokines and CRP, predicts arterial stiffness. Finally, physical activity was explored as a protector against CVD risk by examining whether MVPA mediates the relationship between COVID-19 history and arterial stiffness. This study hypothesized that 1) persons with a history of SARS-CoV-2 infection would have increased ambulatory arterial stiffness measures and systemic inflammatory markers compared to controls; 2) proinflammatory cytokines and CRP would be significantly and positively predictive of arterial stiffness; and 3) MVPA would be significantly mediate the relationship between COVID-19 history and PWV. The main findings of this study were 1) there were no differences in ambulatory measures of arterial stiffness, salivary cytokine levels, or salivary CRP levels between young adults who experienced mild COVID-19 illness and uninfected controls; 2) the combination of proinflammatory cytokine and CRP did not significantly predict PWV; and 3) MVPA does not statistically mediate the relationship between COVID-19 history and PWV. Taken together, these findings suggest that 1) young adults with a history of mild COVID-19 illness may not have increased ambulatory arterial stiffness measures and systemic inflammation compared to controls three-to-four months after infection, 2) systemic inflammation may not be predictive of PWV, and 3) MVPA may not be a mediator for the relationship between COVID-19 history and CVD risk in young adults.

Cardiovascular Disease Risk

This study did not find significant differences in ambulatory measures of arterial stiffness between individuals recovering from COVID-19 and uninfected controls. These findings suggest that young adults who experienced mild COVID-19 illness may not have an accelerated risk for CVD three-to-four months after infection, according to our measure of CVD risk. Interestingly, these results are in contrast to initial investigations from Ratchford et al. [23] and Szeghy et al. [24] that both examined vascular function in young adults three-to-four weeks after SARS-CoV-2 infection. Ratchford et al. found significantly increased cfPWV in young adults who had SARS-CoV-2 infection compared to a control group [23]. Despite this, it was noted that the cfPWV measures were still within the expected ranges and the observed difference was only 0.75 m/s, which may not have clinical relevance [23]. Furthermore, Szeghy et al. found lower carotid distensibility, carotid compliance, and higher aortic AIx in the SARS-CoV-2 group compared to a control group, suggesting that carotid and aortic stiffness is increased in young adults with previous SARS-CoV-2 infection [24]. In contrast to these studies, our study did not find significant differences in arterial stiffness measures, as studied through ABPM with simultaneous PWA, between the SARS-CoV-2 and control groups. These findings suggest that young adults with a history of mild COVID-19 illness may not have an accelerated risk for CVD three-to-four months after infection.

The different results between this study and the investigations from Ratchford et al. and Szeghy et al. may be explained through differences in study design and methodology. First, the individuals recovering from COVID-19 in the studies from Ratchford et al. and Szeghy et al. were studied three-to-four weeks after testing positive for SARS-CoV-2 infection [23, 24], while individuals recovering from COVID-19 in this investigation were studied three-to-four months after their positive infection date. As such, the findings from this study may be more reflective of

an increased time for vascular recovery. However, it is important to consider that our investigation did not find a relationship between PWV and the number of days since testing positive for SARS-CoV-2, suggesting that these differential results may be due to other factors.

Second, data collection for our SARS-CoV-2 and control groups were conducted concurrently, while control groups in the investigations from Ratchford et al. and Szeghy et al. were studied prior to the COVID-19 pandemic [23, 24]. Data collection on control groups prior to the COVID-19 pandemic threatens internal validity through differing history effects. Social distancing guidelines during the COVID-19 pandemic present outside influences on study outcomes that are different between groups studied before and after the pandemic. The pandemic itself may influence cardiovascular function through increased sedentary behavior [167], changes in sleep quality [168], and worsened mental health outcomes [169]. These are important confounders to cardiovascular function that were not controlled for in the investigations by Ratchford et al. and Szeghy et al. [23, 24]. That being said, our SARS-CoV-2 and control groups did not significantly differ in sleep quality, mental health symptomology, or physical activity and sedentary behavior, which may have contributed to the similar results between the two groups.

Finally, the different results in our investigation may be explained by the devices used to measure PWV, our proxy of CVD risk. Our study utilized the oscillometric Mobil-O-Graph device for estimating PWV over a 24-hour period, while investigations from Ratchford et al. and Szeghy et al. utilized gold standard cfPWV methods [23, 24]. Gold standard cfPWV methods may be more adept for detecting signs of early vascular aging from diseased patients with similar age and blood pressures than the Mobil-O-Graph device [170]. This is because the Mobil-O-Graph device derives PWV from an ARCSolver algorithm that incorporates age and SBP [170, 171]. In fact, the variance in Mobil-O-Graph PWV is almost totally explained by age and SBP

[171]. Given that our SARS-CoV-2 and control groups were matched for age and did not significantly differ in SBP readings, this may explain why we did not see any differences in PWV between groups.

It is also worth noting that this study did not find any significant differences in blood pressure between the SARS-CoV-2 and control groups, with both groups being normotensive. The only other study to examine 24-hour blood pressure in COVID-19 was a case study of a 55-year obese male who had experienced severe COVID-19 disease [172]. This individual was hospitalized and admitted to the intensive care unit after experiencing severe multiorgan failure and hypoxemia [172]. After spending 40 days in the hospital, he was admitted to a rehabilitation center where, after showing persistent hypertension and tachycardia from in-clinic measures, he underwent ABPM [172]. ABPM revealed out-of-clinic elevated ambulatory BP measures, suggesting that, in this individual, severe COVID-19 illness may have led to worsened ABPM measures [172]. In contrast to the case study, our study was completed in mostly young, normal weight adults who experienced mild-to-moderate COVID-19 illness. These findings suggest that younger individuals with a milder course of disease may not be affected by adverse ABPM measures after SARS-CoV-2 infection.

Systemic Inflammation

This study did not find differences in proinflammatory cytokine or CRP levels between individuals recovering from COVID-19 and uninfected controls. These findings suggest that young adults who experienced mild COVID-19 illness may not have higher systemic inflammation compared to young adults without a history of COVID-19. In considering these findings, the lack of differences in systemic inflammatory levels between our two groups may be explained by the mild course of disease our SARS-CoV-2 group experienced for recovery.

Although initial studies characterizing the inflammatory response to COVID-19 have shown increased CRP and cytokine levels in those infected with COVID-19, most of these studies were conducted on hospitalized patients during more severe cases of COVID-19 illness [11, 56-64]. In contrast, participants in our SARS-CoV-2 group generally had mild COVID-19 illness, with cases that ranged from asymptomatic to moderate, which may explain why we did not observe higher systemic inflammatory levels in our individuals recovering from COVID-19. Milder courses of illness are absent of the hyperinflammatory phase that characterizes more severe disease, where there is a "cytokine storm" and a marked increase in CRP levels [173]. None of our SARS-CoV-2 infected participants experienced severe disease, with most experiencing mild illness, likely meaning they did not reach the hyperinflammatory phase of COVID-19 disease [173]. As our participants likely did not experience this hyperinflammatory state, this may explain why we did not see a difference in systemic inflammatory levels between our two groups.

Additionally, the lack of differences in systemic inflammation between our individuals recovering from COVID-19 and uninfected controls may be explained by the time course for recovery. Systemic inflammatory markers may peak when viral load is at its highest before normalizing into recovery once viral load has subsided [174, 175]. Initial evidence has shown that proinflammatory cytokine levels are at their highest during peak viral load before returning to normal during recovery [174, 175]. This may be exemplified with IL-8, as even moderate and severe COVID-19 patients have shown low serum IL-8 levels into recovery, suggesting that it may return to normal early into recovery [174]. In addition, when examining IL-6 levels after COVID-19 illness, no differences were observed for IL-6 levels in recovered COVID-19 patients and uninfected controls [174]. These findings are supported by initial investigations in CRP

levels during recovery, with COVID-19 patients who were younger and had a milder course of disease having serum CRP levels that return to normal values after a two-to-three week follow up [176]. More research is needed to characterize systemic inflammatory levels during COVID-19 recovery. Taken together, our findings suggest that young adults recovering from mild COVID-19 illness may not differ in systemic inflammatory levels compared to uninfected controls.

Systemic Inflammation and CVD Risk

To explore potential interactions between systemic inflammation and CVD risk, this study explored proinflammatory cytokine and CRP levels as predictors of arterial stiffness. We did not find the combination of IL-8, IL-1β, IL-6, TNF-α, and CRP to be predictive of PWV, with individual systemic inflammatory markers also not being found to significantly contribute to the predictive model. These findings should be contextualized by the method this study used to measure PWV. As previously mentioned, this study utilized an oscillometric method for estimating PWV using the Mobil-O-Graph device. To estimate PWV, the Mobil-O-Graph device uses an ARCSolver algorithm that uses age and SBP [170, 171]. Consequently, age and SBP almost totally account for the variance in oscillometric estimations of PWV [171]. Thus, we may not have seen our measures of systemic inflammation to be predictors of PWV, because the variance in our PWV estimates was likely due to age and SBP. Although previous studies examining interactions between systemic inflammation and arterial stiffness have found systemic inflammatory markers, especially CRP, to be significant and independent predictors of arterial stiffness, these studies utilized gold standard methods for measuring PWV [8, 177]. Future studies should utilize gold standard methods for measuring PWV to better understand interactions between systemic inflammation and arterial stiffness in the context of COVID-19.

Physical Activity

The protective effects of PA for reducing CVD risk in persons with a history of SARS-CoV-2 infection were examined by exploring MVPA as a mediator for the relationship between COVID-19 history and arterial stiffness. This study did not find a significant indirect effect of COVID-19 history through MVPA on PWV, suggesting that PA levels may not mediate the relationship between COVID-19 history and arterial stiffness. Cross-sectional studies examining the relationship between MVPA and arterial stiffness have observed that individuals who engage in more MVPA have lower arterial stiffness [27, 28, 146], and longitudinal studies have observed that regular MVPA is effective in slowing the progression of arterial stiffness [143-145]. However, studies examining these relationships used gold standard measures for assessing cfPWV [27, 28, 138-141, 181], while this study utilized an oscillometric method for estimating PWV. As differences in oscillometric measured PWV are more related to age and SBP [171], the device may not be as sensitive to differences in PWV with increased engagement in MVPA.

Additionally, this study explored MVPA as a mediator for the relationship between COVID-19 history and CRP levels. This study did not find a significant indirect effect of COVID-19 history through MVPA on CRP levels, suggesting that PA levels may not mediate the relationship between COVID-19 history and systemic inflammation. Although inverse associations have been noted for physical activity levels with CRP [25, 132-134], whether these associations are directly related to higher physical activity levels remains to be determined [178]. Studies examining the effect of increasing physical activity levels on systemic inflammation have also reported improvements in body composition [178]. Body fat percentage seems to be an important factor in low-grade systemic inflammation, meaning that physical activity related improvements in CRP levels may be dependent upon changes in body fat [178]. As our

participants generally had normal BMI values and healthy body fat percentages [179], this may explain why we did not find MVPA to mediate the relationship between COVID-19 history and CRP levels. Future research should examine the impact of increasing MVPA levels in obese individuals recovering from COVID-19 to better understand the impact of PA on the relationship between COVID-19 history and systemic inflammation.

Findings from this study should also be contextualized based on the PA levels of our participants. In our cohort, 82% of the participants met the recommended physical activity guidelines based on the 2nd edition of the Physical Activity Guidelines for Americans [180], with, on average, our participants engaging in more than 35 minutes of MVPA per day, placing them well above the physical activity guidelines of 150 minutes of MVPA per week [180]. The 150 minutes of recommended MVPA per week is considered the level of PA shown to provide substantial health benefits [180], with MVPA levels beyond that amount not necessarily providing additional health benefits [181]. Thus, our non-findings may have been representative of our participants already engaging in PA levels above the recommended guidelines and, as a whole, already experiencing the protective benefits of PA levels above the recommended guidelines. Future studies should longitudinally examine the efficacy for PA interventions to improve arterial stiffness and systemic inflammation, using gold standard methods for measuring PWV, in inactive and obese individuals recovering from COVID-19.

COVID-19 Disease Severity

Although not a primary aim of this study, we also examined associations for arterial stiffness, systemic inflammation, and PA levels with aspects of COVID-19 history. This study did not find any significant associations for PWV, proinflammatory cytokine and CRP levels, or MVPA with COVID-19 disease severity, the number of COVID-19 symptoms, or days since testing positive

for SARS-CoV-2 infection. Overall, these findings may be explained by the mild disease severity of our participants. Disease severities in our cohort ranged from asymptomatic to moderate, while studies examining associations for arterial stiffness and systemic inflammatory levels with COVID-19 disease severity have mostly been in hospitalized patients with more severe courses of COVID-19 illness [18, 19, 174, 176]. Inclusion of more severe COVID-19 cases may lead to stronger associations for arterial stiffness and systemic inflammation with COVID-19 disease severity, as the hyperinflammatory state associated with more severe cases of COVID-19 may lead to large increases systemic inflammation that may then lead to arterial stiffening [173]. In turn, the inclusion of these more severe cases may lead to stronger associations for systemic inflammation and arterial stiffness with COVID-19 history. Furthermore, our participants were very active, with our SARS-CoV-2 and control groups averaging MVPA levels that are well above recommended PA guidelines [180]. PA levels above recommended guidelines may have had a protective effect against more severe COVID-19 illness [182], and it may be prudent to see if stronger associations for PA with COVID-19 history are observed in physically inactive individuals.

Limitations and Future Directions

Although this study provides novel insight into the effect of SARS-CoV-2 infection on cardiovascular function, it is not without limitations. First, this study utilized cross-sectional and correlational analysis, so causation cannot be implied from these findings. Furthermore, participants who tested positive for SARS-CoV-2 infection were asked to self-report their COVID-19 symptomology and, given the difference in time between SARS-CoV-2 infection and participation in this study, this study may be subject to the limitations of recall bias.

Another limitation of this study is the usage of an estimated measure for PWV. Although the Mobil-O-Graph device has been validated gold standard invasive and non-invasive measures of PWV, the derivation of PWV is dependent on age and SBP, which limits its ability to accurately estimate arterial stiffness [170, 171]. That being said, Mobil-O-Graph PWV may still be useful for CVD risk stratification, as ambulatory PWV may add prognostic value in assessing CVD risk. 24-hour PWV is an independent predictor of cardiovascular morbidity and mortality and is associated with different indices of arterial damage [151]. As such, despite being an estimated measure of PWV, ambulatory PWV may still be useful in assessing CVD risk stratification in individuals recovering from COVID-19.

In addition, our study population is predominantly White, as such, the findings from this study may not be generalizable to other racial and ethnic groups. Reports from the CDC show that other racial and ethnic minority groups have been disproportionately affected by the COVID-19 pandemic, with these minority groups having increased rates of hospitalization for SARS-CoV-2 infection compared to their White counterparts [183]. These populations already carry an excessive cardiovascular burden [184-187] that will likely be magnified with the disproportionate affect the COVID-19 pandemic has had on these racial/ethnic groups [183, 188]. COVID-19 disease severity is acutely associated with an increased risk for CVD events [189], and considering that racial and ethnic minority groups are more likely to experience severe COVID-19 illness [183], this suggests that future CVD events may disproportionately increase in these populations in the future. Thus, future studies should examine the impact of SARS-CoV-2 infection in American Indians, Black or African Americans, and Hispanic or Latino Persons to better understand how the COVID-19 pandemic may affect the future cardiovascular burden of these racial/ethnic groups.

We recognize that SARS-CoV-2 antibody tests may not be the most efficacious in identifying persons with previous SARS-CoV-2 infection. More than half of persons with SARS-CoV-2 infection will lose their antibodies within 2 months of being infected, with even greater incidences among asymptomatic cases [190]. That being said, all but two of the participants in the control group were on-campus students and faculty at Syracuse University. On-campus students and faculty at Syracuse University underwent required testing for SARS-CoV-2 infection biweekly during the Fall 2020 semester and weekly during the Spring 2021 semester. Given the frequent viral testing of this population, this strengthens our control group as it allows us to limit the number of potential unidentified asymptomatic cases and be reasonably confident that the control group does actually consist of persons who have never been infected with SARS-CoV-2.

Finally, most of our participants in this study were enrolled prior to the discovery of the new SARS-CoV-2 delta variant. The delta variant has been reported to be increasingly infecting young adults and eliciting more severe cases of COVID-19 [191, 192]. Additionally, the delta variant may have an increased resistance to SARS-CoV-2 antibodies, meaning that vaccines may be less effective against this specific COVID-19 variant [193]. Consequently, future research is needed to determine CVD risk in individuals infected with the delta variant.

Conclusion

This study provides novel insight into the effect of SARS-CoV-2 infection on arterial stiffness and systemic inflammation in young adults. This study found no differences in ambulatory measures of arterial stiffness between individuals recovering from COVID-19 illness and uninfected controls, suggesting that young adults who experienced mild COVID-19 illness may not be at an accelerated risk for CVD three-to-four months after infection, according to our

ambulatory measures. In addition, this study found no differences in salivary cytokine and CRP levels in individuals recovering from COVID-19 compared to uninfected controls, with the combination of these systemic inflammatory markers also not being significant predictors of arterial stiffness. These findings suggest that young adults recovering from mild COVID-19 may not have higher systemic inflammation than those without a history COVID-19, and that systemic inflammatory levels may not be predictive of CVD risk. Finally, this study examined PA as a cardioprotective lifestyle factor for individuals recovering COVID-19 by examining MVPA as a mediator for the relationship between COVID-19 history and arterial stiffness. MVPA was not found to significantly mediate the relationship between COVID-19 history and arterial stiffness. Future studies should longitudinally examine PA levels in individuals recovering from COVID-19 to better understand the utility of PA as a cardioprotective lifestyle factors for individuals recovering from COVID-19.

Illustrative Materials

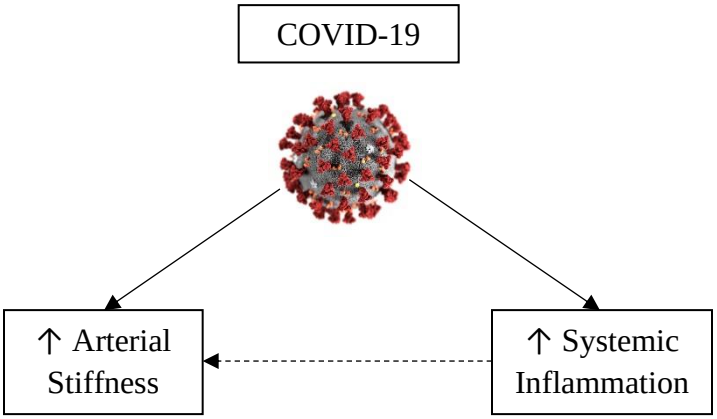
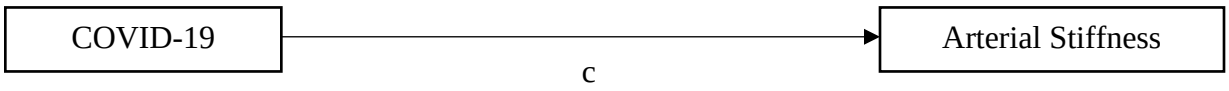


Figure 1. Theoretical model.

Unmediated Model



Mediation Hypothesis

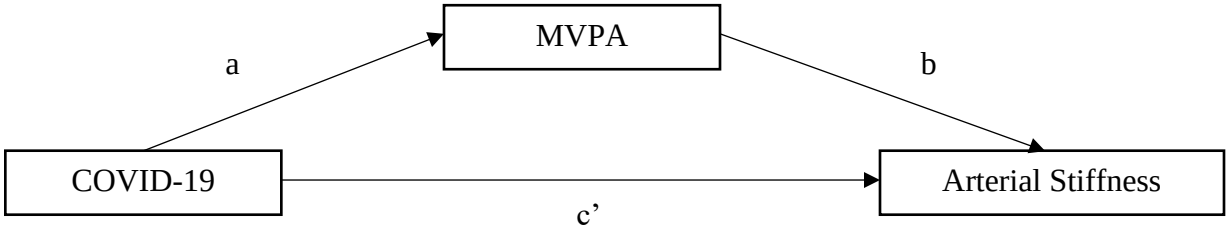
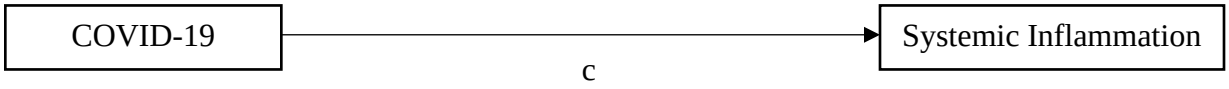


Figure 2. Mediation model for the indirect effect of COVID-19 history through MVPA on arterial stiffness.

Unmediated Model



Mediation Hypothesis

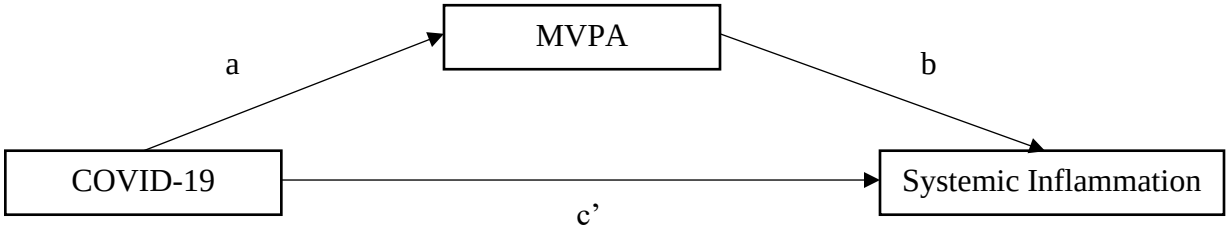


Figure 3. Mediation model for the indirect effect of COVID-19 history through MVPA on systemic inflammation.

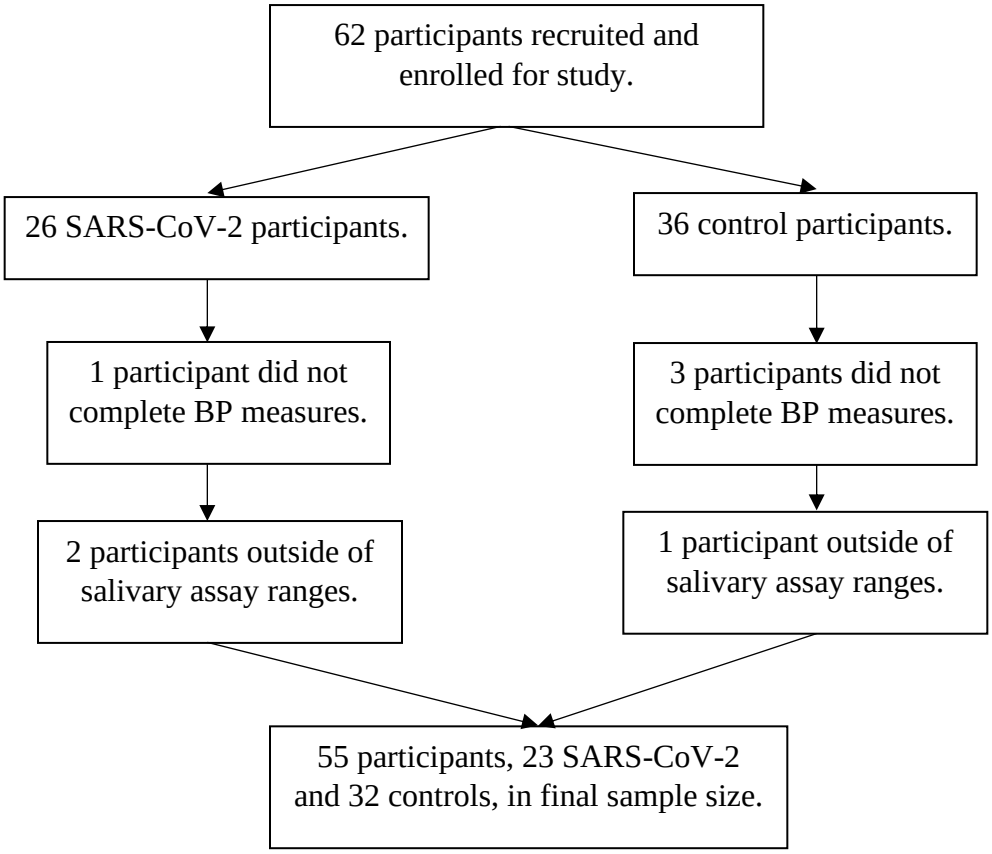


Figure 4. Flow-chart of enrollment and participants throughout study for final sample size.

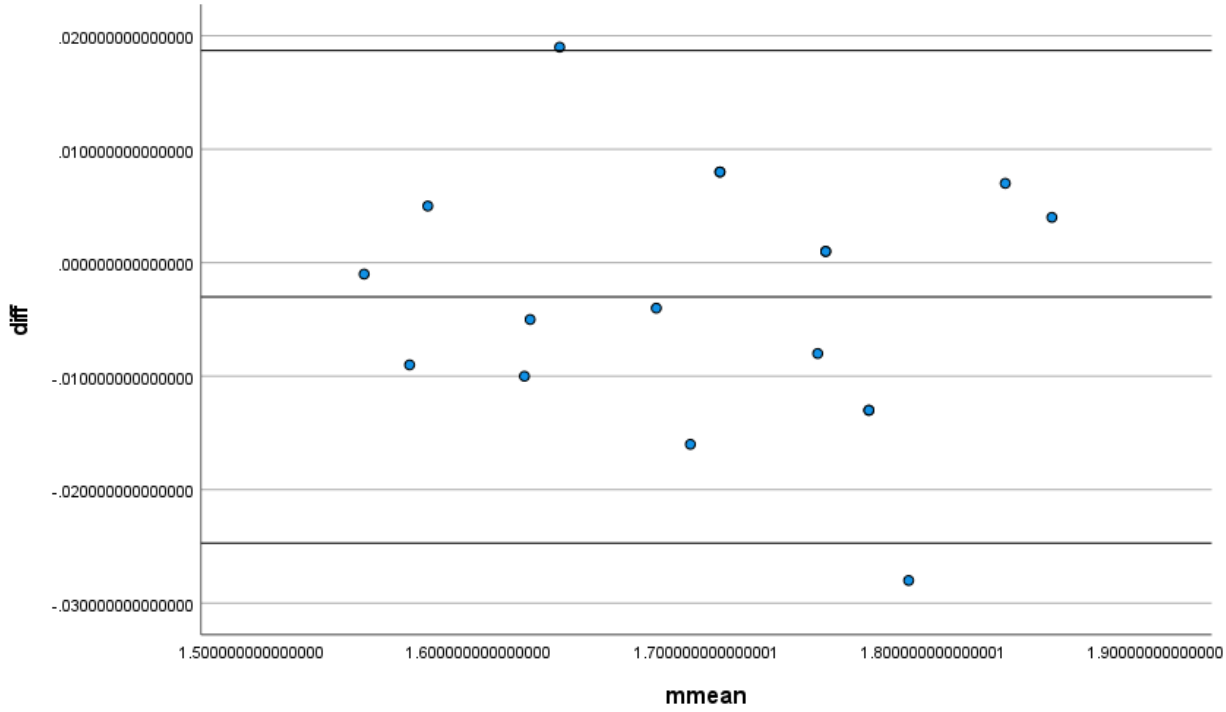


Figure 5. Bland-Altman plot for inter-rater agreement analysis between stadiometer measured (criterion) height and self-reported height. The middle line represents the mean difference of measured and self-reported height, while the upper and lower lines represents the upper and low limits of agreement, respectively.

Table 1.
Clinical characteristics of cytokine levels in COVID-19 Patients.

Study	Participants (N)	Age (years)	Cytokines measured	Results
Chen et al., 2020	21	56	IL-1β, IL-2R, IL-6, IL-8, IL-10 and TNF-α	Significant increases were found for IL-6, IL-10 and TNF-α.
Huang et al., 2020	41	49	IL-1β, IL-1RA, IL-2, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IFN-γ, TNF-α and VEGF	Plasma levels of IL-1β, IL-1RA, IL-6, IL-7, IL-8, IL-9, IL-10. IFN-γ, TNF-α and VEGF were higher in COVID-19 patients than healthy adults.
Wang et al., 2020	65	57	IL-1β, IL-2R, IL-6, IL-8, IL-10, and TNF-α	IL-2R, IL-6 and IL-10 increased with severity of COVID-19 illness. IL-6 and IL-10 were significantly higher in extremely severe patients than mild patients.
Xiong et al., 2020	6 (3 COVID-19 patients, 3 controls)	Not reported	IL-33, IL-18, IL-10, IL-6	Pro-inflammatory cytokines induced by SARS-CoV-2 infection included IL33 in BALF and IL18, IL10 in PBMC. Additionally, IL-6 was increased in 2 of 3 COVID-19 patients.
Wu et al., 2020	71	61	IL-2, IL-4, IL-6, IL-10, IFN-γ and TNF-α	IL-6 was elevated in all COVID-19 patients, but especially in those with severe illness. IL-10 was increased in severe cases, while IL-2 and IL-4 had nonsignificant increases.
Tian et al., 2020	751	64	IL-1β, IL-2R, IL-6, IL-8, IL-10 and TNF-α	IL-6 and TNF-α were identified as risk factors for COVID-19 disease severity. IL-6, TNF-α and IL-2R were also higher in patients with cancer than those without.
Qin et al., 2020	453	58	IL-1β, IL-2R, IL-6, IL-8, IL-10, IFN-γ and TNF-α	COVID-19 patients had severely elevated IL-2R, IL-6 and TNF-α levels. IL-2R, IL-6, IL-8, IL-10 and TNF-α were all elevated in severe versus non-severe cases.
Liu et al., 2020	40	48	IL-2, IL-4, IL-6, IL-10, IFN-γ and TNF-α	IL-2, IL-6, IL-10 and IFN-γ were significantly increased in severe versus mild COVID-19 cases.
Diao et al., 2020	562	Not reported	IL-2, IL-4, IL-6, IL-10, IFN-γ and TNF-α	Only IL-6, IL-10 and TNF-α were significantly increased in infected patients versus healthy controls.
Gao et al., 2020	43	43	IL-6	IL-6 levels were higher in those with severe COVID-19 versus mild. IL-6 also had high specificity and sensitivity for predicting COVID-19 disease severity.

Abbreviations: IL = interleukin; IFN = interferon; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor

Table 2.
Myocardial injury in COVID-19 patients.

Study	Participants (N)	Age (years)	Measure of myocardial injury	Results
Zhou et al., 2020	181	56	hs-cTnT > 99th percentile upper reference limit	17% of patients exhibited acute myocardial injury
Chen et al., 2020	274	62	hs-cTnT > 99th percentile upper reference limit	72% of deceased patients and 14% of recovered patients had increased hs-cTnT levels.
Shi et al., 2020	416	64	hs-TNI > 99th percentile upper reference limit	19.7% of patients exhibited cardiac injury.
Wang et al., 2020	138	56	hs-TNI > 99th percentile upper reference limit	7.2% of patients exhibited acute cardiac injury.
Huang et al., 2020	41	49	hs-TNI > 99th percentile upper reference limit	12% of patients exhibited acute cardiac injury.
Guo et al., 2020	187	58	TnT > 99th percentile upper reference limit	27.8% of patients developed myocardial injury, with this being exacerbated among patients who also had underlying CVD.

Abbreviations: hs-cTnT = high-sensitivity cardiac troponin T; hs-TNI = high-sensitivity troponin I; TNT = serum levels of troponin T; CVD = cardiovascular disease

Table 3.
Association between arterial stiffness and cardiovascular disease risk.

Design	Study	Participants (N)	Age (years)	Arterial stiffness measure	Results
CS					
	Sutton-Tyrrell et al., 2005	2,488	74	cf-PWV	Greater aortic PWV was associated with higher CV mortality, CHD and stroke in generally healthy, community-dwelling older adults.
	Blacher et al., 1999	710	67	cf-PWV	Greater aortic PWV was strongly associated with atherosclerosis and is a predictor of CV risk in hypertensive patients.
	Lee et al., 2014	76	62	ba-PWV	Greater arterial stiffness was associated with functional outcome of acute ischemic stroke.
Long.					
	Mitchell et al., 2010	2,232	63	cf-PWV	Greater aortic PWV was associated with increased risk for a first CV event in community-dwelling older adults during a 7.8 year follow-up.
	Mattace-Ruso et al., 2006	2,835	≥ 55	cf-PWV	Greater aortic PWV was independently associated with risk for CHD and stroke.
	Wang et al., 2010	1,272	52	cf-PWV, AIx, Pa and Pb	Greater aortic PWV, Pa and Pb predicted CV mortality in men and women, while AIx was predictive in only men.

Abbreviations: CS = cross-sectional; Long. = longitudinal; CV = cardiovascular; CHD = coronary heart disease; cf-PWV = carotid-femoral pulse wave velocity; ba-PWV – brachial-ankle pulse wave velocity; AIx = augmentation index; Pa = augmented pressure; Pb = reflected wave amplitude

Table 4.
Association between arterial stiffness and measures of systemic inflammation.

Study	Participants (N)	Age (years)	Inflammatory Measure	Arterial Stiffness Measure	Results
Tuttolomondo et al., 2015	108	N/A	Cytokines: IL-6, IL-1β, and TNF-α.	cf-PWV	Greater aortic PWV was associated with greater plasma levels of IL-6, IL-1β, and TNF-α in patients with SpA.
Mahmud et al., 2005	78	47	Cytokines: IL-6 and TNF-α. CRP: hs-CRP	cf-PWV AIx	Greater aortic PWV and AIx were associated with increased levels of plasma CRP, IL-6 and TNF-α in patients with hypertension.
Arnold et al., 2017	13,724	54	Cytokines: IL-1RA, IL-18 CRP	SI	Greater SI was associated with increased IL-1RA
Johansen et al., 2012	3,769	48	Cytokines: IL-1RA and IL-6 CRP: hs-CRP	cf-PWV	CRP, IL-6 and IL-1RA were found to predict aortic stiffness in both sexes in a 16 year follow-up.
McEniery et al., 2010	825	74	CRP	cf-PWV	CRP was an independent baseline predictor of aortic PWV in a 20 year follow-up.
Vlachopoulos et al., 2005	100	N/A	Cytokines: IL-6 CRP: hs-CRP	cf-PWV	Greater aortic PWV was associated with increases in hs-CRP and IL-6, suggesting acute systemic inflammation causes an increase in aortic stiffness.
Jae et al., 2013	40	N/A	Cytokines: IL-6 CRP	cf-PWV	Greater aortic PWV was associated with increases in CRP and IL-6, suggesting acute inflammation causes an increase in arterial stiffness

Abbreviations: IL = interleukin; IL-1RA = interleukin-1 receptor antagonist; TNF = tumor necrosis factor; hs-CRP – high-sensitivity C-reactive protein; cf-PWV = carotid-femoral pulse wave velocity; AIx = augmentation index; SI = stiffness index; SpA = seronegative spondyloarthritis

Table 5.
Association between physical activity and measures of inflammation.

Study	Participants (N)	Age (years)	Inflammatory measures	Physical activity measure	Results
Wiener et al., 2018	142	26	Cytokines: IL-6, IL-10 and TNF-α	Self-reported leisure-time PA (minutes/week)	Greater PA was associated with increased levels of IL-10 and decreased levels of TNF-α.
Pischon et al., 2003	859	Men: 60 Women: 42	Cytokines: sTNF-R1, sTNF-R2 and IL-6 CRP	Self-reported total PA (MET-hours/week)	Greater PA was inversely associated with plasma levels of sTNF-R1, sTNF-R2, IL-6 and CRP.
Parsons et al., 2017	1,629	78	Cytokines: IL-6 CRP	Accelerometer measured PA (activity counts per minute)	Greater total PA, MVPA and LPA were inversely associated with IL-6 and CRP in community-dwelling older men.
Loprinzi et al., 2013	2,912	48	CRP: hs-CRP	Accelerometer measured PA (activity counts per minute)	Objectively-measured MVPA is associated with lower CRP in adults.
Nicklas et al., 2016	167	66	Cytokines: IL-6 CRP: hs-CRP	Accelerometer measured PA (minutes/day)	Habitual PA was inversely associated with Il-6 and hs-CRP. During a PA intervention, larger increases in PA were associated with greater declines in IL-6 and hs-CRP.
Hamer et al., 2014	112	64	Cytokines: IL-6 and IL-1RA	Accelerometer measured PA (minutes/day)	MVPA was inversely associated with IL-6 and IL-1RA.

Abbreviations: PA = physical activity; MVPA – moderate-to-vigorous physical activity; LPA = light physical activity; IL = interleukin; IL-1RA = interleukin-1 receptor antagonist; TNF = tumor necrosis factor; sTNF-R = soluble tumor necrosis factor receptor; CRP = C-reactive protein; hs-CRP = high-sensitivity C-reactive protein

Table 6.
Association between physical activity and arterial stiffness.

Design	Study	N	Age (years)	Arterial stiffness measure	Physical activity measure	Results
CS						
	Gando et al., 2010	109	63	cf-PWV	Accelerometer measured PA (minutes/day)	More time spent in LPA was associated with lower aortic PWV in older subjects.
	Endes et al., 2016	2,605	63	ba-PWV	Self-reported PA (minutes/week)	Increased MVPA was associated with lower arterial stiffness in older adults.
	Horta et al., 2015	1,241	30	cf-PWV	Accelerometer measured PA (minutes/day)	Increased time spent in MVPA was associated with lower aortic PWV in young adults.
Long.						
	Ahmadi-Abhari et al., 2017	3,797	65	cf-PWV	Self-reported PA (hours/week)	Higher levels of MVPA are associated with a slower rate of aortic stiffening.
	Tanaka et al., 2018	1,747	75	cf-PWV cPP	Self-reported PA (minutes/week)	Higher MVPA in late-life, and habitual physical activity from mid- to late-life, is associated with lower aortic PWV and cPP in community-dwelling older adults.
	Aoyagi et al., 2010	198		cf-PWV, bt-PWV, cc-PWV, cb-PWV and ft-PWV	Accelerometer measured PA (min/day) Pedometer measured PA (steps/day)	Increased PA and step count were associated with lower bt-PWV and aortic PWV, while higher step count was also associated with lower cc-PWV in older adults.

Abbreviations: CS = cross-sectional; Long. = longitudinal; N = number of participants; PA = physical activity; MVPA = moderate-to-vigorous physical activity; LPA = light physical activity; cf=PWV = carotid-femoral pulse wave velocity; ba-PWV = brachial-ankle pulse wave velocity; cPP = central pulse pressure; bt-PWV = brachial-tibial pulse wave velocity; cc-PWV = cardio-carotid pulse wave velocity; cb-PWV = cardio-brachial pulse wave velocity; ft-PWV = femoral-tibial pulse wave velocity

Table 7.
Participant characteristics.

Characteristic	SARS-CoV-2 (*n* = 23)	Controls (*n* = 32)	p-value
Age (years)	25.0 ± 8.9	24.4 ± 6.5	0.792
Female sex (*n*, %)	15, 65.2	18, 56.3	0.503
Race (*n*, %)			0.858
White	19, 82.6	25, 78.1	
Latino	0, 0	1, 3.1	
Black	2, 8.7	3, 9.4	
Pacific Islander	2, 8.7	3, 9.4	
BMI (kg/m^2)	24.1 ± 3.5	25.1 ± 3.5	0.295
Body Fat (%)	22.1 ± 4.6	22.6 ± 4.4	0.645
SaO_2 (%)	97.9 ± 0.7	98.1 ± 0.7	0.299
Questionnaire Scores			
PSQI	6.0 ± 3.7	5.7 ± 2.8	0.781
GAD-7	4.7 ± 5.9	5.2 ± 4.7	0.779
CES-D	13.6 ± 13.5	9.7 ± 6.2	0.233
PSS-10	16.1 ± 7.1	15.0 ± 4.8	0.525
PCL-5	7.8 ± 10.8	10.1 ± 8.9	0.421
Oral Health	6.0 ± 1.5	6.0 ± 1.3	0.839
24-Hour Blood Pressure			
Systolic (mmHg)	118.0 ± 11.8	119.6 ± 10.8	0.606
Diastolic (mmHg)	71.1 ± 8.8	72.3 ± 8.4	0.615
MAP (mmHg)	92.6 ± 9.6	93.9 ± 8.8	0.607
Heart Rate (bpm)	65.5 ± 9.0	66.8 ± 8.5	0.581
Physical Activity	85.7%	80.0%	0.667
% PA Guidelines			
COVID-19 History			
Days Since Positive Test (days)	111.6 ± 118.3 Range: 15 – 484		
Number of Symptoms	5.2 ± 3.8		
COVID-19 Disease Severity (1 – 5)	2.1 ± 0.7 Range: 1 – 3		

Abbreviations: BMI = body mass index; SaO_2 = arterial oxygen saturation; PSQI = Pittsburgh sleep quality index score; GAD-7 = generalized anxiety disorder-7 score; CES-D = center for epidemiological studies depression scale score; PSS-10 = 10-item perceived stress scale score; PCL-5 = PTSD checklist for DSM-5 score; MAP = mean arterial pressure

Table 8.

Independent samples t-test results for measures of arterial stiffness, systemic inflammation, and physical activity between the SARS-CoV-2 and control groups.

Variable	SARS-CoV-2	Controls	*p*-value
24-Hour Pulse Wave Analysis			
PWV (m/s)	5.0 ± 0.5	5.1 ± 0.5	0.402
Systemic Inflammation			
IL-8 (pg/mL)	821.1 ± 772.6	843.8 ± 958.4	0.580
IL-1β (pg/mL)	126.3 ± 102.2	143.6 ± 157.9	0.824
IL-6 (pg/mL)	11.7 ± 25.4	5.9 ± 7.9	0.581
TNF-α (pg/mL)	4.8 ± 3.9	5.1 ± 5.9	0.443
CRP (pg/mL)	765.4 ± 672.9	526.3 ± 674.8	0.052
Physical Activity			
MVPA (min/day)	38.6 ± 13.6	35.7 ± 16.8	0.599

*$p < 0.05$

Abbreviations: PWV = pulse wave velocity; IL = interleukin; TNF = tumor necrosis factor; CRP = C-reactive protein; MVPA = moderate-to-vigorous physical activity

Table 9.
Model summary table for simultaneous multiple regression analysis of systemic inflammation as a predictor of arterial stiffness.

Model	*R*	Adjusted R^2	SE	F	*p*-value
1	0.243	-0.039	0.5195	0.602	0.699

Predictors: IL-8, IL-1β, IL-6, TNF-α, CRP
Dependent Variable: PWV

Table 10.
Coefficients table for simultaneous multiple regression analysis of systemic inflammation as a predictor of arterial stiffness.

Variable	Unstandardized β	SE	Standardized Coefficient β	t	*p*-value
IL-8	0.307	0.394	0.230	0.780	0.439
IL-1β	0.127	0.254	0.096	0.502	0.618
IL-6	0.203	0.221	0.176	0.919	0.363
TNF-α	-0.549	0.376	-0.402	-1.461	0.150
CRP	-0.107	0.158	-0.104	-0.679	0.501

Dependent Variable: PWV
Abbreviations: IL = interleukin; TNF = tumor necrosis factor; CRP = C-reactive protein; PWV = pulse wave velocity

Table 11.
Mediational analysis results for the indirect effect of COVID-19 history through MVPA on arterial stiffness.

Model Summary

Path	*R*	R^2	F	*p*-value
a	0.0935	0.0087	0.2825	0.5988
b and c’	0.2263	0.0512	0.8363	0.4429
c	0.1155	0.0133	0.4327	0.5154

Indirect effect of X on Y

Variable	Effect	Boot SE	95% Confidence Interval	
			LLCI	ULCI
MVPA	0.0220	0.0739	-0.0488	0.2427

Table 12.
Mediational analysis results for the indirect effect of COVID-19 history through MVPA on CRP levels.

Model Summary

Path	*R*	R^2	F	*p*-value
a	0.0954	0.0091	0.2848	0.5974
b and c’	0.3152	0.0993	1.6545	0.2082
c	0.1739	0.302	0.9665	0.3332

Indirect effect of X on Y

Variable	Effect	Boot SE	95% Confidence Interval	
			LLCI	ULCI
MVPA	0.0254	0.0569	-0.0675	0.1646

Table 13.
Relationships between arterial stiffness, systemic inflammation, and physical activity with COVID-19 history.

Variable	COVID-19 Disease Severity (Range: 1 – 5)	Number of Symptoms	Days Since Positive
24-Hour Pulse Wave Analysis			
PWV (m/s)	0.226	0.264	0.049
Systemic Inflammation			
logIL-8	-0.074	0.260	0.164
logIL-1β	0.287	0.399	0.059
logIL-6	0.271	0.287	0.133
logTNF-α	0.091	0.274	0.154
logCRP	0.139	-0.059	-0.173
Physical Activity			
MVPA (min/day)	-0.106	-0.003	0.064

*Correlation is significant at the 0.05 level
**Correlation is significant at the 0.01 level
Abbreviations: PWV = pulse wave velocity; IL = interleukin; TNF = tumor necrosis factor; CRP = C-reactive protein; MVPA = moderate-to-vigorous physical activity

Appendix 1

Supplemental Table

Independent samples t-test results for measures of arterial stiffness, systemic inflammation, and physical activity between the post-acute and post-long COVID-19 groups

Variable	Post-acute[1] (n = 11)	Post-long[2] (n =11)	*p*-value
24-Hour Pulse Wave Analysis			
PWV (m/s)	4.8 ± 0.5	5.1 ± 0.5	0.167
Systemic Inflammation			
IL-8 (pg/mL)	756.9 ± 635.7	924.6 ± 934.9	0.628
IL-1β (pg/mL)	118.1 ± 87.5	137.9 ± 122.3	0.668
IL-6 (pg/mL)	3.9 ± 2.6	10.8 ± 21.3	0.301
TNF-α (pg/mL)	3.9 ± 2.1	5.9 ± 5.1	0.234
CRP (pg/mL)	866.0 ± 834.4	699.4 ± 519.1	0.580
Physical Activity			
MVPA (min/day)	41.7 ± 15.3	35.4 ± 11.9	0.407

[1]post-acute = <84.5 days since positive test date, mean: 31.6 ± 17.3 days.

[2]post-long = >84.5 days since positive test date, mean: 191.7 ± 122.3 days

*$p < 0.05$

Abbreviations: PWV = pulse wave velocity; IL = interleukin; TNF = tumor necrosis factor; CRP = C-reactive protein; MVPA = moderate-to-vigorous physical activity

Appendix 2

Protocol Title: Cardiorespiratory Function in Young Adults with a History of COVID-19

Principal Investigator/Key Research Personnel:

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Introduction:
The purpose of this form is to provide you with information about participation in a research study and offer you the opportunity to decide whether or not you wish to participate. You can take as much time as you wish to decide and can ask any questions you may have now, during, or after the research is complete by contacting the research team: Kevin Heffernan (ksheffer@syr.edu), Andrew Heckel (arheckel@syr.edu), Danielle Arcidiacono (dmarcidi@syr.edu), or Jacob DeBlois (jpdebloi@syr.edu). Your participation is voluntary.

Purpose:

- The defense of the body against COVID-19 is characterized by an inflammatory response that may lead to reduced cardiovascular and respiratory functioning. How long possible reductions in cardiovascular and respiratory function extend into recovery, and whether individuals with asymptomatic or mild cases have reduced function, remains unknown.
- We want to explore how asymptomatic/mild cases of COVID-19 in young adults affects cardiovascular function, lung function and inflammation (the body's response to infection) compared to young adults without a history of COVID-19.
- You may participate in this research study if you are 18-35 years of age; do not have a pacemaker; are currently willing and able to participate in video calls via Zoom with the researchers of this study; have tested positive for COVID-19 (viral or antibody test) or negative (viral test in the past two weeks) for COVID-19, but are not currently infected. If you have previously tested positive for COVID-19, you must be willing to provide documentation of a negative viral test to show that you are no longer infected with COVID-19 and be allowed to participate in this study.

- In addition, you must not have experienced any COVID-19 symptoms, have been in close contact with someone who has COVID-19, or have traveled out of state in the past two weeks to be able to participate in this study.

You will be asked to…

- Read this document with the investigator and provide oral consent to participate in the study. While reading this document with the investigator, the investigator will go over the key points of the document and answer any questions you may have.
- Schedule 2 visits to the Human Performance Laboratory (Women's Building Rm 306, 820 Comstock Avenue, Syracuse, NY 13202) on the Syracuse University campus and 1 Zoom meeting (which will be done remotely) for the collection of study measurements.

Visit 1

- In light of COVID-19, we are conducting this research with limited face-to-face interactions. This means that you will not have direct contact with research personnel and the visit will be completed in a socially distanced manner (6 feet apart, physical barriers between participant and researcher). The remainder of the study interactions will be conducted remotely via Zoom. We will ask that you complete this visit following a 12 hour (overnight) fast (no food, sugary drinks and caffeinated or alcohol beverages).
- You will first be asked to complete a COVID-19 finger stick antibody test. For this, you will be asked to sit in a reclining chair while we clean your finger with an alcohol pad and obtain a drop of blood using a small, finger stick device. Due to the close nature of this test, you and the researcher will be separated via a plastic partition with a hole cut out in the bottom, where we will perform the test. The COVID-19 antibody test has been approved by the U.S. Food and Drug Administration and will allow us to determine whether you have previously had COVID-19. This measure will take approximately 30 seconds.
- After the antibody test, we will show you to the Human Performance Lab conference room. All of the equipment you need will be on the table. The equipment will already be programmed, ready to use, and have been disinfected. You will also be provided with gloves and disinfectant wipes so that you may disinfect the equipment again prior to use. We will also have a Zoom meeting already set up (on a laptop/computer), and we will communicate with you virtually via an adjoining room to explain how to use all of equipment and to collect some study measures. We will coach you through all of the equipment and measures step-by-step and we will ask that you report the measures from this equipment back to us via Zoom. The Zoom sessions will NOT be recorded.
- You will first be asked to measure your height using an automatic stadiometer. We will ask that you stand under the stadiometer and report the measurement back to us. This will take approximately 30 seconds. Then we will ask that you step on a digital scale with no

socks on (barefoot). This scale provides us with an estimation of the amount of fat on your body. The measurement takes approximately 30 seconds.

- You will be asked to wear a fingertip pulse oximeter (show the fingertip pulse oximeter in the Zoom meeting; place on finger to show how it is worn) that is placed over the tip of your finger to record your blood oxygen levels. This reading will take less than 30 seconds and we will ask that you report the measurement to us over Zoom.
- We will also ask that you complete a passive drool test using a saliva collection kit. The saliva collection kit will come with a saliva collection aid (i.e., straw) and a collection vial (show the saliva collection aid and cryovial in the Zoom meeting; show how it will be attached and demonstrate how it will be used). You will be asked to passively drool through the saliva collection aid and into the collection vial for approximately 3 minutes. Afterwards, we will ask that you place the vial into the provided Ziploc bag and dispose of the saliva collection aid. We will review the process together in detail over Zoom. We will measure markers of inflammation in the saliva (markers that provide insight into how well the body can fight infections). This measure will take approximately 3 minutes.
- We will also ask that you measure your chest circumference using a portable tape measure by wrapping the tape measure around your chest. We will ask that you report the value back to us via Zoom. This measure should take approximately 30 seconds and we will use this measure to help better fit you into a chest harness during the second inperson visit.
- Additionally, we will ask that you wear a portable (ambulatory blood pressure monitor for a full 24 hours (show the blood pressure monitor and cuff in the Zoom meeting; show roughly where the blood pressure cuff will be worn). This means that you will walk out of the lab wearing the blood pressure monitor. You may wear the blood pressure cuff on whichever arm you prefer. We will show you over Zoom how to properly wear the blood pressure cuff. The monitor will be programmed to take a blood pressure reading every 20 minutes between 7am and 10pm, and every 30 minutes between 10pm and 7am. That means that we will be asking you to wear the blood pressure monitor at night while you sleep as well. We will ask that you take on blood pressure measurement over the Zoom meeting. This measurement will take approximately 3 minutes.
- Exercise can have an impact on blood pressure for an extended period of time. Therefore, we will ask that you do not exercise for 24 hours while you are wearing the monitor.
- In addition, you'll be asked to wear an activity monitor around your waist for 9 days (show the activity monitor in the Zoom meeting; show roughly where the monitor will be worn over your clothing) You'll wear it for a full 24 hours each day, including at night while you sleep. You will only take it off when you shower or do any other water-based activity where it could get very wet. We will ask that you put the activity monitor on during the Zoom meeting. This will take less than 30 seconds.
- Finally, there will be a box of equipment on the table that we will ask that you take home with you. All of the equipment will be programmed, ready to use and disinfected. We will schedule a Zoom meeting to go over this equipment and collect some study measures.

Additionally, we will schedule a second visit to the Human Performance Lab so that you may return the equipment and complete the respiratory measures. We will schedule this visit for 24 hours after visit 1 (once you have completed wearing the blood pressure cuff for 24 hours).

Zoom Meeting

- For this Zoom meeting for the box of equipment, we will ask that you undergo a 3 hour fast (no food or sugary drinks) and avoid alcohol and caffeinated beverages for 12 hours before the meeting. We will also ask that you avoid exercising for the 24 hours prior to this meeting.
- You will first be asked to wear a heart rate monitor (show the heart rate monitor in the Zoom meeting; place in front of your chest roughly where the monitor is worn; show over your clothing so it is easier to see). The heart rate monitor is connected to two stickers that you will place under the right collarbone and on the left ribcage. As breathing changes heart rate, we will ask you to breathe at a rate of 12 breaths per minute, a rate that is common as most people breathe between 8 and 15 breaths per minute. We will play a metronome and instruct you on pacing your breathing. This measurement will take 15 minutes (10-minute rest, 5 minutes for the breathing exercise).
- While wearing the blood pressure monitor for 24 hours, we will ask that you complete a series of online questionnaires. These questionnaires consist of a medical history form, questionnaires about COVID-19 symptomology and disease severity, general health status, mental health, sleep quality, socioeconomic status, physical activity, and quality of life. The questionnaires should take about 45 minutes to complete.

Visit 2

- After wearing the blood pressure monitor for 24 hours, we will conduct the second inperson visit. We will once again ask that you undergo a 3 hour fast (no food or sugary drinks) and avoid alcohol and caffeinated beverages for 12 hours before the visit. We will also ask that you return all of the equipment in the box, except for the activity monitor.
- The visit will, again, be conducted in socially distanced manner. You will be in the Human Performance Lab conference room. All of the equipment you need will be on the table. The equipment will already be programmed, ready to use, and have been disinfected. You will also be provided with gloves and disinfectant wipes so that you may disinfect the equipment. We will also have a Zoom meeting already set up (on a laptop/computer), and we will communicate with you virtually via an adjoining room to explain how to use all of equipment and to collect some study measures. We will coach you through all of the equipment and measures step-by-step and we will ask that you report the measures from this equipment back to us via Zoom. Once again, the Zoom sessions will NOT be recorded.
- We will then ask for you to a complete two separate lung function tests. You will first be given a spirometer with a disposable mouthpiece, a filter and a nose clip (show the

spirometer in the Zoom meeting; show how to properly attach disposable mouthpiece and filter). The spirometer will already come attached with a viral filter (show filter in Zoom meeting) to prevent the spread of any airborne particles. We will show you how to attach the disposable mouthpiece and how to put on the nose clip. You will then breathe into the spirometer for 3-seconds so that we can assess your lung function. You will be asked to do this test 3 times. This measure will take approximately 7 minutes.

- Then, we will also ask that you breathe into a separate small, handheld device with a disposable mouthpiece (show the device and disposable mouthpiece in the Zoom meeting) that will provide a specific assessment of your lung inflammation. The disposable mouthpiece has a viral filter inside of it to help prevent the spread of any airborne particles. This is a standard measure used for children with asthma. Like the previous test, we will instruct you on how to attach the disposable mouthpiece. This measure will take approximately 2 minutes.
- In total, both lung function tests will take approximately 9 minutes.

Finally, you will be asked to wear a belt with sensors on the lower chest with a wire attached to a fingertip pulse oximeter (show the sensor belt and fingertip pulse oximeter device in the Zoom meeting; show roughly how to wear over clothing so that it will be easier to see). The sensor belt will be worn around the chest, while the wired fingertip pulse oximeter will be worn on the edge of your index finger. We will ask that you wear the device home with you, for 4 continuous hours, so that we can collect blood oxygen levels.

- After the 4 hours of wearing the chest belt and 9 days of wearing the activity monitor are completed, we will coordinate a time so that you may drop off the equipment to the lab, or so that we may pick up the activity monitor and the chest belt from you. If coming to the lab, we will ask that you drop off the equipment in the Human Performance Lab conference room. If we are picking up the equipment, we will ask that you leave the equipment on your doorstep and we will take it from there. All research personnel will be wearing gloves and a mask covering the nose and mouth when picking up any equipment.

There are some possible risks of participation in this research study.

- You should use caution while handling the disinfectant wipes. Like standard cleaning wipes (e.g. Clorox wipes) these wipes may cause moderate eye irritation and may be harmful if absorbed through the skin (long-term use). We are providing you with nitrile (not latex) gloves to wear while handling the wipes. Please be sure to use these gloves when you are wiping down the equipment with the provided wipes. If the wipes come into contact with your skin, rinse the skin with plenty of water for 15-20 minutes and call a local poison control center, as recommended by the manufacturer.
- Blood pressure recordings will be measured using an automated device over a 24-hour period. The compression associated with the cuff inflation may be uncomfortable but is very brief (30 seconds).

- When wearing the blood pressure monitor for 24 hours, the cuff will inflate every 30 minutes during the night. Inflation of the cuff may disrupt your sleep. This is a standard clinical and research technique that will only be done once.
- When wearing the heart rate monitor, the two electrode stickers that are attached may irritate or cause redness to the skin. The electrode sticker will only be worn for 15 minutes and may be removed if it is causing any irritation or redness.
- During the paced breathing with the heart rate measurement, you might feel that the pace is too fast or too slow. We will help you maintain the pace. If it is too fast or too slow and you cannot maintain the pace, you may simply resume breathing at a pace that is comfortable for you.
- The Sensor Belt that goes around the chest will be fitted to the body and may provide some discomfort. The Sensor Belt is adjustable to reduce this discomfort. You may remove the device if you notice any development of skin irritation or rash. Additionally, the fingertip pulse oximeter may cause some discomfort over time.
- During spirometry testing, you will be asked to take deep breaths and forceful exhalations. These actions may cause discomfort accompanied by possible coughing, shortness of breath, or dizziness. However, these tests will last less than 1 minute, so any discomfort will be brief and you are free to stop at any time.
- Wearing the activity monitor on the waist for 9 days may result in discomfort/rubbing/redness under the elastic band. This band can be worn above or underneath the clothing to limit this risk. The elastic band is adjustable to ensure appropriate fit and reduce the risk of discomfort/rubbing/redness.
- When completing the online questionnaires, you may feel uncomfortable, anxious or distressed while answer questions about traumatic experiences, anxiety, or depression. If this occurs, you may stop the questionnaire at any time. We will provide you with a list of local resources for dependency and mental health services with their contact information.
- When collecting data using online questionnaires, we cannot guarantee privacy and confidentiality as online data may be hacked or intercepted. Anytime you share information online there are risks involved. We are using multi-stage password protection (password-locked computers and spreadsheets) and a secure system (RedCap) that is specifically designed for managing surveys for research studies. RedCap is a secure, password-protected system and all information will be stored using a RedCap-generated study ID. Your privacy and confidentiality will be maintained by assigning a unique study ID to your responses. Your email address and survey responses will be stored in separate REDCap databases. Each database has customizable security/access settings so that only the research team will have access to your email address and data. Therefore, the risk that your data may be linked to your email address is minimal.
- Whenever one works with e-mail or the internet there is always the risk of compromising privacy, confidentiality and/or anonymity. Your confidentiality will be maintained to the degree permitted by the technology being used. It is important for you to understand that

no guarantees can be made regarding the interception of data sent via the internet by third parties.

- We cannot guarantee your privacy throughout your study participation. During the Zoom meetings, there is the risk that someone in your residence may see or overhear you in the meeting. We will conduct all Zoom meetings in a quiet, secure, and private area of our homes to limit the risk of someone in our residence seeing or overhearing you, and we recommend you do the same. All Zoom meetings will be password-protected and will use the "waiting room" feature, where only the host of the meeting (the research staff) can allow access to the meeting. We will also be using the "lock meeting" feature to ensure that no one else can enter the Zoom meeting once it has begun. These features will allow us to control who is in the meeting to ensure that we maintain your privacy as best as possible.

Additionally, the Human Performance Laboratory is located in a public building. People in the building may see you enter/exit the laboratory and connect you to the research study. Students that conduct research in the HPL not affiliated with this study have access to the Lab. We will try to maintain a confidential and private space by hanging "Research Study in Progress" signs on the doors.

The possible benefits of participation in this research study are…

- There are no general benefits associated with participation in this research study.
- As a result of your participation in this study, you may learn if you have previously had COVID-19 through the COVID-19 finger stick antibody test.
- You will learn about your blood pressure and how your blood pressure changes over the course of a full day.
- You will also learn about your lung function by learning about how much air you inhale and exhale, as well as how quickly you exhale.
- Additionally, you will learn about your body fat.
- All of these measures will inform you about your overall health.

Your privacy will be protected by…

- The research records from this study will be confidential. Confidentiality means that it is our responsibility to keep any information you provide private and safe.
- Only members of the trained research staff for this study may look over your research records.
- The results and records will be kept in a password-protected spreadsheet that only the researchers will have access to.
- You will be given a study identifier (3 random letters and 3 numbers) and this will be entered into all research computers used to collect data. Your name will not appear anywhere on these computers or the data output from these computers.

- All information stored on computers requires a password to access it. Only members of the research team will have this password.
- The collection vials will have bar-coded labels containing only the study ID and your participant study identifier. No identifying information will be included on the bar-coded label.
- The data and research record will be stored for up to 10 years.
- Your individual results will not be used in any way (we will average all results and display group averages only when presenting findings in papers and presentations).
- If data are shared, it will be done electronically. If/when necessary to share, the data will not include identifying information. Your specific ID number will not be shared with anybody. All data files are password protected and the password will not be included in emails sent between Dr. Kevin Heffernan, Mr. Andrew Heckel, Ms. Danielle Arcidiacono and Mr. Jacob DeBlois.
- Personal identifiers will be removed from the identifiable private information you provide. After removal of personal identifiers, the information could be used for future research studies or distributed to another investigator for future research studies without additional consent from you.

Your data will be maintained to ensure confidentiality by…

- Your individual results will not be used in any way (we will average all results and display group averages only when presenting findings in papers and presentations).
- All computers that contain data and spreadsheets will be password protected. All data (questionnaires, software data entry, and electronic data result spreadsheets) will be deidentified and will only include your participant ID number.
- If data are shared, it will be done electronically. If/when necessary to share, the data will not include identifying information. Your specific ID number will not be shared with anybody. All data files are password protected and the password will not be included in emails sent between Dr. Kevin Heffernan, Mr. Andrew Heckel, Ms. Danielle Arcidiacono and Mr. Jacob DeBlois
- We will generate a list of random 3 letters and 3-digit numbers to which your data will be assigned a number. This will become your confidential participant ID. The database that associates participant names and ID numbers will be separate, password protected, and accessible only by Dr. Kevin Heffernan, Mr. Andrew Heckel, Ms. Danielle Arcidiacono and Mr. Jacob DeBlois
- If data are shared, it will be done electronically. If/when necessary to share, the data will not include identifying information. Your specific ID number will not be shared with anybody. All data files are password protected and the password will not be included in emails sent between Dr. Kevin Heffernan, Mr. Andrew Heckel, Ms. Danielle Arcidiacono and Mr. Jacob DeBlois

- Personal identifiers will be removed from the identifiable private information you provide. After removal of personal identifiers, the information could be used for future research studies or distributed to another investigator for future research studies without additional consent from you.

Will compensation be awarded for participation?

- You will not be awarded compensation for participation in this study.

As a research participants you have the following rights…

- Your participation is voluntary.
- You may skip and/or refuse to answer any question for any reason.
- You are free to withdraw from this research study at any time without penalty.

If you have questions…

- For questions, concerns or more information regarding this research you may contact Kevin Heffernan, Ph.D. (ksheffer@syr.edu), Andrew Heckel, B.S. (arheckel@syr.edu), Danielle Arcidiacono, B.S. (dmarcidi@syr.edu) or Jacob DeBlois, M.S (jpdebloi@syr.edu).
- If you have questions or concerns about your rights as a research participant you may contact the Syracuse University Institutional Review Board at (315) 443-3013.

Do you have any questions?

Are you 18 to 35 years in age?

Do you consent to participate in this research study?

May we contact you for future research studies you may be eligible to participate in?

Appendix 3

Syracuse University

INSTITUTIONAL REVIEW BOARD
MEMORANDUM

TO: Kevin Heffernan

DATE: February 25, 2021

SUBJECT: Expedited Protocol Review - Approval of Human Participants IRB #: 21-030
TITLE: *Cardiorespiratory Function in Young Adults with a History of COVID-19*

The above referenced protocol was reviewed by the Syracuse University Institutional Review Board for the Protection of Human Subjects (IRB) and has been given **expedited approval.** The protocol has been determined to be of no more than minimal risk and has been evaluated for the following:

1. the rights and welfare of the individual(s) under investigation; 2. appropriate methods to secure informed consent; and

3. risks and potential benefits of the investigation.

This protocol is approved as of **February 23, 2021.** An Expedited Status Report will be requested annually, until you request your study be closed.

It is important to note that federal regulations require that each participant indicate their willingness to participate through the informed consent process and be provided with a copy of the consent form. Regulations require that you keep a copy of this document for a minimum of three years after your study is closed.

Your consent form has been date stamped with the approval date. If at any time during the course of your research, a revised consent document is submitted to the IRB via an amendment, it will be stamped with the date the amendment is approved.

Formal amendment requests are required for any changes to the initially approved protocol. It is important to note that changes cannot be initiated **prior** to IRB review and approval; except when such changes are essential to eliminate apparent immediate harm to the participants. In this instance, changes must be reported to the IRB within five days. All protocol changes must be submitted on an amendment request form available on the IRB web site at: AmendmentRequest-Form.doc.

Any unanticipated problems involving risks to subjects or others must be reported to the IRB within 10 working days of occurrence on the Report of Unanticipated Problems form located on the IRB website at: Report-of-UnanticipatedProblems.doc.

Thank you for your cooperation in our shared efforts to assure that the rights and welfare of people participating in research are protected.

Katherine McDonald

IRB Chair

DEPT: Exercise Science, 820 Comstock Ave, Room 207B **STUDENTS:** Andrew Heckel, Danielle Arcidiacono, Jacob DeBlois

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Education

2019 – Present	Syracuse University, Syracuse, NY Master of Science in Exercise Science *Master's Thesis*: Cardiovascular Disease Risk in Young Adults Following COVID-19 Advisor: Kevin S. Heffernan, PhD
2015 – 2019	Florida State University, Tallahassee, FL Bachelor of Science in Exercise Physiology; Magna Cum Laude
2011 – 2015	Stanton College Preparatory School, Jacksonville, FL High School Diploma International Baccalaureate Diploma

Academic/Professional Experience

08/2019 – 05/2021	**Teaching Assistant**, Syracuse University, Department of Exercise Science, School of Education, Syracuse, NY PPE/EXE 295 Introduction to Exercise Science
08/2019 – 05/2021	**Graduate Research Assistant**, Syracuse University, Department of Exercise Science, School of Education, Syracuse, NY Dr. Kevin S. Heffernan's Human Performance Lab
08/2015 – 12/2017	**Undergraduate Research Assistant**, Florida State University, Tallahassee, FL

Publications

Heckel AR, Cilhoroz BT, Rosenberg J. "The Vascular Response to Acute Hyperglycemia: What is the Role of Exercise Capacity?" *The Journal of Physiology*. 2021.

Manuscripts in Preparation

Heckel AR, Garcia J, Wilmoth JM, Barreira TV, Heffernan, KS. "Depression and Estimated Pulse Wave Velocity in Older Male Veterans: The Moderating Effect of Physical Activity." Manuscript in preparation for *Journal of Aging and Physical Activity*.

Presentations

Heckel AR, DeBlois JP, Keller A, Heffernan KS. "Arterial Stiffness and Wave Reflections Predict Postural Sway in Young Adults." Presented at the American College of Sports Medicine's 68th Annual Meeting, June 1 – June 5, 2021.

***Heckel AR**, Arcidiacono DA, Deblois JP, Glasgow AC, Heffernan KS. "The Effect of SARS-CoV-2 Infection on Systemic Vascular Function: Preliminary Findings." Presented at the 10th Annual North American Artery Conference, May 21 – May 22, 2021.

Heckel AR, , Arcidiacono DA, Coonan KA, DeBlois JP Glasgow AC, Heffernan KS. "The Effect of SARS-CoV-2 Infection on Cardiovascular Function." Presented at the Falk Student Research Celebration, May 4 – May 7, 2021.

Heckel AR, DeBlois JP, Keller A, Heffernan KS. "Wave Reflections Predict Postural Sway in Women." Presented at the 3rd Annual Women's Cardiovascular and Brain Health Symposium, April 16, 2021.

Heckel AR, DeBlois JP, Keller A, Heffernan KS. "Arterial Stiffness and Wave Reflections Predict Postural Sway in Young Adults." Presented at Syracuse University's 7th Annual Neuroscience Research Day, April 9, 2021.

*__Heckel AR__, DeBlois JP, Keller A, Glasgow AC, Heffernan KS. "Arterial Stiffness and Wave Reflections Predict Postural Sway in Young Adults." Presented at the Mid-Atlantic Regional Conference American College of Sports Medicine, November 6, 2020.

Heckel AR, Garcia J, Barreira TV, Heffernan KS. "Association of Physical Activity with Depression and Estimated Pulse Wave Velocity in Older Male Veterans." Presented at the American College of Sports Medicine's 67th Annual Meeting, May 26 – May 30, 2020.

Heckel AR, Rodriguez M, Snowden P, Mason J. "The Effects of Aerobic Exercise on Driving in Healthy Older Men and Women." Presented at the Florida State University Undergraduate Research Symposium, April 6, 2016.

*Slide Presentation

Book Chapters in Preparation

Heckel AR. COVID-19, Endothelial Dysfunction and Cardiovascular Disease Risk. Chapter in preparation for *COVID-19: A Systems Perspective*.

Guest Lectures

Heckel AR and Curry K. Introduction to Metabolism. Presented to PPE 295: Introduction to Exercise Science, Syracuse University, Syracuse, NY, 2020.

Funding Support

Himan-Brown Grant for Mount Everest Study Abroad Program ($3,700) - Accepted
Study abroad program was cancelled due to COVID-19 pandemic

Professional Organizations

American College of Sports Medicine (ACSM), 2019 – Present.

Mid-Atlantic American College of Sports Medicine (MARC), 2019 – Present.

Honors

ADI Free Choice Research Award, American College of Sports Medicine – Noninvasive Physiological Interest Group (ACSM-NIP), 2021

Excellence in Graduate Student Research, Exercise Science, 2021

Falk Graduate School Master's Prize, Syracuse University, 2021

Falk College Graduate Department Marshal, Exercise Science, 2021

Rapid Fire Oral Presentation for Trainees Finalist, North American Artery Conference, 2021

MS Research Award Finalist, Mid-Atlantic Regional Chapter, ACSM, 2020

Graduated Magna Cum Laude, Florida State University, College of Human Sciences, 2019

Phi Eta Sigma, Florida State University, 2015 – 2019

President's List, Florida State University, 2015 – 2019

Dean's List, Florida Stated University, 2015 – 2019