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Cardiorespiratory Function in Young Adults With a History of Covid-19 Infection

Danielle Arcidiacono
Syracuse University

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Abstract

***Objective*.** Respiratory complications may persist several months into the recovery period following COVID-19 infection. This study evaluated respiratory function and oxygen saturation variability between young adults with a history of COVID-19 infection and controls. Associations between cardiorespiratory function with potential biobehavioral correlates of COVID-19 infection were also explored.

Methods. 57 adults ages 18 to 65 participated in this study (24 COVID+, 33 Control). Spirometry was used to assess pulmonary function volumes of forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), FEV1/FVC and peak expiratory flow (PEF). Exhaled nitric oxide (FeNO) was measured using the NiOX VERO, a handheld electrochemical nitric oxide analyzer and taken as a proxy of airway inflammation. Systemic inflammation levels were assessed using salivary concentrations of inflammatory biomarkers. Oxygen saturation variability was quantified via extended continuous oxygen saturation (SpO_2) monitoring using linear and nonlinear analyses. Network physiology analysis was conducted to evaluate cardiorespiratory control between SpO_2, heart rate (HR), respiratory rate and skin temperature signals measured by continuous ambulatory monitoring with an Equivital EQO_2 LifeMonitor. Physical activity levels and sedentary time were assessed using 9-day accelerometry. COVID-19 symptom severity was assessed by participant self-report via questionnaires.

Results. No group differences were observed for pulmonary function of FVC (COVID+: 4.22±1.01, C: 4.43±1.06 L, p=.663), FEV1 (COVID+: 3.45±0.72, C: 3.57±0.92 L, p=.865), PEF (COVID+: 349.63±105.54, C: 373.73±140.61 L/min, p=.370), or FeNO (COVID+: 16.61±13.04, C: 20.03±20.11 ppb, p=.285). Linear and nonlinear oxygen saturation variability did not differ

between adults with a history of COVID-19 infection and controls with no history of infection ($p>0.05$). Cardiorespiratory function measured using network analysis of did not differ between recovering COVID-19 individuals and controls ($p>0.05$). Sedentary time was inversely associated with FEV1 ($r=-.392$, $p=.040$), PEF ($r=-.579$, $p=.003$), and IL-6 concentrations ($r=-.370$, $p=.049$). COVID-19 disease severity was inversely associated with FVC ($r=-.461$, $p=.012$) and FEV1 ($r=-.365$, $p=.040$). Number of symptoms was inversely associated with FVC ($r=-.404$, $p=.025$).

***Conclusions*.** Pulmonary function, inflammation levels and oxygen saturation variability were similar between individuals with a history of COVID-19 infection and controls without a history of COVID-19 infection. Network interactions between regulatory components of the cardiorespiratory system were also similar between recovering COVID-19 individuals and controls. Findings suggest that cardiorespiratory function and dynamic control of SpO_2 may not be impaired following COVID-19 infection in young adults. Moreover, increased sedentary time and disease severity may have negative effects on pulmonary function in individuals recovering from COVID-19.

CARDIORESPIRATORY FUNCTION IN YOUNG ADULTS WITH A HISTORY OF COVID-19 INFECTION

By

DANIELLE M. ARCIDIACONO

B.S., Bridgewater State University, 2019

Thesis
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Glossary of terms

Abbreviation	Term
FeNO	Fractional concentration of exhaled nitric oxide (ppb)
SpO_2	Oxygen saturation (%)
HR	Heart rate (bpm)
COVID-19	Coronavirus of 2019
SARS-CoV	Severe acute respiratory syndrome coronavirus
MERS-CoV	Middle East respiratory syndrome coronavirus
ACE2	Angiotensin Converting Enzyme 2
ARDS	Acute respiratory distress syndrome
FVC	Forced vital capacity (L)
FEV1	Forced expiratory volume in 1 second (L)
PEF	Peak expiratory flow (L/min)
TNF-α	Tumor necrosis factor
DLCO	Diffusion limitation carbon monoxide
IL	Interleukin
CRP	C-reactive protein
COPD	Chronic obstructive pulmonary disease
SD	Standard deviation
RMS	Root mean square
MSE	Multiscale entropy
DFA	Detrended fluctuation analysis
SEM	Sensor electronics module
ECG	Electrocardiogram
SaO_2	Arterial oxygen saturation (%)

Chapter 1: Introduction

The World Health Organization declared the novel coronavirus disease of 2019 (COVID-19) a global pandemic in March of 2020. Since its onset, there have been over 30.5 million cases reported globally, with case reports increasing every day. The United States is ranked number one in cases at over 46.25 million confirmed cases to date.[1] As time progresses, the number of people recovering from COVID-19 increases as well. As we continue to study the epidemiology of the virus in regard to its onset and spread, a focus in research has shifted towards understanding the long-term health effects of COVID-19 on survivors.

The lungs are the organ most affected by COVID-19 and act as a major point of viral entry into the body. The novel strain of coronavirus is caused by SARS-CoV-2 infection, a member of the Coronaviridae family that also encompasses severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). The spike (S) protein of SARS-CoV-2 has a significant binding affinity for the Angiotensin Converting Enzyme 2 (ACE2) receptor that is prevalent in type 2 alveolar epithelial cells of the human lung.[2] ACE2 receptors are also widely distributed outside of the lung, including the heart, kidneys, vasculature, brain and gastrointestinal tract. These ACE2 receptors are sites for viral replication and spread, leaving these organs at high risk for infection.[2]

Viral replication inside the cell causes cellular pyroptosis, a highly inflammatory cell death via infection, which causes the release of damage pattern molecules. Nearby epithelial cells and alveolar macrophages recognize the release of damage pattern molecules and release pro-inflammatory cytokines in response. This cytokine release evokes an immune response via monocytes, macrophages and T cells that continue this pro-inflammatory feedback loop. In some patients, a large and nonspecific immune response to cell damage becomes dysregulated

and there is an over-production of these cytokines. This cytokine release can spread systemically in a "cytokine storm" through the circulation, leading to multi-organ damage.[3] Increased systemic inflammation levels are associated with worsened COVID-19 infection disease severity and therefore degree of cardiorespiratory injury during acute infection. More specifically however, this dysregulated inflammatory response leads to alveolar damage and acute injury to both the lung and its microvasculature. This causes the alveoli and the lungs to fill with blood and plasma fluid rather than air, limiting diffusion capacity and causing respiratory distress.[4] This fluid buildup can lead to pneumonia and in more severe cases, acute respiratory distress syndrome (ARDS). In addition to a prolonged inflammatory state, the exacerbated infections that result from COVID-19 and its subsequent respiratory diseases leads to impaired diffusion capacity and gas exchange resulting in persistent hypoxemia.[5–7] The oxygen deficit as well as the acute injury to the lung also manifests as a diminished overall lung capacity.

The potential sequalae associated with COVID-19 infection can be derived from the acute infection caused by SARS-CoV-2 as well as extrapolated from previous follow-up data associated with SARS-CoV and MERS-CoV survivors.[6,8,9] COVID-19 infection targets the lower respiratory tract, primarily the alveoli of the lungs and its surrounding infrastructure. In moderate to severe cases, the infection causes the development of pneumonia and ARDS, resulting in consolidation of the lung and increased difficulty breathing. These secondary conditions associated with COVID-19 infection concurrent with a heightened inflammatory state cause lasting damage to the alveoli, the pulmonary microvasculature and the lobes of the lung, resulting in scarring and are indicated in the pathogenesis of pulmonary fibrosis.[8,10–13] Further persisting injury and potential fibrogenesis could result in those COVID-19 survivors that required mechanical ventilation during the infection period, exacerbating the existing injury to

the lower respiratory tract [8,11] and causing atrophy of the respiratory muscles.[14] During the infection period, the body's vasculature including the microvessels of the lung exist in a procoagulant state, indicating high risk for future occlusions.[9,10] These structural changes to the lung present as functional changes to the respiratory system primarily including diminished gas exchange and general difficulty breathing. These deficiencies result in persisting symptomology from the infection period to recovery including hypoxemia, dyspnea, cough and fatigue.[5,10,15] Similar to the sequalae reported in SARS-CoV and MERS-CoV infections,[8,9,12] data published on recovering COVID-19 individuals suggests that these pulmonary abnormalities can persist several months into the recovery period.[8,11–13,16] Standard pulmonary function testing can give insight into these changes in general lung function, strength of respiratory muscles and airway obstruction from inflammation or swelling.[17]

A nonconventional approach to assessing airway inflammation that is now being explored in COVID-19 patients is measurement of exhaled nitric oxide. Widely utilized with asthma patients, exhaled nitric oxide gives indication to airway-specific swelling related to inflammation. During pro-inflammatory states such as COVID-19, cytokines stimulate the overproduction of nitric oxide in an attempt to combat reductions in airway caliber due to swelling. The preliminary use of this testing has been both as a supplementary diagnostic tool during active infection as well as a means of evaluating airway inflammation during recovery post-infection. Due to the novelty of exhaled nitric oxide testing in the COVID-19 population, utilization of this measure in the current study will provide insight into a potentially useful clinical indicator directly related to COVID-19 infection that may contribute to ongoing symptomology and respiratory limitation experienced post-infection.

Persisting hypoxemia is one of the most common symptoms during COVID-19 infection and is associated with COVID-19 mortality.[5] While standard intermittent pulse oximetry is beneficial in classifying a hypoxemic state, continuous sampling is more intuitive when studying respiratory function. Oxygen saturation variability can be derived from continuous pulse oximetry measurement, measured both individually and in conjunction with other cardiorespiratory signals in a network approach, and gives holistic insight into physiological control of the oxygenation process as well as the integrity of the cardiorespiratory system.[18] Variability can be defined using traditional linear metrics such as mean and standard deviation (SD) but also quantified using novel nonlinear methods.

Measurement of oxygen saturation variability via nonlinear methodology provides a novel view of hypoxemia, one of the most predictive indicators of COVID-19 infection, allowing a better understanding of the oxygen homeostasis process and the lung injury that may be responsible for its malfunction. SpO_2 as a physiological signal is constantly changing and adapting to overcome stressors and maintain a desired physiological state (normoxia), observed as variability. Linear methods, such as the SD of values around the mean SpO_2, fail to account for these natural fluctuations, and therefore only provide minimal insight into the complex dynamics exhibited by SpO_2. Assessing variability from a nonlinear perspective helps researchers successfully capture and define these natural fluctuations in SpO_2 and therefore more accurately identify exacerbations and hypoxemia in clinical populations, such as those recovering from COVID-19 infection. Nonlinear variability analysis has been extensively studied in other physiological signals such as HR, where reductions of variability are indicative of aging and disease.[19,20] Injuries to gas exchange efficiency observed during COVID-19 infection may be indicated by similar reductions in variability.

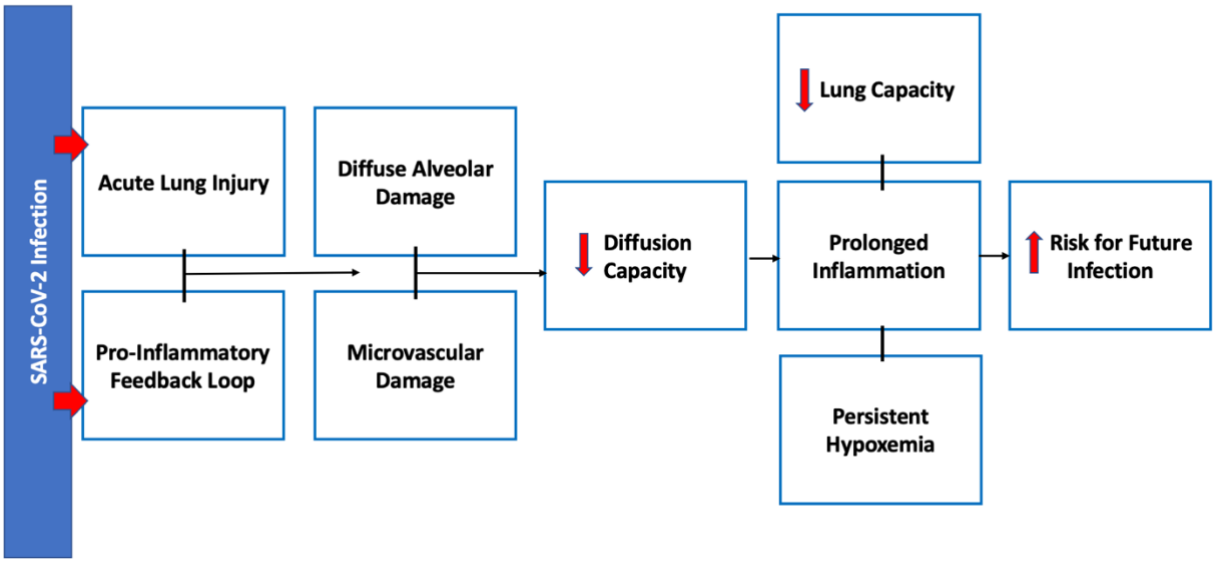
The cardiorespiratory system as a whole is composed of several regulatory components that work together to maintain proper cardiorespiratory function. These components (e.g., the brain and neural circuitry, heart, lungs, circulation, skeletal muscle in the form of the diaphragm) are "coupled" to create an integrated physiological network that is in constant communication using information signaling. Signal outputs generated by these organ systems include heart rate, respiratory rate, skin temperate and oxygen saturation. Network analysis can be used to measure the regulatory signaling between components, and assesses a system's ability to maintain a desired physiological state by adapting to the physiological and pathological stressors placed upon it. Reductions in signaling between components (i.e., systems/signal isolation) is indicative of injury to the system, and occurs in pathology.[21–23] Therefore, network analysis of SpO_2 homeostasis as it relates to other cardiorespiratory signals allows researchers to identify any points of isolation or injury amongst regulatory components of the cardiorespiratory system sustained during COVID-19 infection that may contribute to decreased control of SpO_2. Integration between these signals can further indicate the degree of control one component has on the maintenance of the other (i.e. how much other cardiorespiratory components such as HR contribute to SpO_2 homeostasis), and vice versa. Injuries sustained during COVID-19 infection may result in persisting cardiorespiratory injury that decreases the system's ability to adapt to the stressors placed upon it. This isolation amongst system components can lead to overall dysfunction (i.e. hypoxemia) seen as system uncoupling. These measures work in unison with standard pulmonary function testing and exhaled nitric oxide to give a comprehensive view of an individual's global cardiorespiratory function and can be used to assess recovery status and progression.

There are many biobehavioral factors that may favorably or detrimentally modulate cardiorespiratory function. Physical activity levels and amount of sedentary time are two notable contributors to cardiorespiratory outcomes. Adequate physical activity levels can be protective for cardiorespiratory function as they have been shown to promote proper pulmonary function and reduce systemic inflammation levels.[24–28] Conversely, lower levels of physical activity are associated with increased respiratory symptomology and general disease severity in clinical populations including increased frequencies of exacerbations, gas exchange impairments and elevated inflammatory profiles.[29–33] Similarly, sedentary time may cause a detraining effect on the lungs, including reduction in strength of respiratory muscles and reductions in pulmonary function.[34–36] Increased sedentary time has also been associated with elevated inflammatory profiles in several populations.[37–39] With decreased access to physical activity opportunities with COVID-related restrictions such as quarantine, individuals who have experienced COVID-19 infection may not experience the protective effects of adequate physical activity and suffer from the detraining associated with increased sedentary behaviors. Whether physical activity and sedentary behavior are associated with cardiorespiratory function in a setting of COVID-19 recovery has not been specifically explored.

In summary, measures of respiratory function are predictors of COVID-19 mortality[5] and offer insight into disease severity, progression and recovery. Additionally, these indicators may be used to assess survivor's susceptibility for future respiratory decline as they may be at increased risk for other respiratory conditions due to ongoing inflammation and injury. The proposed relationship between SARS-CoV-2 infection and its long-term implications are depicted in Figure 1. Furthermore, studies utilizing antibody testing reveal that immunoglobulin G antibodies for COVID-19 can dissipate as early as three months after disease recovery,

indicating that these survivors are at risk for repeated COVID-19 infection.[13] Lastly, assessment of prolonged effects of COVID-19 can give insight into rehabilitation needs for these individuals. Continuous SpO_2 monitoring and network analysis of SpO_2 can provide novel insight into components of cardiorespiratory control that contribute to fluctuations in oxygen saturation variability and subsequent cardiorespiratory uncoupling. General assessments of recovering patients are limited during the infection/hospitalization period with fear of virus transmission and lack of resources. The use of at-home testing can eliminate these concerns and allow direct measurement of multiple aspects of lung function. This modality also is beneficial in a follow-up setting, allowing for access to more subjects than with hospital visits alone.

Figure 1. Events From Onset of Infection to Long-Term Respiratory Implications

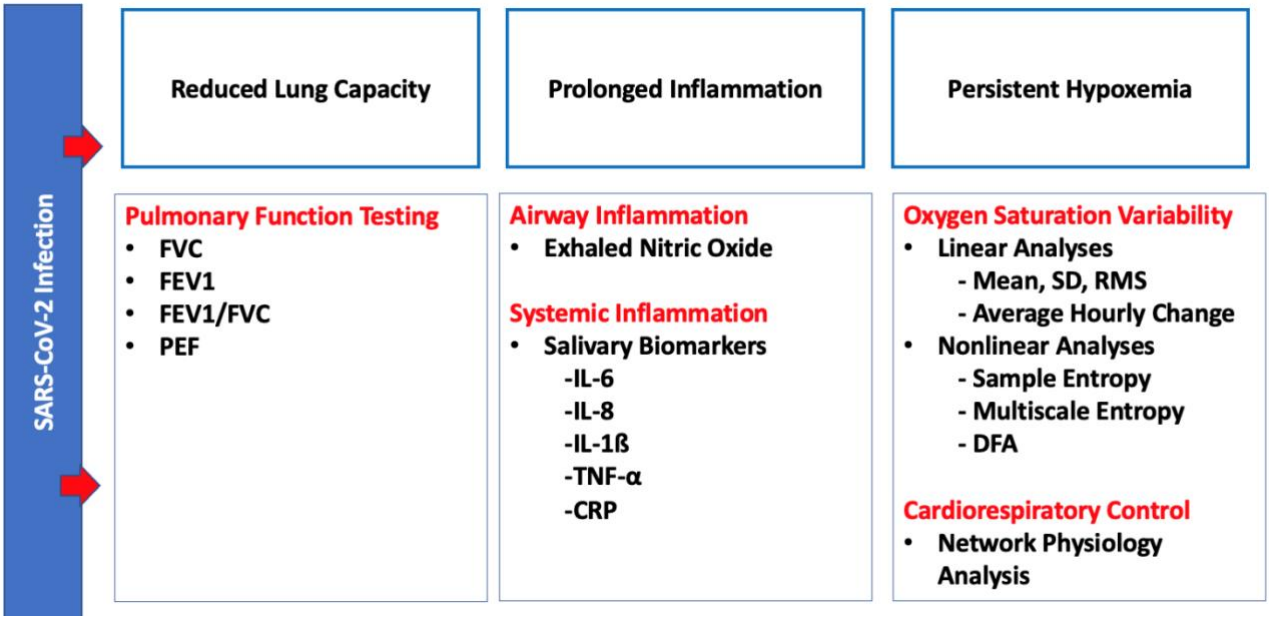


Progression of acute injuries from SARS-CoV-2 viral entry and replication through development of potential future respiratory risk.

Therefore the purpose of this study was 1) to compare pulmonary function, airway inflammation and oxygen saturation variability between individuals with a history of COVID-19 infection and controls with no history of COVID-19 infection using portable pulmonary function testing and extended continuous SpO_2 monitoring, respectively, 2) to understand and compare

exchange of information between regulatory components of the cardiorespiratory system in individuals with a history of COVID-19 infection and controls as measure of dynamic cardiorespiratory control using network analysis of SpO_2, HR, respiratory rate and skin temperature signals, and 3) to explore systemic inflammation, physical activity levels, sedentary time and COVID-19 disease severity as potential biobehavioral correlates of cardiorespiratory function in individuals recovering from COVID-19 infection. It was expected that 1) individuals with a history of COVID-19 will have reduced respiratory function (decreased pulmonary function and increased airway inflammation), and decreased oxygen saturation variability compared to controls suggesting individuals with a history of COVID-19 will have decreased dynamic cardiorespiratory function, 2) there will be decreased information signaling between SpO_2, HR, respiratory rate and skin temperature signals in recovering COVID-19 individuals suggesting isolation within regulatory components of the cardiorespiratory system (i.e., loss of integrated cardiorespiratory control) and 3) that increased systemic inflammation, decreased physical activity, increased sedentary time and increased disease severity will be associated with decreased cardiorespiratory function in recovering COVID-19 individuals. The proposed measures to evaluate the long-term implications of SARS-CoV-2 infection are shown in Figure 2.

Figure 2. Proposed Measures to Evaluate Cardiorespiratory Function following SARS-CoV-2 Infection



Summary of three respiratory sequelae assessed and their associated methodologies.

Chapter 2: Literature Review

The following literature review will introduce concepts related to 1) the pathophysiology of COVID-19 caused by SARS-CoV-2 infection, its clinical manifestations and potential sequelae; and 2) the existing uses and implications of the methodology to be used to assess respiratory function in COVID-19 survivors (Figures 1 and 2). This review provides rationale for a study to explore the potential long-term cardiorespiratory implications associated with COVID-19 infection including measures of pulmonary function, inflammation, and gas exchange in survivors. This review seeks to illustrate the current knowledge provided in the existing literature regarding the largely unknown lasting effects of COVID-19 infection. This study seeks to determine whether there is diminished respiratory function in COVID-19 survivors when compared to those with no history of COVID-19.

SARS-CoV-2 Infection Pathophysiology

On March 11, 2020, the World Health Organization declared COVID-19 a global pandemic.[40] This novel strain of coronavirus is caused my SARS-CoV-2 infection, a member of the Coronaviridae family that also encompasses SARS-CoV and MERS-CoV.[4,7,40] The SARS-CoV-2 pathogen enters the body through respiratory droplets via receptor-mediated endocytosis,[3,7,40] targeting primarily the lower respiratory tract.[3,4,40] The spike (S) protein of SARS-CoV-2 has a significant binding affinity for the ACE2 receptor that is prevalent in type 2 alveolar epithelial cells of the human lung.[3,4,12,41] While most abundant at these type 2 pneumocytes, ACE2 receptors are also widely distributed in extrapulmonary organs including the heart, kidneys, vasculature, brain and gastrointestinal tract.[2] Viral replication inside these cells causes cellular pyroptosis, a highly inflammatory cell death via infection, causing the release of damage pattern

molecules. Type 2 alveolar epithelial cells and macrophages recognize this pattern release using pattern-recognition receptors and respond with a local inflammatory response.[3] This process of cytokine release prompts surrounding pneumocytes to recruit immune cells such as monocytes, macrophages and T cells to the site of inflammation, creating a pro-inflammatory feedback loop.[3,12,42] In some patients, a large and nonspecific immune response to cell damage becomes dysregulated and there is an overproduction of cytokines. This cytokine release can spread systemically in a "cytokine storm" leading to multi-organ damage.[3,7,42] More specifically, however, this dysregulated immune response leads to diffuse alveolar damage and acute injury to both the lung and its microvasculature.[3,6,43,44] Diffuse alveolar damage, as defined by Katzenstein and colleagues (1976), is a nonspecific reaction of the lung to pathogens and other harmful agents, resulting in both alveolar and endothelial injury.[45] The injury causes desquamation of type 2 alveolar cells, resulting in fluid and cellular exudation, limiting diffusion capacity and causing respiratory distress.[3,4,11,42–44,46,47]

Clinical Respiratory Manifestations of COVID-19

Viral infection and replication cause injury to the lung's infrastructure and microvasculature, resulting in impaired functionality. Respiratory distress caused by COVID-19 can be attributed to a wide range of symptomology, extending from mild to life-threatening respiratory indicators. The aforementioned vascular leakage that occurs at the alveolar epithelium causes abnormal gas exchange and inefficient oxygenation of the blood. This is clinically seen as a low blood oxygen SpO_2, defined as hypoxemia at values below 95%.[5,40] In mild to moderate cases, decreased SpO_2 levels combined with pulmonary inflammation manifest as common respiratory symptoms including cough, dyspnea, and fatigue. Additionally, COVID-19 infection causes an aggressive

form of pneumonia[4,7,10,40,42,44] that can lead to exacerbated alveolar damage and in more severe cases, the development of ARDS.[2,3,8,10] These secondary respiratory conditions are often identified from radiological abnormalities, most commonly seen as pneumonia, hyaline membrane formation, lesions, pulmonary consolidation, and pure or mixed ground glass opacities.[4,6,7,9,12,13,40,42,43,47] Patients with persisting hypoxemia or severe secondary infection (i.e. ARDS) may require supplementary oxygen and in some cases mechanical ventilation due to respiratory failure. A study conducted by Wang et al. studying 36 COVID-19 cases requiring admittance to the intensive care unit reported that approximately 89% of patients could not breathe spontaneously and required some form of oxygen supplementation or mechanical ventilation.[48] In summary, the acute lung injury caused by COVID-19 infection manifests most commonly as hypoxemic respiratory distress and its associated symptomology, anatomical alterations seen by radiological scans and the development of secondary respiratory infections. This symptomology may persist long after the infection period and gives insight into potential long-term complications that may arise for these survivors.

Concurrent with respiratory symptomology, the heightened systemic state of inflammation during COVID-19 infection, termed the "cytokine storm," has earned a critical rapport in clinical assessment of COVID-19 individuals. The cytokine storm described in COVID-19 patients was previously defined by researchers as "an activation of auto-amplifying cytokine production due to an unregulated host immune response.[49] The positive inflammatory feedback loop between immune cells and inflammatory molecules has lasting implications for immune function in the host. High levels of cytokines lead to the overactivation of lymphocytes, specifically T cells. T cell exhaustion results in amplified levels of inflammatory molecules such as interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) that play a role in lymphocyte

necrosis.[42] With this, lymphopenia has been observed in COVID-19 patients and leaves them at higher risk for further inflammation as well as increased susceptibility for future infection.[3,40,42] Furthermore, as this hyperinflammation affects the host in a systemic fashion, respiratory function can be indirectly affected by extrapulmonary factors including ongoing cardiac and vascular injury.[6] Finally, the body's vasculature, including the microvessels of the lung, exist in a procoagulant state after infection, indicating high risk for future occlusions.[9,10] These findings indicate that chronic inflammation due to COVID-19 infection results in overactivation of the immune system, ultimately resulting in lymphopenia and increased susceptibility for future respiratory decline.

Potential Sequelae of COVID-19

As the novelty of the COVID-19 pandemic recedes, the population of recovered cases that can and have been accessed to evaluate the long-term physiological implications of COVID-19 infection on survivors becomes increasingly available. To date, much of the potential sequelae associated with COVID-19 can be derived from both the acute infection caused by SARS-CoV-2 as well as extrapolated from previous follow-up data associated with SARS-CoV and MERS-CoV survivors,[6,8,9] and from individuals recovering from the secondary respiratory conditions that may develop during COVID-19 infection, such as ARDS.[43] The secondary infections associated with COVID-19 paired with a heightened inflammatory state cause lasting damage to the alveoli, the pulmonary microvasculature, the lobes of the lung, and the airway. In more critical cases, injuries may result in scarring and are indicated in the pathogenesis of pulmonary fibrosis.[8,11–13] Pulmonary fibrosis is associated with chronic inflammation during fibrogenesis[50] and restricted ventilation and diffusion capacity as a result.[51] A study investigating the

progression of radiological abnormalities one month after discharge in COVID-19 patients found despite the gradual resolution of parenchymal findings (i.e. ground glass opacities), 39% of participants still displayed residual fibrosis.[13] Further persisting injury and potential fibrogenesis could result in COVID-19 patients that require mechanical ventilation[8,11] and cause atrophy of the respiratory muscles.[14,52] A study conducted by Levine and associates (2008) showed that patients who underwent mechanical ventilation who had diaphragmatic inactivity exhibited a more than 50% decrease in muscle cross-sectional area in as little as 18 hours.[53] Mechanical ventilation is also associated with barotrauma and the development of lesions that may lead to edema.[54] Taken together these findings suggest that COVID-19 survivors, especially those with more severe infection experiences (e.g. development of ARDS, ventilation requirement), are at increased risk for lasting respiratory complications following infection including fibrosis and mechanical ventilation injury.

Existing follow-up data suggests that the pulmonary abnormalities seen during COVID-19 infection can persist several months into the recovery period.[8,11–13,16] These deficiencies result in persisting symptomology from infection to recovery including hypoxemia, dyspnea, cough and fatigue.[10] A study conducted with 110 discharged COVID-19 patients of varying disease severity tested pulmonary function three months after hospital release. Diffusion abnormalities were observed in 47.2% of cases (DLCO) followed by diminished total lung capacity in 25% of subjects.[6] These findings were consistent with that of gas exchange efficiency measured as diffusion limitation of carbon monoxide (DLCO) and total lung capacity measurement in SARS-CoV survivors at 0.5-2 years follow-up[55–57] and DLCO measurement alone at 12 months follow-up of MERS-CoV subjects.[58] Another study of 55 recovered COVID-19 patients conducted at three months post-discharge revealed pulmonary function anomalies in

25.45% of subjects as well as radiological abnormalities in 74.55% of survivors. This same study also conducted immunoglobulin G serum antibody testing and found that 14.55% of their cohort tested negative, suggesting that these individuals are susceptible to repeat infection as early as three months after recovery.[12] Despite the growing number of follow-up studies, if and when COVID-19 survivors will return to normal respiratory function and fully recover from the effects of the virus still remains largely unknown. A previous study looking at SARS-CoV survivors reported respiratory abnormalities up to 15 years post-infection, including diminished diffusion capacity in 38.46% and impaired mid-flow maximum expiration (expiratory flow) in 40.38% of patients.[41] Likewise, long-term studies of ARDS patients revealed persistent reduction in health-related quality of life up to five years after hospital discharge,[59,60] correlating with pulmonary dysfunction in these subjects.[61] Overall, current follow-up studies assessing respiratory function in COVID-19 survivors resemble that of existing data of individuals recovering from similar respiratory infections; reporting impaired gas exchange, radiological abnormalities and diminished total lung capacity. Consequently, measures of respiratory function may be valuable predictors of COVID-19 infection severity and symptomology, and therefore disease progression and recovery.

Salivary Inflammatory Biomarkers

The systemic dysregulated inflammatory response known as the cytokine storm is indicative of not only the presence of COVID-19 infection, but also can be used for stratification of COVID-19 disease severity.[3,62] These findings are consistent with the relationship between inflammatory biomarkers and disease severity and outcomes seen in MERS-CoV, SARS-CoV and chronic obstructive pulmonary disease (COPD) patients.[62,63] Researchers have found elevated plasma

levels of chemokines, interleukins (IL) 2, 6, 8, 10 and 1ß, C-reactive protein (CRP), TNF-α and D-Dimer in COVID-19 patients,[7,40,62] with higher levels being indicative of more severe infection. These biomarkers of inflammation can be measured in saliva as a proxy for serum concentrations.[62] While serum levels are representative of systemic inflammation, relationships between inflammatory biomarkers and pulmonary injury have been identified. Elevated inflammatory markers, specifically CRP, were found to be associated with severity of hypoxemia[5] and therefore degree of injury in COVID-19 patients. Similar indicators were shown in a three-month follow-up study where COVID-19 survivors with abnormal computed tomography findings had significantly higher CRP concentrations.[12] This relationship between CRP levels and pulmonary function has previously been reported in patients with COPD as higher CRP concentrations were correlated with decrease lung volumes.[64] Furthermore, IL-1ß has been found to play a strong role in fibrogenesis at the lung[65] while TNF-α has been indirectly associated with the development of pulmonary edema as it contributes to alveolar membrane permeability.[66] Moreover, augmented levels of interleukins 6 and 8 have been found as predictive of poor outcomes in acute lung injury patients.[66,67] More specifically, IL-8 is responsible for upregulation of adhesion molecules[66] as well as functions in altering the integrity of the alveolar membrane.[67] The remainder of these biomarkers are also responsible for the recruitment of additional inflammatory cells including cytokine and chemokine production and neutrophil and macrophage activation.[42,66] A comprehensive overview of these biomarkers and their inflammatory functions as they relate to respiratory injury can be seen in Table 1. Because these biomarkers are indicative of injury, they may be helpful in identifying recovery progress in survivors.

Table 1. Inflammatory Biomarker Functions

IL-6	Predictive of poor outcomes in ALI, inflammatory cell recruitment
IL-8	Predictive of poor outcomes in ALI, upregulates adhesion molecules, alveolar membrane permeability, inflammatory cell recruitment
IL-1ß	Role in pulmonary fibrogenesis, inflammatory cell recruitment
CRP	Associated with decreased lung volumes & severity of hypoxemia, abnormal CT findings, inflammatory cell recruitment
TNF-α	Affects alveolar membrane permeability, contributes to pulmonary edema, inflammatory cell recruitment

IL, interleukin; ALI, acute lung injury; CRP, C-reactive protein; CT, computed tomography; TNF-α, tumor necrosis factor

Pulmonary Function Testing

As previously discussed, current follow-up data on COVID-19 survivors suggests that impaired lung function may extend several months into the recovery period.[6,12] Pulmonary function testing can give insight into general lung capacity, strength of respiratory muscles and airway obstruction from inflammation or swelling.[17] Standard pulmonary function testing utilizes a single spirometry maneuver to capture multiple determinants of pulmonary function including forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), and peak expiratory flow (PEF).[17,68] This manuever is repeated for three trials, and the best score for each pulmonary function volume is taken as a final measure of function. Spirometry is commonly used in clinical practice to assess lung impairment and disease progression in populations with respiratory conditions such as asthma and COPD.[68–70] Individual predicted values are

determined by subject age, height, sex and ethnicity[68,71] as pulmonary function scores are often reported in percentage of predicted value.[17,68–71] FVC is clinically defined as the total volume of air that can be exhaled during a forced, maximal expiration effort following complete inflation of the lungs.[69] Attenuated FVC values are common in restrictive lung diseases such as idiopathic pulmonary fibrosis.[70] FEV1 refers to the amount of air that can be forcibly expired in 1 second following maximal inhalation.[69,70] Reduction is FEV1 values indicates increased airway resistance to expiratory flow[70] and is common in obstructive lung diseases.[70,72] Individual FVC and FEV1 values of greater than or equal to 80% are considered normal[17,69] and these values have been found to be independently associated with mortality in asymptomatic (without obstructive disease) individuals.[73] Additionally, disease severity can be classified by the degree of deficiency (how far a score is below 80%) seen in FEV1 scores.[68] A ratio of FEV1/FVC can be used to distinguish types of lung disease or impairment[69,70] however it must be analyzed in conjunction with individual FEV1 and FVC values.[74] A normal FEV1/FVC ratio has been defined as greater than or equal to 70%. A more pronounced reduction in one value compared to another affects the ratio and can be indicative of types of lung disease as mentioned.[70] However, if both values are reduced to the same extent, the ratio is technically preserved. Despite this presumably normal ratio, this pattern is associated with decreased functional capacity and increased mortality.[74] PEF is a measure of maximum flow achieved during a forced expiration effort following maximal inflation of the lungs. PEF measurement is indicative of lung volume, strength of expiratory muscles, airway caliber and elastic recoil capacity of the lung, where diminished PEF is indicative of airflow obstruction and deficiency in one or more of these areas.[17] According to the British Lung Foundation and American Lung Association, normal PEF scores in adults range from 400 to 700 L/min, or 80% or higher of predicted values for

participant demographic.[71,75] Additional considerations (and potential limitation) regarding these measurements are that they are all contingent on subject effort.[70] Therefore, proper technique, instruction and participant motivation are necessary for accurate data collection.[68,70]

Decreased pulmonary function values have been reported in both COVID-19 survivors and in survivors of other respiratory disease. A three-month follow-up study conducted with COVID-19 patients revealed 25% of participants showed pulmonary function deficiency with approximately half of them showing reduced FVC or FEV1 values.[12] Similar reports were published in COVID-19 patients with abnormally low FEV1 and FVC values at 6 weeks follow-up[76] and at the time of patient discharge.[6] This is consistent with findings of COPD patients showing reduction in both FEV1 and FVC scores independent of markers of inflammation.[63] PEF has also been found to show greater decline in more severe COPD patients, indicating disease progression.[77] Furthermore, a study of ARDS survivors at an average of 30-months post-discharge revealed that 56.3% of subjects displayed an obstructive pattern, restrictive pattern, or combination of the two.[78] These findings indicate that pulmonary function should be utilized to assess COVID-19 patients and identify restrictive flow patterns.[76] The current study will identify if pulmonary dysfunction persists in young adults with a history of infection, and therefore provide indication if this population should be monitored during the recovery period and whether or not these individuals may require therapeutic intervention post-infection.

Exhaled Nitric Oxide

Ongoing inflammation from COVID-19 infection can be measured systemically in the circulation or saliva, but also can be assessed specifically to the airway. Airway inflammation can be measured as concentrations of nitric oxide and may act as a partial source of pulmonary

dysfunction as it affects expiratory flow measures by obstructing the airway. Fractional nitric oxide in exhaled breath, or exhaled nitric oxide (FeNO), measurement is commonly used in patients with respiratory disease[79–81] but has been only preliminarily used with COVID-19 patients.[82–86] FeNO is an indicator of airway caliber and inflammation as nitric oxide is essential in regulating vasomotor tone in the pulmonary circulation[80,87] and has previously been associated with changes in lung function.[79] Aside from its role in broncho- and vasodilation, nitric oxide has also been identified in immune function as it can damage pathogens and recruit T-helper cells to produce antibodies.[81] Under inflammatory conditions, several cytokines that are prevalent in COVID-19 (i.e. IL-1ß, TNF-α, etc.) stimulate the overproduction of nitric oxide in order to combat the change in airway size due to adjustments in vascular tone.[80] Higher concentrations of nitric oxide in expired breath represent increased levels of inflammation in the airway and airway caliber reduction.[79,80] Reference FeNO values are dependent on subject age, sex, anthropometrics, smoking status and dietary habits.[81]

A study conducted on 49 intensive care patients found that exhaled NO values in critically ill patients with pneumonia were elevated compared to those without. This study also found that FeNO values were independent of systemic NO levels, indicating that FeNO values were specific to airway inflammation.[80] These findings were echoed in a recently published study that used FeNO as a rapid screening tool for COVID-19 patients, where it successfully identified 88% of infected subjects.[83] Similarly, Yang and colleagues also applied this modality for COVID-19 diagnostic use. Results of this study were consistent with previous findings and found that FeNO levels were significantly higher in COVID-19 patients compared to healthy controls. It is important, however, to complete additional testing as patients with other chronic diseases (asthma, COPD, etc.) will be classified similarly to COVID-19 subjects in this

assessment.[82] Few studies have evaluated FeNO levels in recovered COVID-19 individuals and have yielded mixed findings.[84–86] Thus, FeNO measurement may be a useful modality in complementing other forms of respiratory evaluation such as pulmonary function testing however more data is necessary to understand its true significance as a measure of respiratory recovery post-infection.

Oxygen Saturation

Diminished lung capacity and functionality due to the acute lung injury caused during infection are largely attributed to reductions in diffusion capacity due to diffuse alveolar damage. The efficiency of gas exchange at the lungs is therefore compromised, and oxygen cannot reach and bind to the red blood cells that pass by in the circulation. This efficiency can be measured as SpO_2, and can fluctuate throughout the day based on a number of things, including ambient oxygen, activity levels, and general lung health. Desaturations due to pathology in the lung can be classified as exacerbations and result in bouts of hypoxemia or low blood SpO_2 levels. These changes in blood oxygen levels can be monitored closely and give important insight into functionality of not only the lung but the entire cardiorespiratory system. While spot check SpO_2 is considered the fifth vital sign, continuous monitoring may be a more effective way of evaluating these fluctuations.

SpO_2 is a measure of the percentage of hemoglobin binding sites that are occupied by oxygen molecules relative to how many hemoglobin binding sites there are total. Each hemoglobin is able to carry four oxygen molecules for transport to the rest of the body.[88] Denoted as a percentage, normal saturation values for a healthy individual at sea level range between 96% and 98%.[89] Persistent hypoxemia is one of the major symptoms associated with

COVID-19, and hypoxemia has been found to be independently associated with in-hospital mortality in COVID-19 patients.[5] SpO_2 measurement has also been shown to be predictive of acute exacerbations in diseased individuals[90] and displayed a high negative predictive value (92-94%) for predicting severe illness in children.[91] Clinically, hypoxemia is defined as an SpO_2 below 95%,[18,92] and severe hypoxemia is defined as an SpO_2 value below 90%.[5,40] Patients experiencing severe hypoxemia require some form of oxygen therapy through supplementation or mechanical ventilation in cases of hypoxemic respiratory failure.[92] Oxygen concentrations in the blood are determined by a variety of factors, including ventilation and gas exchange, as specific to the lung.[89] As previously mentioned, SARS-CoV-2 viral infection and replication in alveolar epithelial cells causes excessive inflammation and acute lung injury, causing fluid exudation into the lung. The most prevalent of these injuries is diffuse alveolar damage, which directly damages the alveolar blood gas barrier at the lung where gas exchange occurs. The potentiated inflammation in this area also damages the pulmonary microvasculature, which includes the alveolar capillaries that transport blood adjacent to the alveoli to allow for oxygenation to occur.[3,6,43,44] This causes limited gas exchange capabilities, which is why oxygen levels in the blood fall below normal.

Spot check pulse oximetry is a standard noninvasive method for measuring oxygen concentration which uses light wavelengths to determine the ratio of oxygenated hemoglobin, providing an instant SpO_2 value for that given point in time.[88] While this is the clinical standard for SpO_2 measurement, continuous measurement of SpO_2 gives more extensive saturation data to analyze than intermittent collection[93,94] and spot check measurement fails to differentiate between natural fluctuations and acute exacerbations of SpO_2.[94] Oxygen saturation variability can be defined as the complex pattern of SpO_2 fluctuations[93] and gives insight into physiological

control of the oxygenation process as well as the integrity of the cardiorespiratory system. Variability analysis can be conducted using both linear and nonlinear methods. Linear analysis consists of basic variability measures such as mean, SD, root mean square (RMS), range, and average hourly change. Nonlinear variability analysis attempts to capture the true pattern of SpO_2 fluctuations through measurement of pattern regularity, complexity and self-similarity.[18,95]

Oxygen Saturation Variability and Complexity

In the field of physiology across all types of biological signals, the concept of homeostasis, or a state of equilibrium, is thought to be the overall goal for an organism and its many systems. However, the most basic understanding of homeostasis suggests that the human body is constantly stable, or at least in a "steady" state. Research has repeatedly demonstrated, however, that the human body is changing physiological states constantly- rest to wake, stress to relaxation, experiencing a wide range of emotions, and continuously adapting to changes to their environment and the stressors placed upon it (i.e. cold weather, exercise). Therefore, the systems use signaling, or regulatory information exchanged between the structural units of a system to elicit a physiological response, usually to maintain/return to a homeostatic state.[96] In contrast to classical concepts of physiologic control and homeostasis, the goal of these signals is not to maintain constancy or equilibrium,[97] but rather to successfully adapt to the constant stressors placed upon it.[98] Adaptation to these stressors is characterized by changes in signaling between regulatory components of the body, spanning from the molecular (cellular) to the systemic level (organ systems).[96] With this, we observe inherent variability in physiological signaling, representing the natural adaptation to ongoing changes in stimuli and stressors.[22,98–101] Variability, from its most basic standpoint, is defined as lack of consistency or fixed pattern.[102]

Therefore, variability observed in physiological signaling suggests responsivity to adaptation to potential stimuli, rather than lack of physiological control. This concept can be observed in HR signaling, where increased heart rate variability is suggestive of better autonomic cardiac regulation.[100,101]

Physiological variability can be observed across systems and their intrinsic signals, demonstrated across multiple time scales. This includes variable patterns seen between just seconds (HR) to months (hormonal cycles). Traditionally, physiological monitoring is conducted under two assumptions- 1) that these signals are largely stable (not variable) and 2) that the signal occurs on a singular time scale (linear). Under these assumptions, variability measurement of these signals is conducted at a singular point in time to provide one "tell-all" value that is compared to a normative value range for classification. As we have now discussed, physiological signaling is variable, and therefore single point measurement fails to account for natural variations in signaling, only giving indication as to what that signal looks like under those specific internal/external conditions. Furthermore, assumptions of linearity when assessing physiological signals limit clinicians from observing the depth of physiological signal patterns across multiple time scales.

Under these assumptions of stability and linearity, traditional metrics of variability such as mean and SD are often used to assess variable patterns in physiological signals. Mean values can be quickly compared to a normative value or range established for a given signal. While SD and other variation metrics do assess variability, these metrics fall under traditional views of homeostasis, where increased variation is thought to be indicative of poor physiological control rather than natural adaptation within a signal. While these signals provide very general and simplified insight into physiological function, these measures fail to recognize the complex and

fractal dynamics within physiological signals. Complexity is used to assess the interaction (information signaling) between regulatory components of a system. Changes in complexity represent an individual's ability to adapt to the stimuli placed upon it, to maintain or transition to a given physiological state.[98,100] In contrast to variability, complexity assesses the patterns created during adaptation to stimuli, rather than simply the level of variation from a steady state (baseline). The concept of variability v. complexity is shown in Figure 3. Variable signals are not necessarily complex, and complex patterns are not necessarily variable (i.e. reductions in variability do not automatically reflect reductions in complexity).[103] Signals can exhibit increased variability without complexity, illustrating a non-calculated and potentially maladaptive response to a stimulus. Furthermore, fractality is an important form of complexity, used to assess self-similarity of signaling across multiple time scales, such that small scale patterns represent larger scale patterns. Fractal patterns improve communication efficiency of information, something observed in the structures of systems (anatomy) and the ability to translate those signals for a physiological effect. Fractals are widely recognized in anatomical structures such as the bronchial and arterial trees, where the part (small) looks like the whole (large).[98] When assessing physiological signals, fractal patterns are those that exhibit the same patterns over small scales (seconds) to long term scales (hours). Examples of fractal patterns in anatomical structures and physiological signaling is shown in Figure 4. Complexity and fractality in physiological signaling has been well documented in HR (electrocardiogram) (ECG), nerve signals (electroencephalogram) (EEG), blood pressure and muscle outputs (electromyography).[98,100,104,105]

Both complexity and fractality acts as indicators of the ability of that system/signal to adapt to the physiological/pathological stimuli placed upon it. That is, loss of complexity and

fractality are characterized by a reduction of interaction between regulatory components, resulting in a hindered adaptive capacity of that signal. Individuals with higher baseline (non-stimulated at rest) levels of complexity exhibit a higher readiness and ability to respond to stressors.[100] Similarly, increased power observed in fractal patterns indicates stronger patterns within the data. With this, reductions in complexity and breakdowns of fractal patterns are usually observed in cases of aging or disease, often due to isolation or injury to one or more of the regulatory components of a physiological system.[100,106] These complex and fractal signals exhibit "hidden information" about physiological patterns that cannot be properly be quantified using conventional linear methods as signaling occurs on both nonlinear and multiple spatio-temporal scales.[97,107] Linear methods suggest that the level of variation will be proportional to the degree of effect (physiological response)[106] which is not the case for most physiological systems. An example of how linear methods can fail to detect the hidden dynamics of physiological signaling is shown in Figure 5 with HR signals. By conventional standards, these individuals exhibit the same level of variability, indicated by similar mean and SD values. In contrast, assessment of complex signaling illustrates the reduction in complexity observed with aging in the old subject, denoted by a lower entropy value. Therefore, to capture more accurate and dynamic variability of physiological signals, nonconventional nonlinear methods of analysis are indicated.[104]

Complex dynamics of a signal are assessed using a nonlinear analytical approach, quantified as entropy.[18,100,108] Contrary to the entropy definition used in physics, entropy from a mathematical and analytical standpoint is used to define the dynamic behavior of a variable, rather than inherent disorder.[98] That is, increased levels of entropy actually indicate a higher degree of physiological control, rather than disorder in signaling. Entropy analysis of

physiological variables began in the early 1990's,[108] but has yet to be defined in many physiological signals. To date, entropy analysis has primarily been used to define nonlinear variability patterns in HR, EEG (brain waves) and electromyography.[19,20,105,109] Analysis of these variables suggests that reductions in entropy are observed with aging and disease, representing the decrease in complexity (regulatory signaling) and compromised ability to adapt to stimuli of these signals/systems. With this, reduced entropy levels have been observed in older populations, individuals with coronary artery disease, Alzheimer's and epilepsy.[18,100,105,110,111] Similar to these pathological conditions, injury to gas exchange during COVID-19 infection may result in reduced entropy levels of SpO_2 signaling. This would suggest a decreased ability to maintain normoxic conditions with the increased stress of injury placed on the cardiorespiratory system, observed as exacerbations or hypoxemic episodes. Entropy analysis can be utilized to assess multiple types of nonlinear dynamics within SpO_2 signals, including patterns of regularity, complexity and fractality. Each of these nonlinear analytical approaches and how they will be used to assess these patterns in SpO_2 signaling will be discussed here.

The most basic form of nonlinear variability analysis, sample entropy is used to quantify the regularity of a variable in a time series, a method that is recognized amongst cardiovascular dynamic variable measurement.[18] The concept of sample entropy is displayed in Figure 6. More irregular signal patterns exhibit higher amounts of information, and therefore increased sample entropy. In contrast, more regular and predictable patterns exhibit less information, and therefore lower sample entropy. More regularity in signaling may be indicative of decreased sensitivity of the signal's regulatory components to stressors, and demonstrated by less informational regulatory inputs/outputs to physiological (SpO_2) signaling.[108] Several studies

have shown that differences in sample entropy can distinguish between normal and diseased participants with reductions in entropy occurring with pathology.[19,112,113] For example, increased regularity has been observed in EEG signals during an epileptic seizure, as well as decreased entropy in ECG signals during heart failure.[98,114] Reductions in entropy may also be predictive of future mortality from pathology.[95,98] Injury to the gas exchange process and therefore SpO_2 signaling observed during/after COVID-19 infection may contribute to increased regularity in signaling, denoted by decreased sample entropy levels in these individuals, but this has not yet been studied.

MSE quantifies the complexity of a variable in a time series and employs cross-scale correlations by calculating the sample entropy of a time series and its multiple derived sub-time series.[18] Multiple time series are created by repeated downsampling of the data by averaging adjacent data points together. That is, variability is quantified as sample entropy levels for "beat to beat" measurement of SpO_2, including analysis of every consecutive SpO_2 value (scale 1) up to analysis of the average of every 10 consecutive SpO_2 values (scale 10). Visualization of the downsampling process is depicted in Figure 7. Interpretation of these sample entropy levels across scale gives indication of complexity v. randomness in a signal. If the downsampling process cancels out sample entropy, a signal exhibits randomness rather than complexity. This suggests that non-physiological "noise" created the variations in the signal, rather than calculated engagement of a system in an effort to adapt to a stressor, creating a false front of complexity that is not consistently observed over different time series. Continuous increases in sample entropy levels across scale despite the downsampling process, however, suggests true complexity within a signal.[110] Complexity observed in SpO_2 signaling indicates that there are patterns to be recognized within the signal, rather than random fluctuations that cannot be quantified. As

previously discussed, complexity has further been defined as dynamic interaction of regulatory components that work together to allow for adaptation to stressors.[18,100] Therefore, this analysis indicates the ability of SpO_2 to properly regulate itself, despite the potential stressors placed upon it (COVID-19 related injury). Previously used to analyze other physiological variables, reduced MSE has been indicated as weakened engagement of the control system (reduced adaptive signaling amongst its regulatory components)[18,95] and has been shown to be predictive of future decline and poor health outcomes.[20] Similarly, as previously mentioned, aging and disease has been shown to result in decreased complexity signaling.[18] Therefore, persisting injuries sustained during COVID-19 infection could emulate that of early aging to the system and its structural units, seen as decreased complexity.

The final nonlinear method, DFA, is used to identify fractal-like (self-similarity) patterns in a fluctuating time-series,[18] where small scale patterns are representative of the larger scale pattern.[100] DFA assesses self-similarity patterns of a variable across multiple time scales using correlations.[18,100] This method further allows researchers to distinguish meaningful fractal properties from potentially random and nonphysiologically influenced patterns,[100,104] adding to the specificity of this analysis. As previously mentioned, fractality in signaling suggests that variations over a small amount of time are representative of longer time series. While smaller scales may be representative of larger scales, this method also allows comparison of complexity levels between scales,[18] which may be a helpful indicator for researchers of future investigations as to what is the most informational and efficient length of signal monitoring is to properly capture and assess these patterns. By confirming that SpO_2 signaling is in fact fractal, it allows researchers to now classify significant patterns in signaling, similar to that of ECG patterns.[106] Similar to other nonlinear metrics, decreased fractality of physiological signals occurs with aging

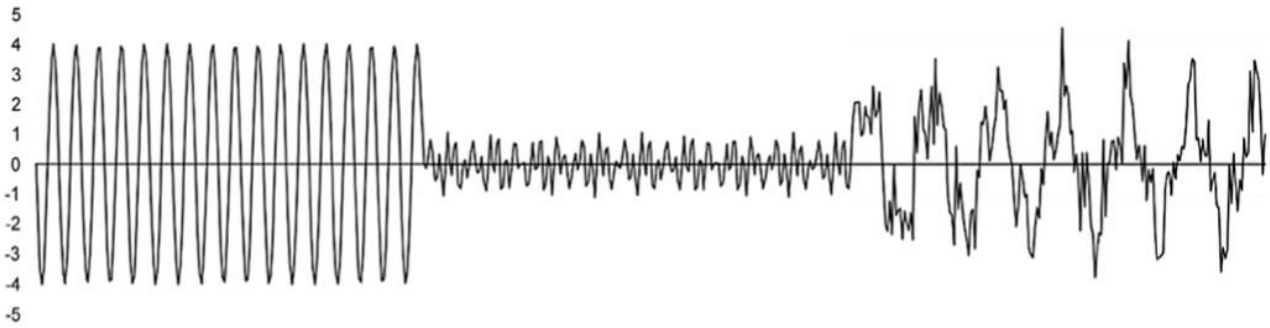
and disease.[100,106,111] This breakdown of fractal patterns may be associated with excessive regularity (similar to that of decreased entropy), classified by repetitive, periodic (and most likely pathological) signaling.[100,104,115] COVID-19 infection could potentially reduce the power observed in fractal patterns of SpO_2, serving as a predictor of adverse outcomes such as future respiratory decline. Collectively, the utilization of nonlinear analysis of oxygen saturation variability to supplement traditional linear methods will provide a novel perspective of complex control of gas exchange and oxygenation homeostasis as it relates to potential persisting injury from COVID-19 infection.

Bhogal et al. was the first to attempt to quantify "normal" oxygen saturation variability using continuous SpO_2 monitoring in adults, as it has only been previously established in infants. This study functioned to provide an important baseline for understanding the intrinsic patterns within oxygen saturation variability, as much of its theory was based upon variability measures of other physiological signals (i.e. heart rate variability). These novel findings showed that oxygen saturation variability exhibits a fractal-like pattern, and that variability is better indicated by long-term measure as denoted by increased complexity in long-term scales. Bhogal also identified an inverse relationship between SpO_2 and sample entropy, where there was higher entropy seen at lower SpO_2 values. Findings suggest that there is tighter control system coupling at lower saturations, further suggesting that system uncoupling seen in aging and disease could be indicated by lower complexity. Thus, Bhogal and colleagues proposed that nonlinear pattern analysis could be utilized to study network physiology, examining how organ systems work together to control a given physiological variable.[18] Another study attempted to observe changes in oxygen saturation variability by introducing healthy individuals to a hypoxic stimulus, anticipating increased signaling between regulatory components (increased complexity) to be

activated to maintain SpO_2. When exposed to graded normobaric hypoxia, individuals displayed increased sample entropy and SD fluctuations in SpO_2. Nonlinear analysis was also found to be more sensitive to the stimulus than linear variables, however linear variability measures were strongly correlated with sample entropy. Furthermore, Costello identified a significant correlation between entropy and dyspnea during hypoxia, and sample entropy proved to be more predictive of hypoxemia than other nonlinear analysis variables.[95]

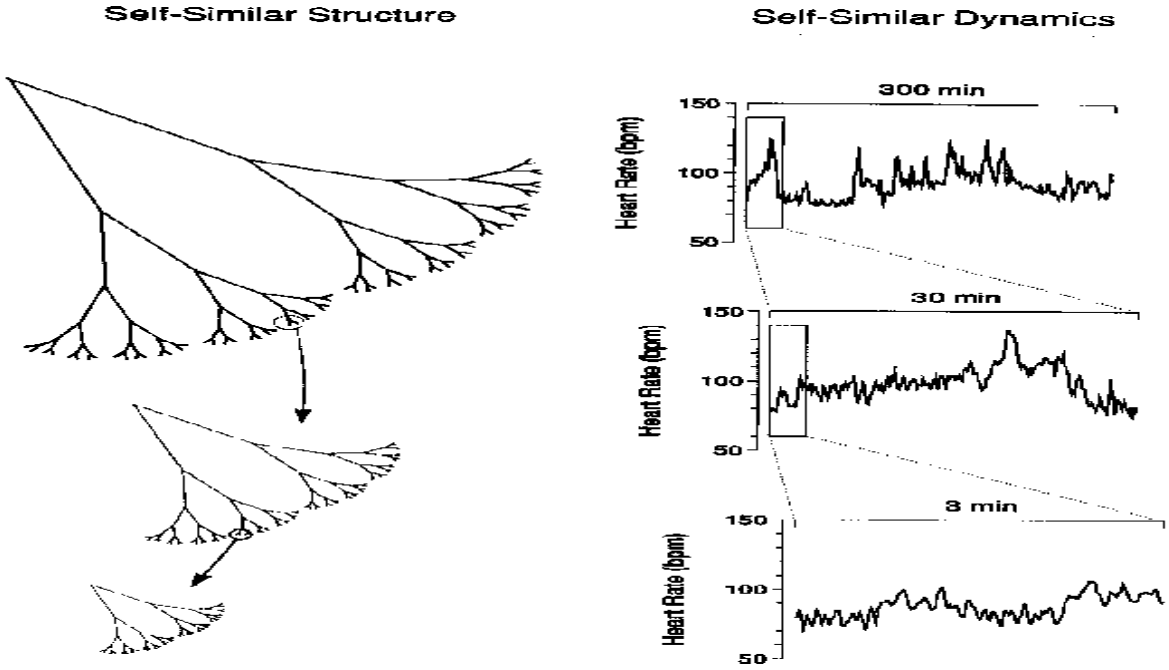
Although complexity analysis has not been conducted in clinical populations, oxygen saturation variability has been studied in these individuals. Buekers et al. collected seven-day continuous pulse oximetry measurement in 20 individuals with COPD. Results revealed an average daily fluctuation of 10.8% for these subjects, with 3.2% fluctuations during 5% of daytime resting values.[94] These findings reiterate previous concerns of false identification of acute exacerbations when discounting natural fluctuations[18,94] and represent the applicability of extended continuous SpO_2 measurement in individuals with respiratory conditions.[94] Therefore, nonlinear variability analysis can be used to observe the natural variability of SpO_2 signaling over an extended period of time, and therefore define true bouts of potential hypoxemia in these populations.

Figure 3. Variability v. Complexity[99]



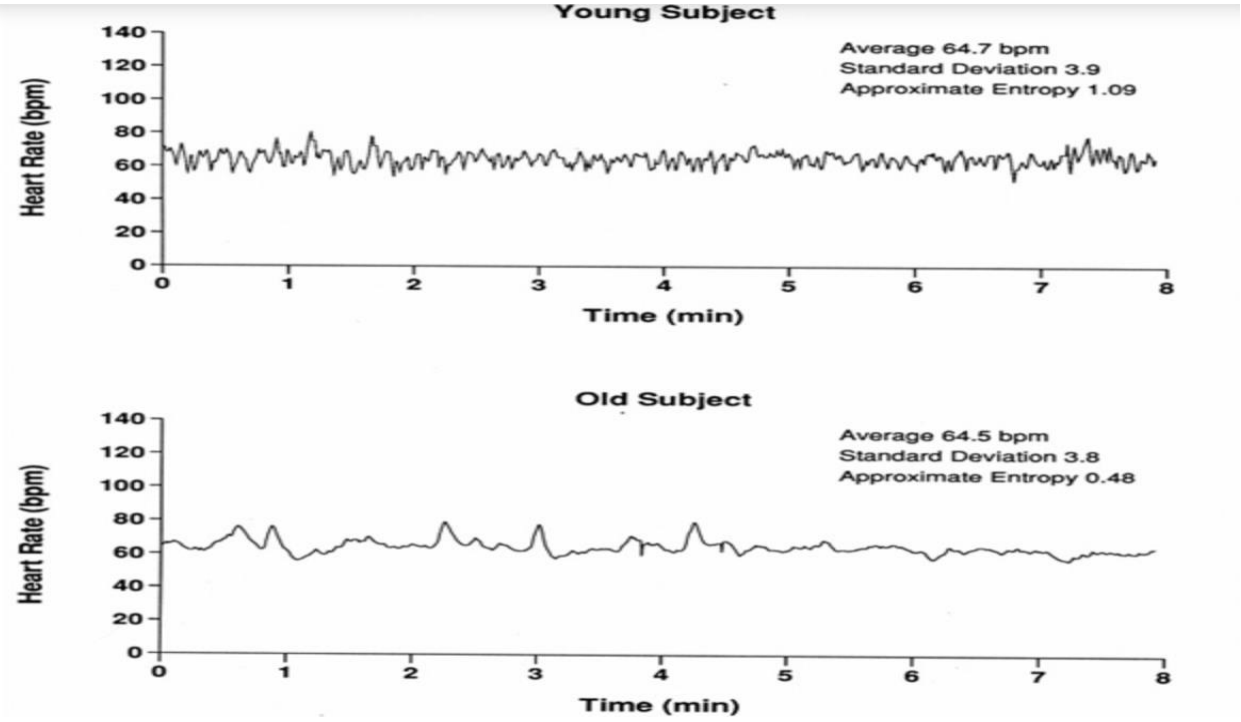
Concepts of variability compared to complexity. Variability in a signal, demonstrated as large deviations from the mean of a sine wave dones not equate to complexity. This concept can be observed in a signal that is variable but not complex (left), complex but not variable (middle), or both variable and complex (right).

Figure 4. Fractals in Anatomical Structures and Physiological Signaling[100]



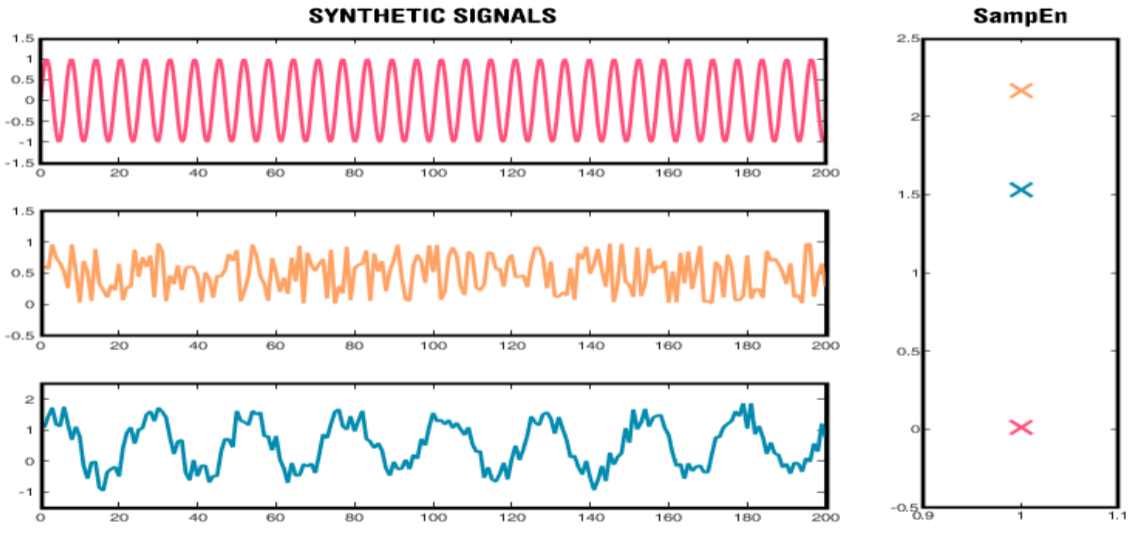
Examples of fractal patterns observed in anatomical structures and physiological signaling. Spatial patterns are observed in anatomical structures such as arterial tree. HR signaling fluctuation patterns appear similar for smaller time scales of three minutes (bottom) as they look at larger scales of 30 minutes (middle) and 300 minutes (top).

Figure 5. Linear and Nonlinear Measurement of Heart Rate Variability[100]



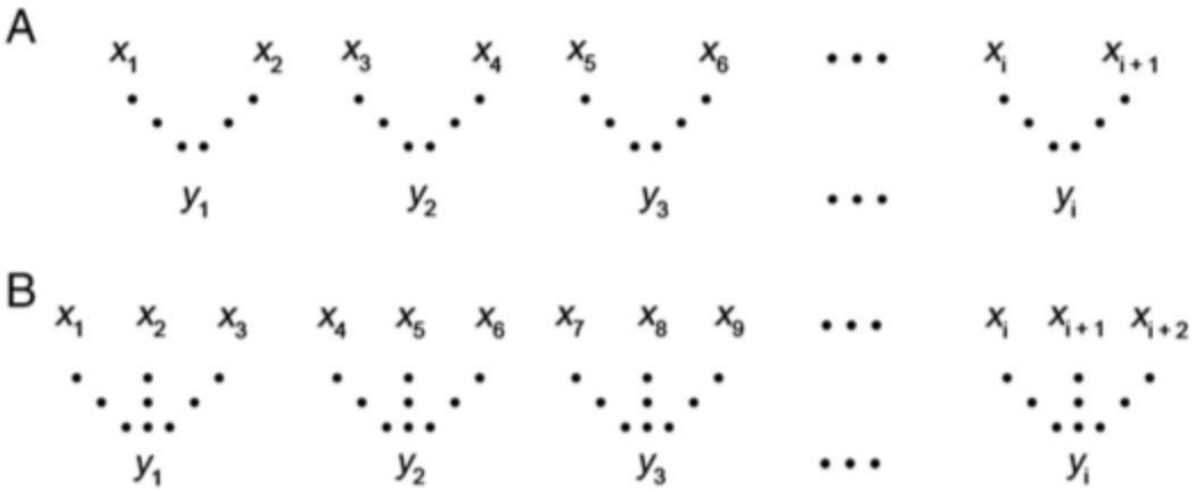
Variability analysis of a heart rate tracing of a young (top) and old (bottom) subject. Linear methods of variability mean and SD suggest nearly identical levels of cardiovascular control between subjects. Nonlinear analysis of variability using entropy, however, suggests that the complex cardiovascular dynamics are much lower in the old subject than the young.

Figure 6. Examples of Sample Entropy[116]



Examples of signals with varying levels of sample entropy. More irregular signals exhibit higher levels of sample entropy (orange) while more predictable signals exhibit lower sample entropy (red).

Figure 7. Downsampling Process Used for MSE Analysis[110]



Examples of the downsampling process for MSE anaylsis for scale 2 (A) and scale 3 (B). Each scale is created by averaging adjacent data points together. For scale 2, every two consecutive data points are averaged together to create a singular value (y1). For scale 3, every three consecutive data points are averaged together to create a singular value. This process is repeated for a given number of scales throughout the time series or data collection period.

Cardiorespiratory Network Physiology Analysis

Just as singular signals have regulatory components that exchange information, organ systems have similar structures of regulatory components that interact for proper function as well. These regulatory components create a physiological network that is in constant communication. An example of a network components and how they interconnect are displayed in Figure 8. The nodes of a network represent each regulatory component while the edges demonstrate the information signaling that connects them. This can be seen as system coupling, and is observed throughout physiological processes as control systems work together to maintain their perspective functions.[23,107] Examples of coupled physiological systems include cardiorespiratory, cardiovascular, neuromuscular, neuroendocrine systems, etc. These integrated systems are again composed of several regulatory components that interact in a network, constantly exchanging information between them to elicit physiological responses. Using exercise as an example, network interactions are stimulated amongst several components of the

cardiorespiratory system, including HR, breathing rate, blood pressure and skin temperature. These components all communicate bidirectionally between each other to maintain adequate blood flow to working muscles, avoid hypoxemia, regulate body temperature, adjust blood pressure to accommodate increases in HR in a simultaneous manner. Injury to one or more of these components therefore can consequently cause an entire physiological network to breakdown. These types of breakdowns are observed during aging and disease and can be classified as system uncoupling. In the example above, this means the cardiovascular and respiratory systems are no longer communicating to allow for adequate adaptation to the stimuli placed upon it, resulting in poor physiological or pathological control of its signals (i.e. tachycardia for HR, hypoxemia for SpO_2, heat stroke for skin temperature).

These network interactions amongst physiological systems can be quantified and compared using network analysis. Similar to sample entropy levels assessed within singular physiological signals, these interactions of information exchange between multiple physiological signals are measured as transfer entropy.[93] Reductions or interruptions in network signaling are demonstrated with disease and aging, and are indicated by reduced transfer entropy levels. In a healthy working network, exposure to physiological or pathological stimuli should evoke an increase in network information signaling amongst regulatory components, denoted by an increase in transfer entropy levels. In cases of aging and disease, degradation of network signaling is observed (reduced transfer entropy), compromising the ability of that system/network to adapt to the given stimulus. While not extensively studied in the cardiorespiratory system, network analysis has been conducted on other physiological systems such as the brain. The brain as a network has been assessed not only at rest, but under several pathological stimuli including network responses to insomnia, depression, mental disorders and

post-traumatic stress disorder.[21,23,109,117,118] These investigations successfully captured significant information processing between components of neurological signaling and indicate that pathological stimuli may result in negative alterations to the system such as reductions in transfer entropy levels within the network. Negative alterations to these networks may be suggestive of systemic risk, observed as vulnerability, symptomology and adaptability.[21–23,98] The results of these studies also suggest that there may be a hierarchy amongst the regulatory components of a system, suggesting that certain parts of a network may be more influential to desired system outputs than others. In the cardiorespiratory system, for example, physiological signals may differentially contribute to the maintenance and regulation of SpO_2, and vice versa. Reductions in network signaling due to aging or disease therefore also may result in modification of this structural hierarchy,[98] where some signals may have to overcompensate for the injury to others, or there is failure to compensate altogether. By identifying points of potential injury and determining the magnitude to which they contribute to other signals, clinicians and researchers may be more equipped to understand and treat signals of interest with therapeutic intervention. In summary, network analysis is used to assess the degree of integration amongst the regulatory components of a system/network, assess the ability of these components to adapt to potential stressors, and to identify a hierarchy of physiological structure and control amongst these components (i.e. the magnitude to which each component contributes to proper function).

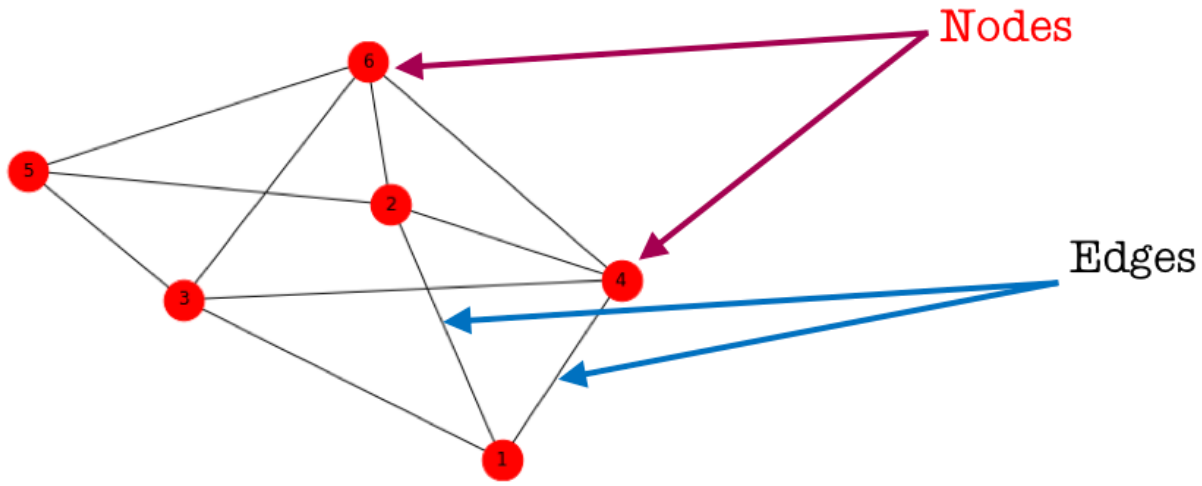
As previously discussed, the cardiorespiratory system is made up of several regulatory components that continuously interact to maintain proper physiological function. These include SpO_2, HR, breathing rate and skin temperature. In cases of cardiorespiratory injury, often observed during and following COVID-19 infection, these regulatory components may not properly compensate for the stressors placed up on them, resulting in dysfunction of the overall

system seen as cardiorespiratory uncoupling. Representation of the signals used for the proposed cardiorespiratory network analysis are displayed in Figure 9. These signals were explored due to their established physiological interdependence as well as due to the potential effect COVID-19 infection may have on each component. Post-COVID effects on HR have been reported due to inflammatory damage to heart tissue at ACE2 receptors and to the endothelial tissue of the heart's microcirculation.[119] These persisting injuries may cause heart palpitations, breathlessness, and autonomic dysfunction of HR regulation.[120,121] Other COVID-related respiratory symptomology include exacerbations of hypoxemia and dyspnea followed by increases in respiratory effort to compensate for low SpO_2 and decreased air flow. These symptomologies simultaneously effect SpO_2, respiratory rate and subsequent HR regulation due to respiratory control. Similar to the heart and lungs, endothelial tissue throughout the body is damaged, leaving the body's microcirculation in a damaged and procoagulant state.[9,10,119,121] These injuries may cause disruptions to thermoregulation in the body, effecting skin temperature as a result. Thermoregulatory signaling may result in changes to respiratory rate and HR, subsequently affecting SpO_2. Furthermore, neurological manifestations of COVID-19 indicate injury to the hypothalamus, a portion of the brain that is responsible for several physiological processes including thermoregulation, respiratory control and cardiovascular regulation- directly impacting each of the proposed signals.[122,123] As the effects of COVID infection extend further than just the lungs, it is important to explore the cardiorespiratory system as a whole when discussing these highly interdependent signals.

Network physiology analysis assessing cardiorespiratory signaling regarding SpO_2 control has only been conducted once prior to the current work. Costello et al. employed a network physiology approach to understanding cardiorespiratory control of oxygen saturation variability,

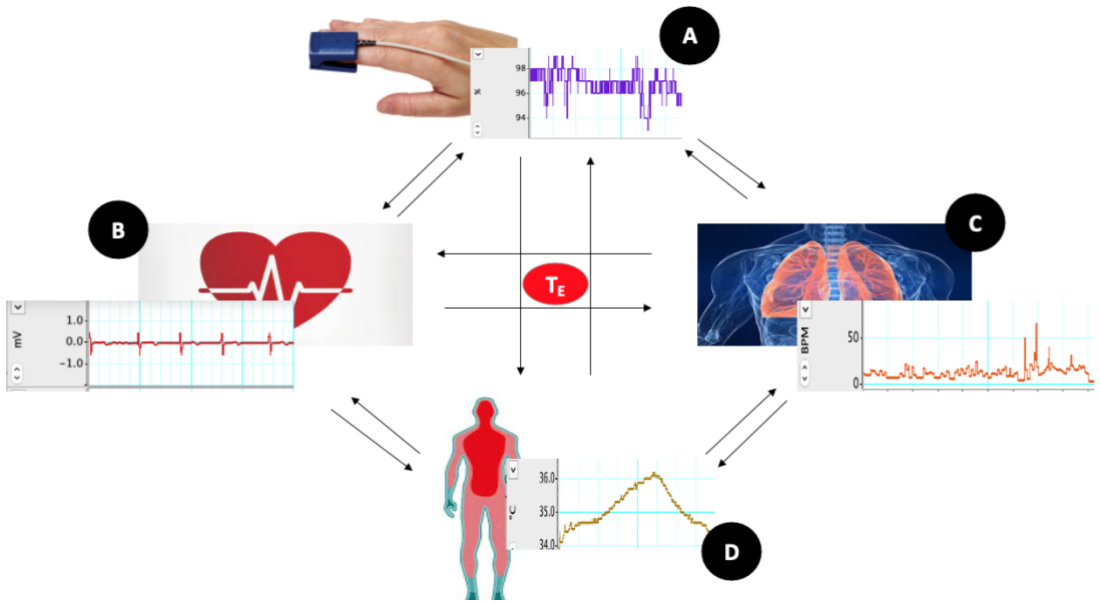
assessing transfer entropy between simultaneous respiratory rate, tidal volume, minute ventilation, HR, SpO_2, and both end-tidal O_2 and CO_2 signals during exposure to graded normobaric hypoxia.[93] This novel analysis revealed a significant exchange of information between these cardiorespiratory signals, giving insight into the integrity of the overall physiological system. The current work will set out to understand the flow of information between similar cardiorespiratory variables (SpO_2, HR, respiratory rate and skin temperature) when challenged with a pathological (previous COVID-19 infection) rather than environmental (graded hypoxia) stimulus.

Figure 8. Example of General Interconnect Network[124]



Example of an interconnected network. Nodes represent the components of a network and edges represent the relationships that connect them. In physiological analysis, nodes represent physiological signals, and edges represent the exchanges of information that are communicated between them.

Figure 9. Proposed Cardiorespiratory Signals for Network Physiology Analysis



Representation of proposed dynamic interaction of transfer entropy (T_E) between cardiorespiratory signals including: SpO_2 (A), ECG (B), respiratory rate (C) and skin temperature (D).

Summary

The acute lung injury, chronic inflammation and subsequent respiratory complications observed in COVID-19 patients have important implications for potential sequelae on the respiratory system and methodology that should be used to assess recovery status in these individuals. Existing follow-up studies evaluating respiratory function in COVID-19 survivors suggest that abnormalities seen during the infection phase may persist months into the recovery period, similar to that seen in survivors of SARS-CoV, MERS-CoV, ARDS and COPD.[6,8,56,57,59,60,76–78,125,9,11–13,41,43,53,55] With this, chronic inflammation poses its own enduring challenges for COVID-19 survivors including a suppressed immune system and increased susceptibility for the development of future infections and general respiratory decline.[3,6,9,40,42,62,63] These attributes of COVID-19 infection and recovery can be assessed both in a clinical and home setting, allowing

for increased accessibility to this population. Standard pulmonary function values identify restrictive lung patterns and elaborate on the source of diminished lung capacity observed in these individuals.[17,55,68,69,71,75,76] Additionally, both systemic inflammation and inflammation specific to the airway can be assessed using salivary biomarker samples[3,5,7,12,40,62,63] and exhaled nitric oxide measurement,[80–82,87,126] respectively. Furthermore, quantifying oxygen saturation variability and transfer entropy in COVID-19 patients will offer mechanistic insight into the persistent hypoxemia that is hallmark to this population and the degree of control exhibited by the cardiorespiratory system as it functions holistically,[5,18,89–91,93–95,100] in contrast to standard spot-check measurement. These methodologies will collectively allow for evaluation of respiratory function in COVID-19 survivors and supplement the forthcoming literature of recovery assessment and prolonged respiratory health in this population.

Chapter 3: Methods

Participants

57 adults (both men and women) ages 18 to 65 were recruited to participate in this study. The participants were allocated to two groups- one case group of participants who have previously been diagnosed with COVID-19 infection (n=24) and a control group with no history of COVID-19 (n=33). The diagnosis of the participants in the case group was confirmed via documentation of a clinical diagnosis including either a positive viral test or positive antibody test. Participants in the control group were required to provide documentation of both a negative viral test to indicate no active infection and a negative antibody test to rule out previous infection. Documentation of one of these negative tests was required in the two weeks prior to the participant enrolling in the study. All participants were required to provide documentation of a negative viral test within two weeks prior to contacting researchers to prevent possible transmission. Sex, age, and race-/ethnicity were similar between recovering COVID-19 individuals and healthy controls. Prior to participation, subjects completed a screening questionnaire for COVID-19 exposure/infection to confirm their eligibility. The screening questionnaire provided a list of common COVID-19 symptoms and asked about their recent travels and contact with persons who had COVID-19 in the last two weeks as well as any symptomology they may have experienced within the last two weeks. All participants provided oral informed consent prior to study involvement in accordance with university institutional review board guidelines.

Exclusion Criteria

Participants were excluded from the study if they were under the age of 18 and/or if they had a pacemaker. Additionally, participants who experienced symptoms, had contact with someone who has COVID-19, or have traveled out of state in the two weeks prior were not allowed to participate in this study. Participants who were unwilling or unable to engage in Zoom calls with the researcher were also excluded from the study. Finally, participants who did not complete both respiratory measures (pulmonary function and exhaled nitric oxide) were excluded from analysis.

Study Design

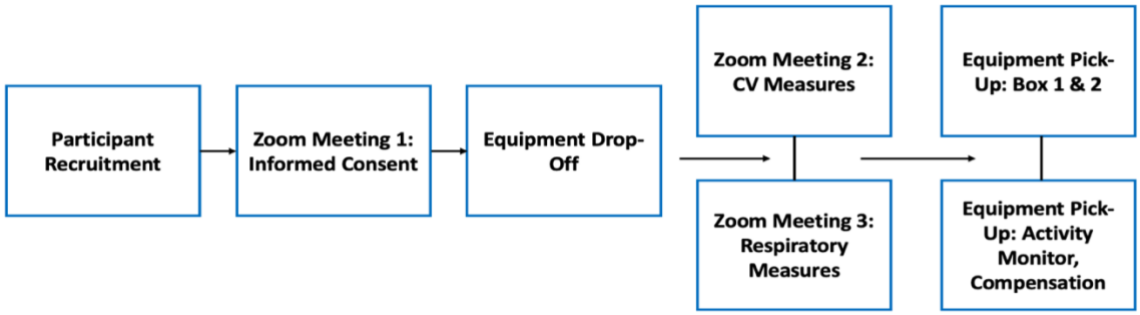
This study used a case-control design, with the case group consisting of participants with a history of COVID-19 infection and a control group of participants with no history of COVID-19. The study was conducted remotely with limited to no face-to-face interaction to prevent potential exposure of both participants and researchers to COVID-19. All physiology measurements were completed by the participant with assistance from research personnel via Zoom. Due to the remote functionality of this study, the consent process included an oral informed consent conducted via Zoom. During this Zoom meeting a member of the research team explained the study purpose and methods to the potential participant. Oral consent was then provided by the participant if they met all inclusion criteria and agreed to participate in the study. All study procedures were approved by the Syracuse University Institutional Review Board.

Upon completing the oral consent process, researchers coordinated a date and time to delivery study equipment to the participant's home. The equipment was delivered in two boxes, one (box 1) with cardiovascular equipment and the other (box 2) with respiratory equipment.

These two boxes corresponded to two additional Zoom meetings. When university research restrictions lifted, two brief, socially distanced 15-minute in-person meetings were held at the Syracuse University Human Performance Laboratory for data collection in place of these two Zoom meetings. Two separate meetings were decided upon to minimize participants burden and to maximize the efficiency of testing. Box 1 containing cardiovascular equipment included a 24-hour ambulatory blood pressure monitor, an activity monitor, a fingertip pulse oximeter, a digital scale, a HR monitor, a saliva collection kit (containing a collection tube and funnel for saliva collection aid) and an instruction sheet for all measures. Box 2 containing respiratory equipment included a handheld spirometer to assess lung function, a handheld NiOX machine to measure airway inflammation, an Equivital EQ02 LifeMonitor (chest bioharness) to assess continuous blood SpO_2 and cardiorespiratory measures and a new instruction sheet.

Prior to both meetings participants were asked to undergo a three-hour fast (no food or sugary drinks for at least three hours) as food or drink containing nitrates may affect FeNO measurement.[127,128] Participants were also asked to refrain from exercise, alcohol, smoking and caffeinated beverages for at least 12 hours. Equipment from each Zoom meeting besides the activity monitor was picked up the day following the meeting. The activity monitor was picked up nine days after the first meeting at the participant's home after completing data collection. At this time, researchers dropped off a standard blood pressure cuff as compensation for participation in the study. Full study design is displayed in Figure 10.

Figure 10. Study Design



Study design from initial participant recruitment through completion of all study measures.

Anthropometrics

Height, weight and body composition were collected for anthropometric data. Height was self-reported by the participant to researchers during remote collection. This measure has been found valid for assessing height in adults under the age of 60.[129] For those who completed in-person data collection, height was also assessed using an automated stadiometer in addition to self-reported measurement. A digital scale equipped with bioelectrical impedance analysis was used to obtain both weight and body fat percentage. Body mass index was calculated from height and weight as kg/m^2.

Pulmonary Function Testing

Lung function was assessed via pulmonary function testing using a handheld CareFusion MicroGP Spirometer (Becton, Dickinson and Company, Franklin Lakes, NJ). This device was successfully applied in home spirometry monitoring in diseased populations where it demonstrated sufficient validity and reliability, as well as highly predictable in early identification of disease progression and FVC decline in this cohort.[130] Additionally, this spirometer has been standardized against both American Thoracic Society and European Respiratory Society guidelines.[131] A Viromax™ bacterial-viral filter (A-M Systems, Sequim, WA) was attached to the spirometer (>99.99% viral filtration efficiency) to prevent the spread of

COVID-19 through airborne aerosol particles.[132] Participants were asked to wear the provided nose clip during the test to ensure all exhaled air is directed through the mouth. They were then asked to assume a seated, upright position with feet uncrossed and flat on the floor. After inserting the viral filter mouthpiece, participants input their subject information (sex, age, height) as well as an ethnic correction factor as directed by the CareFusion MicroGP company. Three trials were completed consisting of one deep breath that provided both absolute and percentile (of expected value for inputted subject information) of four measures of pulmonary function: FVC, FEV1, FEV1/FVC and PEF. FVC is the amount of air exhaled forcefully and quickly out of the lungs after maximal inflation and emptying the lungs completely. FEV1 is a measurement of the amount of air that is expired in the first second (1 second) of the test. FEV1/FVC is the proportion of the total forced vital capacity that is exhaled in 1 second. PEF is a measurement of air flow out of the lungs and the maximal flow of air achieved during the sharp expiration of the test. These four lung function measurements were collected simultaneously during the same spirometry maneuver and give insight into lung function, strength of expiratory muscles and the general condition of the airway. They were then asked to place the nose clip on their nose and take a deep breath, maximally inflating their lungs. Participants then placed the mouthpiece in their mouth and close their lips tightly. Research personnel instructed the participant to exhale as quickly and forcefully as they could into the mouthpiece. To ensure the participant fully emptied their lungs, the researcher encouraged them to exhale for three seconds even after the lungs felt empty to void of any excess air. Participants completed this maneuver for a total of three times, and the highest score of the three trials was taken as their final score for each measure.

Airway Inflammation

Airway inflammation was measured by quantifying exhaled nitric oxide levels using the NiOx VERO™ FeNO device (Circassia, Morrisville, NC). Elevated nitric oxide levels in expired breath are indicative of airway inflammation caused by respiratory distress. This device has shown sufficient validity and reliability in monitoring of patients with asthma.[133] The NiOx VERO™ FeNO device uses a disposable mouthpiece to minimize transmission potential. Although this type of testing is not an aerosol generating procedure, the disposable mouthpiece included a bacterial-viral filter with over 99.97% viral filtration efficiency and has been tested with microbes up to four times smaller than SARS-CoV-2, indicating its efficiency in filtration.[134] Prior to beginning the testing procedure, participants were instructed to assume a seated, upright position with feet uncrossed and flat on the floor. This test was completed by first inhaling through the mouthpiece as directed by the prompt on the display screen followed by exhaling into the mouthpiece at a steady flow rate of 50 mL/s for approximately 10 seconds. The NiOX VERO utilizes an electrochemical sensor, reporting the fractional concentration of exhaled nitric oxide in approximately 60 seconds on the display screen.

Arterial Blood Oxygen Saturation

Noninvasive spot check pulse oximetry at rest was conducted to measure arterial blood oxygen saturation (SaO_2) using a fingertip pulse oximeter on the index finger. The pulse oximeter measures infrared and red-light wavelength absorption v. reflection to determine the ratio of oxygenated hemoglobin to total hemoglobin, reported at as a percentage.

Continuous Cardiorespiratory Monitoring

Continuous SpO_2 monitoring and other continuous cardiorespiratory measures were collected using the Equivital EQ02 LifeMonitor (Equivital Hidalgo, Cambridge, UK). This system is a three-piece ambulatory multi-parameter telemetry device consisting of a bioharness with three textile sensors that was worn around the lower chest attached to a Sensor Electronics Module (SEM) and wired fingertip pulse oximeter. The Equivital EQ02 LifeMonitor has previously demonstrated high validity and reliability for ECG and skin temperature signaling and the feasibility of wear for collecting multiple signals simultaneously makes it desirable for continuous physiological data collection.[135,136] Although the device has not been implemented with diseased populations, it has been used in several cohorts including healthy individuals, military personnel, athletes and occupational measurement.[135–137] Its primary use was for continuous pulse oximetry measurement but was also used to simultaneously collect important variables of cardiorespiratory function including HR derived from ECG, respiratory rate, skin temperature. SpO_2 was collected using the Nonin wired pulse oximeter ancillary sensor, connected directly to the sensor belt, relaying SpO_2 values 1/15 s (2 Hz) directly to the SEM to create one continuous signal. Two chest leads built into the textile sensors of the sensor belt captured ECG at a sampling rate of 256 Hz. HR was then calculated directly from the ECG channel using the cyclic measurement function in LabChart. Respiratory rate was derived using the cyclic measurement function directly from an intrinsic chest expansion signal, captured using the textile sensors located on the chest at sampling rate of 25.6 Hz. Skin temperature was collected every 15 seconds via medical grade infra-red thermometer located on the sensor belt under the left arm.

Prior to device setup participants were asked to remove any nail polish from their index finger, any lotions or oils from their chest region and to wash their hands thoroughly. The researcher instructed the participant in how to set up and wear the device on the Zoom meeting. The participant was instructed to moisten the textile sensors using clean water to help keep close contact with the skin and strengthen all signals. The bioharness was worn with direct contact with the skin under any sort of clothing or undergarment. The pulse oximeter was worn on tip of the index finger of the participant's choice, usually their nondominant hand. Once the device setup was complete the participant was asked to wear the Equivital EQ02 LifeMonitor for four daytime hours during which they completed their normal daily activity. Data collection was standardized to begin between 8:00-10:00 am to account for the circadian rhythm of this signal and was carried out for four continuous hours. Four-hour data collection was chosen as the device previously displayed superior reliability during shorter time periods (four hours v. eight hours) however was still long enough to capture the natural fluctuation of SpO_2 signals. A previous study showed that a minimum of one hour was necessary to observe SpO_2 variability, however this same study also observed that oxygen saturation variability was predominantly made up of long-term variations.[18] Therefore, to see this higher degree of variability, a longer collection window was chosen. Participants were asked to refrain from consumption of caffeine and alcohol, exercise, smoking, prolonged exposure of equipment to direct sunlight, and immersion of any equipment in water as these can affect one or more of the cardiorespiratory signals.

Oxygen Saturation Variability

Oxygen saturation variability was calculated using both linear and nonlinear analysis methodology using the continuous SpO_2 signal captured over four hours. Linear analysis is a more elementary method of looking at variability, including variables such as mean, SD, RMS, and average hourly change. However, to get the true essence of SpO_2 fluctuations, nonlinear analysis looking at entropy patterns on several scales is more representative of true variations in SpO_2. Nonlinear analysis methods include sample entropy assessing the regularity and predictability of the variable, MSE assessing the complexity of the variable, and DFA to identify fractal-like behavior of the variable. Low entropy values of SpO_2 have previously indicated cardiorespiratory uncoupling and lack of physiological control. Finally, network physiology analysis was used to quantify the interaction between four of these cardiorespiratory signals (continuous SpO_2, HR derived from ECG, respiratory rate, and skin temperature) measured as transfer entropy.

SpO_2 Signal Processing

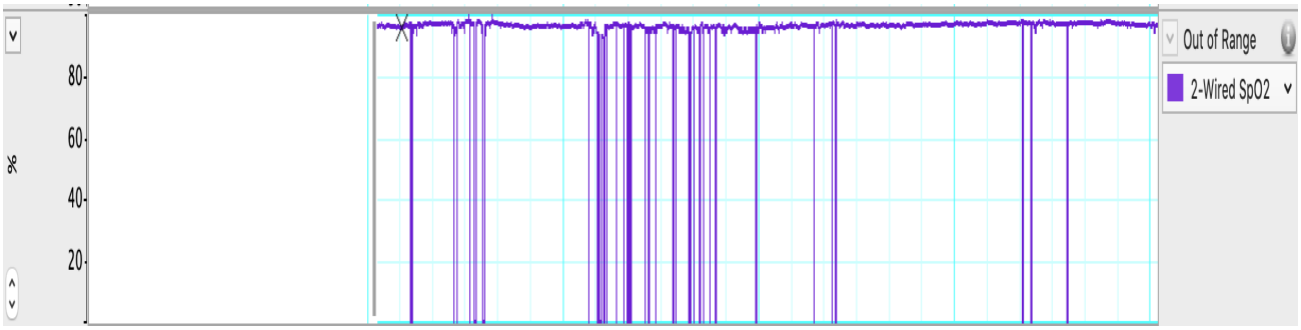
SpO_2 was collected every 15 seconds continuously for four hours at a sampling rate of 2 Hz. All files were downloaded from the device to the Equivital Manager software and subsequently converted to a LabChart files consisting of eight channels.[138] Data files were considered complete if the subject completed at least 3.5 hours of data collection, and the raw signal was not lost for greater than 15 minutes during collection due to device removal or excessive artifact. Complete files were then "cleaned" using a combination of internal arithmetic functions within LabChart. The algorithm included Threshold, Smoothsec, Window and NanRemover functions.[139] A summary of each function and its use for the data can be found in Table 2. A

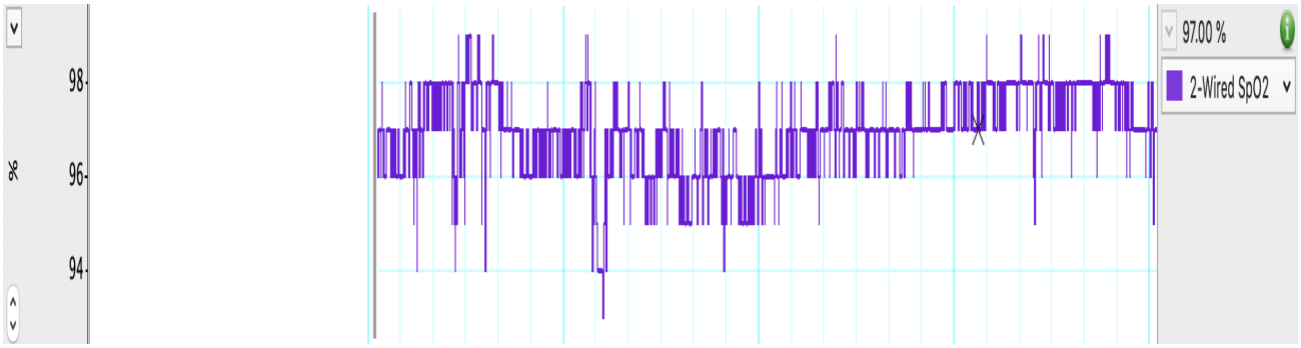
minimum threshold of 90% was chosen for the data file, such that if the raw signal read values of below 90%, the algorithm interpolated the mean value for the overall signal in its place. This threshold was chosen to eliminate artifact and preserve the natural variability of the signal as it can be assumed that none of our participants were experiencing severe hypoxemia such that they would require a supplemental oxygen requirement. Examples of a raw and clean data file can be seen in Figure 11.

Table 2. LabChart Arithmetic Cleaning Functions

Threshold (90)	Detects data points that fall below 90%
Smoothsec (1)	Calculates a moving average in a specified sliding window of one second
Window (90,100)	Detects SpO_2 data within the range of 90-100%, any data points outside this range assigned a zero
NanRemover	Removes Not a Number (Nan) and out of range data from data file to create one continuous signal

Figure 11. Example Raw and Clean SpO_2 File





Examples of raw (top) and cleaned (bottom) extended SpO_2 files for four continuous hours. Cleaned files include data file treated with combination cleaning functions from Table 2.

Linear Analyses

Linear analysis of clean files was conducted directly in LabChart using the DataPad function. SpO_2 mean, SD, and RMS were calculated for the entire four-hour collection period. The range for each of the four hours was calculated individually and these four values were then averaged to derive the average hourly change in SpO_2 for the overall file. An example data output for linear analysis from DataPad can be found in Figure 12.

Figure 12. Example Linear Oxygen Saturation Variability DataPad Output

COVID_00312 Clean.adicht — Data Pad View

3,660,000 points from t=3:11.4062 to t=4:01:28.2812

	A	B	C	D	E	F	G
	2-Wired SpO2 Mean %	SpO2 Standard Deviation %	2-Wired SpO2 RMS %	SpO2 Maximum - Minimum %			
1	96.9283	0.9153	96.9327	6.0			
2	Mean	Std Dev	RMS	Max - Min			
3	%	%	%	%			
4	96.9283366	0.915305801	96.9326582	6.00000005			
5							

Example of four-hour output of linear oxygen saturation variability measures using DataPad function in LabChart.

Nonlinear Analyses

1. *Sample Entropy*. Sample entropy was calculated to assess the degree of regularity of the continuous SpO_2 signal in our time series (four hours). This value calculates the probability that an event (SpO_2 fluctuation) during a set window length described as "m", with tolerance "r", will be repeated later in a time series.[18,140] For the current analysis, sample entropy was calculated where "m" was set at 2 and "r" at 0.2 as described in previous studies.[18,140,141] Low sample entropy indicates regularity in a signal and decreased complexity, and potential isolation of regulatory components of cardiorespiratory control.[140] In contrast, high sample entropy suggests higher irregularity in a time series, and increased engagement of cardiorespiratory control.[18] Sample entropy was calculated using MATLAB codes shared at PhysioNet by Goldberger et al.[97]

2. *Multiscale Entropy.* MSE was assessed by calculating sample entropy across 10 different time series and plotting each value against one another to determine if these scaled sample entropy values are correlated.[18] Each scale functions to evaluate the time series at a progressively lower resolution (termed "coarse graining"), achieved by averaging consecutive SpO_2 values of increasing length. For example, at scale 1, sample entropy is calculated for the original time series. At scale 2, sample entropy is calculated by averaging every two consecutive SpO_2 values, functioning to down sample the signal x2. This process is repeated up to scale 10, where sample entropy is calculated for every 10 consecutive SpO_2 values, and the original time series has been coarse grained x10.[110] The resulting plot displays sample entropy across multiple time series, indicating the change in complexity based on the direction of the change in values. This process also functions to expose randomness (decreased complexity) of a signal through coarse graining, reflected by a decrease in sample entropy as scale increases.[18] In contrast, if sample entropy values remain the same or increase concurrently with scale, this indicates

increased complexity within the signal. MSE analysis was conducted using MATLAB coding shared at PhysioNet.[97,141]

3. *Detrended Fluctuation Analysis.* DFA was conducted to identify fractality (self-similarity) within SpO_2 signals by calculating the RMS of fluctuation across different time series (scales) plotted against one another on a log-log scale.[18] The signal is fractal if this plot exhibits linearity. The slope of this line was then determined as the scaling exponent, denoted as alpha (α), for both short- (α_1) and long-term (α_2) time series. α values of 0.5 indicate uncorrelated random data.[18] α values of greater than 0.5 to 1.0 indicate complexity of a signal,[140] with a slight decrease in complexity at $\alpha > 1.0$. DFA was conducted using software at PhysioNet developed by Goldberger et al.[97]

4. *Network Physiology Analysis.* Network analysis of the cardiorespiratory control system was conducted by calculating bidirectional transfer entropy between continuous physiological signals of SpO_2, HR, respiratory rate and skin temperature for one hour of the data collection period. Transfer entropy reflects both the direction and magnitude of information processing between these regulatory components of the physiological system, defining the interaction of complexity between each signal over the same time series. Network analysis was conducted using an open source function in MATLAB shared at PhysioNet.[97]

Biobehavioral Correlates of Cardiorespiratory Function

Potential biobehavioral correlates of cardiorespiratory function were explored in order to gain insight into potential modifiable factors that could impact cardiorespiratory recovery in a COVID-19 setting. Chronic elevations in inflammation post-infection may contribute to ongoing injury, resulting in reduced respiratory function in recovering individuals. Increased physical

activity levels may be protective to cardiorespiratory function while low physical activity levels and increased sedentary time amongst recovering patients due to persisting symptomology (or non-infection related reasons) may result in worsened inflammatory profiles as the anti-inflammatory effects of activity are not seen. Disease severity may give an indication of whether persisting impairments are related directly to the acute injury caused by infection.

Salivary Biomarkers

Systemic inflammation was assessed using salivary C-reactive protein (CRP) and cytokine (IL-1β, IL-6, IL-8 & TNF-α) levels via a passive drool. These cytokines have been deemed major components of the "cytokine storm" seen in COVID-19 patients and are significantly elevated due to infection. Participants were asked to refrain from eating a major meal for one hour prior to measurement and to rinse their mouth with water 10 minutes before collection. The measurement was conducted using passive drool through the Saliva Collection Aid (Salimetrics, Carlsbad, CA) and into a SalivaBio's 2 mL cryovial. Participants were asked to fill the vial to the line provided, collecting two separate 1.8 mL of samples. Samples were stored at -80 degrees Celsius until they were shipped to Salimetrics, Carlsbad, CA for analysis. Using the Salimetrics ELISA "sandwich" immunoassay kits, saliva samples were analyzed in duplicate and assayed for CRP, IL-1β, IL-6, IL-8, and TNF-α concentrations in pg/mL.

Physical Activity and Sedentary Time

Physical activity levels in all participants were assessed using tri-axial accelerometry. Participants continuously wore an ActiGraph wGT3x-BT accelerometer (ActiGraph LLC, Pensacola, FL) around the waist and above the right hip 24 hours a day (except when participating in water-based activities) for nine consecutive days. Accelerometry data was

downloaded to the ActiLife Software (version 6.13, ActiGraph LLC) to be analyzed. Data was collected at 80 Hz and processed in 60-second epochs. Sleep wear and awake wear time were distinguished using a previously validated algorithm (SAS syntax available at http://www.pbrc.edu/pdf/PBRCSleepEpisodeTimeMacroCode.pdf) developed by Barriera et al. (2018) using SAS version 9.4.[142] Activity was measured as moderate-to-vigorous (MVPA) based on activity counts per minute. MVPA was defined as greater than 2020 activity counts per minute.[143] Sedentary time was defined as any movement ≤25 counts per 15 seconds.[144,145] Non-wear time was determined as 60 or more consecutive minutes of zero activity counts, with the exception of 1-2 minutes of activity counts between 0 and 100.[143] A complete day of accelerometer data collection was determined as at least 10 hours of awake wear time. A minimum of 4 days of complete wear data was considered valid and included in the final analysis.[143]

Infection History and Experience

After the completion of all Zoom meetings, participants were sent an email containing a secure link via Research Electronic Data Capture (REDCap) to an online health survey consisting of a compilation of questionnaires assessing infection experience in participants with a history of COVID-19 disease as well as a general health history. REDCap is a web-based data collection system that allows for secure computerized collection and storage of data as well as stratified randomization algorithms (https://projectredcap.org). COVID-19 infection experience in recovering individuals was assessed using a survey developed by our researchers that asked about symptomology and participant experience with COVID-19 infection, including number of days since positive test, number of symptoms experienced during infection, and disease severity

rated on a five-point scale based on reported symptomology as per the World Health Organization's guidelines.[146] Infection experience will be assessed as a potential covariate of respiratory function, systemic inflammation and physical activity levels within the COVID-19 group. General health history was assessed using a patient health history questionnaire, which asked participants about chronic cardiovascular, respiratory, liver and kidney diseases.

Statistical Analyses

Descriptive statistics were calculated as mean ± SD. All variables were tested for normality of distribution and outliers using Shapiro-Wilks test, Q-Q plots, and histograms. Pulmonary function, exhaled nitric oxide, linear oxygen saturation variability, systemic inflammation and physical activity measures did not meet assumptions for parametric analysis, therefore we proceeded with nonparametric analyses. Nonlinear measures of oxygen saturation variability did meet assumptions for normality, therefore we proceeded with parametric analyses for these variables only. Group differences in pulmonary function, airway inflammation and linear oxygen saturation variability between recovering COVID-19 individuals and controls were assessed using the Mann-Whitney U-Test. Group differences in nonlinear oxygen saturation variability were assessed using independent samples T-test. Two-way ANOVA was used to assess group differences in MSE variability to evaluate the effect of both COVID-19 infection history and scale on MSE only. Categorical group differences were evaluated with Chi-Square. One-Tail Spearman correlation was used to explore any associations between components of respiratory function, oxygen saturation variability and measures of systemic inflammation (CRP and cytokine levels) as well as physical activity (MVPA and sedentary time) in recovering COVID-19 participants. Partial eta-squared (η^2) as a measure of effect size and observed power

were calculated for all variables to give indication of the strength of potential group differences observed based on our sample size. Statistical significance will be set *a priori* at $p<0.05$. All analyses were performed using IBM Statistical Package for Social Sciences (SPSS, version 27, IBM, Chicago IL).

Chapter 4: Results

Participant Characteristics

Fifty-seven participants consented for this study, completed all respiratory function measures and were included for analysis of pulmonary function and inflammation levels. Participant descriptive characteristics are displayed in Table 3. Participants were age, sex, and race-ethnicity matched between COVID-19 and Control study groups ($p>0.05$). Our cohort was generally normotensive, normoxic and of healthy weight-status and these measures of SaO2, BMI and body fat percentage were not different between groups ($p>0.05$).

Table 3. Participant Characteristics

	COVID+ (n=24)	Control (n=33)	*p* value
Age (years)	25.0 ± 9.0	24.0 ± 6.0	.570
Sex (%)			.813
Male	8 (33.3)	12 (36.4)	-
Female	16 (66.7)	21 (63.6)	-
Race/Ethnicity (%)			1.00
White	19 (79.2)	27 (79.4)	-
Hispanic	0 (0)	0 (0)	-
African American	3 (12.5)	4 (11.8)	-
Asian American	2 (8.3)	3 (8.8)	-
SaO_2 (%)	97.92 ± 0.78	97.75 ± 2.26	.403
BMI (kg/m^2)	24.02 ± 3.45	25.07 ± 3.52	.264
Body Fat (%)	22.30 ± 4.57	22.76 ± 4.50	.703
Days Since Positive	94.0 ± 82.0	-	-
COVID Disease Severity	2.2 ± 0.83	-	-
COVID Symptoms	6.0 ± 5.0	-	-

*$p<0.05$
Abbreviations: BMI, body mass index

Respiratory Function

Pulmonary Function and Airway Inflammation

Group differences in respiratory function can be found in Table 4. Both groups exhibited normal pulmonary function, achieving 80% or above of their predicted values for all pulmonary function parameters based on participant sex, age, height, and race-ethnicity. Non-parametric

independent samples analysis revealed that pulmonary function did not differ between our study groups, such that FVC, FEV1, FEV1/FVC and PEF were not significantly different ($p>0.05$) between COVID-19 individuals and controls. Airway inflammation measured as exhaled nitric oxide levels did not significantly differ between our study groups ($p>0.05$). Both groups exhibited slight airway inflammation, but did not reach abnormal levels, minimally surpassing the cut-off for no inflammation (16 ppb).

Table 4. Full Sample Respiratory Function, Systemic Inflammation and Physical Activity

	COVID+ (n=24)	Control (n=33)	*p* value	Partial η^2	Observed Power
Respiratory Function					
FVC, L (%)	4.22 ± 1.01 (102)	4.43 ± 1.06 (102)	.663	.010	.115
FEV1, L (%)	3.45 ± 0.72 (97)	3.57 ± 0.92 (95)	.865	.005	.083
FEV1/FVC (%)	84.88 ± 10.68 (99)	81.27 ± 8.93 (94)	.293	.034	.275
PEF, L/min (%)	349.63 ± 105.54 (81)	372.73 ± 140.61 (80)	.370	.008	.101
Nitric Oxide (ppb)	16.61 ± 13.04	20.03 ± 20.11	.285	.009	.108
Systemic Inflammation					
IL-8 (pg/mL)	1206.90 ± 2021.4	871 ± 944.1	.508	.012	.126
IL-1ß (pg/mL)	302.61 ± 869.4	149.05 ± 155.5	.959	.017	.158
IL-6 (pg/mL)	8.81 ± 16.7	5.98 ± 7.9	.973	.013	.129
TNF-α (pg/mL)	14.86 ± 49.4	5.25 ± 5.9	.364	.021	.185
CRP (pg/mL)	1226.72 ± 1642.1	735.16 ± 1112.9	.067	.032	.254
Physical Activity					
MVPA (min/day)	35.37 ± 17.02	35.65 ± 17.49	.925	.000	.050
Sedentary Time (min/day)	717.91 ± 166.92	717.82 ± 167.08	.763	.000	.050

*$p<0.05$

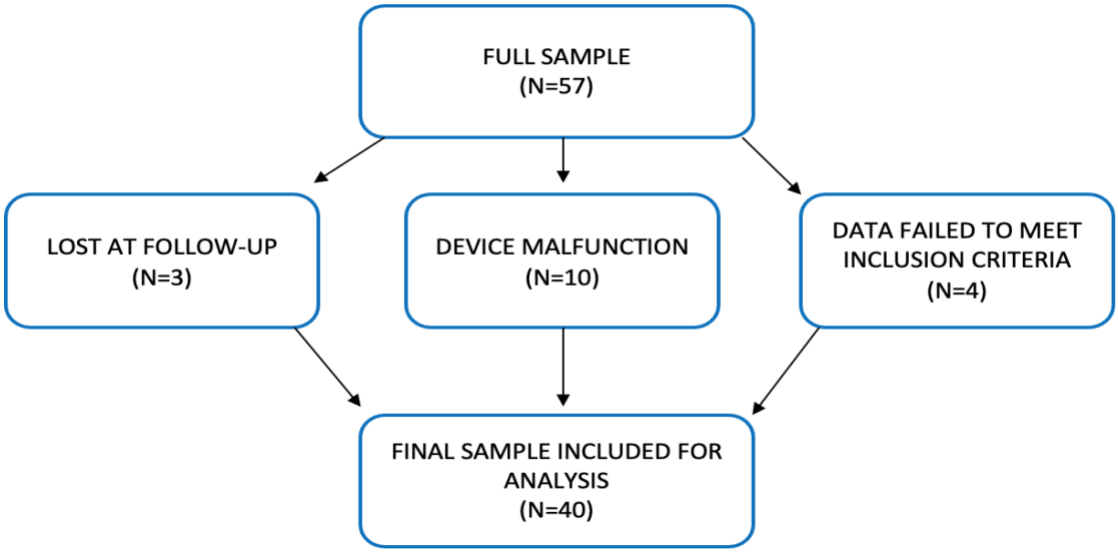
Abbreviations: η_2, partial eta-squared; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; PEF, peak expiratory flow; IL, interleukin; TNF, tumor necrosis factor; CRP, C-reactive protein; MVPA, moderate to vigorous physical activity

Oxygen Saturation Variability

Oxygen saturation variability analysis was conducted on a subsample of 40 participants from our original cohort. Determination of which participants were included in this subsample is depicted in Figure 13. From the original sample, three participants were lost at follow-up, 10 participants were lost due to device malfunction, and four participants' SpO_2 data files failed to meet inclusion criteria, bringing the final sample for SpO_2 analysis to n=40. Device malfunction included participants that wore the Equivital EQ02 LifeMonitor however experienced a failed SpO_2 signal due to connectivity issues between the sensor belt and pulse oximeter (n=10). Participants who did not complete at least 3.5 hours of data collection or had missing data of ≥15 minutes at a time during the collection period due to excessive artifact from activity and/or device removal were not included for analysis (n=4).

Participant characteristics for the subsample included for SpO_2 analysis are displayed in Supplementary Table 2. Similar to our overall sample, age, sex and race-ethnicity were similar between groups ($p>0.05$). Our subsample was normotensive, normoxic and of normal weight-status. Additionally, none of these measures of SaO2, BMI or body fat percentage differed between our study groups ($p>0.05$). Recovering COVID-19 participants in our subsample were measured on average 121.0 ± 132.0 days post-infection, experiencing mild-to-moderate COVID-19 infection with an average of 5.0 ± 5.0 symptoms). Group differences for all respiratory function, systemic inflammation and physical activity measures in our subsample can be found in Supplementary Table 3.

Figure 13. Inclusion Process for Oxygen Saturation Variability Analysis



Breakdown of inclusion process for participants included in oxygen saturation variability analysis. Of the full sample of 57 participants, three were lost at follow-up, 10 did not successfully complete data collection due to device malfunction, and four participants' data files did not meet inclusion criteria for analysis.

Linear Analyses

Group differences in linear oxygen saturation variability are displayed in Table 5. Participants in both groups were normoxic and spot check SaO_2 values did not differ between groups ($p>0.05$). In our subsample, no group differences were observed for any linear oxygen saturation variability parameters such that mean, SD, RMS and average hourly change were not significantly different between groups ($p>0.05$).

Table 5. Linear Oxygen Saturation Linear Variability Group Differences in Subsample

	COVID+ (n=16)	Control (n=24)	*p* value	Partial η^2	Observed Power
Spot SaO_2 (%)	98.00 ± 0.63	97.71 ± 2.56	.503	.005	.072
Full O_2 Mean (%)	97.51 ± 0.62	97.29 ± 0.68	.070	.072	.386
Full O_2 SD (%)	1.12 ± 0.39	1.19 ± 0.41	.469	.006	.076
Full O_2 RMS (%)	97.52 ± 0.62	97.15 ± 0.69	.070	.072	.387
Avg Hourly O_2 Change (%)	5.97 ± 1.21	6.35 ± 1.35	.469	.022	.146

Abbreviations: η_2, partial eta-squared; SaO_2, arterial oxygen saturation; O_2, oxygen; SD, standard deviation; RMS, root mean square; avg, average

Nonlinear Analyses

Group differences for all nonlinear analyses of sample entropy, MSE and DFA oxygen saturation variability can be found in Table 6.

1. *Sample Entropy.* No group differences in sample entropy over the 4-hour collection period were observed between individuals with a history of infection and controls ($p>0.05$).
2. *Multiscale Entropy.* Multiscale entropy analysis revealed that oxygen saturation variability exhibits a complex, but not random, correlated data signal across a 10-scale time series. This relationship is reflected by an increase in sample entropy concurrent with the increase in scale (Figure 14). One-way ANOVA reflected no differences in sample entropy at any time scale ($p>0.05$) between study groups. According to two-way ANOVA, while there was a significant factor effect as previously noted (i.e., sample entropy increases with increasing scale, $p<0.05$), there was not factor-by-group interaction ($p = 0.068$) suggesting that both groups experienced similar increases in sample entropy with increasing scale.
3. *Detrended Fluctuation Analysis.* Based on DFA, oxygen saturation variability exhibits fractality, illustrated by the linear pattern of the scaled signal (Figure 15). DFA was conducted for both short-term and long-term time scales, where we did not observe a significant difference between groups for α_1 or α_2 ($p>0.05$). This analysis did reveal, however, that oxygen saturation variability exhibits more powerful complexity during long-term variation as opposed to short-term, indicated by a mean α_2 closer to 1.0 (in contrast to a mean α_1 value closely approaching 1.5).
4. *Network Physiology Analysis.* Network analysis of SpO_2, HR, respiratory rate and skin

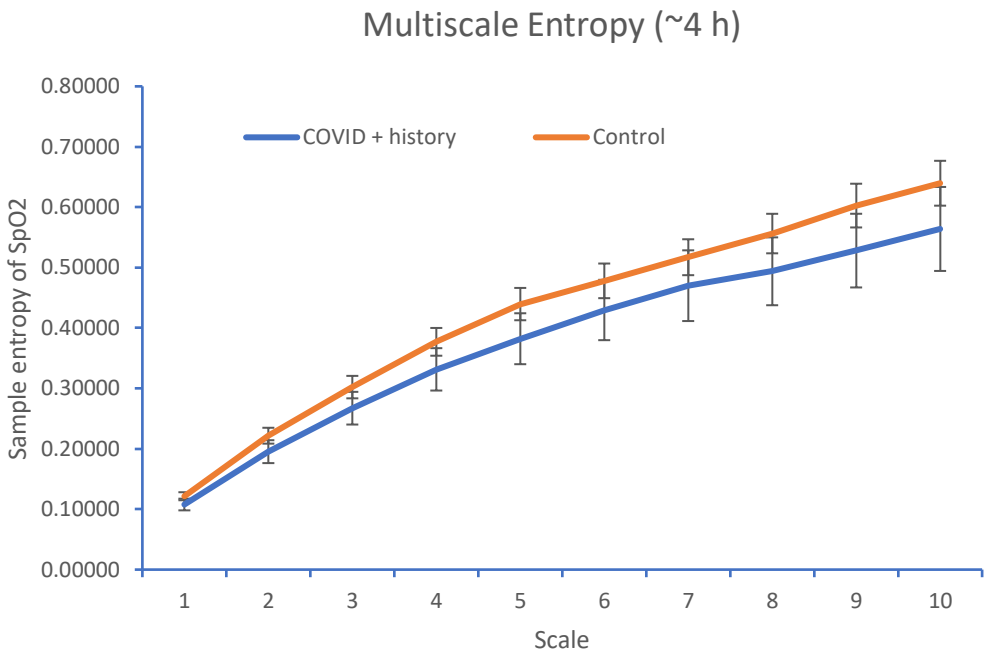
temperature demonstrated significant bidirectional exchanges of information between SpO_2 and other cardiorespiratory signals over the same time series. These exchanges information was quantified as transfer entropy levels between signals, displayed by the graphical representation in Figure 16. The direction and magnitude of transfer entropy between signals are illustrated by the orientation and thickness of the arrows between signals, respectively. Transfer entropy between all signals in either direction was similar between groups ($p>0.05$).

Table 6. Nonlinear Oxygen Saturation Variability Group Differences in Subsample

	COVID+ (n=16)	Control (n=24)	*p* value
Sample Entropy	.119	.134	.240
MSE			
Scale 1	.105	.121	.166
Scale 2	.191	.222	.179
Scale 3	.261	.302	.205
Scale 4	.323	.377	.191
Scale 5	.374	.440	.184
Scale 6	.422	.478	.306
Scale 7	.470	.517	.437
Scale 8	.499	.565	.283
Scale 9	.537	.616	.241
Scale 10	.577	.664	.245
DFA			
α_1	1.40	1.40	.811
α_2	1.01	1.02	.640

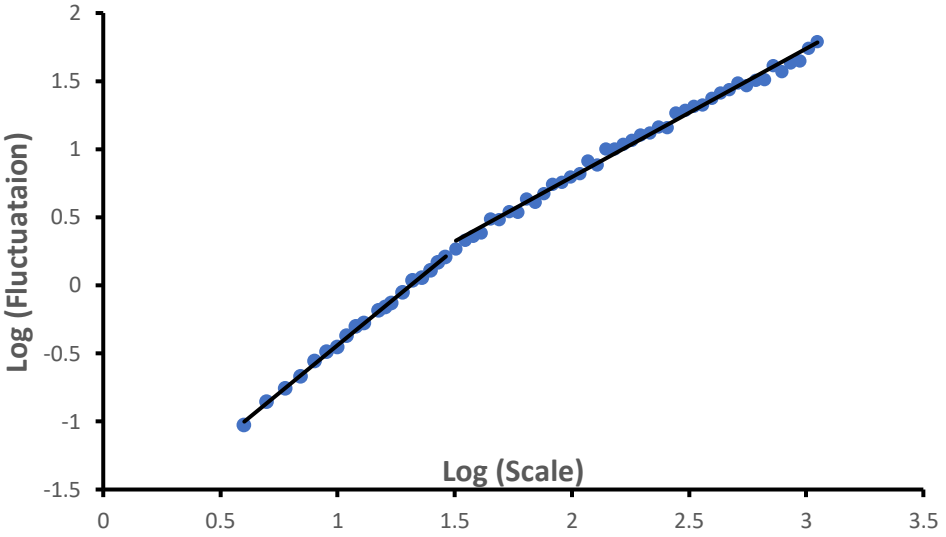
Abbreviations: MSE, multiscale entropy; DFA, detrended fluctuation analysis

Figure 14. Multiscale Entropy Analysis



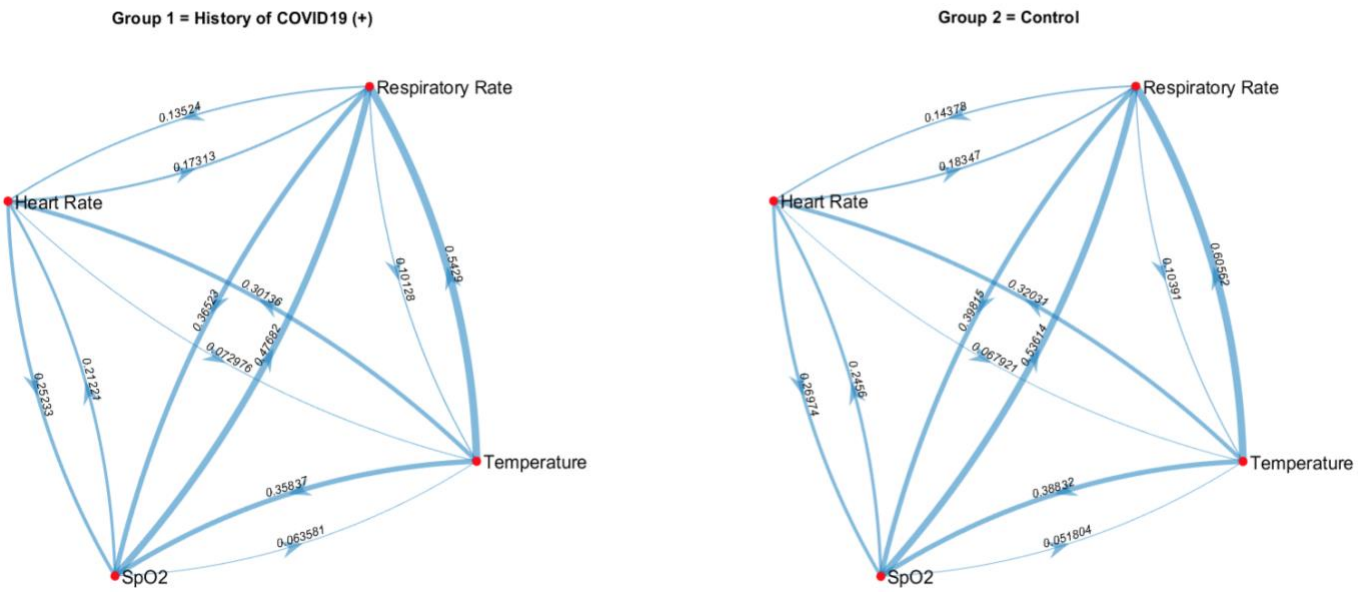
Multiscale entropy analysis of sample entropy levels across 10 scales comparing complexity patterns between individuals with previous COVID-19 infection (blue) and controls (orange) over four hour collection period. Increased sample entropy with increasing scale is suggestive of complexity in both groups.

Figure 15. Detrended Fluctuation Analysis Sample for One Participant



Sample detrended fluctuation analysis for one participant. Plotted calculation of root mean square of fluctuation for short-term (scales 0.5 to 1.5) and long-term (scales 1.5 to 3.0) time scales. Linear shape of graph suggests fractality in variability signaling.

Figure 16. Network Physiology Analysis



Results from network physiology analysis between oxygen saturation, heart rate, respiratory rate and skin temperature signals (nodes) for individuals with previous COVID-19 infection (left) and controls (right) conducted for one hour of monitoring. Exchanges of transfer entropy (edges) represented by the direction of information flow (orientation of arrow) and the magnitude of information flow (thickness of arrow).

Covariates of Infection

Inflammatory Salivary Biomarkers

Group differences in systemic inflammation biomarkers are displayed in Table 4. Systemic inflammation measured with salivary cytokine biomarkers did not differ between our study groups such that IL-8, IL-1ß, IL-6, and TNF-α did not significantly differ between COVID-19 individuals and controls ($p>0.05$). Differences in CRP levels were observed between groups however this difference did not reach significance ($p=.067$). No group differences were observed in systemic inflammation between recovering COVID-19 individuals and controls in our subsample ($p>0.05$) (Supplementary Table 3).

No associations between inflammatory biomarkers and any measures of respiratory function were observed in individuals with a history of COVID-19 infection. IL-6 was inversely

associated with sedentary time (r=-.370, p=.049). For linear metrics of oxygen saturation variability, average hourly change was inversely associated with TNF-α concentrations (r=-.497, p=.025). IL-6 was associated with several nonlinear variability metrics, including short-term DFA variability (α_1) (r=-.593, p=.015), and MSE scales 5 (r=.426, p=.050) and 6 (r=.461, p=.036).

Physical Activity and Sedentary Time

Participants in both groups met suggested physical activity guidelines for MVPA. No group differences were observed for two of our accelerometer-derived physical activity measures such that MVPA and sedentary time were not significantly different between COVID-19 and control groups (p>0.05). All physical activity measures between groups are reported in Table 4. MVPA was positively associated with FeNO levels (r=.473, p=.015). While no group differences were observed, sedentary time was inversely associated with several pulmonary function parameters including FEV1 (r=-.392, p=.040) and PEF (r=-.579, p=.003) in our overall COVID-19 group. Additionally, no group differences were observed in physical activity measures within our subsample (p>0.05) (Supplementary Table 3). Associations between sedentary time with PEF (r=-.533, p=.050) remained in our subsample of COVID-19 individuals. No associations between physical activity or sedentary time with linear or nonlinear oxygen saturation variability were observed.

Infection Experience

Individuals in the COVID-19 group participated on average 94.0 ± 82.0 days since their positive test, experiencing mild-to-moderate COVID-19 infection and an average of 6.0 ± 5.0 symptoms.

Frequencies of specific symptoms experienced by those with a history of COVID-19 infection are displayed in Supplementary Table 1. The most common symptoms experienced were fatigue (79%), headache (75%) , nasal congestion (67%), and loss of smell (63%). Four participants (16%) reported experiencing asymptomatic infection. No participants required hospitalization for infection. Inverse associations were observed between FVC with both disease severity (r=-.461, p=.012) and number of symptoms (r=-.404, p=.025). Additionally, disease severity was negatively associated with FEV1 (r=-.365, p=.040). No associations were found with infection experience and any linear or nonlinear oxygen saturation variability parameters. Number of days since positive infection status was inversely correlated with sedentary time (r=.386, p=.042).

Chapter 5: Discussion

This study primarily sought to assess the effect of COVID-19 infection on respiratory function in young adults by comparing group differences in pulmonary function, airway inflammation, and both linear and nonlinear metrics of oxygen saturation variability between individuals with a history of COVID-19 infection and controls. Furthering our nonlinear analyses, we then conducted a network physiology analysis to understand exchange of information between regulatory components of the cardiorespiratory system by quantifying transfer entropy between continuous SpO_2, HR, respiratory rate and skin temperature signals. This was done to further explore potential cardiorespiratory uncoupling between groups in our sample. Finally, we explored the role of potential correlates of cardiorespiratory function including salivary biomarkers of systemic inflammation, physical activity levels and sedentary time, and infection experience as they relate to respiratory function and oxygen saturation variability in the COVID-19 group. These findings indicate that there are no group differences in any of the primary cardiorespiratory measures, suggesting that respiratory function and oxygen saturation variability (linear and nonlinear) are similar between infection and control groups, in contrast to the study hypothesis. Several associations amongst respiratory function, oxygen saturation variability, systemic inflammation biomarkers, physical activity levels and infection experience were observed and will be discussed below.

Taken together, the results of the current study show similar pulmonary function, airway inflammation levels and oxygen saturation variability between recovering COVID-19 individuals and controls with no history of infection. This suggests that mild-to-moderate COVID-19 infection does not have lasting effects on respiratory function and oxygen saturation variability approximately three months into recovery. Similarly, results from network physiology analysis

show similar levels of exchanges of transfer entropy amongst cardiorespiratory signals between groups. These findings further suggest that cardiorespiratory integration and complexity of control of SpO_2 is not impaired following mild-to-moderate COVID-19 infection, indicating there is no isolation within the cardiorespiratory system in those who have experienced COVID-19 infection.

Group Differences in Respiratory Function

Pulmonary Function

The current study did not find any significant differences in any of our measures of respiratory function; including pulmonary function, exhaled nitric oxide levels, linear and nonlinear measurement of oxygen saturation variability, between individuals with a history of COVID-19 infection and healthy controls. Assessment of pulmonary function via spirometry included measurement of FVC, FEV1, FEV1/FVC and PEF. The lack of group differences observed between our study groups differs from findings of several previous studies, including accounts of pulmonary function anomalies and diminished total lung capacity in 25% of subjects at three months post-discharge from COVID-19 infection.[6,12] In the former study, investigator Zhao reported that approximately half of those exhibiting pulmonary function anomalies showed reduced FVC or FEV1 values.[12] Similarly, abnormally low FVC and FEV1 values were published at 6 weeks follow-up to COVID-19 infection[76] and at the time of patient discharge.[6]

An important distinction to make between the cohort of the current study and those reported in these publications is the severity of COVID-19 infection experience and timeline of measurement relative to infection. The cohort of the current study experienced mild-to-moderate infection, was assessed on average three months into recovery, with no hospitalizations required.

In contrast, the majority of literature evaluating individuals during recovery from infection is reporting on follow-up assessments conducted post-hospitalization, encompassing the residual effects of a far more severe tier of COVID-19 infection. However, one study identified the frequency of pulmonary dysfunction at six months follow-up, revealing low FVC, FEV1, and FEV1/FVC values in only 3%, 8% and 8% of the 89 patients included, respectively, however these participants were also hospitalized during infection.[147] A more representative study of our cohort evaluating non-critical COVID-19 patients with mild-to-moderate infection reported no differences in pulmonary function pre-to-post infection,[148] aligning with our findings. Furthermore, a larger scale study of 661 young adults (mean age 22.6 years) disclosed no differences in mean change in FVC, FEV1 or FEV1/FVC between seronegative and seropositive individuals.[84] Abnormal pulmonary function has been deemed "rare" in children and adolescents who did not experience severe infection.[149] These studies evaluating younger cohorts and those who had a more mild infection experience are more representative of our cohort and report findings seemingly parallel to the current study.

Minimal studies have measured PEF in individuals with a history of COVID-19, however one study reported normal mid-expiratory flow in conjunction with normal FVC and FEV1/FVC values.[85] Two accounts of direct measurement of PEF can be identified, one study yielding normal PEF, FVC, FEV1, FEV1/FVC values in 379 patients four months post-infection (aligning directly with the current study),[150] and another an ongoing clinical trial yet to release their findings.[151] These findings taken together with the current study suggest that pulmonary function is normal in young adults 3 months following mild-to-moderate COVID-19 infection.

Exhaled Nitric Oxide

Our cohort exhibited slightly elevated exhaled nitric oxide (FeNO) levels, with average scores of 16.61 ppb (COVID+) and 20.03 ppb (Control), however these values were not significantly different between groups. Prior to our investigation, few studies had considered FeNO measurement as a means of evaluating post-COVID airway inflammation, as it was primarily used in assessing individuals with asthma and other related respiratory conditions. FeNO may be useful in a COVID_19 setting as several of the inflammatory molecules that compose the cytokine storm characteristic to COVID-19 infection stimulate overproduction of NO in order to contest constrictive changes to the airway[80] and has previously been found to be associated with changes in lung function.[126] Higher FeNO values have been reported in critically ill patients with pneumonia compared to those without.[80] It is important to note, however, that the ailments in this study were not COVID-19 related, although pneumonia is a common condition associated with COVID-19 infection. The Yang et al. study appeared to be the first to use FeNO measurement directly for evaluating respiratory function in COVID-19 patients, reporting significantly higher values compared to healthy controls.[82] Nevertheless, these values were acquired during active infection, and do not give indication about FeNO measurement during recovery.

As the novelty of the pandemic has decreased, more studies have now assessed FeNO in recovering COVID-19 patients, with comparable results to the current study. A large scale study of 661 young adults reported no differences in FeNO values between recovering COVID-19 individuals and controls, including 123 individuals with asthma in the analysis.[84] Another study that evaluated FeNO in a much smaller sample of 20 recovering COVID-19 individuals at 5 months post-infection revealed that 7 (39%) of their cohort had slightly elevated FeNO levels

(25-50 ppb) indicating possible inflammation, but none reaching abnormal levels of over 50 ppb.[85] Furthermore, a study measuring multiple-flow FeNO values reported no significant differences in FeNO levels at a flow rate of 50 mL/s,[86] the same flow rate used for data collection with the NiOX VERO here. These findings suggest that FeNO measurement may be plausible as a diagnostic tool during active COVID-19 infection, but more research is needed to understand its significance during recovery. The findings of the current study indicate that young adults with a history of COVID-19 infection have mild, but not abnormal airway inflammation levels approximately three months into recovery from infection.

Oxygen Saturation Variability

This study employed both linear and nonlinear methods to quantify and compare oxygen saturation variability between individuals with a history of COVID-19 infection and controls. Assessing oxygen saturation variability using continuous monitoring accounts for the natural fluctuations in SpO_2 that spot check measurement cannot. This attempt to capture true variability was conducted through analysis of a four-hour SpO_2 signal, including basic variability measures of mean, SD, RMS and average hourly change in conjunction with novel nonlinear analyses of sample entropy, MSE and DFA. Further insight into cardiorespiratory system control of SpO_2 was provided via network physiology analysis of SpO_2 as it relates to HR, respiratory rate and skin temperature. While no group differences were observed for any linear or nonlinear variability parameters during our continuous collection period, the results of these analyses can be utilized to help define oxygen saturation variability and provide a baseline for young adults both with and without histories of COVID-19 infection moving forward.

A novel approach to SpO_2 measurement, oxygen saturation variability, both linear and nonlinear, has only been calculated in a handful of studies. Prior to the current work, oxygen saturation variability had been quantified in specified populations and/or under unique conditions, including infants,[152] healthy adults,[18] COPD patients,[94] and healthy adults under a graded hypoxic stimulus[95] but had not been evaluated in a COVID setting. The literature suggests that increased linear variability metrics associated with desaturation may be indicative of disease, such as in the extended home monitoring of COPD patients.[94] In contrast, decreased nonlinear variability may indicate lower complexity and control system uncoupling associated with aging and disease.[18] It is important to note that although increased entropy/higher complexity has been associated with tighter control system coupling during pathological or environmental stimuli (desaturation or hypoxia)[18,95] oxygen saturation variability is theoretically more engaged during these conditions where homeostatic intervention by the cardiorespiratory system is required. Despite this, however, oxygen saturation variability may still be decreased compared to healthy counterparts due to injury or isolation to one or more of the regulatory components involved in cardiorespiratory control. In summary, during a stimulus, it appears that increased linear variability and decreased nonlinear variability distinguishes healthy and diseased individuals.[19,94,112,113]

In the current study, no differences were observed in linear or nonlinear oxygen saturation variability between our study groups. Our findings are supported by two recent publications, suggesting that COVID-19 infection may not affect oxygen saturation homeostasis in the same manner as we see in other respiratory conditions such as COPD. Mapelli et al. reported no significant change in SpO_2 at 7-12 days post-discharge (2-hour bout per day) but did not compare these findings to a control population.[153] Moreover, Banzi et al. conducted 8-day

continuous SpO_2 data collection during symptomatic COVID-19 infection, where no patient experienced a 5% or greater decrease in saturation during monitoring.[154]

Network physiology analysis in the current work indicated that while there were significant exchanges of information signaling between regulatory components of the cardiorespiratory system, that the integrity of said control system is not compromised in those with a history of COVID-19 infection when compared to their healthy counterparts. Specifically, two additional studies have discussed similar system control as it relates to SpO_2 monitoring in COVID-19 patients. Mapelli et al. carried out extended at-home seven-day continuous monitoring of identical cardiorespiratory parameters in recently discharged COVID-19 patients, yielding similar results to the current study. No differences in any cardiorespiratory parameters were observed from the day of hospital discharge to the last day of monitoring.[153] It is important to note, however, that these measures were not compared to a control group and the average age of this cohort was 54 years, unlike the current sample. Desaturation during exercise was observed in this same cohort, illustrating the cardiorespiratory dynamics discussed in Michard et al.'s evaluation of continuous cardiorespiratory monitoring in individuals with COVID-19 infection[155] as hypothesized in the current study. While these multisignal interactions were not different between our study groups, the results of our network analysis echo that of previous studies,[93,140] highlighting the exchange of physiological information across the cardiorespiratory system. By successfully capturing this flow of information, we can potentially identify points of isolation amongst regulatory components in the system during/after not only COVID-19 infection, but also other pathological/environmental stimuli.[93]

When discussing the findings of the current work as they relate to the literature, the timing of measurement and target populations should be considered. More specifically, the

current cohort does not fall in a high-risk demographic, in terms of severity of infection and potential age-related comorbidities. As previously highlighted regarding pulmonary function performance, the present sample of healthy young adults experienced mild-to-moderate infection, were assessed approximately three months post-infection, and required no hospitalizations. Furthermore, in a risk assessment of COVID-19 patients, the presence of one or more comorbidities such as diabetes or hypertension was found to be a strong risk factor for hypoxia post-COVID infection.[156] Because of our sample demographic, we may fail to see the ongoing cardiorespiratory injury reported in other populations at similar points in recovery.

While no group differences were observed in any oxygen saturation variability measures, we were able to gain important insight into oxygen saturation variability dynamics overall. Similar to reports by Bhogal et al., SpO_2 exhibited both fractality (self-similarity) and increased long-term complexity in our overall sample.[18] Furthermore, SpO_2 displayed increased long-term complexity when compared to short-term variations, denoted in our DFA, also echoing previous investigations.[18] As the demonstration of the feasibility and accessibility to similar methodologies for home monitoring of SpO_2 and associated measures increases in the COVID-19 population,[153–155] the current work functions to establish points of reference in regards to oxygen saturation variability metrics, allowing for refinement in future methodical approaches and comparison of observed patterns across populations. Our findings suggest that while cardiorespiratory control of oxygen saturation is not impaired in young adults three months into recovery from COVID-19 infection, there is significant information to be acquired within these cardiorespiratory signals.

Biobehavioral Correlates of Cardiorespiratory Function

Salivary biomarkers of inflammation, physical activity, sedentary time and infection experience were explored in our COVID-19 group to assess potential correlates of cardiorespiratory function. Although no group differences were observed in cardiorespiratory function, these correlates were explored as potential mechanisms contributing to pulmonary injury, airway inflammation or hindered oxygen saturation variability during COVID-19 recovery. Elevated inflammatory profiles characteristic of increased disease severity may be responsible for injury to the cardiorespiratory system, resulting in poor performance outcomes. Physical activity metrics (MVPA and sedentary time), on the other hand, were chosen to assess the potential protective effects of activity (or detrimental effects of lack thereof) on the cardiorespiratory system and its functionality. When discussing physical activity metrics, an important distinction to be made is the difference between physical activity and sedentary time, as well as the physiological implications on cardiorespiratory function for each. While closely correlated with one another, it is possible for an individual to be both physically active (meeting physical activity guidelines) while still exhibiting substantial amounts of sedentary time. Additionally, these constructs may offer individual insight into cardiorespiratory function as both exhibit relationships with cardiorespiratory outcomes independent of the other.[34,157–160] Therefore, we distinguished between the two measures and assessed them as potential correlates of cardiorespiratory function individually.

In the current sample, no associations between MVPA and pulmonary function were observed. This lack of association could potentially be attributed to the activity levels of our cohort, as our sample met suggested physical activity guidelines. While no associations were found with pulmonary function, MVPA did exhibit a positive association with FeNO. Higher

levels of physical activity have been found associated with increased FeNO levels in healthy young adults.[161] In contrast to the inflammatory response during infection, activity stimulates endothelial production of eNOS, and releases nitric oxide into the airway, increasing NO bioavailability and raising FeNO levels.[161,162] Unlike MVPA, increased sedentary time exhibited a relationship with respiratory decline, demonstrated by an inverse association with both FEV1 and PEF. Increased bouts of sedentary time have been reported to accelerate age-related decline in lung function, and has previously demonstrated negative relationships with FEV1 and PEF specifically.[35,36] Reductions in cardiorespiratory function characterized by lower pulmonary function may be attributed to a detraining effect at the lungs from lack of activity.[34] Therefore, while similar between our study groups, it is plausible that reductions in pulmonary function may be due to increased bouts of sedentary time rather than the injuries related to acute COVID-19 infection.

Furthermore, sedentary time has previously been associated with elevated inflammatory profiles, as the anti-inflammatory effects of increased activity are not seen. While we did not observe any relationships between sedentary time and the pro-inflammatory cytokines (IL-8, IL-1ß, TNF-α, CRP), we did note an inverse association between sedentary time and IL-6. In contrast to other cytokines, IL-6 has functional roles outside of pro-inflammatory processes, and increased concentrations have been documented in more active populations.[163,164] More specifically, muscle-derived IL-6 produced during activity has documented anti-inflammatory effects, reported to combat pro-inflammatory cytokines such as its measured counterpart IL-1ß.[165] These findings suggest that sedentary time may contribute to an elevated inflammatory profile due to a low production of anti-inflammatory IL-6.

Regarding infection experience, disease severity exhibited a negative impact on pulmonary function, noted by inverse relationships with both FVC and FEV1. Previous studies assessing pulmonary function based on disease severity have yielded mixed findings.[6,148,150,166–168] Interestingly, the relationship between disease severity and pulmonary function appears to be more likely driven by more severe cases that include exacerbated inflammatory responses to secondary injury such as COVID pneumonia, supplemental oxygen requirements or ARDS, however this was not demonstrated in our recovering individuals. Not only did the current sample report mild to moderate infection, but no associations between disease severity and any inflammatory biomarkers were observed. Regardless, the reduction in pulmonary function in our cohort may be due in part to the acute lung injury brought on by infection, independent of inflammation levels. Taken together our findings suggest that both sedentary time and disease severity may be indicative of respiratory decline in young adults recovering from COVID-19 infection. Finally, sedentary time may be a modifiable point of intervention for improving pulmonary function during recovery in this population.

Limitations and Implications

While this study did provide a novel perspective into cardiorespiratory function of young adults recovering from COVID-19 infection, it is not without limitations. Primarily, this study was cross-sectional and correlational in design, and therefore cannot claim causality. Additionally, due to the viral transmission risks posed to both participants and researchers when conducting this study, much of the data collection was completed remotely, adding additional limitation for achieving accurate and complete measurement of study variables. Furthermore, documentation

of COVID-19 infection experience was self-reported up to six months post infection, and therefore may not be entirely accurate.

Due to the novelty of the Equivital LifeMonitor device, there were several unforeseen complications and extraneous variables that led to the failed sampling of several of our participants, causing them to be excluded from our secondary analysis for oxygen saturation variability metrics. Prior to its use in the current study, the Equivital was piloted in a small-scale study, where it exhibited good reliability for eight-hour continuous measurement. However, due to the remote functionality of our study, the set-up of the device was done over Zoom and removal of device unsupervised, so neither could be physically conducted by a researcher. Additionally, as has been mentioned in the few studies where the same device was used, the quality of the data and successful collection by the device heavily relies on participant cooperation with the device restrictions, as the pulse oximeter easily generates artifact during higher activity levels and fidgeting with the device connection. Furthermore, our sample and subsample were both predominantly white for our secondary analysis that decreased the generalizability of our findings. However, it is important to note that there are no established racial differences in oxygen saturation metrics per se, so it may not have contributed to the lack of group differences observed in our cohort.

This study highlighted several measures that may be beneficial to managing recovery of COVID-19 patients after infection. Future studies should continue to evaluate these components of respiratory function in populations of varying infection severity and age. Additionally, pre-to-post measurement of individuals who contract COVID-19 would allow for a more direct comparison of changes in respiratory function, and repeated measurement throughout recovery would be beneficial. More research is needed to quantify, understand, and compare oxygen

saturation variability metrics and cardiorespiratory function in all populations, and may be valuable in more severely impacted populations. The current work provides an important baseline to be referenced moving forward when discussing oxygen saturation variability and cardiorespiratory dynamics in any future bodies of work. As vaccination rates increase and transmission risk decreases, more accurate and direct measurement of these populations should become more plausible.

Conclusion

Respiratory function measured as pulmonary function, exhaled nitric oxide and oxygen saturation variability does not differ between young adults who experienced mild-to-moderate COVID-19 infection and healthy controls. Moreover, the significant exchange of information (transfer entropy) between SpO_2, HR, respiratory rate and skin temperature did not differ between groups suggesting that both complexity and integrity (i.e., dynamic integration) within the cardiorespiratory control system is not compromised during recovery from COVID-19. Increased sedentary time and disease severity may have negative effects on pulmonary function in this population. In conclusion, young adults who have experienced mild-to-moderate COVID-19 infection do not appear to be at increased risk for future respiratory decline.

Supplementary Table 1. Frequency of COVID-19 Symptomology

Symptom	Number of Participants
Fever	10
Cough	12
Fatigue	19
Loss of Appetite	11
Shortness of Breath	9
Hyperventilation	1
Muscle Pain or Ache	13
Joint Pain or Ache	9
Sore Throat	12
Nasal Congestion or Runny Nose	16
Headache	18
Diarrehea	3
Nausea or Vomiting	4
Loss of Smell	15
Loss of Taste	11
Dehydration	5
Reduced Alertness	6
Purple Lesions on Hands or Feet	0
Persistent Chest Pain or Pressure	3
Confusion	3
Inability to Wake/Stay Awake	3
Blush Lips or Face	0
Other	3
Asymptomatic	4
Hospitalization	0

Supplementary Table 2. Subsample Participant Characteristics for Secondary Analysis

	COVID+ (n=16)	Controls (n=24)	*p* value
Age (years)	26.6 ± 10.3	24.5 ± 7.1	.733
Sex (%)			.505
Male	6 (37.5)	10 (41.7)	-
Female	10 (62.5)	14 (58.3)	-
Race/Ethnicity (%)			.191
White	13 (81.3)	23 (95.8)	-
Hispanic	0 (0)	0 (0)	-
African American	1 (6.2)	1 (4.2)	-
Asian American	2 (12.5)	0 (0)	-
BMI (kg/m2)	23.69 ± 3.62	25.45 ± 3.79	.062
Body Fat (%)	22.47 ± 5.11	23.07 ± 4.69	.389
Questionnaire Data			
Days Since Positive	120.88 ± 132.20	-	-
COVID Disease Severity	2.06 ± 0.85	-	-
COVID Symptoms	5.0 ± 5.0	-	-

Abbreviations: BMI, body mass index

Supplementary Table 3. Subsample Group Differences in Respiratory Function, Systemic Inflammation and Physical Activity

	COVID+ (n=16)	Control (n=24)	*p* value	Partial η^2	Observed Power
Respiratory Function					
FVC, L (%)	4.28 ± 1.04 (105)	4.60 ± 1.11 (106)	.557	.021	.143
FEV1, L (%)	3.49 ± 0.77 (100)	3.71 ± 0.87 (99)	.672	.020	.136
FEV1/FVC (%)	85.06 ± 11.26 (99)	81.96 ± 8.43 (95)	.345	.025	.163
PEF, L/min (%)	363.75 ± 118.05 (88)	394.05 ± 141.85 (86)	.389	.013	.106
Nitric Oxide(ppb)	12.33 ± 4.44	21.21 ± 23.24	.123	.054	.294
Systemic Inflammation					
IL-8 (pg/mL)	711.44 ± 750.4	954.41 ± 1039.2	.641	.017	.122
IL-1ß (pg/mL)	104.87 ± 112.6	161.75 ± 174.9	.177	.034	.200
IL-6 (pg/mL)	3.95 ± 3.7	5.14 ± 4.8	.454	.019	.129
TNF-α (pg/mL)	4.53 ± 4.2	5.11 ± 5.8	.641	.003	.063
CRP (pg/mL)	942.86 ± 1395.3	483.37 ± 563.8	.159	.052	.285
Physical Activity					
MVPA (min/day)	29.69 ± 17.71	34.85 ± 19.04	.500	.020	.121
Sedentary Time (min/day)	710.62 ± 171.90	719.46 ± 170.78	.796	.001	.052

Abbreviations: FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; PEF, peak expiratory flow; IL, interleukin; TNF, tumor necrosis factor; CRP, C-reactive protein; MVPA, moderate to vigorous physical activity

Supplementary Table 4. Participant Characteristics for Included v. Excluded Sample for Secondary Analysis

	Included (n=40)	Excluded (n=17)	*p* value
Age (years)	25.4 ± 8.5	21.5 ± 2.8	.011*
Sex (%)			.874
Male	15 (37.5)	6 (35.3)	-
Female	25 (62.5)	11 (64.7)	-
Race/Ethnicity (%)			.007*
White	36 (90.0)	9 (52.9)	-
Hispanic	0 (0)	0 (0)	-
African American	2 (5.0)	5 (29.4)	-
Asian American	2 (5.0)	3 (17.6)	-
BMI (kg/m2)	24.75 ± 3.8	24.55 ± 2.86	.875
Body Fat (%)	22.83 ± 4.81	22.07 ± 3.77	.601
Questionnaire Data			
Days Since Positive	121.0 ± 132.0	89.0 ± 62.0	1.00
COVID Disease Severity	2.06 ± 0.85	2.50 ± 0.76	.264
COVID Symptoms	5.0 ± 5.0	7.0 ± 4.0	.214

*p value <0.05
Abbreviations: BMI, body mass index

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CURRICULUM VITAE

Danielle Arcidiacono
dmarcidi@syr.edu
37 Pine Street Bellingham, MA
Bellingham, MA 02019
508-254-1757

Education

2019-Present Master of Science in Exercise Science
Syracuse University, Syracuse NY
Thesis: Cardiorespiratory Function in Young Adults with a History of COVID-19
GPA: 3.93 | December 2021

2015-2019 Bachelor of Science in Physical Education
Concentration in Sport & Exercise Science
Bridgewater State University, Bridgewater, MA
GPA: 3.61

2011-2015 Bellingham High School, Bellingham, MA
High School Diploma
GPA: 3.72

Academic/Professional Experience

08/2019-05/2021 **Teaching Assistant**, Syracuse University, Department of Exercise Science, Falk College of Sport & Human Dynamics, Syracuse, NY

- EXE 500 Principles of Exercise Testing
- EXE 295 Intro to Exercise Science
- EXE 483 Scientific Principles of Strength & Conditioning
- EXE 500 Kinesiology, Performance & Injury Prevention

08/2019-05/2021 **Graduate Research Assistant**, Syracuse University, Department of Exercise Science, Falk College of Sport & Human Dynamics, Syracuse, NY

- Human Performance Laboratory
- Altitude Simulation Laboratory

Publications

- **Arcidiacono DM**, Heffernan, KS VanderDrift, LE Lefferts WK, Wolf DA. Social Isolation and Subclinical Vascular Pathways to Cerebrovascular Disease. *In prep for submission to Physiology and Behavior.*
- **Arcidiacono DM**, Heffernan KS. Respiratory Function in COVID-19 Survivors. Book Chapter for "COVID-19: A Systems Perspective." *Under review.*

National Presentations

- ***Arcidiacono DM,** Lefferts WK, Gump BB, Heffernan KS. Sex Differences in Subclinical Carotid Atherosclerotic Cardiovascular Risk in Children and Adolescents. Presented at Women's Cardiovascular and Brain Health Symposium. April 16, 2021.

- ***Arcidiacono DM**, Heffernan KS, VanderDrift LE, Lefferts WK, Wolf DA. Social Isolation and Subclinical Atherosclerotic Cardiovascular Disease Risk. Presented at North American Artery 10th Annual Meeting. May 21-22, 2021.
- Heckel AR, **Arcidiacono DM**, Deblois JP, Glasgow AC, Heffernan KS. The Effect of SARS-CoV-2 Infection on Systemic Vascular Function: Preliminary Findings. Presented at North American Artery 10th Annual Meeting. May 21-22, 2021.
- **Arcidiacono DM**, Lefferts WK, Gump BB, Heffernan KS. Associations Between Physical Activity, Body Mass Index and Carotid Extra-Medial Thickness in Children. Presented at American College of Sports Medicine 68th Annual Meeting. June 1-5 2021.
- *† **Arcidiacono, DM,** Lefferts, WK, Gump BB, Heffernan KS. Associations Between Physical Activity, Body Mass Index and Carotid Extra-Medial Thickness in Children. Presented at American College of Sports Medicine Non-Invasive Physiology Special Interest Group. June 3, 2021.

Regional Presentations

- †**Arcidiacono DM,** Lefferts WK, Gump BB, Heffernan KS. Sex Differences in Subclinical Carotid Atherosclerotic Cerebrovascular Risk in Children and Adolescents. Presented at Syracuse University Neuroscience Research Day. April 9, 2021.
- †**Arcidiacono DM,** Lefferts WK, Gump BB, Heffernan KS. Associations Between Physical Activity, Body Mass Index and Carotid Extra-Medial Thickness in Children. Presented at Syracuse University 2021 Falk Student Research Celebration. May 4-7, 2021.
- Heckel AR, **Arcidiacono DM**, Coonan KA, DeBlois JP Glasgow AC, Heffernan KS. The Effect of SARS-CoV-2 Infection on Cardiovascular Function. Presented at Syracuse University 2021 Falk Student Research Celebration. May 4-7, 2021.

*Oral presentation; †Award recipient

Funding Support

Himan-Brown Grant for Mount Everest Study Abroad Program ($3,700)- Accepted

- Program cancelled due to COVID-19 pandemic

Professional Organizations

Mid-Atlantic American College of Sports Medicine (MARC), 2019-present

Certifications/Honors/Awards

- Master's Student Research Award, American College of Sports Medicine Non-Invasive Physiology Special Interest Group, 2021
- Rapid Fire Oral Presentation for Trainees Finalist, North American Artery Conference, 2021
- Trainee Oral Presentation Competition Finalist, Women's Cardiovascular and Brain Health Symposium, 2021
- Graduate Research Poster Award, Syracuse University Falk Student Research Celebration, 2021
- 3rd Place Graduate Research Poster Award, Syracuse University Neuroscience Research Day, 2021

- Citi Program Training | Biomedical Research- Human Subjects Research, 2020-Present
- Graduated Summa Cum Laude, Bridgewater State University, Physical Education- Sport and Exercise Science, 2019
- Sigma Alpha Chi Chapter | National College Athlete Honors Society, 2019
- Captain of Women's Basketball Team, Bridgewater State University, 2018-2019
- Dean's List, Bridgewater State University, 2015-2019
- John Abigail Adams Scholarship Recipient, 2015
- American Red Cross CPR

Technical Skills

- Data analysis with Statistical Package of Social Sciences (SPSS)
- Human performance and exercise testing
- Study protocol design and Institutional Review Board (IRB) submissions
- Participant recruitment and consent
- Data collection of biological samples (blood, saliva)
- Data acquisition/analysis with ADI LabChart, Equivital LifeMonitor EQO2 System, spirometry, NiOX VERO, ultrasound, accelerometry, body composition assessment
- Data acquisition with Research Electronic Data Capture (REDCap)
- Preparation and submission of research manuscripts