THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF KINESIOLOGY

Physical Activity and Microvascular Endothelial Dysfunction After SARS-CoV-2 Infection

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SPRING 2022

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Kinesiology
with honors in Kinesiology

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ABSTRACT

Infection with the SARS-CoV-2 virus can affect the body in many ways with vascular dysfunction being reported as one change in the body. The purpose of this project is to examine the impacts of physical activity behavior and SARS-CoV-2 infection on cutaneous microvascular function in healthy, young adults. It was hypothesized that young adults recovering from COVID-19 will have impaired microvascular function compared to adults that did not have COVID-19. A cross-sectional study was performed including 10 (5 men/5 women, 24 ± 4 yr) healthy control (HC) adults who were unvaccinated for COVID-19, 11 (4 men/7 women, 25 ± 6 yr) healthy vaccinated (HV) adults, and 12 (5 men/7 women, 22 ± 3 yr) post-COVID-19 (PC, 19 ± 14 wk) adults. Physical activity behavior over a week was quantified using accelerometers (Actigraph GT9X, LLC, Pensacola, FL). A standardized 39°C local heating protocol was used to assess NO-dependent vasodilation via perfusion (intradermal microdialysis) of 15 mM N^G-nitro-l-arginine methyl ester during the plateau of the heating response. Red blood cell flux was measured (laser-Doppler flowmetry) and cutaneous vascular conductance (CVC = flux/mmHg) was expressed as a percentage of maximum (28 mM sodium nitroprusside + 43°C). We found that the physical activity behavior was not different among groups (sedentary: p=0.30, light: p=0.89, m-vv: p=0.10). The local heating plateau (HC: 61 ± 20%, HV: 60 ± 19%, PC: 67 ± 19%, $P = 0.80$) and NO-dependent vasodilation (HC: 77 ± 9%, HV: 71 ± 7%, PC: 70 ± 10%, $P = 0.36$) were not different among groups. Neither symptom severity (25 ± 12 AU) nor time since diagnosis correlated with the NO-dependent vasodilation ($r = 0.46$, $P = 0.13$; $r = 0.41$, $P = 0.19$, respectively). In conclusion, healthy adults who have had mild-to-moderate COVID-19 do not have impaired cutaneous microvascular function regardless of physical activity behavior.

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ACKNOWLEDGEMENTS

I would first like to thank Dr. Lacy Alexander for the guidance throughout my Schreyer Honors College experience. Thank you for pushing me to take advantage of all the opportunities available to me. Your knowledge and passion for your field has been inspiring to me and you have showed me how to be a strong woman in science.

I would also like to thank Gabie Dillion for allowing me to be a part of your research and helping me through the entire process of writing this thesis. Your enthusiasm and excitement for research made my time in the lab very enjoyable.

Thank you, Dr. Larry Kenney, for the positive experience in Noll lab and for taking the time to be the faculty reader for my thesis.

Chapter 1

Literature Review

For many years, vascular endothelial dysfunction has been used as a model for overall cardiovascular health. Early in the research of vascular endothelial function the coronary arteries were studied using coronary angiography and coronary Doppler flow. This technique was invasive, so less-invasive techniques using peripheral vasculature were developed. Studies have shown that peripheral vascular endothelium and coronary artery endothelium show similar dysfunction, so peripheral vasculature can be examined instead of the coronary arteries. Risk factors that are associated with atherosclerosis like hypertension, diabetes, and hyperlipidemia create oxidative stress on the endothelium by reducing vascular nitric oxide availability. The nitric oxide mediated response of the skin microvasculature will be reduced by this stress, which can be used as an early indicator of cardiovascular dysfunction (1).

The SARS-CoV-2 (COVID-19) virus largely affects the respiratory system, but there is increasing evidence that infection with the virus also affects the cardiovascular system. Infection with the virus causes a hyperinflammatory response which can affect the endothelium. Organs in the body that are highly vascularized like the kidneys, liver, heart, and brain, are affected by the virus which shows the virus can affect human blood vessels. Since endothelial dysfunction can be a predictor of overall cardiovascular dysfunction, it is important to look into endothelial dysfunction after infection with the virus (2).

Infection with COVID-19 comes with a large, systemic inflammation response. An acute "cytokine storm" is seen early after infection and this high level of proinflammatory cytokines can be damaging to organs in multiple systems in the body. The inflammatory response continues chronically and continues to damage organs, specifically endothelial cells in the vasculature of organs (3). Endothelial dysfunction in vascular beds of different organs across three different subjects with severe reactions infections with the SAR-CoV-2 virus was explored through histological analyses of tissues from the kidney, heart, lungs, and intestines. Inflammation of the endothelium, known as endothelialitis, was seen in the organs (4). Inflammation of the endothelium can lead to dysfunction (5).

The severity of symptoms that can come from infection with the SARS-CoV-2 virus varies between people. Comorbidities like cardiovascular disease, hypertension, and diabetes were associated with patients with severe reactions that were hospitalized from the symptoms of the virus in many countries (6). A specific example comes from data from a hospital in Bangladesh where cardiovascular disease was involved with 91% of the fatalities from COVID-19, and the highest fatality rate was seen with patients who had the combination of COVID-19, cardiovascular disease, and diabetes (7). In another part of the world, patient data from Wuhan Union Hospital from February 2020 was analyzed to see the severity of symptoms from the virus. Data was used from patients that had cardiovascular disease and patients that did not have cardiovascular disease. Patients with cardiovascular disease showed more serious lung injuries and poor prognosis that could be from higher risk of tissue injury-related enzymes release, excessive uncontrolled inflammation responses, and hypercoagulable state (8).

Underlying comorbidities can worsen the prognosis of COVID-19, but infection with the virus can also cause issues within the cardiovascular system. Infection with the SARS-CoV-2 virus can induce myocardial injury, myocarditis, arrhythmias, sudden cardiac arrest, and heart failure. There is a “bidirectional interaction” between COVID-19 and cardiovascular disease (Figure 1) (6).

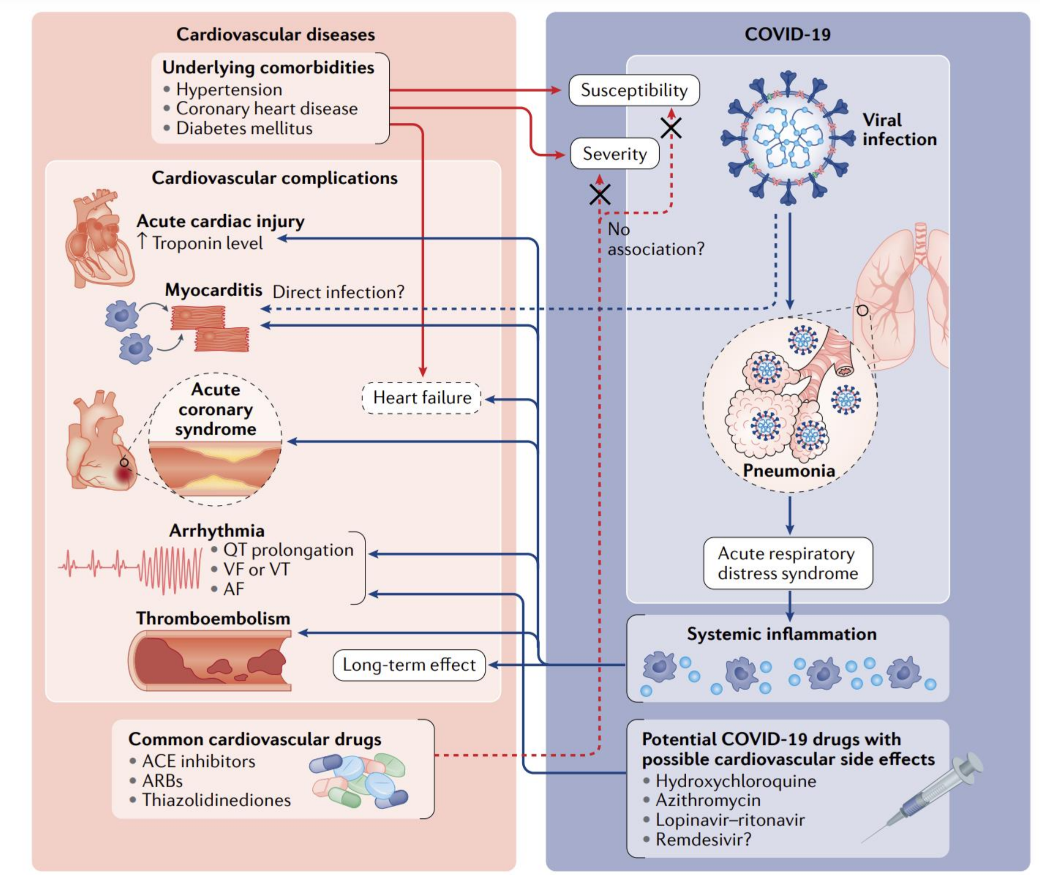

Figure 1: “Bidirectional Interaction” of COVID-19 and Cardiovascular Disease (6).

The long-term effects on the cardiovascular disease from the virus are still unknown, especially in healthy people without comorbidities before infection. Assessing vascular function in otherwise healthy individuals after infection with the SARS-CoV-2 virus can show early signs of long-term cardiovascular dysfunction. A study used flow mediated dilation (FMD), reactive hyperemia (RH), and single passive limb (sPLM) movement to assess vascular function in participants before and a couple weeks after contracting COVID-19. They hypothesized that vascular function would be reduced. Their data shows that brachial artery FMD was significantly lower in the patients after contracting the SARS-CoV-2 virus, but reactive hyperemia was similar between the healthy control group and the COVID-19 group. This study showed microvascular dysfunction in young, healthy adults after being infected with the virus (11).

Another study looked at both peripheral and cerebral vasodilator function in young, otherwise healthy adults greater than 4 weeks past recovery from SARS-CoV-2 infection. The participants in the study underwent FMD and reactive hyperemia to assess peripheral vasodilator function as well as carotid-femoral pulse wave velocity (PWV) and pulse wave analysis (PWA) to assess central artery stiffness. This study found that the cerebral vasodilator function was not different between the control group and the group that tested positive for COVID-19. They found a blunted microvascular response in participants that were still symptomatic at the time of testing (12).

Other long-term effects from the virus have been observed and are continually being researched. Some people experience symptoms that last longer than other symptoms. A survey sent to patients who were hospitalized after infection with the virus have found that cardiopulmonary symptoms worsened after discharge from the hospital, and some were re-hospitalized because of the symptoms that persisted. Stress from hospitalization and the pandemic lifestyle was also seen through this survey (9). Mental health issues and stress that is not necessarily physiological will be important to consider moving forward as the pandemic lockdowns and other restrictions are lifting. Another questionnaire administered in the UK found that in low-risk individuals, there were chronic symptoms and mild impairment in the heart, lungs, liver, and kidney four months after having COVID-19. They also found that cardiac impairment was more common with people who had more severe post COVID-19 symptoms (10).

A retrospective cohort study was performed with medical record data from John's Hopkins Hospital to compare sex differences in the prognosis of COVID-19. After adjusting for underlying comorbidities and clinical confounds men still had an increased risk for myocardial injury and death following COVID-19 infection. Men also had higher amounts of inflammatory markers while in the hospital with COVID-19, but in the outpatient setting, women had higher amounts of inflammatory markers. Even though men had higher amounts of inflammatory markers acutely, men and women had similar increased risks of myocardial injury and mortality. Men are more likely to have a hyper-inflammatory response, but that may not be the only reason there is a difference in mortality rates and cardiac outcomes for men (13).

Regular physical activity has many benefits, one specific benefit is the ability to reduce the risk of cardiovascular disease. As COVID-19 became a larger threat to communities, restrictions were put into place and a new lifestyle came with the stay-at-home orders. Many normal opportunities for physical activity were not available because people were not leaving their houses and gym facilities were closed. Physical inactivity could be linked to the severity of symptoms people experienced with the SARS-CoV-2 virus. The severity of COVID-19 symptoms and the duration of symptoms were evaluated through a cross-sectional study using data from patients who came to the respiratory emergency department at a university hospital. Physical inactivity was significantly associated with severity of COVID-19 and suggested that people with lower levels of physical activity experienced a longer duration of symptoms. Moderate and high levels of physical activity did not have a significant difference in severity of COVID-19 (14).

Relating self-reported physical fitness levels prior to COVID-19 infection and the severity of the COVID-19 symptoms provides insight to the impact physical activity can have on the short-term and long-term effects of the virus. A study found that a small increase in self-reported cardiorespiratory fitness reduced the odds of hospitalization from COVID-19, and as the self-reported cardiorespiratory fitness increased the odds of hospitalization also increased, but the findings were not statistically significant. They attribute this to the healthy status of the participants of the study, so making small improvements to physical activity levels did not have as large of an impact compared to participants with lower levels of physical activity. A majority of the participants (80%) had a BMI less than 30, and 57% of participants had self-reported higher than moderate levels of physical activity. The important take away from this study is that small improvements to cardiorespiratory fitness can reduce the chances of being hospitalized by COVID-19 (15).

Although conventional opportunities for physical activity were affected by the COVID-19 pandemic shutdowns, physical activity levels might not have changed for everyone. People who were physically active before the pandemic were motivated to remain physically active during the pandemic. A study looked at physical activity levels of Spanish university students before the pandemic started (January 2020) and during the pandemic lockdowns (April 2020). Questionnaires about demographics, alcohol consumption and smoking habits, diet, and self-reported physical activity were administered to the students at both time periods. Sitting time increased during the pandemic, but they also saw an increase in physical activity in many of the groups the participants were categorized into. The groups that did not see a difference were the groups that were highly sedentary before the lockdown. An important point is made in the study that healthy habits that were ingrained in the population were not influenced or changed by the lockdown (16).

Chapter 2

Introduction

In December of 2019 the SARS-CoV-2 virus was found in Wuhan China, and in the subsequent months, the virus spread around the world. This virus causes the coronavirus disease known as COVID-19. The virus mainly affects the respiratory system, but many of the other systems in the body can be affected by the widespread inflammatory response in the body. Symptoms like cough, fever, loss of taste and smell, congestion, and stuffy nose, along with other symptoms. These symptoms can last about 2 weeks or people can be asymptomatic and not experience any symptoms. Some people experience symptoms that last a longer amount of time (17). The long-term effects of COVID-19 on the body, especially the cardiovascular system, are continually being researched.

According to the American Heart Association, heart attacks are the leading cause of death for Americans and cardiovascular disease affects half of adults in America (18). One benefit of regular physical activity is reducing the risk of cardiovascular disease and other cardiovascular complications (19). Comorbidities like cardiovascular disease, or hypertension can make the prognosis of COVID-19 worse (6). Even though it is known that physical activity can reduce the risk for these comorbidities, the effects of regular physical activity on symptom severity of the virus are still being researched (14,15).

With the outbreak of the COVID-19 pandemic and the subsequent lockdowns, conventional opportunities for physical activity were limited and people had to change their regular physical activity habits. These changes are important to consider when looking at the impact of COVID-19. A common way to study physical activity behavior is by having the participants self-report physical through a survey. Accelerometers are a wearable device that measure physical activity behavior by measuring acceleration in three different directions and an accurate alternative to self-reporting physical activity (20).

Two prior studies with conflicting findings, (Ratchford 2021, Nandadeva 2021) looked at vascular health and COVID-19, but did not take into consideration the physical activity behavior of their participants (11,12). Since physical activity behavior may have been impacted by the pandemic, and increased physical activity is beneficial to vascular health, it is important to include it in the analysis of vascular health.

This project was a part of a larger study (21) and the goal was to examine the impacts of physical activity behavior and SARS-CoV-2 on cutaneous microvascular function in otherwise young, healthy adults. During a 7-day ambulatory period an accelerometer was worn to collect data on physical activity behavior. Microdialysis coupled with laser doppler flowmetry was performed to analyze nitric- oxide mediated vasodilation in the cutaneous microvasculature. It is hypothesized that those who recovered from COVID-19 will have attenuated cutaneous microvascular function compared to age-matched healthy adults independent of amount of physical activity.

Chapter 3

Methods

Participants

All participants underwent a complete medical screening, including a blood chemistry analysis (Quest Diagnostics, Pittsburgh, PA). Participants were nonobese, did not use tobacco products, and were not taking any prescription medications with primary or secondary cardiovascular effects (e.g., antihypertensives, statins, anticoagulants, etc.). Ten women were on birth control (oral contraceptives HC: n = 2, PC: n = 2, HV: n = 2; intrauterine devices HC: n = 0, PC: n = 2, HV: n = 2). All women were premenopausal; thus, a urine pregnancy test confirmed the absence of pregnancy before experimental visits. The phase of menstrual cycle was not controlled among women participants (21).

Participants were separated into three groups based on COVID-19 infection and vaccination status. The healthy control (HC) group included participants who were never diagnosed with COVID-19, never reported COVID-19 symptoms, and were not vaccinated for COVID-19. The post-COVID-19 (PC) group included participants who tested positive for COVID-19 (have a positive antibody test) and are at least 14 days recovered from infection. The healthy vaccinated control (HV) group included participants that never had COVID-19 and are vaccinated for COVID-19.

Screening Visit

During the screening visit, subjects were given accelerometers (Actigraph GT9X, LLC, Pensacola, FL) and instructions on how to wear the accelerometer correctly. Vaccinated and post-COVID-19 participants completed a COVID-19 symptoms severity survey, which lists the top 18 most common symptoms to COVID-19. Participants rated each symptom on a scale of 0–100 of increasing severity. The values for each symptom were totaled and averaged. Post-COVID-19 participants were asked to recall the severity of their symptoms during peak COVID-19 illness and for current symptoms at time of testing. Healthy vaccinated adults were asked to recall peak symptoms post-complete vaccination.

Physical Activity Behavior Measurements

Participants wore the accelerometer around their waist on the side of their dominant leg for 7 days leading up to their visit to the lab. Participants were instructed to wear the accelerometer during waking hours and to remove them nightly at bedtime and when doing activities that could damage the accelerometer (swimming, bathing, contact sports, etc.). A minimum of 400 minutes a day (~7 hrs/day) for at least 4 days of wear time was required to obtain valid data from the accelerometers. A total of 24 out of 33 participants (HC n = 9, PC n = 8, and VAC n = 7) had sufficient data for analysis. The participants were blinded (i.e., the screen of the device was turned off) to the ongoing recording of data (e.g., steps taken, active minutes, etc.) to eliminate behavioral reactivity.

The data from the accelerometers was categorized into percentage of day and minutes per day spent sedentary, and percentage of day doing light, moderate, vigorous, and very vigorous physical activity. Moderate to very vigorous physical activity categories were combined into one category for analysis due to low. Percentages and minutes from each category were averaged over the days of valid data for each participant. Step counts were also recorded during wear time and averaged over the days of valid data for each participant.

COVID-19 Symptoms and Vaccination Status

Vaccinated and post-COVID-19 participants completed a COVID-19 symptoms survey which lists the top 18 symptoms most common to COVID-19 (11, 22). Participants rated each symptom on a scale of 0-100 of increasing severity. The values for each symptom were totaled and averaged. Post-COVID-19 completed the survey for peak symptoms during COVID-19 illness and again for current symptoms at time of testing. Healthy vaccinated adults completed the survey for peak symptoms post-complete vaccination (21).

Skin Blood Flow Measurements

Before each experimental session, participants were instructed to abstain from caffeine, alcohol, and strenuous physical activity for at least 12 h before arrival at the laboratory. An intradermal microdialysis fiber (CMA Linear 31 probe, 55 kDa, Harvard Apparatus, Holliston, MA) was inserted into the ventral forearm skin for the local delivery of pharmacological agents, as previously described (23-25). Cutaneous red blood cell flux was continuously measured directly over the microdialysis site with an integrated laser Doppler flowmetry probe placed in a local heating unit (VP12 and VHP2; Moor Instruments, Wilmington, DE). Pharmacological agents were mixed just before use, dissolved in lactated Ringer’s solution, sterilized using syringe microfilters (Acrodisc; Pall, Port Washington, NY), and wrapped in foil to prevent degradation due to light exposure (FDA IND No. 120058). All solutions were perfused through microdialysis fibers at a rate of 2 mL/min (Bee Hive controller and Baby Bee microinfusion pumps; Bioanalytical Systems, West Lafayette, IN). After placement of microdialysis fiber, 60–90 min were allowed for hyperemia associated with fiber placement to resolve. Baseline data were then collected (~10 min) before beginning a standardized local heating (39ºC) protocol, as described previously (26). This local heating protocol elicits an initial axon reflex-mediated peak skin blood flow response, followed by a brief nadir, after which there is a gradual rise and eventual blood flow plateau (after ~40 min). After observing a stable local heating plateau, 15 mM NG-nitro-L-arginine methyl ester (L-NAME; NO synthase inhibitor) was perfused, allowing for quantification of NO-dependent vasodilation (%NO) (23–26). After observing a stable L-NAME plateau, 28 mM sodium nitroprusside (SNP; USP, Rockland, MD) was perfused and local temperature was increased to 43ºC to elicit maximal vasodilation (27, 28). Automated brachial BP (Cardiocap; GE Healthcare, Milwaukee, WI; Connex Spot Monitor, Welch Allyn, Skaneateles Falls, NY) was measured at each stage (e.g., baseline, initial axon reflex, local heating plateau, L-NAME plateau, and maximal vasodilation) throughout the protocol (21).

Data Analysis

Data were recorded at 40 Hz and stored for offline analysis (Powerlab/LabChart, ADInstruments, Bella Vista, NSW, Australia; WINDAQ, DATAQ Instruments, Akron, OH). Average values for red cell flux (perfusion units) were obtained during baseline and at each phase of the local heating protocol. Cutaneous vascular conductance (CVC) was calculated as red cell flux divided by mean arterial pressure. Due to the heterogeneity of capillary density at each microdialysis site, CVC was normalized as a percentage of the site-specific maximum ($\text{CVC\%}_{\text{max}}$). Participant characteristics were analyzed using a one-way ANOVA (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY; GraphPad Prism version 9.0.0 for Windows, GraphPad Software, San Diego, California USA). Responses to local heating were analyzed using a two-way repeated-measures ANOVA to evaluate group (healthy control, post-COVID-19, and healthy vaccinated) and phase (baseline, plateau, L-NAME, and maximum) effects. Linear regression analyses were performed using a Pearson's correlation coefficient. Significance was set *a priori* at $\alpha<0.05$. All results are presented as mean ± standard deviation (minimum-maximum). Participant characteristics were analyzed using a one-way ANOVA (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY; GraphPad Prism version 9.0.0 for Windows, GraphPad Software, San Diego, California USA). Responses to local heating were analyzed using a two-way repeated-measures ANOVA to evaluate group (healthy control, post-COVID-19, and healthy vaccinated) and phase (baseline, plateau, L-NAME, and maximum) effects. Linear regression analyses were performed using a Pearson's correlation coefficient. Significance was set *a priori* at $\alpha<0.05$. All results are presented as mean ± standard deviation (minimum-maximum) (21).

Chapter 4

Results

Participants

A total of 33 young adults participated in the study (healthy controls n=10, post-COVID-19 n=12, vaccinated n=11). Participant characteristics did not differ among groups (all p>0.05, Table 1). All healthy control participants had a confirmed negative COVID-19 antibody test, while all healthy vaccinated and post-COVID-19 participants had positive COVID-19 antibody tests. Two post-COVID-19 had received the first dose of the Pfizer-BioNTech before testing positive for COVID-19 but were fully vaccinated before the experimental visit; and five post-COVID-19 were fully vaccinated after testing positive for COVID-19, but before the experimental visit. Removing the post-COVID-19 who were either partially or fully vaccinated when getting COVID-19 did not alter the results (all p>0.05). All post-COVID-19 were symptom-free at the time of testing.

Table 1. Participant Characteristics

	Healthy Control	Post-COVID-19	Healthy Vaccinated
N (M/W)	10 (5/5)	12 (5/7)	11 (4/7)
Age (years)	24 ± 4 (19-31)	22 ± 3 (19-27)	25 ± 6 (19-35)
BMI (kg/m^2)	25 ± 3 (20-29)	24 ± 3 (16-28)	24 ± 4 (20-32)
SBP (mmHg)	114 ± 8 (100-128)	112 ± 13 (95-132)	108 ± 11 (89-122)
DBP (mmHg)	68 ± 8 (50-78)	74 ± 7 (64-82)	66 ± 6 (54-74)
HR (bpm)	62 ± 7 (47-75)	69 ± 12 (52-91)	71 ± 9 (55-84)
HDL (mg/dL)	59 ± 10 (46-79)	60 ± 7 (46-80)	51 ± 11 (39-69)
LDL (mg/dL)	91 ± 16 (65-119)	87 ± 27 (26-109)	87 ± 16 (47-104)
Total CHO (mg/dL)	169 ± 18 (136-183)	163 ± 29 (104-195)	159 ± 13 (135-176)
HbA1c (%)	4.9 ± 0.3 (4.4-5.4)	4.7 ± 0.3 (4.3-5.2)	4.8 ± 0.2 (4.4-5.1)
Race/Ethnicity (n)			
Asian/Indian	1	1	2
NH Black	1	0	2
NH White	7	9	6
Latino	0	2	1
Mixed (2+ listed above)	1	0	0
Days since…		**Diagnosis**	**Vaccine**
		131 ± 97 (12-357)	81 ± 36 (43-139)

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CHO, cholesterol; HbA1c, hemoglobin A1c; NH, non-hispanic.

Physical Activity Behavior

Of the 33 participants, 25 participants (health control n=8, post-COVID n=8, vaccinated n=9) returned the accelerometer with valid data. Participants spent a majority of their awake time sedentary (HC: 64 ± 5%, HV: 67 ± 3%, CR: 70 ± 10%). Participants spent the least amount of time in moderate-to-very vigorous exercise (Table 2). There were no differences in physical activity behavior among the three subject groups (Figure 2).

Table 2. Physical Activity Behavior

Physical Activity (min/week)	**Healthy Control**	**Post-COVID-19**	**Healthy Vaccinated**
Sedentary	3638 ± 434 (3189-4251)	3387 ± 974 (2184-5107)	3317 ± 772 (1853-3936)
Light	1566 ± 295 (1282-1952)	1153 ± 470 (737-2108)	1314 ± 81 (928-1585)
Moderate-to-Very Vigorous	478 ± 264 (132-901)	211 ± 107 (34-360)*	279 ± 220 (48-520)
Physical Activity (% of awake time)			
Sedentary	64 ± 5 (57-71)	70 ± 10 (53-83)	67 ± 3 (64-73)
Light	28 ± 5 (22-35)	25 ± 10 (16-44)	27.7 ± (16-44)
Moderate-to-Very Vigorous	8 ± 4 (2-15)	4 ± 3 (1-8)	6 ± 3 (1-9)

*p=0.04 Post-COVID-19 vs. Healthy Control.

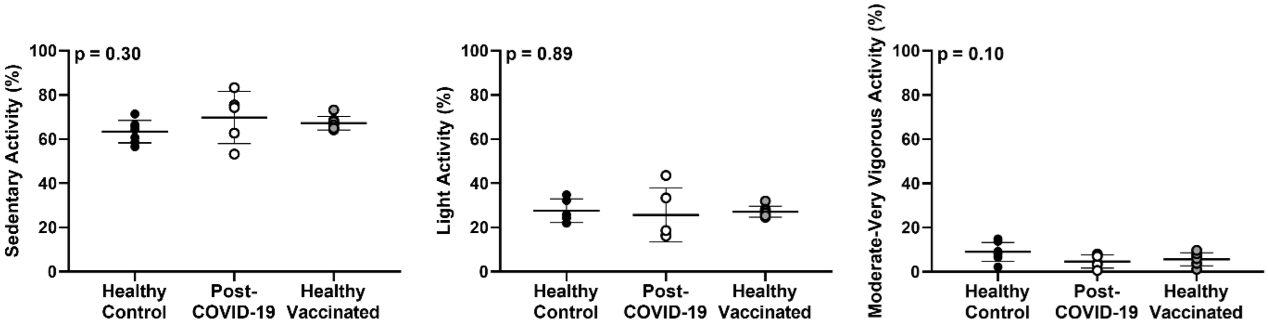

Figure 2. Physical Activity Behavior Among Groups.

Percentage of week spent sedentary, doing light activity, and moderate to very vigorous activity was not different (p=0.30, p=0.89, p=0.10, respectfully) in healthy control (n=8), post-COVID-19 (n=8), and healthy vaccinated (n=9) participants.

Skin Blood Flow

Baseline (healthy controls: 0.25±0.15, post-COVID-19: 0.36±0.26, healthy vaccinated: 0.27± 0.18, flux/mmHg, p=0.38),maximum CVC (healthy controls: 1.83±0.57, post-COVID-19: 1.65±0.74, healthy vaccinated: 1.86±0.92, flux/mmHg, p=0.77), and the initial axon reflex-mediated peak were not different among groups (healthy controls: 54.4±22.6, post-COVID-19: 58.2±15.0, healthy vaccinated: 47.6±21.8, $CVC\%_{max}$, p=0.50). There were no differences among groups in the local heating plateau or the post-L-NAME plateau, regardless of whether those responses were presented as absolute CVC or normalized to a percentage of maximal CVC. Similarly, the NO-contribution to the local heating response did not differ among groups (Figure 3).

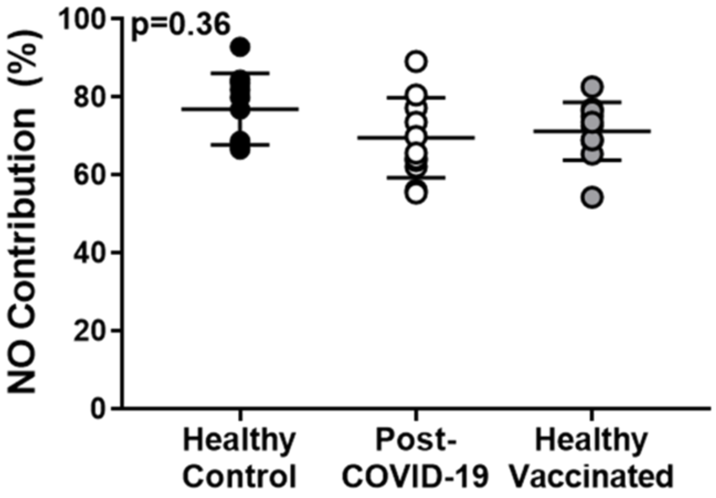

Figure 3. NO Contribution Among Groups.

The nitric oxide (NO) contribution to the local heating response in healthy controls (closed circles, *n* = 10), post-COVID-19 adults (open circles, *n* = 12), and healthy vaccinated adults (gray circles, *n* = 11) did not differ among groups.

Physical Activity and Skin Blood Flow

Physical activity behavior represented as percentage of day was not predictive of the NO-contribution to the local heating response (Figure 4) in all participants regardless of COVID-19 status (sedentary: $R^2<0.01$, light: $R^2<0.01$,moderate-very vigorous: $R^2=0.03$). Physical activity behavior was not predictive of the NO-contribution to the local heating response in participants after having COVID-19 (sedentary: $R^2<0.01$, light: $R^2=0.04$, moderate-very vigorous: $R^2=0.35$) (Figure 5). Average step counts were not predictive of NO contribution ($R^2=0.05$, p=0.26) (Figure 6).

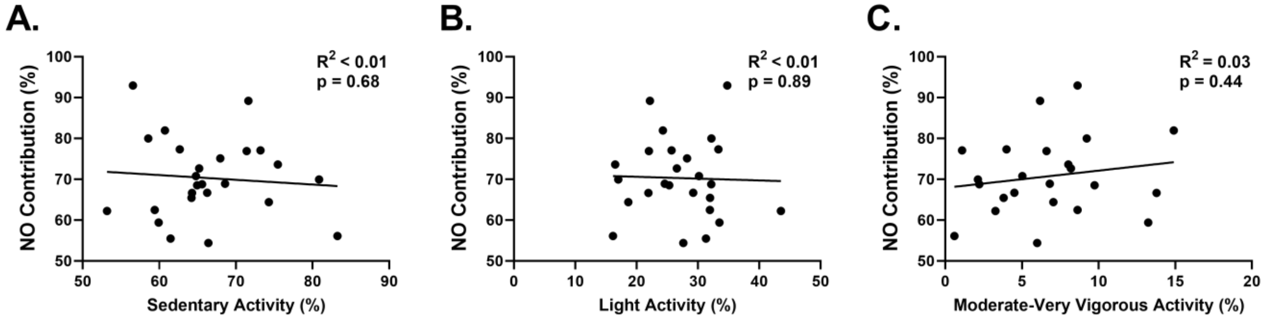

Figure 4. Physical Activity Behavior Predicting NO Contribution.

The nitric oxide (NO)- contribution to local heating was not related to percentage of time spent in sedentary (panel A), light (panel B), or moderate-very vigorous activity (panel C) per day.

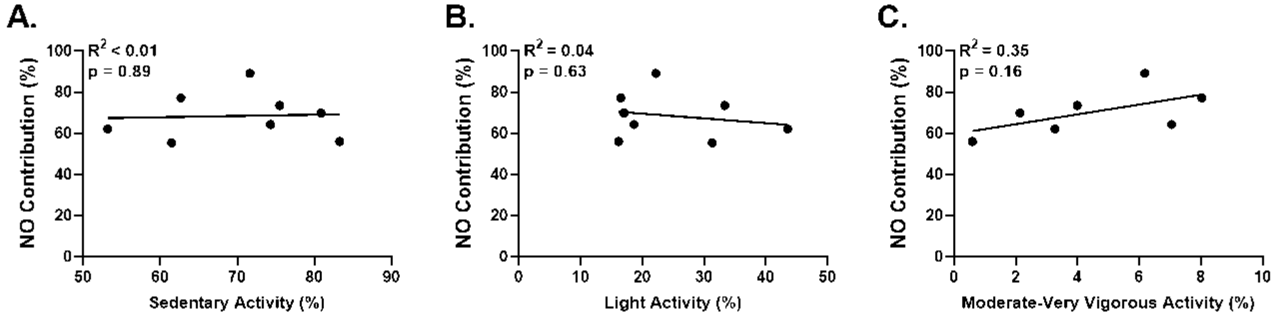

Figure 5. Physical Activity Behavior Predicting NO Contribution in post-COVID-19 Group.

The nitric oxide (NO)- contribution to local heating was not related to percentage of time spent in sedentary (panel A), light (panel B), or moderate-very vigorous activity (panel C) per day.

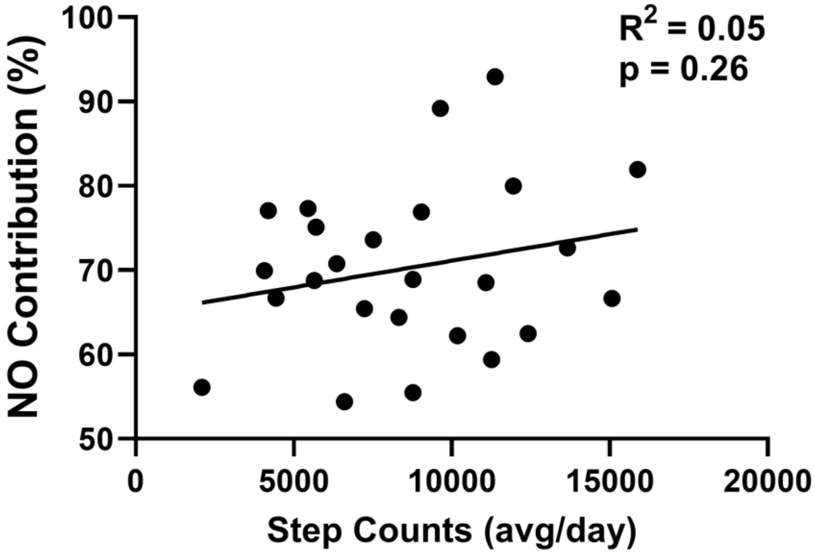

Figure 6. NO Contribution and Step Counts.

The nitric oxide (NO)- contribution to local heating was not related to the average number of steps taken per day.

COVID-19 and Skin Blood Flow

The time from COVID-19 diagnosis (Table 1), the number of COVID-19 symptoms (10±4 symptoms, 1-14,), and the average symptom severity (25±12 AU, 6-44) were not related to the magnitude of the local heating plateau (time from diagnosis: R^2=0.12, p=0.27; number of symptoms: R^2=0.17, p=0.18; average symptom severity: R^2=0.08, p=0.37) or the NO-contribution (Figure 6). The time from COVID-19 vaccine (Table 1), the number of vaccine symptoms (4±4 symptoms, 0-10), and the average symptom severity (4±6 AU, 0-17) were not related to the magnitude of the local heating plateau (time from diagnosis: R^2<0.01, p=0.99; number of symptoms: R^2=0.14, p=0.25; average symptom severity: R^2=0.29, p=0.10) or the NO-contribution (time from diagnosis: $R^2<0.01$, p=0.95; number of symptoms: $R^2=0.01$, p=0.79; average symptom severity: $R^2=0.03$, p=0.61).

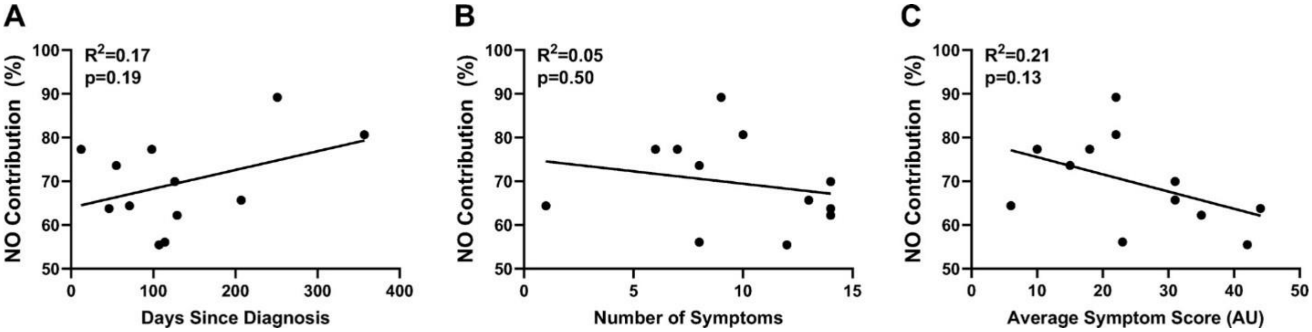

Figure 7. COVID-19 and NO Contribution.

Time from diagnosis, number of symptoms and average symptom score (AU) was not predictive of NO contribution.

Chapter 5

Discussion

This project has demonstrated that infection with the SARS-CoV-2 virus does not have an effect on nitric oxide dependent endothelial microvascular function regardless of physical activity behavior in otherwise healthy, young adults.

The accelerometer data allowed us to consider physical activity as an additional factor related to vascular heath. According to the Physical Activity Guidelines for Americans (2nd edition) (29), young adults should do 300 minutes of moderate exercise per week to see health benefits. The participant's moderate to very vigorous physical activity was slightly below this recommendation, but as shown in table 1, the participants in this study were healthy according to other markers (BMI, BP, HBA1C, etc.). These parameters demonstrate that the vascular health of the participants would not be attenuated because of their physical health. Since physical activity behaviors were not different among the three participant groups, one of the causes for microvascular dysfunction would be infection with the virus, which was not found.

This study has demonstrated that nitric oxide mediated cutaneous microvascular function was not reduced regardless of COVID-19 infection or vaccination status in healthy, young adults. The intensity of symptoms, or time from COVID-19 diagnosis also did not affect microvascular function. Vaccination status did not affect microvascular function either. The findings of this study can be attributed to the many redundant mechanisms that healthy, young adults in their cutaneous microvasculature to help respond to an infection like COVID-19.

Overall, this study adds to the literature currently available about the SARS-CoV-2 virus and the effect it has on microvascular function. As seen during the past two years, data about COVID-19 is continuously being published as the newest findings come out. The results from this study conflict with the prior study by Nandadeva et. al (2021), which found that peripheral microvascular function was blunted in their post- COVID-19 group (12). This study was performed closer to the date of COVID-19 diagnosis which may explain the differences in the results. In the study by Ratchford et. al (2021), there were no differences in microvascular function found between the post-COVID-19 group and the control group which aligns with results from our study (11).

This project had several limitations. The participants that were involved with the study were overall very healthy, the findings of this study may not generalize to populations of people who have other health concerns. The majority of the physical activity data was collected during the spring and summer months in a climate with variable weather during the different seasons. Physical activity behavior of the participants may be different during colder months with less opportunities for exercise outside. The accelerometers used in this project were not waterproof which meant that swimming workouts were not able to be counted toward physical activity, so participants that rely on swimming as a main form of physical activity had to be excluded from the physical activity analysis as well.

Future studies should consider investigating vascular function farther out from COVID-19 diagnosis or vaccination. Also, people who have been infected with the virus more than once should be studied to see the effect reinfection would have on vascular health. Cutaneous microvascular health can be affected by aging, kidney disease, hypertension, heart disease, and other diseases. Future studies should look at special populations of people who had COVID-19 along with other comorbidities that may affect their vascular health. Older adults who are otherwise healthy but have had COVID-19 should also be studied.

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ACADEMIC VITA

EDUCATION

The Pennsylvania State University

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Bachelor of Science, Kinesiology (Movement Science)

RESEARCH

The Pennsylvania State University Department of Kinesiology, Muscle Function and Locomotion

Undergraduate Research Assistant, Fall 2019- Spring 2020

- Hands on work in the lab, with guinea fowl
- Analyzed videos from past experiments
- Attended bi-weekly team meetings
- Wrote a literature review about foot-strike type and related multiple papers in the review

The Pennsylvania State University Department of Kinesiology, Thermoregulation and Microvascular Function

Undergraduate Research Assistant, Spring 2021- Spring 2022

- Assisted with experiments in the microvascular function lab
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HONORS

Penn State Schreyer Honors College Scholar
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AWARDS

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