

HONORS THESIS RESEARCH PROJECT: EMPLOYING THE VENDYS DIGITAL
THERMAL MONITORING AND HAIR SAMPLES TO DETERMINE THE
EFFECTS OF LONG COVID ON ENDOTHELIAL FUNCTION
AND CORTISOL LEVELS IN COLLEGE STUDENTS

by

Hannah Le

Submitted in partial fulfillment of the requirements for Departmental Honors

The Harris College of Nursing

Texas Christian University

Fort Worth, TX

May 5, 2025

HONORS THESIS RESEARCH PROJECT: EMPLOYING THE VENDYS DIGITAL
THERMAL MONITORING AND HAIR SAMPLES TO DETERMINE THE
EFFECTS OF LONG COVID ON ENDOTHELIAL FUNCTION
AND CORTISOL LEVELS IN COLLEGE STUDENTS

Project Approved:

Supervising Professor: Dennis Cheek RN, PhD

Department of Nursing

Vaughna Galvin DNAP, CRNA

Department of Nurse Anesthesia

Ann Johnson PhD, APRN, CPNP-PC

Department of Nursing

ABSTRACT

The long-term effects of COVID-19, commonly referred to as long COVID, have generated increasing concern regarding its impact on cardiovascular health and chronic stress regulation. While much of the existing research has focused on older adults or individuals with pre-existing conditions, young adults, particularly college students, remain an understudied population in this area. Given the unique physiological and psychological stressors that students experience, it is critical to explore whether long COVID contributes to measurable changes in stress biomarkers and vascular function, both of which have implications for long-term health outcomes.

This study aims to examine the relationship between long COVID, cortisol levels, and endothelial function in college students by utilizing two key physiological assessment methods: the VENDYS II Digital Thermal Monitoring (DTM) system to evaluate endothelial function and hair cortisol analysis to assess chronic stress levels. The study included participants who self-reported long COVID symptoms and those who did not, allowing for comparative analysis between the two groups. The primary outcome measures were cortisol levels, which provide insight into prolonged activation of the hypothalamic-pituitary-adrenal (HPA) axis due to chronic stress, and the vascular reactivity index (VRI), which serves as a marker of endothelial function and overall cardiovascular health.

Findings from this study indicate that students with long COVID exhibited higher cortisol levels compared to their counterparts without long COVID, suggesting a prolonged physiological stress response. However, while an upward trend in cortisol levels was observed, statistical significance was not reached ($p = 0.08$), potentially due to sample size limitations or variability in individual stress responses. More strikingly, the vascular reactivity index was significantly

lower in the long COVID group ($p = 0.05$), indicating compromised endothelial function. This finding is particularly concerning as impaired endothelial function is an early marker of cardiovascular disease, suggesting that even in a young, otherwise healthy population, long COVID may contribute to long-term vascular impairment.

These results underscore the necessity for early detection, lifestyle modifications, and targeted interventions to mitigate both cardiovascular and stress-related risks in young adults recovering from COVID-19. Given the increasing prevalence of long COVID and its potential to affect daily functioning, college students may benefit from institutional policies that incorporate screening measures, mental health support, and education on stress management and cardiovascular health. Nurses play a pivotal role in addressing these concerns through patient education, advocacy, and the development of care strategies aimed at minimizing long COVID-related complications.

Future research should focus on expanding sample sizes to improve statistical power, incorporating additional biomarkers to enhance the understanding of long COVID's physiological impact, and conducting longitudinal studies to determine whether observed vascular and stress-related impairments persist over time or improve with targeted interventions. Understanding the long-term effects of long COVID in young adults will be essential for developing effective public health strategies and ensuring that this population receives appropriate medical and psychological support.

Acknowledgments

I would like to express my deepest gratitude to my committee members for their guidance, encouragement, and mentorship throughout my honors thesis journey:

Dr. Cheek, thank you for being an exceptional and inspiring primary supervisor. Your wealth of experience in the lab and dedication to teaching made this process seamless and enjoyable. From the very beginning, you welcomed me into the lab and created an environment where I felt comfortable learning and asking questions. You took the time to explain complex concepts with clarity and patience, and your encouragement gave me the confidence to tackle challenges I initially found intimidating. Your ability to blend expertise with approachability not only made the lab work an invaluable experience but also deepened my appreciation for research in nursing. You have profoundly influenced my growth as a student, researcher, and future nurse, and I am grateful for your mentorship.

Dr. Galvin, thank you for sharing your incredible knowledge and expertise in support of my honors thesis. Your insights were instrumental in shaping this project, and your dedication to excellence inspired me to push my boundaries. I am grateful for your contributions and guidance throughout this process.

Dr. Johnson, thank you for your support and mentorship throughout my honors thesis journey. Your encouragement and insightful feedback pushed me to strengthen my research and approach challenges with confidence. Your passion for teaching and commitment to student success have been truly inspiring, and I feel fortunate to have learned from you.

Contents

Introduction

COVID-19, a global pandemic, has profoundly disrupted lives across the world, leaving a lasting impact on the physical and mental health of many. While the acute symptoms of the virus are widely recognized, a growing number of patients experience prolonged complications beyond the initial phase of infection. This condition, known as long COVID, is emerging as a significant public health challenge. Long COVID manifests with a wide range of symptoms, including persistent fatigue, muscle pain, cognitive dysfunction (commonly referred to as brain fog), and sleep disturbances (Zheng et al., 2024). In addition to these physical symptoms, long COVID can also have profound emotional and psychological effects, further complicating the recovery process for those affected (Zheng et al., 2024).

Beyond these symptoms, long COVID has been shown to affect cardiovascular health. Among the most common cardiovascular symptoms is exertional dyspnea, which can significantly limit physical activity and quality of life (Vafa et al., 2023). More alarmingly, long COVID has been associated with severe cardiovascular complications, such as myocarditis, myocardial infarction, acute heart failure, arrhythmias, and venous thromboembolism (Vafa et al., 2023).

College students are a unique population, navigating the challenges of academic success, personal growth, and the transition to adulthood. With demanding schedules filled with exams, internships, extracurricular activities, and financial pressures, they are particularly vulnerable to stress-related complications (Clayborn & Kerr, 2023). Investigating the effects of long COVID

in this demographic is critical, as this is a formative time when lifelong habits and health patterns are established.

Hair cortisol concentrations offer a retrospective measurement of chronic cortisol production, serving as a promising biomarker for physiological stress. By analyzing cortisol levels in hair samples, researchers can better understand the stress experienced over weeks to months (Torrecillia & Barrantes-Vidal, 2021). Endothelial dysfunction, which disrupts the permeability of the endothelial barrier, plays a significant role in the inflammatory processes that contribute to cardiovascular diseases (Sun et al., 2020).

While previous studies have examined the impact of long COVID on various populations, including the elderly, pregnant individuals, and specific ethnic groups such as Brazilian and Black Americans, there is little research focusing on college students. Furthermore, the association between long COVID, cortisol levels, and endothelial function in this population remains largely unexplored. Understanding these relationships can empower college students to recognize potential risks and implement strategies for symptom management.

Despite the growing body of literature on long COVID, there is a critical gap in research focusing on its impact on college students. This study aims to investigate the relationship between long COVID, cortisol levels, and endothelial function in this population. The findings will provide valuable insights into the physiological and psychological effects of long COVID, enabling college students to recognize and manage potential risks effectively.

The project research question is: Is there an association between endothelial function and cortisol levels in college students with long COVID compared to those without?

Theoretical Framework

The theoretical framework guiding this study is the **Biopsychosocial Model**, which emphasizes the interplay between biological, psychological, and social factors in health outcomes. Long COVID is a multifaceted condition that affects individuals not only physiologically, as evidenced by endothelial dysfunction and elevated cortisol levels, but also psychologically and socially, influencing mental health and overall quality of life (Engel, 1977; Wade & Halligan, 2017). This model is particularly relevant as it allows for a holistic understanding of how stress (evidenced by cortisol levels) and vascular health (endothelial function) interact to impact the well-being of college students with long COVID. By integrating these dimensions, the Biopsychosocial Model supports the study's aim to explore the relationship between long COVID symptoms, physiological stress markers, and vascular reactivity. This approach highlights the importance of addressing both the physical and psychological aspects of health in this population, fostering a deeper understanding of long COVID's comprehensive impact and guiding potential interventions (Engel, 1977).

Review of Literature by Themes

Cortisol and Physiological Stress

Cortisol, often referred to as the "stress hormone," plays a critical role in the body's stress response and overall homeostasis. A systematic review by Xiang et al. (2023) demonstrated a significant negative association between psychological resilience and hair cortisol concentration in a study of 1,064 adults. Notably, this association was stronger in individuals aged 40 years or younger, indicating that younger populations may exhibit heightened cortisol responses to

stressors. Xiang et al. (2023) concluded that further research is necessary to validate the utility of hair cortisol as a biomarker for psychological resilience.

Chronic stress is a state of prolonged physiological or psychological strain that can disrupt homeostasis and increase the risk of numerous health conditions, including hypertension, diabetes, gastrointestinal disorders, cardiovascular diseases, and asthma (James et al., 2023; Dhama et al., 2019). Stress is also a known contributor to mental health disorders, such as anxiety and depression, further emphasizing the importance of identifying and mitigating stress in vulnerable populations.

In a literature review conducted by Botschek et al. (2023), hair cortisol concentration was identified as a promising biomarker for tracking the trajectory of interventions in conditions such as PTSD, depressive disorders, and chronic social stress. However, the review highlighted inconsistencies and heterogeneity in findings, underscoring the need for additional research. Despite the extensive literature on cortisol and stress, research specifically examining cortisol levels in college students is notably absent. Addressing this gap is critical to understanding the intersection of long COVID, physiological stress, and the unique challenges faced by this population.

Long COVID and Vascular Endothelial Function

Endothelial dysfunction is a hallmark of long COVID and is associated with significant health risks. Turner et al. (2024) found that patients with long COVID exhibited elevated levels of inflammatory and endothelial biomarkers, including von Willebrand factor (VWF), platelet factor 4 (PF4), serum amyloid A (SAA), and endothelial-leukocyte adhesion molecules such as E-selectin and Platelet endothelial cell adhesion molecule. These findings suggest the presence

of thrombotic endothelialitis, a condition characterized by the formation of anomalous fibrinoid microclots, hyperactivated platelets, and elevated levels of prothrombotic inflammatory molecules.

Importantly, standard pathology tests often fail to detect these abnormalities because the inflammatory molecules are trapped within microclots rather than circulating freely in the blood (Turner et al., 2024). This limitation highlights the need for advanced diagnostic tools to accurately assess endothelial dysfunction in long COVID patients.

Brambilla et al. (2025) further supported these findings by demonstrating increased levels of proinflammatory cytokines, platelet adhesion, aggregation, and thrombin generation in plasma samples from long COVID patients. These changes contribute to a hypercoagulable state, increasing the risk of thrombotic events and other cardiovascular complications.

Methodology

Study Design and Setting

This study will employ a quasi-experimental design to compare cortisol levels and endothelial function between college students with and without long COVID. Data collection will take place at the TCU Harris Nursing Research Laboratory.

Sample

This study recruited men and women between the ages of 18-22 years of age who have never received a positive COVID test and another group between the ages 18-22 years of age who have

never had a previous positive COVID test. The control group is referred to as Non-COVID and experimental is Long COVID.

The inclusion criteria for the control group will be young adults (18-30 years old) without a previous positive COVID test and for the experimental group will be young adults (18-30) with a previous positive COVID test. Participants will self-identify if they have or have not had a positive COVID test. For the participants who have had a previous positive COVID test, they will have to meet at least 2 symptoms of long COVID including post-exertional malaise, fatigue, brain fog, dizziness, gastrointestinal symptoms, palpitations, change in sexual desire or capacity, loss of or change in smell or taste, chronic cough, thirst, chest pain, and abnormal movements (Thaweethai et al, 2023).

Exclusion criteria would be subjects who have a diagnosis of heart disease, taking medication that impacts the vascular endothelium (ex: statins and vasodilators) or have been diagnosed with Raynaud's disease. Subjects who have had a previous positive COVID test, but are not experiencing any symptoms of long COVID will be excluded as well.

Recruitment:

A recruitment email was sent out to students at Texas Christian University. Potential participants indicated their interest in the study by responding to the email, to prevent the possibility of influence or coercion, potential participants will be informed that participation is completely voluntary and that they may withdraw from the study at any time without penalty. Pre Screening will include a questionnaire requesting potential participants to indicate if they have Raynaud's disease, a wig or extensions, or heart medications. If a potential participant has any questions they can be answered via phone, email or in person in Bass 2212.

Consent procedures

The prescreened participants upon hearing about the study will be invited to Bass 2212 the Nursing Laboratory and the potential participants will be provided with the TCU IRB approved consent document to review and ask any questions with regard to participation. Participation is completely voluntary, and participants may withdraw at any time without penalty. Upon signing the consents in-person, the participant will receive a copy of the signed consent and the original will be retained by the principal investigator. A participant can withdraw by informing Hannah Le and Dr. Dennis Cheek that she or he does not wish to continue participation. Dr. Dennis J. Cheek can be reached by email at (d.cheek@tcu.edu) or in person, Hannah Le can be reached by email (hannah.m.le@tcu.edu), or in person. There will be incentive or compensation to participants in this study.

Privacy and Confidentiality:

All data will be stored in a locked cabinet in the Nursing Laboratory-Bass2212, only the principal and co-investigators will have access to the stored data. The data will be stored for at least 3 years. Electronic data will be stored without any identifying information in a password protected computer. The only people with access to the data (both electronic and hard copies) will be the principal investigator. The data will be presented and published without any identifying information.

Data Collection

Hair Collection

Hair samples were collected following the standardized procedure outlined by Meyer et al. (2014). Each sample was obtained by a trained researcher or faculty member to ensure consistency and accuracy. To minimize external contamination, excess hair was clipped up, and a small section—no more than half the width of a pencil—was carefully cut from the back of the participant's head, as close to the scalp as possible. This location was specifically chosen due to its reduced exposure to environmental factors such as sunlight and hair products, which could potentially alter cortisol levels.

To capture an accurate representation of cortisol deposition over the past three months, at least three centimeters of hair was collected, based on the assumption that human hair grows at an average rate of one centimeter per month. Once collected, the hair sample was secured onto a piece of aluminum foil with the scalp end clearly marked for orientation. The aluminum foil was then labeled with the participant's identification code to ensure proper tracking and analysis.

Hair processing

Hair will be processed for cortisol in the Texas Christian University Harris College of Nursing and Health Sciences Research Lab using the adapted protocol described in the paper of Meyer et al. (2014). From the hair samples, the team will utilize the first 3 cm from the root end of each sample. Each centimeter of hair represents the prior month and will be analyzed to view the average stress response over 3 months. Hair longer than 3 cm will be laid next to a ruler and cut to be 3 cm. A screw-cap polypropylene tube was weighed on a Sartorius balance. The hair sample will be placed in the tube and weighed again to get the sample weight, which could range from 10 to 50mg of hair. The hair samples were then immersed in Salimetric immunoassay diluent buffer to facilitate cortisol extraction. Two 6 mm chrome steel beads were added to each

sample tube to aid in homogenization. The tubes were then placed in a Thermo-Scientific SpeedVac centrifuge to concentrate the samples by evaporating the solvent. A SPEX® SamplePrep Homogenizer will be used to pulverize the hair for at least a minute. Once the sample is in a powder form, 2.0 mL of HPLC- grade methanol will be added to the polypropylene tube and will be left to incubate for 24 hours at room temperature on a Labnet Rocker. The methanol will be removed using a speed vac centrifuge, 1.0 mL of the supernatant will be transferred to a microcentrifuge tube. The team will ensure not to dip the pipet into the powdered hair. After the methanol is extracted from the tubes, the remaining cortisol extract is reconstituted using immunoassay cortisol analysis diluent buffer (Meyer et al., 2014).

Utilizing the Salimetrics ELISA kit, the reagents will be prepared, and the standards, controls, and hair samples are laid out on the plate and run in the microplate reader (Salimetrics, & SalivaBio, 2013). The microplate reader software analyzes the cortisol levels in $\mu\text{g}/\text{dL}$ and then converted to pg/mg using the formula provided (Meyer et al., 2014).

Digital Thermal Monitoring:

The digital thermal monitoring device or Vendys II (Endothelix; www.endothelix.com) is a Federal Drug Administration (FDA) approved device for measuring vascular reactivity using digital thermal measures. The device is completely non-invasive and utilizes a standard blood pressure cuff and temperature finger probes for the right and left index finger. The temperature probes will begin to measure the fingertip temperature and the device with its intuitive software will determine the vascular reactivity. There are three phases of the test. During the first stabilization section, the temperature output for both hands will be displayed as a stable baseline. In the following section, the red line for the right hand should display a linear temperature drop, while the blue (left hand-control) line should remain stable. In the third section, the red line

should show a quick return to the baseline temperature after occlusion. The first phase lasts 5 minutes known as the stabilization phase. If you are feeling cold please let the operator know. The second phase lasts 5 minutes after the first phase is complete, the program will emit a soft sound alert the patient that the occlusion phase is about to start. In this phase, you may feel numbness and tickling in your right hand due to occlusion via the blood pressure cuff staying inflated. There are normal symptoms such as tingling, or numbness participants may experience during the second phase lasts 5 minutes. The deflation phase is the third final phase 5 minutes when the blood pressure cuff on the right arm deflates rapidly. You will notice your right lower arm and hand may become turning red due to the restoration flow of the warm blood. During rapid deflation, there will be more blood rushing to your forearm and hand than usual. After 1-2 minutes. The redness will disappear, and your hand and your arm will return to normal. The total amount of time required for the participants will be about 40 minutes in a one-time visit.

Analytical Plan

The following represents the data analysis plan for the objectives of the proposed honors project.

- Descriptive statistics and an unpaired t-test was conducted to examine the differences in mean cortisol levels between college students without COVID versus those with Long-COVID.
- Descriptive statistics and an unpaired t-test was conducted to examine the differences in mean Vascular Reactivity Index between college students without COVID versus those with Long-COVID.

RESULTS

When comparing the Long COVID group ($n = 7$) to the Non-COVID group ($n = 8$), cortisol levels were higher in the Long COVID group ($M = 24.26$, $SD = 17.87$) than in the non-COVID

group (M = 11.42, SD = 6.62). The unpaired t-test indicated that this difference trended towards statistical significance ($p = 0.08$). (See figure 3)

For vascular reactivity index (VRI) values, the Long COVID group exhibited lower values (M = 2.32, SD = 0.16) compared to the Non-COVID group (M = 2.68, SD = 0.37). The unpaired t-test showed this difference was statistically significant ($p = 0.05$). (See figure 3)

Height ($p=0.24$) not statistically significant. Weight ($p=0.05$) statistically significant. Age ($p=0.79$) not statistically significant. BMI ($p=0.36$) not statistically significant. (See figure 1)
Diastolic ($p=0.13$) not statistically significant. Systolic ($p=0.7$) not statistically significant. (See figure 2)

DISCUSSION

The purpose of this study was to evaluate the potential relationship between cortisol levels and endothelial function, as measured by the vascular reactivity index (VRI), in college students with and without long COVID symptoms. The findings contribute to the growing body of research on long COVID by focusing on a younger population, which has been underrepresented in prior studies.

Cortisol Levels and Stress Response

The COVID group, comprising individuals with long COVID symptoms, demonstrated higher mean cortisol levels (M = 24.26, SD = 17.87) compared to the control group (M = 11.42, SD = 6.62). While this difference did not reach statistical significance ($P = 0.08$), the trend suggests a potential relationship between long COVID and elevated physiological stress levels. These findings align with previous research indicating that cortisol serves as a key biomarker for

chronic stress and inflammation (Xiang et al., 2024; Botschek et al., 2023). Elevated cortisol levels in individuals with long COVID may reflect the body's prolonged activation of the hypothalamic-pituitary-adrenal (HPA) axis in response to ongoing symptoms such as fatigue, brain fog, or other stress-inducing conditions.

However, the substantial variability in cortisol levels within the intervention group ($SD = 17.87$) indicates the need for further research to identify potential moderators, such as the duration of long COVID symptoms, individual resilience, or pre-existing health conditions. The absence of statistical significance may also be attributed to the small sample size ($n = 15$), limiting the study's power to detect more subtle differences. Future research with larger cohorts and more homogeneous symptom profiles could help clarify these trends and provide stronger evidence for the association between long COVID and cortisol.

Endothelial Function

In contrast to cortisol levels, VRI values showed a statistically significant difference between the Long-COVID group ($M = 2.32$, $SD = 0.16$) and the Non-COVID group ($M = 2.68$, $SD = 0.37$; $P = 0.05$). This finding suggests that endothelial dysfunction may be more pronounced in individuals with long COVID. Reduced VRI values are indicative of impaired vascular reactivity, which has been linked to inflammatory processes and a heightened risk of cardiovascular complications (Sun et al., 2019; Turner et al., 2024). The statistically significant reduction in VRI among the Long-COVID group aligns with emerging evidence that long COVID can contribute to persistent endothelial inflammation, potentially mediated by increased cytokine levels and microthrombi formation (Mroueh et al., 2023). SARS-CoV-2 induces an

inflammatory response in endothelial cells, leading to increased vascular permeability and disruption of blood flow (Libby & Lüscher, 2020).

This finding is particularly relevant for younger populations, as endothelial dysfunction is often a precursor to long-term cardiovascular issues. The results underscore the importance of early identification and management of vascular abnormalities in college students experiencing long COVID symptoms. Interventions such as lifestyle modifications, exercise regimens, and targeted pharmacological therapies may help mitigate the risk of progression to chronic cardiovascular conditions.

Limitations

Several limitations must be acknowledged. First, the small sample size limits the generalizability of the findings and reduces statistical power. Second, self-reported data on long COVID symptoms may introduce bias or inaccuracies. Objective measures of symptom severity and duration would strengthen the reliability of future studies. Third, the exclusion of participants with pre-existing cardiovascular conditions or those on medications affecting vascular function may limit the applicability of the findings to a broader population.

NURSING IMPLICATIONS

The findings of this study have significant implications for nursing practice, particularly in addressing the long-term effects of long COVID in college students. Nurses are uniquely positioned to play a pivotal role in early detection, education, and intervention to mitigate the physiological and psychological impact of long COVID.

1. Screening and Early Detection

Nurses can integrate routine assessments of endothelial function and stress biomarkers, such as cortisol levels, into care protocols for patients reporting long COVID symptoms. Identifying early signs of endothelial dysfunction can help prevent the progression to more severe cardiovascular conditions. This may involve collaboration with interdisciplinary teams to ensure appropriate diagnostic tests, such as vascular reactivity measurements or stress biomarker analyses, are conducted.

2. Patient Education

Nurses must educate young adult patients, including college students, about the potential long-term health implications of long COVID. Key topics include:

- Recognizing early symptoms of cardiovascular and stress-related conditions.
- The importance of regular physical activity, a healthy diet, and stress reduction techniques in promoting endothelial health.
- Encouraging adherence to follow-up care and recommended interventions.
- Nurses can help patients manage fatigue by teaching them about energy conservation techniques and how to pace activities (American Nurses Association, 2025).
- Nurses can also offer guidance related to breathing exercises to build respiratory capacity and aid anxiety management (American Nurses Association, 2025).

By providing education tailored to the needs and concerns of this population, nurses can empower patients to take proactive steps in managing their health.

3. Psychosocial Support

The psychological impact of long COVID, including chronic stress and fatigue, underscores the need for nurses to provide holistic care. Nurses should:

- Advocate for access to counseling and mental health resources to address psychological resilience and coping strategies.
- Facilitate support groups or peer networks where young adults can share their experiences and challenges related to long COVID.
- Promote strategies for reducing academic and social stress, particularly for college students managing multiple responsibilities.

4. Development of Prevention Programs

Based on the evidence linking endothelial dysfunction to long COVID, nurses can advocate for and participate in the development of prevention and wellness programs. These programs may include:

- Health screenings at college health centers.
- Workshops focused on stress management, mindfulness, and cardiovascular health.
- Collaborating with public health initiatives to increase awareness of long COVID among college-aged populations.

5. Clinical Advocacy and Research

Nurses should advocate for policies and practices that address the unique needs of young adults with long COVID. This includes:

- Advocating for funding to expand research on long COVID, particularly in younger populations.
- Encouraging the inclusion of long COVID-related content in nursing education to better prepare future nurses for addressing this emerging health issue.
- Supporting research initiatives that explore innovative interventions to improve vascular health and reduce chronic stress in long COVID patients.

6. Personalized Care Plans

Given the diverse manifestations of long COVID, nurses must collaborate with patients to develop personalized care plans. These plans should account for:

- Individual symptom presentations, such as fatigue, brain fog, or cardiovascular issues.
- Unique stressors faced by college students, including academic and social pressures.
- Interdisciplinary approaches that incorporate physical, psychological, and social interventions.

Figure 1

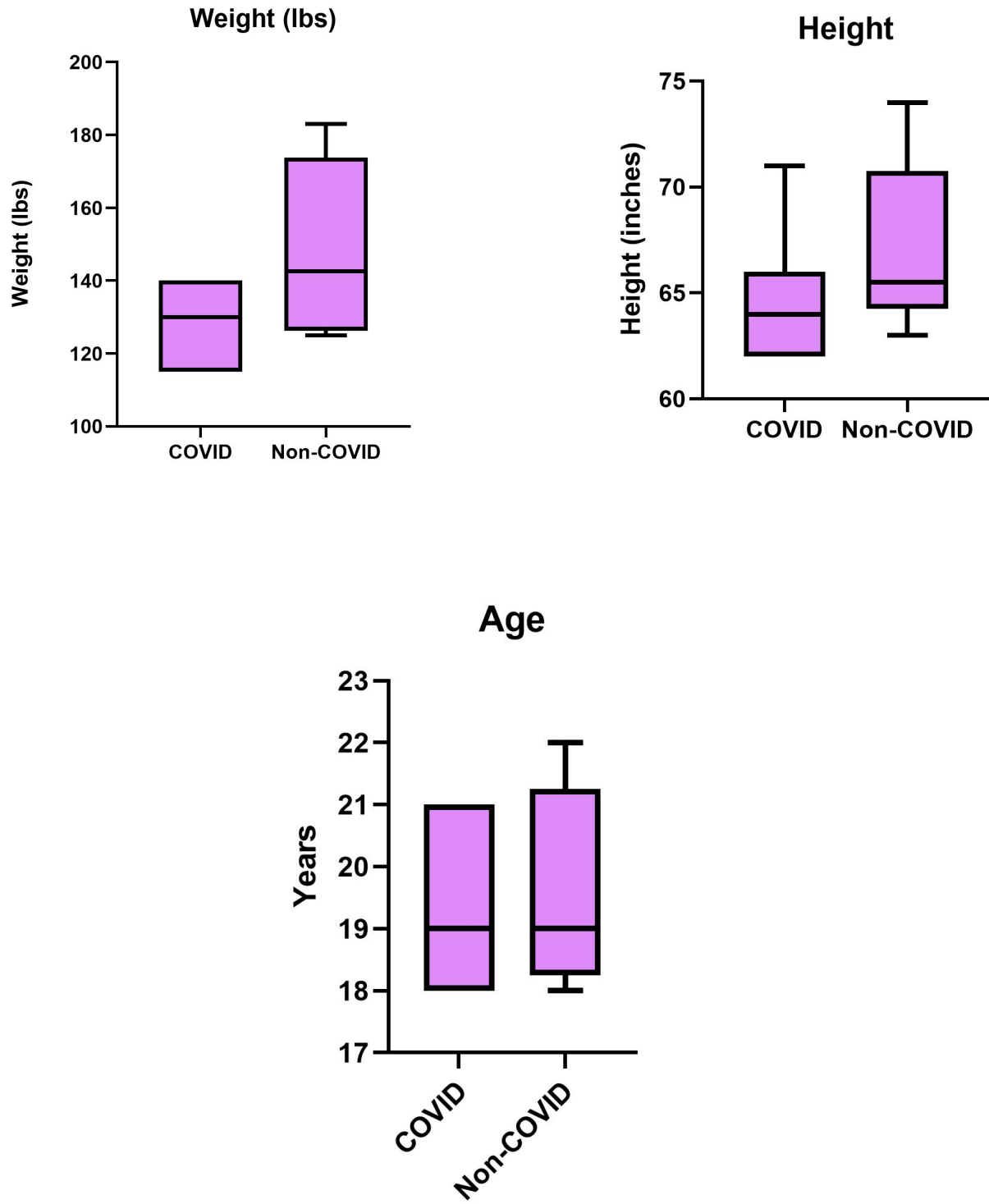


Figure 2

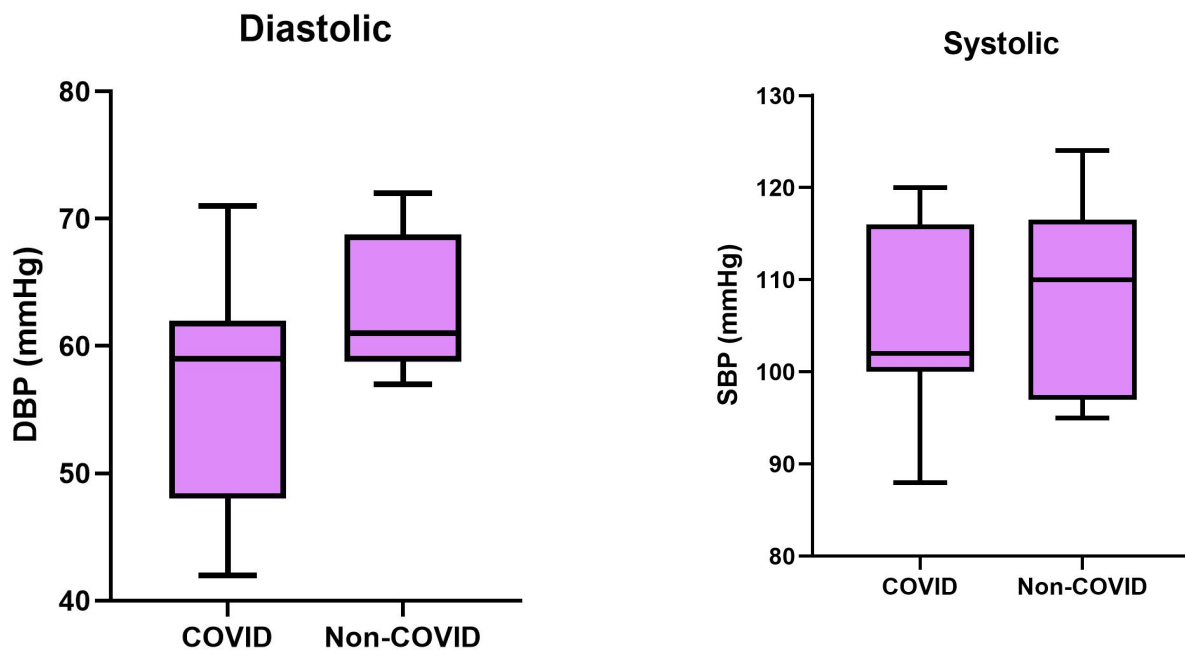
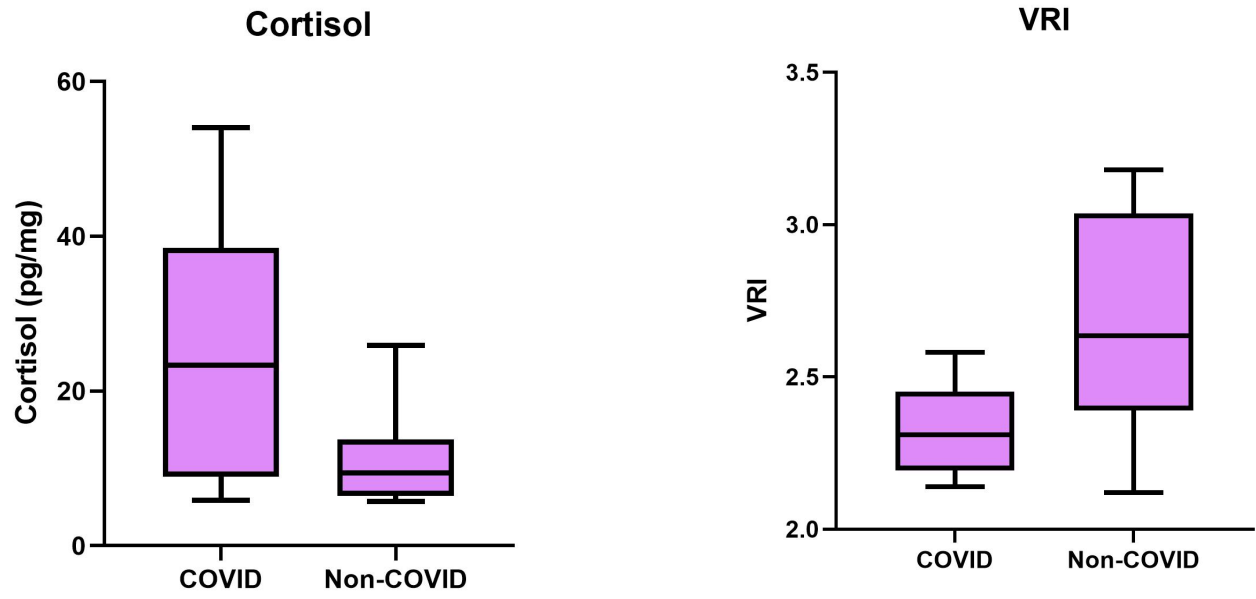


Figure 3



References

- Botschek, T., Schommer, N. C., Schmidt, J., Kolassa, S., & Kolassa, I. T. (2023). Hair cortisol as outcome parameter for psychological and neuropsychiatric interventions—a literature review. *Frontiers in Psychiatry, 14*, 1227153. <https://doi.org/10.3389/fpsy.2023.1227153>
- Brambilla, M., Marini, C., Paglionico, A., Cianflone, D., & Colombo, G. I. (2025). Low-grade inflammation and platelet activation in long COVID syndrome. *JACC: Basic to Translational Science, 10*(1), 20–39. <https://doi.org/10.1016/j.jacbts.2024.11.003>
- Brown, S. L., Nobiling, B. D., Teufel, J., & Birch, D. A. (2011). Are kids too busy? Early adolescents' perceptions of discretionary activities, overscheduling, and stress. *The Journal of School Health, 81*(9), 574–580. <https://doi.org/10.1111/j.1746-1561.2011.00629.x>
- Center for Disease Control, (2019, May 29). *Recess*.
<https://www.cdc.gov/healthyschools/physicalactivity/recess.htm>
- Clark, L., & Rhea, D. (2017). The LiiNK project®: Comparisons of recess, physical activity, and positive emotional states in grades K-2 children. *International Journal of Child Health and Nutrition, 6*(2), 54-61. <https://doi.org/10.6000/1929-4247.2017.06.02.1>
- Condon, E. M. (2018). Chronic stress in children and adolescents: A review of biomarkers for use in pediatric research. *Biological Research For Nursing, 20*(5), 473-496.
<https://doi.org/10.1177/1099800418779214>
- Darling-Hammond, L., & Cook-Harvey, C. M. (2018). *Educating the whole child: Improving school climate to support student success*. Learning Policy Institute.
https://learningpolicyinstitute.org/sites/default/files/product-files/Educating_Whole_Child_REPORT.pdf

- Dubose, K. D., & Mckune, A. J. (2014). The relationship between objectively measured physical activity, salivary cortisol, and the metabolic syndrome score in girls. *Pediatric Exercise Science*, 26(3), 221-230. <https://doi.org/10.1123/pes.2013-0109>
- Emmanouil, C., Bacopoulou, F., Vlachakis, D., Chrousos, G. P., & Darviri, C. (2020). Validation of the Stress in Children (SiC) Questionnaire in a Sample of Greek Pupils. *Journal of molecular biochemistry*, 9(1), 74–79.
- Engel, G. L. (1977). The need for a new medical model: A challenge for biomedicine. *Science*, 196(4286), 129–136. <https://doi.org/10.1126/science.847460>
- Eythorsdottir, D. Y., Frederiksen, P., Larsen, S. C., Olsen, N. J., & Heitmann, B. L. (2020). Associations between objective measures of physical activity, sleep and stress levels among preschool children. *BMC Pediatrics*, 20(1).
<https://doi.org/10.1186/s12887-020-02108-7>
- Fox, S. E., Levitt, P., & Nelson, C. A., 3rd (2010). How the timing and quality of early experiences influence the development of brain architecture. *Child development*, 81(1), 28-40.
<https://doi.org/10.1111/j.1467-8624.2009.01380.x>
- Granger, D. A., Johnson, S. B., Szanton, S. L., Out, D., & Schumann, L. L. (2012). Incorporating salivary biomarkers into nursing research: an overview and review of best practices. *Biological research for nursing*, 14(4), 347–356.
<https://doi.org/10.1177/1099800412443892>
- Groeneveld, M. G., Vermeer, H. J., Linting, M., Noppe, G., van Rossum, E. F., & van IJzendoorn, M. H. (2013). Children's hair cortisol as a biomarker of stress at school entry. *Stress (Amsterdam, Netherlands)*, 16(6), 711-715. <https://doi.org/10.3109/10253890.2013.817553>

- IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.
- Kohl, H. W., & Cook, H. D. (2013). *Educating the student body taking physical activity and Physical Education to school*. National Academies Press.
- Lazarus, R. S., & Folkman, S. (1984). *Stress, appraisal, and coping*. Springer Publishing Company.
- Libby, P., & Lüscher, T. (2020). COVID-19 is, in the end, an endothelial disease. *European Heart Journal*, 41(32), 3038–3044. <https://doi.org/10.1093/eurheartj/ehaa623>
- Martikainen, S., Pesonen, A., Lahti, J., Heinonen, K., Feldt, K., Pyhälä, R., Tammelin, T., Kajantie, E., Eriksson, J. G., Strandberg, T. E., & Räikkönen, K. (2013). Higher levels of physical activity are associated with lower hypothalamic-pituitary-adrenocortical axis reactivity to psychosocial stress in children, *The Journal of Clinical Endocrinology & Metabolism*, 98(4), 619–627. <https://doi.org/10.1210/jc.2012-3745>
- Meyer, J., Novak, M., Hamel, A., & Rosenberg, K. (2014). Extraction and analysis of cortisol from human and monkey hair. *Journal of visualized experiments: JoVE*, (83), e50882. <https://doi.org/10.3791/50882>
- Naya, C. H., Zink, J., Huh, J., Dunton, G. F., & Belcher., B. R. (2021) Examining the same-day relationship between morning cortisol after awakening, perceived stress in the morning, and physical activity in youth. *Stress*, 24(3), 338-347. <https://doi.org/10.1080/10253890.2020.1804852>
- Noppe, G., Van Rossum, E. F., Koper, J. W., Manenschijn, L., Bruining, G. J., de Rijke, Y. B., & van den Akker, E. L. (2014). Validation and reference ranges of hair cortisol measurement

in healthy children. *Hormone research in paediatrics*, 82(2), 97–102.

<https://doi.org/10.1159/000362519>

Osika, W., Friberg, P., & Wahrborg, P. (2007). A new short self-rating questionnaire to assess stress in children. *International Journal of behavioral medicine*, 14(2), 108–117.

<https://doi.org/10.1007/BF03004176>

Pervanidou, P., & Chrousos, G. P. (2018). Early-life stress: From neuroendocrine mechanisms to stress-related disorders. *Hormone research in pediatrics*, 89(5), 372–379.

<https://doi.org/10.1159/000488468>

Rhea, D.J. (2015). *Putting Play, Character Development, Creative Time, and Academic Content in Perspective*. Kinesiology, Texas Christian University. <https://liinkproject.tcu.edu/wp-content/uploads/2020/05/U-S-Play-Coalition-Featured-Presentation-Feb-2015-Rhea.pdf>

Rhea, D. J. (2016) Recess: The forgotten classroom. *Instructional Leader*, 29(1), 1-4.

Rhea, D. J., & Bauml, M. (2018). An innovative whole child approach to learning: The liink project. *Childhood Education*, 94(2), 56–63.

<https://doi.org/10.1080/00094056.2018.1451691>

Rhea, D. J., & Rivchun, A. P. (2018). The liiNK project®: Effects of multiple recesses and character curriculum on classroom behaviors and listening skills in grades K–2 children.

Frontiers in Education, 3. <https://doi.org/10.3389/educ.2018.00009>

Salimetrics, L. L. C., & SalivaBio, L. L. C. (2013). Saliva Collection and Handling Advice.

Southard, E.P. . (2025). Navigating long COVID. *American Nurse Journal*, 20(1), [page 12].

- Stults-Kolehmainen, M. A., & Sinha, R. (2014). The effects of stress on physical activity and exercise. *Sports medicine*, 44(1), 81–121. <https://doi.org/10.1007/s40279-013-0090-5>
- Tajik E., Zulkefli N. A, Baharom A., Minhat H. S., Latiff L.A. (2014). Contributing factors of obesity among stressed adolescents. *Electronic Physician*. 6(1), 771-778.
<https://doi.org/10.14661/2014.771-778>
- Turner, S., Naidoo, C. A., Usher, T. J., Kruger, A., Venter, C., Laubscher, G. J., Khan, M. A., Kell, D. B., & Pretorius, E. (2024). Increased levels of inflammatory and endothelial biomarkers in blood of Long COVID patients point to thrombotic endothelialitis. *Seminars in Thrombosis and Hemostasis*, 50(2), 288–294. <https://doi.org/10.1055/s-0043-1769014>
- Vafa, R. G., Heydarzadeh, R., Rahmani, M., Tavan, A., Mansorkhani, S. K., Zamiri, B., Amiri, F., Azadian, A., Khademolhosseini, A., Montaseri, M., Hosseini, N., Hosseini, S. A., & Kojuri, J. (2023). The long-term effects of the COVID-19 infection on cardiac symptoms. *BMC Cardiovascular Disorders*, 23(1), 292. <https://doi.org/10.1186/s12872-023-03322-8>
- Wade, D. T., & Halligan, P. W. (2017). The biopsychosocial model of illness: A model whose time has come. *Clinical Rehabilitation*, 31(8), 995–1004.
<https://doi.org/10.1177/0269215517709890>
- Wood, C. J., Clow, A., Hucklebridge, F., Law, R., & Smyth, N. (2018). Physical fitness and prior physical activity are both associated with less cortisol secretion during psychosocial stress. *Anxiety, stress, and coping*, 31(2), 135–145.
<https://doi.org/10.1080/10615806.2017.1390083>

Xiang, J., Zhang, L., Zhou, W., & Wang, Z. (2023). The association between psychological resilience and hair cortisol concentration in adults: A systematic review and meta-analysis.

Psychoneuroendocrinology, *157*, 105315. <https://doi.org/10.1016/j.psyneuen.2023.105315>

Yaribeygi, H., Panahi, Y., Sahraei, H., Johnston, T. P., & Sahebkar, A. (2017). The impact of stress on body function: A review. *EXCLI journal*, *16*, 1057–1072.

<https://doi.org/10.17179/excli2017-480>