Examination of Healthcare Workers' Response to Rotating Shift Work During the COVID-19 Pandemic in Greater Victoria Care Sites

by

Marisa A. Harrington
BSc Kinesiology (Hons), University of Victoria, 2019

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

MASTER OF SCIENCE

in the School of Exercise Science, Physical and Health Education

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Abstract

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Nurses are already exposed to plenty of stressors while at work, one of which being the unavoidable nature of rotating shift work scheduling which can have profound physiological effects carrying heightened long-term health risks. Working on the frontlines of the COVID-19 pandemic has introduced new stressors while further exacerbating the effects of pre-existing ones in this already understudied group of essential workers. The purpose of this research was to examine physiological markers of stress and health in nurses during the COVID-19 pandemic. Nine subjects (mean age 32.11 ± 7.25 years) from two hospitals in the Greater Victoria region collected data over an eight-day shift roster consisting of two 12-hour day shifts, two 12-hour night shifts, and four days off in two separate collection periods; remote data collection was used to adhere to COVID-19 safety guidelines. Salimetrics ELISA kits were used to conduct analyses for salivary cortisol, melatonin, and interleukin-6 (IL-6) content. Frequency domain heart rate variability (HRV) was collected with a Polar H10 Chest Strap and Polar Ignite Activity Tracker. A salivary sample and 5-minute HRV recording were obtained upon waking or shortly thereafter on each day; a second saliva sample was obtained after work for the four working days. The Expanded Nursing Stress Scale (ENSS) was completed at the end of the last night shift in each period. There were no significant differences between IL-6 concentrations across the eight days within each

period; the same was observed for cortisol. Additionally, no difference was apparent between the morning and evening salivary cortisol concentrations, thus demonstrating a blunting of the diurnal release pattern. Evening salivary cortisol concentrations remained elevated near the level of morning samples and were consistently above reference values for the population age group. Morning salivary melatonin concentrations significantly differed by day ($F(5, 25) = 6.626, p < 0.001$) but not period; melatonin concentrations were lowest following night shifts, showing a suppression in release due to participants being exposed to light at night with shift work. No statistically significant differences were apparent between any frequency domain HRV parameters in either Period 1 or Period 2. Perceived occupational stress was heightened in comparison to previously published pre-pandemic research using the ENSS. The results of this research reveal alterations to the circadian nature of cortisol and melatonin alongside elevated perceived occupational stress; these physiological and psychological effects can compound the risk for adverse health outcomes. While it is difficult to discern the root cause of these responses, it nevertheless reveals insight into the effects of nurses working during the COVID-19 pandemic and raises concern for potentially related disease risk.

Table of Contents

Supervisory Committee ii
Abstract iii
Table of Contents v
List of Tables vii
List of Figures viii
Acknowledgements ix
Chapter 1: Introduction 1
1.1 Rationale 1
1.2 Purpose 4
1.3 Research Questions & Hypotheses 4
1.4 Delimitations 5
1.5 Limitations 5
1.6 Assumptions 6
1.7 Operational Definitions 6
Chapter 2: Review of Literature 8
2.1 Circadian Rhythm & Disruption 9
2.1.1 Melatonin 9
2.1.2 Cortisol 12
2.1.3 Heart Rate Variability 14
2.1.4 Interleukin-6 17
2.1.5 Psychological & Cognitive Constructs 19
2.2 Population Considerations Regarding Sex 20
2.2.1 Physiological Responses and the Differences Between Sexes 21
2.3 Recovery from Shift Work 23
2.4 Perceived Occupational Stress 27
2.4.1 Physiological Responses and Health Risks 28
2.4.2 Special Considerations with COVID-19 29
2.5 Current Nursing Research 30
2.5.1 Epidemiological Trends & Disease Risk 30
Chapter 3: Methodology 33
3.1 Participants and Recruitment 33
3.2 Study Design 34
3.3 Instrumentation 35
3.3.1 Melatonin, Cortisol, and Interleukin-6 (Salimetrics ELISA) 36
3.3.2 Heart Rate Variability (Polar H10 Monitor and Polar Ignite Activity Tracker) 37

3.3.3 Chronotype (Morningness-Eveningness Questionnaire) 38
3.3.4 Demographics (Participant Demographics Questionnaire) 38
3.3.5 Subjective Stress (Expanded Nursing Stress Scale) 38

3.4 Procedure 39

3.5 Analyses 41
3.5.1 Subjective Stress 41
3.5.2 Heart Rate Variability 41
3.5.3 Salivary Biomarkers 43

Chapter 4: Results 47

4.1 Sample Population Characteristics 47

4.2 Salivary Biomarkers 50
4.2.1 Melatonin 50
4.2.2 Cortisol 52
4.2.3 Interleukin-6 55

4.3 Expanded Nursing Stress Scale 57

4.4 Heart Rate Variability 59

Chapter 5: Discussion 68

5.1 Objective and Subjective Assessment of Stress 68

5.2 Frequency Domain Heart Rate Variability 77

5.3 Eight Day Shift Roster & Potential for Chronic Change 82

Chapter 6: Conclusion 90

6.1 Limitations 92

6.2 Directions for Future Research 94

References 96

Appendix A: Recruitment Poster 106

Appendix B: Informed Consent Package & Consent Log 107

Appendix C: Ethics Certificate 118

Appendix D: Salimetrics ELISA – Manufacturer's Information 119

Appendix E: Heart Rate Log 120

Appendix F: Morningness-Eveningness Questionnaire 121

Appendix G: Demographics Questionnaire 127

Appendix H: Expanded Nursing Stress Scale 130

Appendix I: Study Website 132

Appendix J: COVID-19 Safety Plan 133

List of Tables

Table 3.3.1 Instrumentation and Measurable Outcomes Related to Each Variable of Interest 35
Table 4.1.1 Physical Characteristics of Participants 48
Table 4.1.2 Occupational History of Participants 49
Table 4.1.3 Morningness-Eveningness Questionnaire Scores for Participants 50
Table 4.2.1.1 Mean Salivary Melatonin Concentrations (pg/mL) in Period 1 and Period 2 51
Table 4.2.2.1 Mean Salivary Cortisol Concentrations (µg/dL) on Working Days in Periods 1 and 2 53
Table 4.2.2.2 Mean Salivary Cortisol Concentrations (µg/dL) on Off Days in Period 1 and Period 2 53
Table 4.2.2.3 Change in Cortisol Concentrations (µg/dL) from Morning to Evening on Working Days in Period 1 and Period 2......55
Table 4.2.3.1 Mean Salivary Interleukin-6 Concentrations (pg/mL) in Period 1 and Period 2......56
Table 4.4.1.1 Mean Heart Rate Variability Measurements in Period 1 60
Table 4.4.2.1 Mean Heart Rate Variability Measurements for Period 2 of Data Collection 63

List of Figures

Figure 3.2.1 Shift Roster Schedule for Data Collection ……………………….…….35
Figure 3.3.1.1 Collection of Salivary Variables ....………………………………………37
Figure 3.5.2.1 Period 1 Frequency Domain Heart Rate Variability Statistical Analyses………………………………………………………………….42
Figure 3.5.2.2 Period 2 Frequency Domain Heart Rate Variability Statistical Analyses………………………………………………………………….42
Figure 3.5.2.3 Statistical Analyses for Comparison of Frequency Domain Heart Rate Variability Between Day and Night Shifts in Period 1 and Period 2 ……43
Figure 3.5.3.1 Analyses of Morning Salivary Biomarkers in Period 1 and Period 2 …...44
Figure 3.5.3.2 Comparison of Cortisol Differences During Working Days in Period 1 and Period 2 ....…………………………………………………………..45
Figure 4.1.1 Recruitment Process with Number of Participants Involved in Each Stage ....………………………………………………………………47
Figure 4.2.1.1 Average (Period 1 and Period 2 Inclusive) Morning Melatonin Concentrations ………………………………………………………….52
Figure 4.2.2.1 Average (Period 1 and Period 2 Inclusive) Morning Cortisol Concentrations ………………………………………………………….54
Figure 4.2.2.2 Difference Between Morning and Evening Cortisol Concentrations on Working Days in Period 1 and Period 2 ....…………………………….…55
Figure 4.2.3.1 Average (Period 1 and Period 2 Inclusive) Morning Interleukin-6 Concentrations ……………………………….……………………57
Figure 4.3.1 Average Answer Scores for Expanded Nursing Stress Scale Responses ....………………………………………………………….…58
Figure 4.3.2 Summed Scores for Each Subcategory of the Expanded Nursing Stress Scale in Period 1 and Period 2 ....…………………………………….…59
Figure 4.4.1.1 $LFms^2$ and $HFms^2$ Trends on Day 2, Night 2, and Off 2 in Period 1 ……61
Figure 4.4.1.2 LFnu and HFnu Trends on Day 2, Night 2, and Off 2 in Period 1………61
Figure 4.4.1.3 LF/HF Trend on Day 2, Night 2, and Off 2 in Period 1 ....………………62
Figure 4.4.2.1 $LFms^2$ and $HFms^2$ Trends on Day 1, Day 2, Night 1, Night 2, Off 3, and Off 4 in Period 2 ....………………………………………………….64
Figure 4.4.2.2 LFnu and HFnu Trends on Day 1, Day 2, Night 1, Night 2, Off 3, and Off 4 in Period 2 ....………………………………………………….64
Figure 4.4.2.3 LF/HF Trend on Day 1, Day 2, Night 1, Night 2, Off 3, and Off 4 in Period 2 ....……………………………………………………………65
Figure 4.4.3.1 $LFms^2$ and $HFms^2$ as seen on Day 2 and Night 2 in Period 1 and Period 2 ....………………………………………………………………66
Figure 4.4.3.2 LFnu and HFnu as seen on Day 2 and Night 2 in Period 1 and Period 2 ....………………………………………………………………67
Figure 4.4.3.3 LF/HF as seen on Day 2 and Night 2 in Period 1 and Period 2 ....………67

Acknowledgements

I would like to thank everyone at Island Health who made it possible for us to conduct this research during the COVID-19 pandemic. To the nurses who volunteered their time to be a part of this, I cannot thank you enough for your willingness to participate. Not only do I thank you for your involvement with our research, but for also working on the frontlines of the COVID-19 pandemic. The last 18 months have been anything but normal, and I hope we can shine a light/bring attention to what you have been going through while highlighting the importance of your work.

Thank you to WorkSafe BC for the research training award to help fund this research. Thank you to the University of Victoria for scholarship funding.

Thank you to my support network of friends and coworkers, who I cannot possibly name all of. Greg and Tom, thank you for the logistical help and advice, and for lending your expertise to the development and execution of this research. Amelia, Cailyn, Kim, Nastja, your willingness to help whenever needed does not go unnoticed and I appreciate you all so much. Sylvia, you were always there to listen and made sure I was never too hard on myself. Thank you for being there throughout the highs and lows of this all.

Dr. Coehoorn, thank you for your incredibly valuable insight and assistance throughout this process. I have thoroughly enjoyed working with you, and even though it has only been a short amount of time, I feel that my abilities as a researcher and critical thinker have grown immensely under your guidance. Thank you for your willingness to help with this research remotely from another country and for the fresh perspectives that you brought to this.

Dr. Stuart-Hill, you have played a massive role in not only my scholastic, but also personal development and I am forever indebted to your willingness to take me on as one of your students and help me grow over the last five years. You have provided the utmost support and guidance as an academic supervisor and mentor within the field. I have learned so much from working with you and thank you for always being in my corner.

To my family, thank you for motivating me and helping me keep my perspective. Mom and Dad, you have done more than I can write in here but thank you for being the best support system, for listening to me and dealing with whatever baggage I brought along with me, and for teaching me the importance of hard work. My educational journey has been long, and you have always been by my side to help assure me along the way. Lastly, Nana and Pa, thank you for always believing in me and for investing in my future. This would not have been possible without your love and support. I miss you; this is dedicated to you both.

Chapter 1: Introduction

1.1 Rationale

Nurses are an integral part of the healthcare system and are consistently exposed to stressors while at the workplace (Canadian Institute for Health Information, 2019). Working on the frontlines of the COVID-19 pandemic understandably presents its challenges which can potentially further compound physiological responses to occupational stress, thus placing nurses at increased risk for negative acute and long-term health outcomes (Lai et al., 2020; Maben & Bridges, 2020; Said & El-Shafei, 2021; X. Shen et al., 2020). Documenting and understanding the effects of working on the frontlines of the COVID-19 pandemic is crucial for ensuring employee health and safety, which, in turn, can help improve patient care strategies.

Nurses experience plenty of occupational stressors that can greatly influence their health and job performance (B. B. Arnetz et al., 2017; Purdy et al., 2010; Sarafis et al., 2016; Van Bogaert et al., 2014). Shift work is a necessary aspect of nursing, but exposure to a rapidly-rotating schedule, such as what is seen as Island Health, can have detrimental mental effects on nurses that could extend to patient care (Ganesan et al., 2019; Han et al., 2014; Molzof et al., 2019). The circadian dysfunction associated with shift work further affects various biological rhythms in nurses, and alterations to these can place nurses at an increased risk for chronic diseases, cancer, obesity, and mental health disorders (Peplonska et al., 2015; Ramin et al., 2015; Wegrzyn et al., 2017). The suppression of melatonin release associated with increased light at night due to shift work has been closely linked with decreased cognitive proficiency and an increased risk for breast cancer in female nurses (Davis et al., 2012; Ganesan et al., 2019; Knower et al., 2012; Schernhammer & Hakinson, 2009). A 2007 ruling by the International Agency for Research on Cancer (IARC) listed shift work as “probably carcinogenic to humans”

(Carcinogen Class 2A) resultant from circadian dysfunction; this was further updated in 2019 to include night shift work as the main definition based on recent studies linking human models to cancer (Ward et al., 2019). In British Columbia, shift work is the most prevalent carcinogen that employees are exposed to in the workplace, with nearly 275,000 workers vulnerable (CAREX Canada, 2015).

Perceived stress is elevated in healthcare professions, including nurses, which is related to alterations in circulating cortisol and interleukin-6 (IL-6) levels and can further contribute to negative health outcomes (B. B. Arnetz et al., 2017; J. Arnetz et al., 2019; Hung et al., 2016). Occupational stress is also associated with decreases in heart rate variability (HRV) (van Amelsvoort et al., 2000). Additionally, patient care duties and work environments are highly related to the mental health and wellbeing of nurses. Difficult patients and/or families, poor team cohesion in nursing units, and a lack of perceived autonomy can lead to unfavourable working conditions and thus impact mental health and lead to increased rates of burnout among nurses (Maben & Bridges, 2020; Van Bogaert et al., 2014).

The COVID-19 pandemic has introduced several novel occupational stressors in the profession of nursing (Canadian Nursing Association, 2020a; Maben & Bridges, 2020). An increased threat of exposure to COVID-19 at the workplace and fear of transmission from close contacts have subsequently led to implementation and adherence of social isolation protocols whenever possible; this can extend into decreased socialization with coworkers, families, and friends. Personal protective equipment (PPE) further introduces issues with comfort, security, and stress, as well as physical limitations and restraints that could impact pre-existing protocols for various patient care duties (Canadian Nurses Association, 2020b). Self-isolation combined with stress from working in an environment with the constant threat of exposure to COVID-19

and caring for those that have the disease can have a high burden on nurses' psychological health and wellbeing (Lai et al., 2020; Maben & Bridges, 2020; Neto et al., 2020; X. Shen et al., 2020). Furthermore, there is a heightened potential for an observed increase in long-term disability claims resultant from working during the COVID-19 pandemic (e.g., PTSD).

The added stress of working during the COVID-19 pandemic in an already stressful working environment can lead to myriad physiological changes with affiliated lasting health impacts. In "normal" work conditions (i.e., no pandemic), cortisol and IL-6 increase in times of elevated perceived stress (B. B. Arnetz et al., 2017; J. Arnetz et al., 2019; Yang et al., 2001). However, if stress is constantly elevated at the workplace due to unavoidable conditions and precautions associated with COVID-19, cortisol and IL-6 levels can become chronically raised alongside a corresponding decrease in HRV that can lead to a higher risk for inflammatory and cardiovascular disease, myocardial infarction, and all-cause mortality (M. Kumari et al., 2011; Nijm & Jonasson, 2009; Tsuji et al., 1996). Shift work naturally decreases melatonin production due to increased exposure to light at night, and can further affect physiological and cognitive responses; this can be reflected in impairments to nurses' alertness and vigilance, which can further impact patient care duties (Ganesan et al., 2019). This is not optimal during a pandemic, as nurses need the ability to make quick and efficient decisions to protect themselves and patients from COVID-19.

Previous occupational research has been concerned with examining physiological and psychological effects in nurses while on-shift and immediately before or following a shift; however, little investigation has occurred to determine if the effects remain apparent on days off from work. Current guidelines suggest having at least 11 hours between shifts involving work at night, but research has found that this may not be sufficient for the recovery of some

physiological variables (Davis et al., 2012; Kecklund & Axelsson, 2016). If employees are unable to return to baseline physiologically and psychologically, this could then present a greater risk for negative long-term health outcomes.

1.2 Purpose

The COVID-19 pandemic presents a unique opportunity to gain insight into nurses' physiological responses to stress and health in a global emergency. There is a noted lack of research concerned with examining nurses while on-shift and on days off in a non-pandemic environment; working on the frontlines of the COVID-19 pandemic offers the potential to both exacerbate and add to pre-existing stressors inherent within the profession. Furthermore, only one study has used a subset population of nurses from British Columbia, thus emphasizing the need for greater insight into local/regional nurses (Grundy et al., 2013). The purpose of this study was to conduct a comprehensive analysis measuring physiological indicators of stress and health in nurses on rotating shift work schedules during the COVID-19 pandemic at hospitals in the Greater Victoria region. Nurses' neuroendocrine and autonomic profiles were assessed through analyses of melatonin, cortisol, IL-6, and HRV.

1.3 Research Questions & Hypotheses

The following research questions were evaluated with this study. Related hypotheses are included below.

1. Does working rotating shift work during the COVID-19 pandemic objectively result in greater physiological stress placed on female nurses at Greater Victoria hospitals as measured through cortisol and IL-6 levels, and does this align with heightened perceived stress?

$H1_o$: Working during the COVID-19 pandemic will not result in elevated cortisol and IL-6 levels or higher perceived stress as seen with high Expanded Nursing Stress Scale (ENSS) scores.

2. Does rotating shift work during the COVID-19 pandemic affect autonomic function as measured with frequency domain HRV (i.e., $LFms^2$, $HFms^2$, LFnu, HFnu, LF/HF).?

   $H2_o$: Shift work will not affect autonomic function as measured by HRV in the frequency domain.

3. Is a circadian disruption or heightened stress response still present on days off as indicated through changes in concentrations of melatonin, cortisol, or IL-6?

   $H3_o$: Circadian disruptions and elevated stress responses will not be apparent on days off for employees.

1.4 Delimitations

1. Participating nurses were regulated nurses (i.e., registered nurses, registered nurse practitioners, registered psychiatric nurses, licensed practical nurses) employed by Island Health at Royal Jubilee Hospital or Victoria General Hospital.
2. Nurses were 20 years of age or older, female, did not have cardiovascular or neuroendocrine disease, were not pregnant or nursing.
3. Data was collected at participants' homes before and after shift and while on-shift.
4. Nurses carried out normal duties on-shift and engage in normal daily activities while off-shift.

1.5 Limitations

1. This study could not control for activities on participants' days off. This could potentially impact circadian re-adaptation/phenomena observed on days off.

2. It was not possible to control for events experienced on shift. Some participants may be exposed to more stressful events than others, which could impact corresponding physiological responses.
3. There was an inherent potential for selection bias, as this study accepted participants who voluntarily elected to participate.
4. Participants were collecting data independently (on themselves) without direct researcher supervision; instead, video and written instructions were provided, and participants were able to contact the researchers at any time. This could impact quality of samples collected and resultant data analyses.

1.6 Assumptions

1. Participants followed guidelines related to the study that are appropriate for data collection.
2. The distribution of age and experience among the study population was representative of the larger pool of British Columbian and Canadian nurses.
3. None of the accepted nurses had self-reported pre-diagnosed mental or physical conditions that prevent them from fully participating in the study.

1.7 Operational Definitions

1. Shift work: rapidly rotating schedule used by nurses employed by Island Health consisting of two 12-hour day shifts (0700-1900) followed by two 12-hour night shifts (1900-0700), then four days off.
2. Nurse: employed regulated nurse (i.e., registered nurse, registered nurse practitioner, registered psychiatric nurse, licensed practical nurse) with Island Health.

3. Heart rate variability (HRV): beat-beat variation in heart rhythm as determined by R-R intervals.
4. Circadian rhythm: physiological, mental, and behavioural responses that follow a ~24-hour rhythmic cycle corresponding to environmental change.
5. Stress: the sum of the biological reactions to any adverse stimulus, physical, mental or emotional, internal or external, that tends to disturb an individual's homeostasis.

Chapter 2: Review of Literature

Shift work is a hallmark of nursing in Canada. Shift work, which is a Class 2A carcinogen, poses a risk to nurses' health and safety both acutely and chronically (Ward et al., 2019; Wong et al., 2011). Compromises to nurses' health and functioning can impact their ability to take care of society's sick and vulnerable populations (B. B. Arnetz et al., 2017; Ganesan et al., 2019; Molzof et al., 2019; Purdy et al., 2010). While shift work cannot be eliminated due to the nature of the profession, it is crucial to understand how current shift systems are affecting nurses to examine the potential occupational health and safety risks. The additional stress of working during the COVID-19 pandemic can greatly impact nurses through alterations of various indicators of stress and health (Maben & Bridges, 2020). While it is unknown how long these unique stressors will be present in nurses' workplaces, their effects have the potential to impact nurses' acute and chronic health outcomes.

The following review of literature is composed of five main sections. First, the natural human circadian rhythm and how shift work can lead to subsequent dysregulation will be discussed from a physiological perspective; this will be related to the variables of interest for this study (i.e., melatonin, cortisol, HRV, IL-6) along with cognitive processing. The differences in responses to shift work by sex will then be reviewed, along with the need for population-specific considerations to be accounted for in this study. The effects of occupational stress concerning these physiological variables in conjunction with COVID-19 will also be considered. This will be followed by examining research concerned with recovery from shift work, or the lack of consensus thereof. The review will conclude with a look into the current epidemiological trends observed in nursing research and the apparent strengths and limitations of these.

2.1 Circadian Rhythm & Disruption

Humans follow a diurnal pattern of alertness resultant from entrainment of the circadian rhythm and the biological response to the light-dark cycle (Kalsbeek et al., 2012; Kecklund & Axelsson, 2016; Klerman et al., 2002; Potter et al., 2016). Shift work alters this natural circadian response and affects varying physiological and psychological processes, which can negatively impact health and functioning. It can also lead to an increased prevalence of sleep deprivation and sleep disorders (Kecklund & Axelsson, 2016).

Circadian rhythm is largely derived from the suprachiasmatic nucleus (SCN) within the hypothalamus (Evans, 2016; Kalsbeek et al., 2012; Potter et al., 2016). The SCN controls the cyclic production of melatonin in the pineal gland that is responsible for dictating the multitude of physiological responses to the light-dark cycle through autonomic control of peripheral cell oscillators. These peripheral cells control natural rhythms corresponding to core body temperature (CBT), hormone release, and the stimulation of various organ systems (Klerman et al., 2002). Alterations to circadian rhythms can potentially result in negative acute and chronic health outcomes (Kecklund & Axelsson, 2016).

2.1.1 Melatonin

Melatonin is the main hormone involved in regulating the human light-dark circadian cycle and the related sleep response by serving as the primary stimulus for the SCN (Knower et al., 2012; Wright et al., 2005). Intrinsically photosensitive retinal ganglionic cells (iPRGCs) within the eye contain the light-sensitive photoprotein melanopsin, which is further involved in entraining the circadian release of melatonin (Brainard et al., 2001). iPRCGs contain projections to the SCN via the retionohypothalamic tract along with cone and rod cells; these projections alert the SCN of the presence of light and affect resultant melatonin production (Evans, 2016).

Melatonin also affects the central nervous system and immune system functioning and provides additional protection from oxidative stress through removing extraneous free-radical molecules. Melatonin synthesis in the pineal gland is controlled by the endogenous circadian clock and environmental light exposure; peak melatonin production occurs during the night and low levels are seen during the day (Klerman et al., 2002).

Shift work results in increased exposure to light at night, and employees in assorted working environments have been found to have altered melatonin release patterns. The consensus in the literature shows that employees who work shift work schedules across various industries have suppressed melatonin production at night and a delay in the peak timing in comparison to permanent day workers (Amirian et al., 2015; Burch et al., 2005; Daugaard et al., 2017; Davis et al., 2012; Jensen et al., 2016; Schernhammer & Hakinson, 2009). Exposure to light for as little as 10 minutes at night is significant enough to affect melatonin patterns (Daugaard et al., 2017). This altered melatonin release can have various consequences for health and job performance. Impaired cognitive processing has been linked to suppressed melatonin production at night, and sleep disturbances and mental health symptoms become more pronounced with an increase in consecutive night shifts worked (Burch et al., 2005; Dijk et al., 2012; Ganesan et al., 2019).

Melatonin suppression is associated with an increased risk for hormone-related cancers, including breast cancer (Schernhammer & Hakinson, 2009). Melatonin blocks estrogen production in fibroblasts within breast adipose tissue; specifically, melatonin inhibits excess local estrogen production in the fibroblast, which further inhibits the CYP19A1 gene that is responsible for aromatase production and thus reduces the risk for malignant tumour growth (Knower et al., 2012). When melatonin production is dampened, estrogen production in the

breast can increase and lead to uncontrolled growth. Chronic exposure to night shift work has notably been linked to an increased risk of breast cancer development (Åkerstedt et al., 2015; Cordina-Duverger et al., 2018; Hansen & Stevens, 2012; Lie et al., 2006; Schernhammer & Hakinson, 2009; Wegrzyn et al., 2017).

Measurement of melatonin concentration provides quality insight into circadian function (Klerman et al., 2002). Plasma melatonin measurement allows for high resolution and sensitivity of absolute concentrations but is not recommended for field research due to its invasive nature (Benloucif et al., 2008; De Almeida et al., 2011). Instead, salivary or urinary collection procedures are preferred. Salivary levels represent free, unbound plasma melatonin levels and share similar rhythmicity of concentrations seen in plasma, with oscillations corresponding to evident circadian rhythms. That said, salivary melatonin concentrations account for approximately 30% of total melatonin concentration; a salivary dim-light melatonin onset (DLMO) reading of 3 pg/mL would be similar to a plasma DLMO measurement of 10 pg/mL (Benloucif et al., 2008; Kennaway, 2019). Both mediums of analyses are similar in capturing the circadian response, though serum measurements offer greater depth of measurement regarding the total amount of melatonin produced in comparison to the onset of production which is best reflected in salivary analyses (Kennaway, 2019). Peak values are observed at night, which is also seen in urinary samples that evaluate the presence of melatonin's main metabolite, 6-sulfatoxymelatonin (aMT6s) (Schernhammer et al., 2004).

Analyses of salivary melatonin content additionally provide insight into a subject's melatonin variability over a 24-hour period and can be used to compare responses between day and night shifts (Grundy et al., 2011). Differences in salivary melatonin levels have been observed between permanent day workers and night workers in both sexes across varying

industries (Daugaard et al., 2017). However, research examining female nurses exclusively has found that no difference is apparent (Bracci et al., 2016; Grundy et al., 2011). Grundy et al. (2011) tested a population of Canadian female nurses employed in Kingston, Ontario that followed a similar shift schedule as to what is currently used by Island Health (i.e., two 12-hour day shifts followed by two 12-hour night shifts) and found no difference in melatonin concentrations within subjects. It is known though that the specific work environment can influence the subsequent melatonin response, which can account for contrasting findings and emphasizes the need to test a local group of nurses (Wright et al., 2005).

2.1.2 Cortisol

Cortisol is a glucocorticoid hormone that encourages energy production in response to a stressor that is threatening homeostasis through acting on the hypothalamic-pituitary-adrenal (HPA) axis (Kalsbeek et al., 2012; M. Kumari et al., 2011; Morgan et al., 2002). Cortisol patterns are also affected by dysregulation in human circadian rhythms. The medial parvocellular aspect of the hypothalamic paraventricular nucleus (PVN) synthesizes corticotrophin releasing hormone (CRH) that serves as the driver of the HPA axis; CRH release will stimulate adrenocorticotropic hormone (ACTH) in the anterior pituitary to control cortisol release in the zona fasciculata of the adrenal cortex (Kalsbeek et al., 2012). The SCN controls cortisol release through two main mechanisms, as it directly acts on the HPA axis and impacts CRH release, while autonomic control of the adrenal glands affects the sensitivity of the adrenal cortex to incoming ACTH release to establish responsiveness of the adrenal glands to ACTH.

Cortisol follows a diurnal rhythm in humans, with its release being the highest at waking time in the morning and steadily declining until reaching the lowest point at midnight (Boudreau et al., 2012; Dorn et al., 2007; Kalsbeek et al., 2012; M. Kumari et al., 2011). While melatonin is

the best biomarker for indicating the human circadian response, cortisol offers further understanding when evaluated alongside melatonin and additionally provides insight into the stress response (Dorn et al., 2007; Kalsbeek et al., 2012; Klerman et al., 2002; M. Kumari et al., 2011; Morgan et al., 2002). Cortisol concentrations sharply increase at the time of stress and remain elevated for 24 hours following removal of the stressor, thus demonstrating a significant positive association (Morgan et al., 2002). When the presence of a stressor is chronic, or an individual is consistently exposed to various stressors for an extended period of time, cortisol production remains elevated, and new baseline levels become heightened to establish a new homeostatic level (M. Kumari et al., 2011). Sustained stresses can lead to implications for health due to the constant elevations in cortisol. In the Whitehall II cohort study of British office workers, Kumari et al. (2011) found that sustained levels of cortisol, as resultant in a flattened diurnal curve with heightened evening concentrations, was related to an increase in risk for cardiovascular disease and overall mortality. Research has also found that nurses and physicians experience an increase in cortisol release when experiencing elevated perceived job stress (B. B. Arnetz et al., 2017; Yang et al., 2001).

Salivary cortisol collection is preferred for field research due to feasibility while also maintaining a high level of validity and reliability (Calvi et al., 2017; Dorn et al., 2007). Salivary analyses of male and female early shift workers in vehicle manufacturing found that subjects had a diurnal cortisol release pattern as expected, although cortisol was elevated in the evening on working days in comparison to days off, which was hypothesized to be linked to work-related stress (Šušoliaková et al., 2018). Female nurses at an Italian hospital on rotating shift schedules also had an unaffected diurnal pattern, except peak cortisol levels were lower in comparison to permanent day nurses (Bracci et al., 2016). This aligns with findings in surgeons, as Amirian et

al. (2015) found a decrease in morning salivary cortisol levels from pre-call to on-call days from 6.2 ng to 1.3 ng. Urinalysis of cortisol has demonstrated similar results, as Canadian female hospital employees on rotating shift work schedules had a flatter diurnal curve and overall produced less cortisol (Hung et al., 2016). These rotating shift workers exhibited a second peak in cortisol release 17 hours after awakening. However, Hung et al. (2016) suggest that these alterations are likely temporary, as no difference in cortisol patterns was observed when both permanent day and rotating shift workers were both working day shifts. It is known that stress is a crucial factor influencing cortisol release patterns, which could explain varied findings in different occupations and locations of employment and further strengthens the need to examine a local cohort of nurses.

2.1.3 Heart Rate Variability

HRV is a non-invasive method employed to assess autonomic functioning through measuring the effects of the autonomic nervous system on cardiovascular control (Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology, 1996). The heart's intrinsic rhythm is determined by the sinoatrial node within the right atrium and is set at approximately 100 bpm; this can be modulated through regulation of sympathetic or parasympathetic outflow to either increase or decrease heart rate respectively (Ernst, 2017). This dual innervation allows for appropriate cardiovascular responses to occur in response to internal and environmental factors.

The timescale of HRV measurement dictates whether frequency or time domain measures should be used for analysis. Time domain measurements are used for measurements that are longer in duration, including 24-hour readings (Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology, 1996). Frequency

domain measures are used for shorter duration recordings of two to five minutes Three frequency bands are used for power spectral analysis, which includes Very Low Frequency (VLF) of 0.003 Hz – 0.04 Hz, Low Frequency (LF) of 0.04 Hz – 0.15 Hz, and High Frequency (HF) of 0.15 Hz – 0.4 Hz (Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology, 1996). LF power and HF power are the best-understood frequency bands and therefore are most often used in analyses; HF power is indicative of parasympathetic outflow, whereas LF power is more debated as to whether it is exclusively sympathetic outflow or a mix of both autonomic divisions (Ernst, 2017). LF/HF ratio is generally used to determine the involvement of sympathetic control with higher values indicative of greater sympathetic output (Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology, 1996). Situations of high stress or injury can lead to prolonged and heightened sympathetic activation, as seen in a less variable HRV reading; this can be predictive for the development of cardiovascular disease (Tsuji et al., 1996).

The autonomic control of the heart is under circadian control similar to melatonin and cortisol production and can be observed through HRV analysis. HRV generally decreases during the day as sympathetic activation rises, then increases during the evening alongside a corresponding switch to parasympathetic dominance (Sammito et al., 2016). Peak LF/HF values are seen in the morning, demonstrating that sympathetic activation is highest when awakening from sleep; this coincides with the natural release pattern of cortisol (Boudreau et al., 2012). Sleep-wake cycles and circadian processes influence autonomic cardiovascular control in tandem, as it is theorized that both sympathetic and parasympathetic neuronal projections from the SCN and natural diurnal cortisol secretion influence HRV. Similar research has found that social jetlag, which is resultant from inconsistent sleep/wake times, can lead to increases in heart

rate in apparently healthy individuals as the sympathetic nervous system remains activated for a longer duration in relation to the stress response (Rutters et al., 2014). This further illustrates the interplay between the HPA axis and cardiovascular autonomic control.

Shift type can influence HRV, as S. H. Shen et al. (2016) found that female nurses on rotating shift schedules had greater impairments to cardiovascular autonomic control in comparison to permanent day and night shift nurses through a decrease in parasympathetic-associated HRV variables. S. H. Shen et al. (2016) also report that poor sleep quality and increased risk of cardiac death were seen alongside the impaired HRV profile in the rotating shift work nurses. These responses were only seen in nurses on rotating shift rosters, as no difference was apparent between nurses working permanent days and permanent nights. Changing shifts to be more ergonomically beneficial for workers has shown improvements in HRV with significant decreases in LF power and the LF/HF ratio, along with a significant increase in HF power (Järvelin-Pasanen et al., 2013).

HRV during sleep periods in particular has been under scrutiny, as autonomic dysregulation is apparent in healthcare workers following shiftwork (Burch et al., 2019; Chung et al., 2011; Neufeld et al., 2017). Shift work has been found to result in shorter and more frequently interrupted sleeps coinciding with increased sympathetic activation lasting into days off, which further increases the risk for cardiovascular disease (Chung et al., 2011; Neufeld et al., 2017). Furthermore, having shift workers attempting to induce an adaptation to sleep-wake cycles (i.e., working at night, sleeping during the day) could lead to an increased risk for cardiovascular disease as daytime sleep is associated with greater sympathetic and lower parasympathetic modulation of the heart (Boudreau et al., 2012).

2.1.4 Interleukin-6

IL-6 is a proinflammatory cytokine that stimulates immune activity and neural mechanisms (Nijm & Jonasson, 2009; Zhang & An, 2007). It also interacts with cortisol and the related HPA axis to modulate the stress response in humans. IL-6 synergistically works with interleukin-1 and tumor necrosis factor alpha (TNF-α) to increase glucocorticoid secretion at the adrenal cortex by stimulating synthesis and release of CRH and ACTH in the hypothalamus and pituitary respectively (Nijm & Jonasson, 2009). In turn, cortisol inhibits IL-6 production through a negative feedback loop and prevents it from exerting its effect on target tissues. IL-6 can also exhibit anti-inflammatory properties through controlling the immune response itself (Scheller et al., 2011; Xing et al., 1998).

There is evidence alluding to IL-6 exhibiting diurnal variation like melatonin, cortisol, and HRV. A meta-analysis conducted by Nilsonne et al. (2016) characterized a best-fit model of IL-6 release in which levels were lowest in the morning (at approximately 0900) and peaking in the evening with a second peak occurring near 0200. The low morning values are hypothesized to be related to the cortisol acrophase that also occurs in the morning and the decrease in monocyte and lymphocyte concentrations in peripheral blood (Nijm & Jonasson, 2009; Nilsonne et al., 2016). The peak of IL-6 during sleep is echoed by other interleukins, thus emphasizing the importance of sleep in modulating the immune and stress responses (Lange et al., 2010). Disturbances to sleep can consequently impair IL-6 release and increase the risk for negative health outcomes and all-cause mortality (Vgontzas et al., 1999).

Shift work is known to cause disturbances in sleep and circadian rhythms which can potentially extend to IL-6 levels, though there is still a lack of generalizability apparent in the literature. Nevertheless, some research has demonstrated that IL-6 release patterns can be altered

by exposure to light at night, as a large cohort of permanent night workers in varying industries in Japan had altered IL-6 rhythms (Amano et al., 2018). However, employees on shift work schedules exhibited no alterations in IL-6 rhythmicity, and when analyses were adjusted for job stress this difference was still only apparent in permanent night workers. Amano et al. (2018) state that socioeconomic status could influence results, as permanent night workers within this cohort came from a lower socioeconomic background which often has increases in chronic stress not related to work, which could in turn result in elevations in IL-6. A German study found that shift workers displayed greater subjective reporting of sleep disturbances and poor sleep quality but no accompanying objective impact on IL-6 levels (Van Mark et al., 2010). Although, the authors do not state which industries workers are from along with information pertaining to shift type (e.g., shift duration, consecutive nights worked, etc.); these two aspects must be considered as it is known that stress impacts IL-6, and not every occupation and work environment elicit similar levels of stress. This absence of a consistent definition for shift work can contribute to a lack of homogeneity in the findings.

Perceived stress ultimately has a greater role in impacting IL-6 release than circadian disruptions from shift work (Slavish et al., 2015). A positive correlation has been observed between work stress and serum IL-6 concentrations in nurses and emergency medicine residents and physicians (B. B. Arnetz et al., 2017; J. Arnetz et al., 2019). B. B. Arnetz et al. (2017) further demonstrated that both serum IL-6 ($r = 0.37$) and TNF-α ($r = 0.67$) measurements were related to “near misses” in the emergency department, thus illustrating the link between job performance, stress, and the cytokine response. Ultimately, this can place both attending medical staff (e.g., altered immune response) and patients at risk (e.g., resultant job performance) for negative health outcomes.

Serum measurements are often used in research to obtain peripheral blood levels of IL-6 (Amano et al., 2018; B. B. Arnetz et al., 2017; J. Arnetz et al., 2019; Pejovic et al., 2013; Van Mark et al., 2010; Vgontzas et al., 1999). The invasive nature of serum measurements poses an obstacle for field research, and, like melatonin and cortisol, salivary methods are often used instead. IL-6 levels in serum and saliva share similar associations and response profiles to psychosocial resources and risk factors, although serum concentrations are often smaller due to only capturing IL-6 release from macrophages and monocytes (Sjögren et al., 2006; Van Snick, 1990). Specifically, serum and saliva IL-6 have similar positive associations to stress and therefore can to capture similar responses in this regard (Slavish et al., 2015).

2.1.5 Psychological & Cognitive Constructs

Dysregulated circadian rhythmicity can impact the aforementioned physiological variables and can also be extended to psychological states including mood, decision making, cognition, and alertness. Shift work, which is known to affect sleep status, can exacerbate sleep deprivation in workers and potentially lead to lasting negative health outcomes (Burch et al., 2005, 2019; Ganesan et al., 2019; Kecklund & Axelsson, 2016; Molzof et al., 2019; Neufeld et al., 2017; S. H. Shen et al., 2016). Sleep deprivation induced from shift work is additionally linked with impaired working and short-term memory, impaired executive functioning, and poor emotional functioning (Kecklund & Axelsson, 2016).

Nurses on shift work schedules experience disruptions to circadian rhythms which can affect job performance. Female nurses that work 12-hour shifts in a rotating fashion, which is similar to the current method used at Island Health, experience heightened fatigue (Han et al., 2014). American nurses on shift work schedules experienced greater sleep fragmentation and exhibited a greater discrepancy between waking time and CBT minimum on work days in

comparison to permanent day nurses, thus illustrating circadian dysregulation (Molzof et al., 2019). Working night shifts further results in worse quality of sleep, increased bouts of sleepiness during the day, and greater prevalence of insomnia and anxiety in nurses, and also contributes to decreased cognitive performance as seen through slower cognitive proficiency and impaired reaction times (Ganesan et al., 2019; Molzof et al., 2019; S. H. Shen et al., 2016).

Melatonin levels are also related to mental performance. In a simulated shift work study, Dijk et al. (2012) found that the expected decline in melatonin levels seen in shift work was related to decreases in alertness and performance on a numerical addition task, with greater suppression of melatonin linked to worse performance. Male and female nursing staff working rotating night shift schedules had the urinary aMT6s acrophase associated with higher self-reported bouts of sleepiness during the day and increased reaction times (Ganesan et al., 2019). This response was enhanced when shift cessation was closer to the timing of aMT6s acrophase, with greater impairments in alertness and performance observed. Workers with altered melatonin rhythmic production, as seen in shift work, also have a higher prevalence of sleep, fatigue, and mental health symptoms and disorders (Burch et al., 2005).

2.2 Population Considerations Regarding Sex

The majority of Canadian nurses (92%) are female (Canadian Institute of Health Information, 2019). This means that special consideration must be given to this employee group, as females have unique physiological characteristics that need to be accounted for when evaluating occupational health and safety. It has already been determined that women on shift work schedules are at greater risk for developing hormone-related cancers due to circadian endocrine disruptions (Åkerstedt et al., 2015; Schernhammer & Hakinson, 2009; Wegrzyn et al., 2017). Highly controlled lab-based research has demonstrated that women experienced a more

pronounced decrease in subjective alertness at night than men (Boivin et al., 2016). While this finding occurred in a limited scope of a small number of participants in a laboratory, Boivin et al. (2016) theorize that women could exhibit worse tolerance to shift work along with an increased risk for occupational injury. This is seen in field-based data, as Wong et al. (2011) found that Canadian female workers on rotating night shifts had a heightened odds ratio of 2.29 [1.37, 3.82] for occupational injury compared to their male counterparts. This illustrates that employee health and safety, along with job performance which in the case of nurses is crucial for patient care, can be negatively impacted and place individuals at greater risk for disease or injury and that this can be a heightened risk in working female populations.

2.2.1 Physiological Responses and the Differences Between Sexes

Population makeup can account for conflicting results observed in salivary melatonin analyses. Field-based studies involving either exclusively male or mixed male and female subjects have found that exposure to shift work decreased melatonin levels observed in saliva while on shift (Daugaard et al., 2017; Jensen et al., 2016). Meanwhile, no difference in salivary melatonin levels was apparent in female nurses working permanent days and those on shift work schedules (Bracci et al., 2016; Grundy et al., 2011). This was also seen in lab-based research consisting of both male and female participants, as no difference existed between sexes in melatonin rhythms in a controlled sleep-wake cycle (Boivin et al., 2016).

Varying results have also been observed regarding cortisol release patterns between sexes. Both male and female participants in a simulated shift work environment had elevated fasting serum cortisol concentrations, but females had higher levels than males (Rutters et al., 2014). Male police officers on shift work schedules had a delay in the phase of cortisol release rather than the magnitude of response itself (Jensen et al., 2016). Contrarily, participant pools

composed of both men and women on shift work rosters found that cortisol's diurnal rhythm was maintained, rather the amplitude of results was affected (Amirian et al., 2015; Hung et al., 2016; Šušoliaková et al., 2018).

Cortisol release increases upon the emergence of perceived stress; however, sex can influence the degree of elevation. A subgroup of workers in the British Whitehall II study found that men and women experienced similar salivary cortisol concentrations upon waking, although the waking cortisol response, which measures the change in cortisol from waking to 30 minutes following, was greater in women (Kunz-Ebrecht et al., 2004). Contrastingly, an American laboratory-based study found that men had greater elevations in cortisol throughout the day compared to women (Lovallo et al., 2019). Lovallo et al. (2019) also examined the influence of women taking hormonal contraceptives on cortisol response; morning cortisol levels were higher in women taking hormonal contraceptives than those who were not, but this response tapered off as the day progressed with both groups exhibiting similar responses. This was a laboratory-based study though which limits the generalizability of findings to real-world nursing demands. Additionally, both Kunz-Ebrecht et al. (2004) and Lovallo et al. (2019) tested vastly different age groups (47 – 59 years and 18 – 30 years, respectively).

Men and women in a laboratory-based sleep deprivation study had similar increased IL-6 production in the morning immediately following sleep restrictions (Irwin et al., 2010). However, IL-6 production increased in women as the day progressed, whereas a decrease was seen in men. Field research has shown that populations composed of both male and female workers experienced a similar IL-6 response to perceived stress, although the authors do state that their samples were predominately composed of male participants (B. B. Arnetz et al., 2017;

Van Mark et al., 2010). Additionally, both studies found similar circadian responses between day workers and shift workers.

Sex differences are also apparent in shift workers' HRV profiles. Men shift workers from diverse industries in Denmark experienced a greater maladaptation than their female counterparts, as evidenced through increased total power, while women had a lower LF/HF ratio (Hulsegge et al., 2018). Conversely, male and female shift workers in a Norwegian residential care home did not exhibit any differences in HRV, rather differences existed between age or the sheer fact of being at work or not (Goffeng et al., 2018). The impact of the work environment cannot be overstated when conducting occupational research, which could explain these different findings. It has also been shown that both male and female shift workers in the medical field (e.g., nurses and emergency medical technicians) can have altered HRV responses during sleep, as seen with decreases in HF power (Burch et al., 2019; Neufeld et al., 2017; S. H. Shen et al., 2016). This aligns with findings from both male and female shift workers across various industries who had decreased HRV during sleep periods (van Amelsvoort et al., 2000).

Boivin et al. (2016) found a circadian advancement for CBT in women rather than men, which was also associated with advanced sleep schedules and related subjective alertness. The authors suggest that women are therefore more susceptible to maladaptation to night shift work as observed with decreased nighttime alertness. When applied to shift working populations, this can have a great impact on the occupational health and safety of employees and their coworkers and patients under their care.

2.3 Recovery from Shift Work

Current occupational recommendations suggest that at least 11 hours should be allotted for workers for recovery between shifts (Kecklund & Axelsson, 2016). However, it is unknown

whether the neuroendocrine (i.e., melatonin, cortisol, IL-6) or autonomic (i.e., HRV) responses experience full recovery to pre-baseline levels within this timeframe in female nursing staff populations. This lack of research is concerning, as it is known that the detrimental psychological and physiological effects from shift work can significantly impact human health while on shift, and a potential extension into days off can further hinder health and functioning.

It is possible for employees to re-adapt to initial sleep-wake cycles on days off and achieve baseline melatonin production in various extended shift conditions (Daugaard et al., 2017; Jensen et al., 2016). No difference in melatonin levels was observed in permanent day and night shift male and female employees in various employment sectors; although, when shift work employees' results were compared amongst each other, it was found that melatonin levels were 15.4% lower when on shift compared to days off (Daugaard et al., 2017). Additionally, an increase in nights worked is inversely related to melatonin production in male police officers without change in the peak timing of the circadian rhythmicity (Jensen et al., 2016). However, both Daugaard et al. (2017) and Jensen et al. (2016) did not test a homogenous sample of nurses and did not collect samples each day on recovery to examine if these effects remain blunted throughout or drastically improve on each subsequent day off.

Similar results were seen in Norwegian oil rig workers, as long-term night shift work led to greater dysregulation and re-adaptation to cortisol's baseline levels took longer with an increase in nights worked (Harris et al., 2010). Oil rig workers on a swing roster (i.e., reversal in schedule from one week of permanent nights to one week of permanent days) took one week to recover, whereas workers on two consecutive weeks of night shifts did not exhibit cortisol levels re-approaching a circadian baseline within one week off. A study of surgeons found that cortisol rhythms decreased from pre-call to on-call, but levels were quickly re-adapted on post-call shifts

showing that an adaptation to shift work and type is possible (Amirian et al., 2015). This was also seen in male police officers on shift work schedules, as a recovery back to a circadian baseline for cortisol was seen in all three shift work conditions (Jensen et al., 2016). It should be noted that these studies did not test nurses and had either mixed male and female or exclusively male participant pools, again alluding to how sex and occupation can influence observed results.

A laboratory-based study of young adult men and women found that two days of extended recovery sleeps following a five-day sleep restriction schedule akin to shift work is enough to lower cortisol levels and restore IL-6 to baseline concentrations (Pejovic et al., 2013). The circadian rhythmicity of cortisol was restored while the peak plasma concentration remained reduced. While physiological markers of stress and inflammation were able to exhibit recovery following two "recovery sleeps" (similar to two days off), participants' performance on a motor vigilance task remained impaired throughout the sleep restriction and recovery days. This is of concern for nurses, as physiological markers may indicate readiness to return to work, yet job performance could still be impacted; however, motor vigilance tasks may not be the best for ensuring generalizability with occupational patient care duties. Laboratory-based research has also investigated the effectiveness of using naps for recovering from sleep restriction, and promising results have been seen with IL-6 returning to baseline measurements in young adult males (Faraut et al., 2015). However, both studies were laboratory-based in a highly controlled environment and do not use a sleep restriction that is like the shift work used with Island Health scheduling. Additionally, both sample populations were comprised of young adults and had male subjects, which limits generalizability to female nurses.

Research in nurses specifically related to the physiological happenings on days off from shift work has been limited. Decreases in melatonin production can be lowered into the sleep

after a night shift, yet whether this exists in prolonged recovery (e.g., 4 days off as what is seen with Island Health) remains to be seen (Davis et al., 2012). This conflicts with findings from nurses at an Italian hospital, where no differences in melatonin levels in permanent day nurses and rotating shift work nurses were seen both on-shift and into days off (Bracci et al., 2016). Bracci et al. (2016) did find that cortisol levels remained depressed after shift work, and that 48 hours of rest was not sufficient for shift working nurses to restore cortisol levels like those of daytime nurses.

Autonomic recovery from shift work in terms of measuring HRV has not been thoroughly examined with nurses on shift work rosters. Norwegian nurses in a residential care setting working a shift scheme consisting of four 14-hour day shifts followed by seven days off and then three day shifts with seven days off had higher cardiovascular stress (as seen with an increased LF/HF ratio) on the first day of recovery compared to the fourth day showing that HRV recovery is possible (Goffeng et al., 2018). Similar findings have also been seen in female nurses in Taiwan working a rotating shift schedule, as a recovery in HRV (specifically a decrease in LF/HF ratio and an increase in HF frequency) was apparent following two days off (Chung et al., 2011). However, these studies examined different shift schedules than what is used with nurses employed by Island Health which further emphasizes the need for greater investigation.

While current guidelines and literature are lacking in consensus for determining appropriate recovery guidelines, the benefits of following creating an ergonomically-sound shift schedule can greatly improve and limit negative effects of shift work. An intervention in Finland conducted by Järvelin-Pasanen et al. (2013) found that changes to work schedules for the benefit of the worker decreased sympathetic drive observed through decreases in LF power and LF/HF ratio, along with a significant increase in HF power. While most of the authors'

recommendations for effective shift work scheduling align with current policies used by Island Health, there is one glaring outlier: Järvelin-Pasanen et al. (2013) suggested that extended shifts greater than eight hours are only acceptable if the nature of work and workload are suitable, and complete recovery after work is possible. This is further emphasized by the Canadian Centre for Occupational Health and Safety (2019), who suggest that when rotating shift work is used it should follow a gradual rotation (i.e., morning-afternoon-night) rather than morning-night extremes. This shows that the current study can provide great benefit as it can be determined if the current methods are appropriate or not for the working population of interest.

2.4 Perceived Occupational Stress

Healthcare professionals experience high levels of stress at work (Purdy et al., 2010; Sarafis et al., 2016; Van Bogaert et al., 2014; Yang et al., 2001). Incidences of occupational stress can lead to negative acute and chronic health problems in healthcare workers, and can further impact performance of patient care duties and ultimately outcomes of hospital stays (B. B. Arnetz et al., 2017; J. Arnetz et al., 2019; Sarafis et al., 2016). Emergency medicine resident physicians' ratings of a "near miss" (i.e., similar to a medical event but ultimately did not affect a patient, employee, or visitor) were positively associated with perceived stress as well as systemic inflammation as seen with IL-6 and TNF-α (B. B. Arnetz et al., 2017). Questionnaires and surveys have been developed to best assess healthcare workers' perceived stress levels; the ENSS, developed by French et al. (2000), is a valid and reliable measure that is commonly employed by researchers to quantify the subjective stress seen in nurses. There are nine subcategories on the ENSS, and higher scores are representative of higher perceived individual stress. ENSS scores have been correlated with mental health as well as patient health outcomes (Sarafis et al., 2016).

2.4.1 Physiological Responses and Health Risks

Cortisol is intimately related to stress. When an individual is acutely stressed, cortisol levels spike and remain elevated for up to 24 hours, and when stress remains chronic, cortisol production stays elevated while new homeostatic baselines are set to heightened levels (M. Kumari et al., 2011; Morgan et al., 2002). Hypercortisolism (i.e., chronic exposure to elevated cortisol levels) is also associated with a flatter diurnal rhythm (Nijm & Jonasson, 2009). The resultant tempered cortisol response to stress and flattened diurnal pattern are due to reduced HPA axis activity from the negative feedback loop's reaction to excess circulating cortisol. Chronic cortisol elevation is associated with many negative health outcomes, including cardiovascular disease and all-cause mortality (M. Kumari et al., 2011; Nijm & Jonasson, 2009). In patients with coronary artery disease, a decreased diurnal reduction of cortisol is closely linked with systemic inflammation (Nijm & Jonasson, 2009).

Consistent cortisol elevations in turn increase sympathetic outflow, thus affecting cardiovascular autonomic control (Rutters et al., 2014). This in turn decreases HRV, as the body enters a state of sympathetic dominance associated with heightened catecholamine and cortisol levels. This is seen in situations with elevated occupational stress in workers from diverse industries (van Amelsvoort et al., 2000). Increases in chronic stress can lead to reductions in HRV, which can have negative implications to health including a heightened risk for myocardial infarction and all-cause mortality (Tsuji et al., 1996).

IL-6 shares a positive association with psychosocial stress through its role as a proinflammatory cytokine (Sjögren et al., 2006; Slavish et al., 2015). Proinflammatory states, as seen with elevated IL-6, are closely related to atherosclerosis and cardiovascular disease (Nijm & Jonasson, 2009). In states absent of chronic stress, IL-6 typically opposes cortisol action;

however, if stress were to remain elevated for a prolonged period and caused a hyporesponsive HPA axis, both cortisol and IL-6 would rise (Nijm & Jonasson, 2009). Exposure to situations inducing chronic stress drastically increase IL-6 and set heightened homeostatic baselines, and further increases risks for negative health outcomes and mortality (Kiecolt-Glaser et al., 2003). Chronic elevation of IL-6 concentrations can work to stimulate both ACTH and cortisol release, thus compounding the resultant stress response (Nijm & Jonasson, 2009).

2.4.2 Special Considerations with COVID-19

COVID-19 has introduced new challenges into the medical field that have transformed the traditional working environment in a short amount of time (Maben & Bridges, 2020). This has forced healthcare workers and medical systems to rapidly adapt to the ever-changing pandemic landscape to ensure their own safety and that of their patients. The stress and wellbeing of nurses, particularly those on the frontlines of hospitals with confirmed COVID-19 cases, have been under an immense burden that has greatly affected psychological well-being and mental health (Lai et al., 2020; Maben & Bridges, 2020; X. Shen et al., 2020). For example, nurses working in the intensive care unit of Wuhan Pulmonary Hospital, the main COVID-19 treatment site for critical patients in the city, have reported increases in indigestion, fatigue, sleep disturbances, nervousness, crying, and even suicidal thoughts; these are exaggerated in younger nurses who have limited experience in caring for critically ill patients (X. Shen et al., 2020). A larger cohort of healthcare workers across China further exhibited a high prevalence of mental health symptoms, including depression, anxiety, stress, and insomnia (Lai et al., 2020). These symptoms were more severe in nurses and female healthcare workers, and those working in the Hubei province and Wuhan especially.

2.5 Current Nursing Research

Nurses are a vital part of the healthcare system, and their job performance is critical to ensuring patient safety. In 2018, 431,769 regulated nurses were working in Canada, and 52,996 in British Columbia (Canadian Institute of Health Information, 2019). Shift work, which is known to induce a state of circadian disruption, can affect biological rhythms responsible for individual health and wellbeing for nurses and can also potentially extend to the performance of patient care duties. The COVID-19 pandemic can further exacerbate these detriments in health and job performance.

2.5.1 Epidemiological Trends & Disease Risk

Previous research has found a link between night shift work and an increased risk for various disease states in nurses; these include obesity, diabetes, and cancer (Åkerstedt et al., 2015; Cordina-Duverger et al., 2018; Hansen & Stevens, 2012; Peplonska et al., 2015; Ramin et al., 2015; Schernhammer et al., 2001; Wegrzyn et al., 2017). The link between cancer risk and night shift work has been under scrutiny and led to the classification of night shift work as a Class 2A Carcinogen by the IARC (Ward et al., 2019). Breast cancer is the most widely researched case, as the link between melatonin suppression from increased light at night in shift work and the related rising estrogen levels in breast adipose tissue is the best-understood mechanism behind the link of circadian dysfunction and disease state (Knower et al., 2012). It is generally accepted that female nurses with greater lifetime exposure to shift work (e.g., between 20 – 30 years) have an increased risk for cancer proliferation (Cordina-Duverger et al., 2018; Hansen & Stevens, 2012; Schernhammer et al., 2001; Wegrzyn et al., 2017). A recent Canadian report found that approximately 470 – 1,200 diagnosed breast cancer cases in women are

possibly related to night shift work each year, which accounts for 2 to 5% of overall Canadian cases annually (Pahwa et al., 2019).

These findings are not without limitations. The lack of a standardized definition for "shift work" poses trouble for a clear interpretation of findings. A 2018 pooled analysis conducted by Cordina-Duverger et al. (2018) noted that the included retrospective studies all had different criteria for what categorized "shift work", which could have led to misclassification of information for study participants. The type of shift work performed (i.e., rotating, permanent nights) must also be considered when reviewing results, as this could also influence findings. Rotating shift work, which is used in the current population of interest, has been linked to an increased risk for breast cancer in many international cohort groups (Hansen & Stevens, 2012; Wegrzyn et al., 2017).

The data used to classify disease risk is mainly retrospective in nature and relies heavily on case reports and survey data, as a lack of a comprehensive analysis of neuroendocrine and autonomic factors among others leaves the exact relationship between shift work and risk for various diseases up for debate. The currently accepted hypothesis is that melatonin suppression from shift work leads to overstimulation of the estrogen cascade in the breast, thus affecting fibroblasts (Knower et al., 2012). However, it cannot be stated with absolute certainty as shift work studies examining this link have not used direct physiological measurement methods from participants; instead, surveys and healthcare records have been the preferred method of collection (Grundy et al., 2013; Hansen & Stevens, 2012; Wegrzyn et al., 2017). There is also potential for inherent recall bias within survey collection, as participants may have altered their self-reported exposures to shift work if they were made aware of the IARC rulings regarding night shift work. Many studies have additionally failed to examine sleep habits or ambient

exposure to light along with biomarkers of human circadian dysfunction (Cordina-Duverger et al., 2018).

Plenty of research has focused on examining individual neuroendocrine or autonomic responses to shift work, but a comprehensive analysis of the physiological impact of shift work on a poorly functioning circadian rhythm and elevated stress needs greater attention to see how these factors interact and ultimately affect nurses' health and job performance. The population of nurses within Canada is also in need of greater investigation. Studies have been conducted on nursing cohorts within Canada, but only one has used a sample of nurses from British Columbia which relied on retrospective data to confirm cancer risk (Grundy et al., 2013). There has been more research examining nurses in Ontario, but insight into the recovery (i.e., days off) from shift work alongside a comprehensive analysis remains lacking. Moreover, the COVID-19 pandemic provides a unique opportunity to research our vital frontline healthcare workers responsible for keeping society safe and healthy.

Chapter 3: Methodology

3.1 Participants and Recruitment

This study featured no physical interaction between researchers and participants to adhere to provincial health orders and reduce potential exposure to COVID-19. Participants were recruited from Royal Jubilee Hospital and Victoria General Hospital in the Greater Victoria region via posters sent to a mutual third-party contact employed with Island Health who distributed them to nursing wards in both hospitals and the respective emergency departments (Appendix A). This study used continuous recruitment of participants throughout the data collection timeframe (e.g., rolling recruitment). The group of interest were female nurses employed with Island health, minimum 20 years of age, and working rotating shift schedules at Royal Jubilee Hospital or Victoria General Hospital. Participants were selected for this study with no preference for race or religion.

Participants were emailed the informed consent form for this study upon indication of interest for enrollment (Appendix B). The research team coordinated a phone call with the participant to review the contents of the consent form and to address any questions/concerns. If a participant provided their verbal consent in agreement to participate, their information was recorded in a consent log (Appendix B). Researchers reviewed the verbal consent with participants prior to participating in the second period of data collection; if participants decided not to participate in the second period, their data was destroyed. Ethical Approval was granted by the Island Health Research Ethics Board (#H20-00575) (Appendix C).

Potential participants were excluded if they were pregnant or nursing; currently had or were undergoing medical treatment for endocrine, autoimmune, or cardiac disorders; had been diagnosed with a primary sleep disorder other than shift work sleep disorder; or had reported

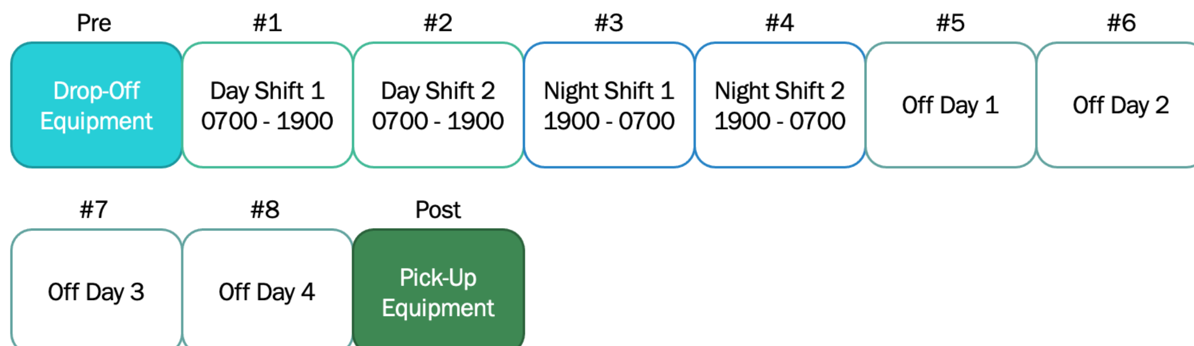
current use of sleep medication or melatonin supplementation. Both full-time and part-time nurses were recruited, as nurses were eligible to participate if they would be working one complete 8-day shift roster within both data collection periods. Twelve nurses originally expressed interest in participating; one was deemed ineligible and two dropped out, thus leaving nine nurses who participated in this study. The same group of participants were in both periods of data collection.

3.2 Study Design

This project followed an observational cross-sectional design. Data was collected by participants for eight consecutive days, with no intervention, at their home and workplace for two collection periods (Period 1 from October – December 2020, Period 2 from January – March 2021). The two collection periods happened to align with widespread provincial health orders; that is, Period 1 was largely collected prior to implementation of restrictions related to the "second wave" of COVID-19 infections within British Columbia, and Period 2 was collected in the midst of the "second wave" during the imposed restrictions along with the beginning of the province-wide vaccine rollout (Judd & Zussman, 2021; Kotyk, 2021; Lindsay, 2020). Participants were instructed to carry out the normal duties of their workday while on-shift and to engage in normal activities while off-shift. Data collection occurred over eight days (four on-shift, four off-shift) (Figure 3.2.1).

Figure 3.2.1

Shift Roster Schedule for Data Collection



3.3 Instrumentation

Table 3.3.1 below all instruments used for data collection in the present study.

Table 3.3.1

Instrumentation and Measurable Outcomes Related to Each Variable of Interest

Variable	Instrument (reliability/validity)	Measurable Outcome
Melatonin	Salimetrics ELISA Immunoassay (Kennaway, 2019)	Circadian dysfunction (Benloucif et al., 2008; De Almeida et al., 2011; Voultsios et al., 1997)
Cortisol	Salimetrics ELISA Immunoassay (Calvi et al., 2017)	Circadian dysfunction (Dorn et al., 2007) Stress response (B. B. Arnetz et al., 2017; Lovallo et al., 2019; Yang et al., 2001)
IL-6	Salimetrics ELISA Immunoassay (Slavish et al., 2015)	Stress response (J. Arnetz et al., 2019; Sjögren et al., 2006)
HRV	Polar H10 Monitor (Gilgen-Ammann et al., 2019)	Autonomic cardiovascular control (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996)

Variable	Instrument (reliability/validity)	Measurable Outcome
Individual chronotype	MEQ (Horne & Ostberg, 1976)	Score out of 86 (lower score = evening chronotype) (Horne & Ostberg, 1976; Sack et al., 2007)
Subjective Stress	ENSS (French et al., 2000)	Likert scaling of subjective relation of perceived stress in nurses in psychological, physical, and social environments (Alomari et al., 2021; French et al., 2000; McGilton et al., 2007; Said & El-Shafei, 2021; Sarafis et al., 2016)

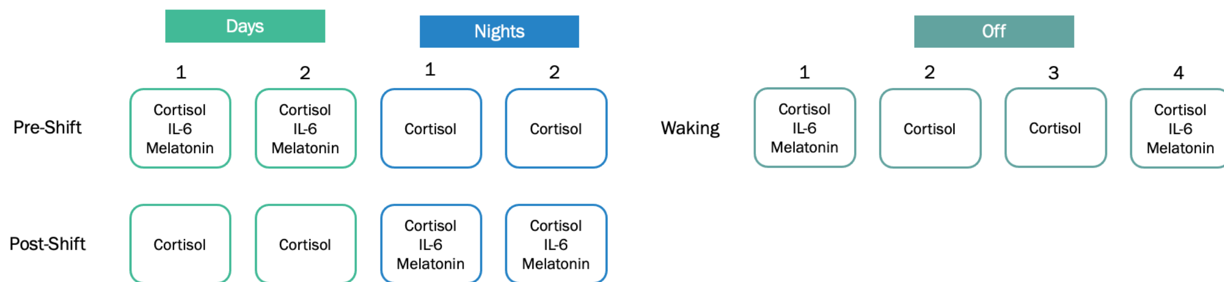
3.3.1 Melatonin, Cortisol, and Interleukin-6 (Salimetrics ELISA)

Salivary samples were collected by a passive drool protocol using Salimetrics Enzyme-Linked Immunoassay (ELISA) kit (State College, PA, USA) (Boudreau et al., 2012; Calvi et al., 2017; Dorn et al., 2007; Kennaway, 2019; Slavish et al., 2015; Yang et al., 2001). Participants collected samples both pre-shift (i.e., upon waking or shortly thereafter) and post-shift, as well as upon waking or shortly thereafter on days off as per manufacturer guidelines (Appendix D). All samples obtained in the morning (i.e., pre-shift for day shifts, post-shift for night shifts, morning for days off) were analyzed for melatonin, cortisol, and IL-6; evening samples (i.e., post-shift for day shifts, pre-shift for night shifts) were analyzed for cortisol content only (Figure 3.3.1.1). Participants were instructed to try and collect samples at roughly equal times (i.e., ± 30 minutes) for all morning and evening collections Participants collected samples with equipment provided by the researchers, which included 12 salivary collection aids, 12 collection vials, and one storage box. Participants placed the samples in a home freezer for storage until the researchers came to retrieve the samples after the last day of collection. Salivary samples were then transported on ice from the participants' homes to the University of Victoria for storage. Samples were frozen at -21^{0} C to maintain viability until assays could be completed. Assays were

conducted by a doctoral student at the Centre for Advanced Materials and Related Technology at the University of Victoria in June 2021.

Figure 3.3.1.1

Collection of Salivary Variables



Note: Collection schedule was the same for both Period 1 and Period 2.

3.3.2 Heart Rate Variability (Polar H10 Monitor and Polar Ignite Activity Tracker)

Heart rate was continuously monitored with a 3-lead Polar H10 chest strap monitor (Kempele, Finland) for 5 minutes upon waking or shortly thereafter for each of the eight days of collection. Participants were instructed to lie quietly in an undisturbed fashion for the duration of collection. The H10 monitor was paired with the Polar Ignite Activity Tracker (Kempele, Finland) worn on the wrists of participants' non-dominant hand to receive data.

Participants synced the Polar Ignite Activity Tracker to a Samsung Galaxy tablet (Seoul, South Korea) containing the Polar Flow application (Version 4.4.6, Kempele, Finland) at the end of each day. Participants were assigned a unique account username and password created by the researchers to allow for storage of data on the device. Researchers were able to access this data through linkage of accounts to a pre-assigned study group through the Polar Flow website. Once the eight days of data collection were complete, participants returned the chest strap and activity

tracker to the researchers. Data was downloaded from the Polar Flow Application and converted from RR intervals to HRV using Kubios HRV Standard (Version 3.4.2, Kuopio, Finland) software on a secure computer. A daily log was provided to participants to allow them to track any strenuous, stressful, or other activities that could impact resultant HRV profiles (Appendix E).

3.3.3 Chronotype (Morningness-Eveningness Questionnaire)

The Morningness-Eveningness Questionnaire (MEQ) is a valid and reliable measure used to subjectively assess individual chronotype developed by Horne & Ostberg (1976). It consists of 19 questions; scores range from 16 to 86. There are five ranges for scores: 16 – 30 is defined as "definitely evening type", 31 – 41 as "moderately evening type", 42 – 58 as "neither type", 59 – 69 as "moderately morning type, and 70 – 86 as "definitely morning type". Participants completed this questionnaire online prior to sample collection during Period 1 of data collection only (Appendix F).

3.3.4 Demographics (Participant Demographics Questionnaire)

A questionnaire was developed by the researchers to obtain information pertaining participants' occupational and medical histories (Appendix G). This served as a further screening mechanism for participants and provided additional demographic and occupational history for participants. This was completed online prior to commencement of data collection during Period 1 only.

3.3.5 Subjective Stress (Expanded Nursing Stress Scale)

Participants' perceived occupational stress was evaluated using the ENSS (French et al., 2000). It is composed of 57 questions divided into nine subcategories, which include nursing-specific questions related to: Death and Dying, Conflict with Physicians, Inadequate Preparation,

Problems with Peers, Problems with Supervisors, Workload, Uncertainty with Treatment, Patients and Families, and Discrimination (Appendix H). The ENSS was completed online following the last worked shift (i.e., after the last night shift (Night 2) in the eight-day shift roster). This was completed in both Period 1 and Period 2 of data collection. Participants' responses were averaged for each question and subcategory, and question averages were also summed within each subcategory.

3.4 Procedure

Participants completed data collection at their homes. This study was contactless in nature to ensure that government-mandated provincial health orders were adhered to, thus lessening the potential exposure to COVID-19 for researchers and participants. The research team developed a website containing relevant information pertaining to this study along with instructional data collection videos and links to the three questionnaires (Appendix I). All interaction between participants and researchers occurred remotely (i.e., email, text message, phone call).

Participants completed the MEQ and Participant Demographics Questionnaire on an online platform (hostedincanadasurveys.com; Market Access Canada, Ottawa, Ontario) prior to the first shift of data collection. Links to these questionnaires were provided in an email that was sent to participants prior to data collection and were also available on the study website. The necessary equipment and instructions for data collection were provided to participants through a mobile delivery service; the research team conducted a contactless drop-off to participants at a pre-arranged time and location to best serve the participant. Appropriate precautions and safety measures surrounding COVID-19 were followed (Appendix J).

On-shift data was collected over a series of four shifts: two consecutive day shifts (0700 – 1900) and two consecutive night shifts (1900 – 0700). Pre-shift salivary samples were collected and analyzed for cortisol, IL-6, and melatonin content (Day 1 and Day 2) or cortisol only (Night 1 and 2). Saliva samples were stored in a home freezer immediately after collection at -21^{0} C. HRV was monitored for a 5-minute period upon waking or shortly thereafter; participants used the Polar H10 chest strap and Polar Ignite Activity Tracker watch. Participants were able to decide whether to collect a salivary sample or HRV recording first; it was recommended that they follow the same order was followed throughout the eight days. When participants returned home from their shift, a post-shift saliva sample was collected to be analyzed for cortisol (Day 1 and Day 2) or cortisol, IL-6, and melatonin content (Night 1 and Night 2); the same collection and storage protocols as the pre-shift sample were followed (Figure 3.3.1.1). When participants finished their last scheduled working shift (Night 2), they completed the ENSS online. The questionnaire link was sent to participants via email.

Off-shift data was collected immediately upon participant waking as per on-day shifts. Cortisol, IL-6, and melatonin were collected through salivary analyses like on-day shifts (Figure 3.3.1.1). Resting HRV was obtained from the Polar H10 chest strap and Polar Ignite Activity Tracker in a similar 5-minute time block upon waking or shortly thereafter.

Participants and researchers once again remotely interacted to arrange a pick-up location for the samples akin to the drop-off process. Participants were provided with equipment pick-up instructions through email. Similar COVID-19 precautions were followed during the equipment pick-up process (Appendix J).

3.5 Analyses

All statistical analyses were conducted using SPSS (Version 26.0, Chicago, IL, USA). The α level for significance was set at $p < 0.05$.

3.5.1 Subjective Stress

ENSS responses were downloaded from the online platform into a .xlsx file for organization into Microsoft Excel (Version 16.50, Redmond, WA, USA). There were issues surrounding data retention, which limited the ability to conduct statistical tests. Instead, average responses were tabulated for each question and subcategory to be used for visual comparison of the data.

3.5.2 Heart Rate Variability

HRV was analyzed using Kubios HRV Standard (Version 3.4.2, Kuopio, Finland). A filter setting of "strong" was used to eliminate excess artifact and extraneous data points; specifically, RR intervals larger than 0.15 sec were removed and replaced with interpolated data from a cubic-spline interpolation (Taravainen et al., 2021). HRV was analyzed using the frequency-domain, which was based on Fast-Fourier Transform (FFT) waveforms to allow for separation of the signal into the components of very low frequency (VLF), low frequency (LF), and high frequency (HF). The parameters of interest for the present study included LFms^2, LFnu, HFms^2, HFnu, and LF/HF. The first and last 30 seconds of the recording were discarded from the recording session; a four-minute interval was therefore analyzed from 0:30-4:30. This removed participant acclimation at the start of the recording and getting up/stopping the recording at the end. The parameters of interest were recorded into Excel and then exported to SPSS for future analyses.

Statistical analyses were conducted using SPSS (IBM; Version 26.0, Chicago, IL). Repeated measures ANOVAs were conducted to examine if any differences were apparent between day shift, night shift, and off-days frequency-domain HRV variables. This was done for Period 1 (Figure 3.5.2.1) and Period 2 (Figure 3.5.2.2) of collection; sample sizes and days of analyses differed due to constraints surrounding missing data. Two-way repeated measures ANOVAs were also conducted to compare the differences between Period 1 and Period 2 in Day 2 and Night 2 (timepoints selected due to limitations regarding missing data) (Figure 3.5.2.3). Significance was set at $p < 0.05$.

Figure 3.5.2.1

Period 1 Frequency Domain Heart Rate Variability Statistical Analyses

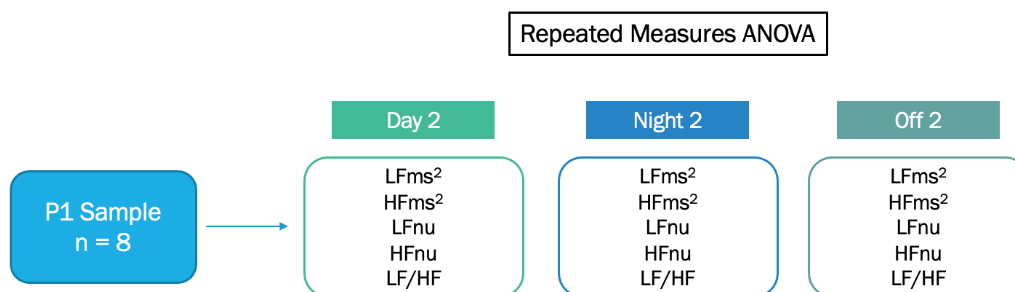


Figure 3.5.2.2

Period 2 Frequency Domain Heart Rate Variability Statistical Analyses

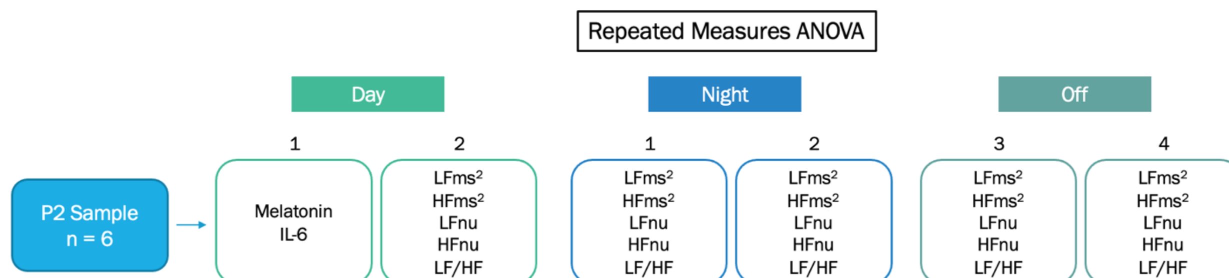
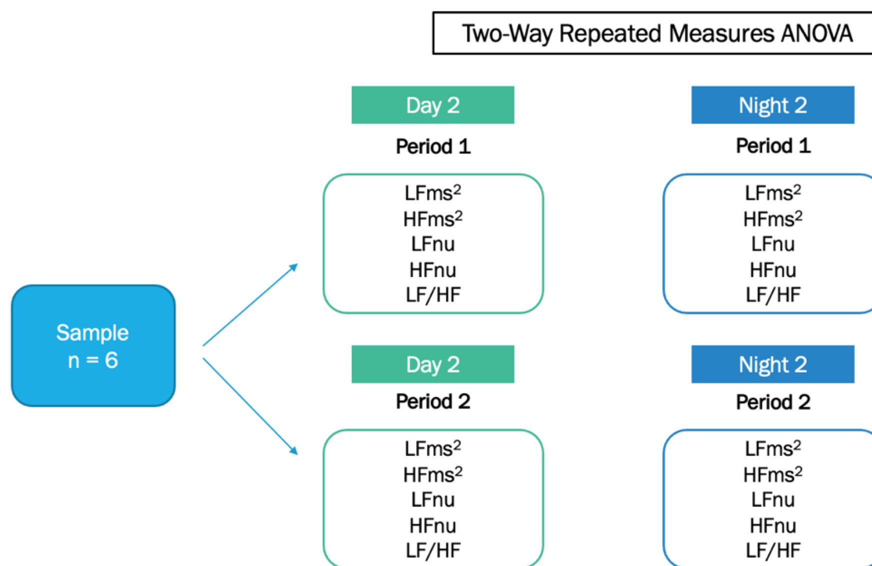


Figure 3.5.2.3

Statistical Analyses for Comparison of Frequency Domain Heart Rate Variability Between Day and Night Shifts in Period 1 and Period 2



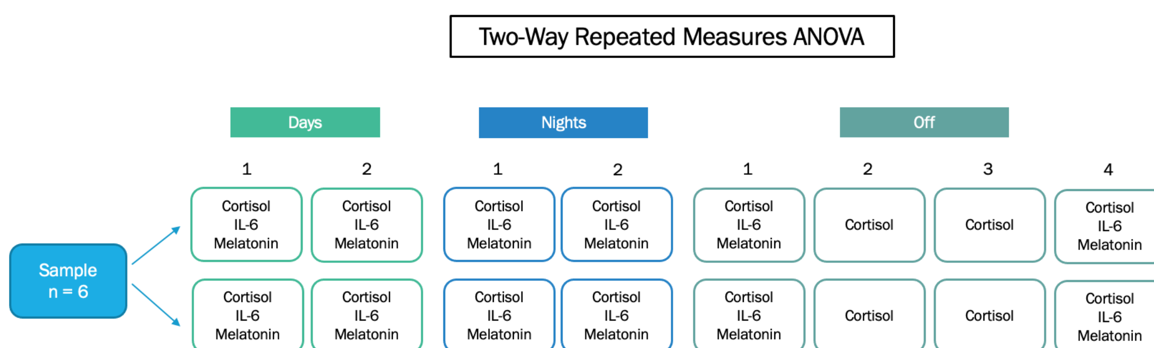
3.5.3 Salivary Biomarkers

All samples were stored at -21^{0} C until each participant completed their involvement in the study; this allowed for the entirety of the sampling wells to be filled and to adhere to COVID-19 safe work restrictions within the Centre for Advanced Materials and Related Technology at the University of Victoria. Samples were analyzed in June 2021. Salimetrics ELISA kits were used for determining the concentration of each biomarker of interest (i.e., cortisol, IL-6, melatonin) and manufacturer instructions were followed for each respective assay (Appendix D). All participant samples were on plates that ran in singlet (due to equipment and funding constraints), with the exception being for standards, controls, zeroes, and non-specific binding (NSB) that were ran in doublet. The raw optical densities (OD) were first attained, after which a wavelength as indicated in each biomarker's assay kit was used to remove incidental OD that was unrelated to the biomarker of concern. The averages for all doublets were then obtained.

All concentration calculations were conducted in Microsoft Excel, and SPSS was used for all statistical analyses. Two-way repeated measures ANOVAs were conducted for the morning concentrations of each biomarker in both Period 1 and Period 2 of collection (Figure 3.5.3.1).

Figure 3.5.3.1

Analyses of Morning Salivary Biomarkers in Period 1 and Period 2



Note: A 2 x 6 repeated measures ANOVA was conducted for IL-6 and melatonin, while a 2 x 8 repeated measures ANOVA was conducted for cortisol due to available days for analysis.

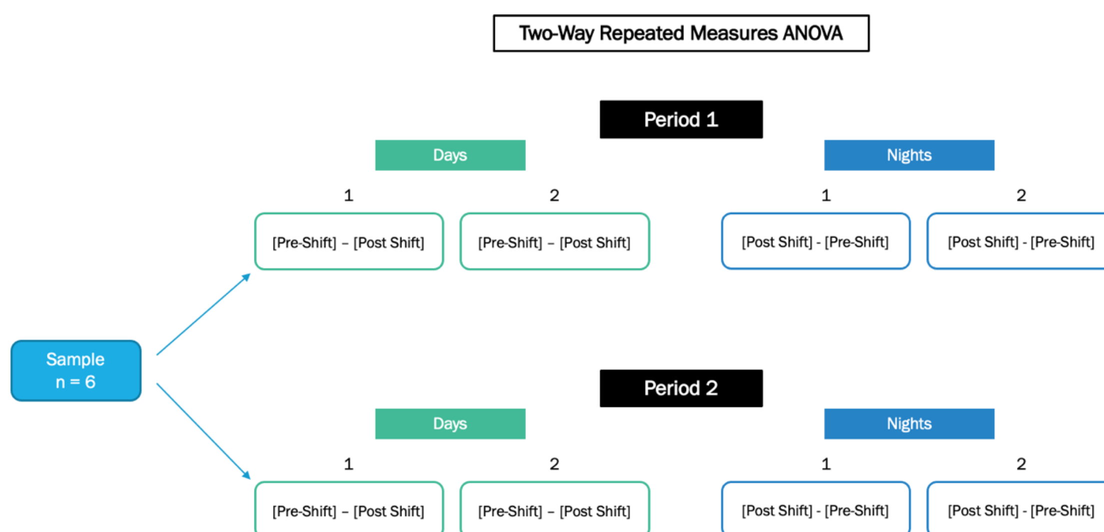
Cortisol concentrations (µg/dL) in each salivary sample were measured with Salimetrics ELISAs as per manufacturer instructions (Appendix D). Two plates were used for analysis; the intraassay variability for Plate 1 was 3.8% and for Plate 2 it was 7.1%. The average OD was calculated for the wells ran in doublet (i.e., zeroes, standards, controls, NSB); this was then subtracted by the OD of the doublet wells to obtain true OD values. The OD of the standards and controls were then divided by the OD of the zero to determine the bound to unbound ratio (B/B_o). A 4-parameter non-linear regression curve was then calculated using the 6 standards

provided and was used to extrapolate the concentrations of each sample. The r^2 for the curve for Plate 1 was 0.9996, and for Plate 2 r^2 was 0.9999.

The differences in morning cortisol values were also compared across the eight days in both Period 1 and Period 2 through use of a two-way repeated measures ANOVA; this is demonstrated above in Figure 3.5.3.1. The rate of change in cortisol concentrations were examined between working shift type (i.e., Day 1, Day 2, Night 1, Night 2). This was done by subtracting evening concentrations from morning concentrations (Yang et al., 2001). The rate of change for each working shift in both Period 1 and Period 2 were evaluated using a two-way repeated measures ANOVA (Figure 3.5.3.2).

Figure 3.5.3.2

Comparison of Cortisol Differences During Working Days in Period 1 and Period 2



IL-6 concentrations (pg/mL) were obtained following Salimetrics ELISA protocols (Appendix D). One plate was used for analysis, with an intraassay variation of 7.9%. The average OD was calculated for all duplicate wells (i.e., standards, controls, zeroes). A 4-parameter non-linear regression curve was then calculated to obtain concentrations of the

controls and saliva samples; $r^2 = 0.9998$. A dilution factor of 5 was multiplied to each concentration to obtain final IL-6 concentrations in each sample. A two-way repeated measures ANOVA was conducted to determine if any differences existed in morning concentration of IL-6 across the six days in question (Day 1, Day 2, Night 1, Night 2, Off 1, Off 4) in Period 1 and Period 2. This is depicted in Figure 3.5.3.1.

Salimetrics ELISA protocols were followed to obtain concentrations of melatonin (pg/mL) (Appendix D). The average OD was calculated for all doublet wells (i.e., zero, NSB, controls, standards). The B/B_o was obtained by dividing the OD of each well by the average OD for the zero. The melatonin concentrations of the controls and participant samples were determined using a 4-parameter non-linear regression curve ($r^2 = 0.9983$). One plate was used for analysis, with an intraassay variation of 2.66%. A two-way repeated measures ANOVA was performed on SPSS to examine the changes of melatonin values in the morning samples obtained on Day 1, Day 2, Night 1, Night 2, Off 1, and Off 4 in Period 1 and Period 2 (Figure 3.5.3.1).

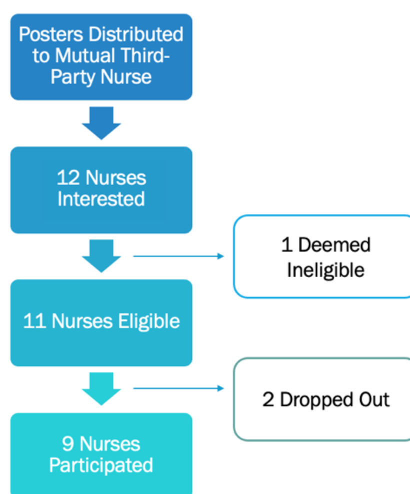
Chapter 4: Results

4.1 Sample Population Characteristics

Nine female nurses from two hospitals in the Greater Victoria Region (Royal Jubilee Hospital and Victoria General Hospital) participated in this study. The initial research plan was designed for 20 nurses to participate and to offer optimal statistical analyses to be conducted; however, due to the unavoidable constraints of COVID-19, the participant pool had to decrease in size. Twelve nurses had expressed interest in participating; one dropped out, one did not meet the eligibility criteria, and one did not return interest from the researchers (Figure 4.1.1).

Figure 4.1.1

Recruitment Process with Number of Participants Involved in Each Stage



All nurses who participated in this research were female; this aligns with current occupational statistics from the Canadian Institution of Health Information (2019) revealing that 95% of the nursing workforce in Canada is women. None of the participants were currently pregnant, none used melatonin supplementation, none had cardiovascular, neuroendocrine, or autonomic diseases or disorders, none were diagnosed with sleep disorders other than shift work

sleep disorder and did not use medication to assist with sleep. The average age of participants was 32.11 years (± 7.25), average height was 167.33 cm (± 8.09), average weight was 67.44 kg (± 17.54), and average BMI was 23.97 kg/m^2 (± 5.53) (Table 4.1.1).

Table 4.1.1

Physical Characteristics of Participants

Participant	Age	Height (cm)	Weight (kg)	BMI (kg/m^2)
1	24	165	52	19.1
2	45	154	52	21.9
3	30	180	84	25.9
4	29	167	104	37.3
5	33	178	76	24.0
6	38	161	66	25.5
7	39	163	55	20.7
8	27	168	58	20.5
9	24	170	60	20.8
Mean	32.11	167.33	67.44	23.97
SD	7.25	8.09	17.54	5.53

Occupational information was also obtained from participants (Table 4.1.2). Eight registered nurses (RNs) and one licensed practical nurse (LPN) participated in this study; four nurses were from Victoria General Hospital and five from Royal Jubilee Hospital. This is of note as Royal Jubilee Hospital is the region's COVID-19 frontline hospital (Farrell, 2020). Shift work history was also obtained from participants through average exposure to night shift work on a weekly and monthly basis, along with cumulative years of work involving night shifts.

Table 4.1.2

Occupational History of Participants

Participant	Occupation	Ward	Hospital	Night Shift Exposure: Average number of night shifts worked per week	Night Shift Exposure: Average number of night shifts worked per month	Night Shift Exposure: Total years spent working night shift
1	RN	Rapid Assessment Discharge Unit Emergency Department	RJH	2	8	1
2	RN	Neonatal Intensive Care Unit	VGH	2	6.5	25
3	RN	Intensive Care Unit	VGH	2	8	3
4	RN	Wounds, Burns, & Urology	RJH	3	10	6
5	RN	Emergency Post Anesthetic Care Unit	VGH	2	6	10
6	RN	Acute Medicine	VGH	2	8	5.5
7	LPN	Complex Thoracic & Vascular Surgery	RJH	1.5	5	8
8	RN	Renal Medicine Rapid Assessment Discharge Unit Clinical Teaching Unit Medical & Surgical Overflow Oncology Respiratory Medicine & Palliative Care	RJH	1	5	4
9	RN	Renal Medicine	RJH	2.5	7.5	3
			Mean	1.90	7.10	7.80
			SD	0.60	1.60	7.20

Note: RN = Registered Nurse, LPN = Licensed Practical Nurse, RJH = Royal Jubilee Hospital, VGH = Victoria General Hospital.

Participants completed the MEQ as self-assessment of individual chronotype (Table 4.2.1). Scores are used from these to determine if an individual self-identifies as a “morning-type” or “evening-type” and can be further used alongside melatonin analyses to determine circadian preference/states (Horne & Ostberg, 1976; Sack et al., 2007).

Table 4.1.3

Morningness-Eveningness Questionnaire Scores for Participants

Participant	Score	Chronotype
1	59	Moderately Morning
2	69	Moderately Morning
3	45	Intermediate
4	39	Moderately Evening
5	63	Moderately Morning
6	55	Intermediate
7	54	Intermediate
8	63	Moderately Morning
9	46	Intermediate
Mean	55	
SD	9.9	

4.2 Salivary Biomarkers

Viable salivary samples were available for analyses from six participants. Melatonin and IL-6 were able to be analyzed for six of the eight days (Days 1 and 2, Nights 1 and 2, Off 1 and 4); funding restrictions prevented the analyses of all melatonin and IL-6 samples for the six participants on Off 2 and Off 3. Cortisol samples were available for analyses on each of the eight days. Other participant samples had to be discarded due to high presence of solute within collected samples, which made the samples invalid for analyses.

4.2.1 Melatonin

Morning salivary melatonin concentrations were obtained pre-shift on Days 1 and 2, post-shift on Nights 1 and 2, and upon waking or shortly thereafter on Off 1 and Off 4. Mean concentrations are presented below in Table 4.2.1.1.

Table 4.2.1.1

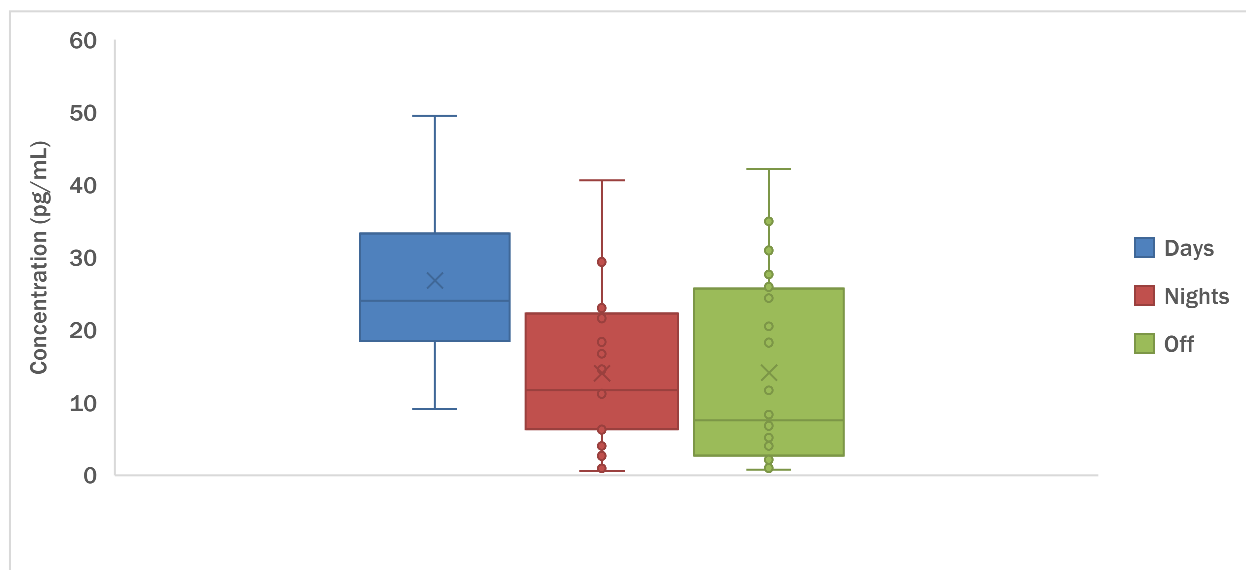
Mean Salivary Melatonin Concentrations (pg/mL) in Period 1 and Period 2

	Day 1		Day 2		Night 1	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Period 1	28.46	13.81	24.83	13.86	14.214	11.00
Period 2	23.59	7.40	30.57	15.14	8.09	8.02
	Night 2		**Off 1**		**Off 4**	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Period 1	17.82	15.72	7.79	11.35	20.26	16.00
Period 2	16.02	6.77	10.33	9.34	18.34	14.55

Shapiro-Wilk tests were performed for each of the days and the assumption of normality was met on each day with one exception being Off 1 in Period 1; this is due to the presence of outliers specific to this date. A two-way repeated measures ANOVA was conducted, and Mauchly's test of sphericity was satisfied for both day and day*period interaction. A significant difference was seen for day of collection ($F(5, 25) = 6.626$, $p < 0.001$); that is, there was a difference between morning salivary melatonin concentrations across the eight days of collection regardless of period. Therefore, a Bonferroni correction was conducted but failed to find significant differences between days of analysis. Meanwhile no significant difference was found between Period 1 and Period 2 overall ($F(1, 5) = 0.182$, $p = 0.687$) as well as for the day*period interaction ($F(5, 25) = 0.780$, $p = 0.573$) in which Mauchly's assumption of sphericity was met.

Figure 4.2.1.1

Average (Period 1 and Period 2 Inclusive) Morning Melatonin Concentrations



When observing Figure 4.2.1.1, the differences between combined days (i.e., Day 1 and Day 2 concentrations), combined nights (i.e., Night 1 and Night 2 concentrations), and combined days off (i.e., Off 1 and Off 4) are presented. The average value for Day 1 concentration was 26.03 pg/mL and remained relatively constant as an overall average of 27.70 pg/mL on Day 2; in Period 1, the average increased from Day 1 to Day 2, while it dropped in Period 2. The overall average then sharply dropped to 11.15 pg/mL in Night 1, and slightly increased to 16.92 pg/mL on Night 2 before dropping again to 9.058 pg/mL on Off 1. This then increased to 19.27 pg/mL on Off 4.

4.2.2 Cortisol

Cortisol was evaluated for each of the eight days in the Island Health shift roster. Table 4.2.2.1 shows mean cortisol concentrations (μg/dL) and 95% CIs in both Period 1 and Period 2 for working days, while Table 4.2.2.2 shows cortisol concentrations on days off.

Table 4.2.2.1

Mean Salivary Cortisol Concentrations (µg/dL) on Working Days in Periods 1 and 2

	Day 1				Day 2			
	Pre-Shift (morning)		Post-Shift (night)		Pre-Shift (morning)		Post-Shift (night)	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Period 1	0.30	0.16	0.48	0.28	0.49	0.81	0.26	0.13
Period 2	0.31	0.29	0.28	0.22	0.16	0.14	0.19	0.10
	Night 1				Night 2			
	Pre-Shift (night)		Pre-Shift (morning)		Pre-Shift (night)		Pre-Shift (morning)	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Period 1	0.47	0.81	0.32	0.12	0.26	0.18	0.34	0.20
Period 2	0.32	0.21	0.31	0.19	0.36	0.21	0.21	0.14

Table 4.2.2.2

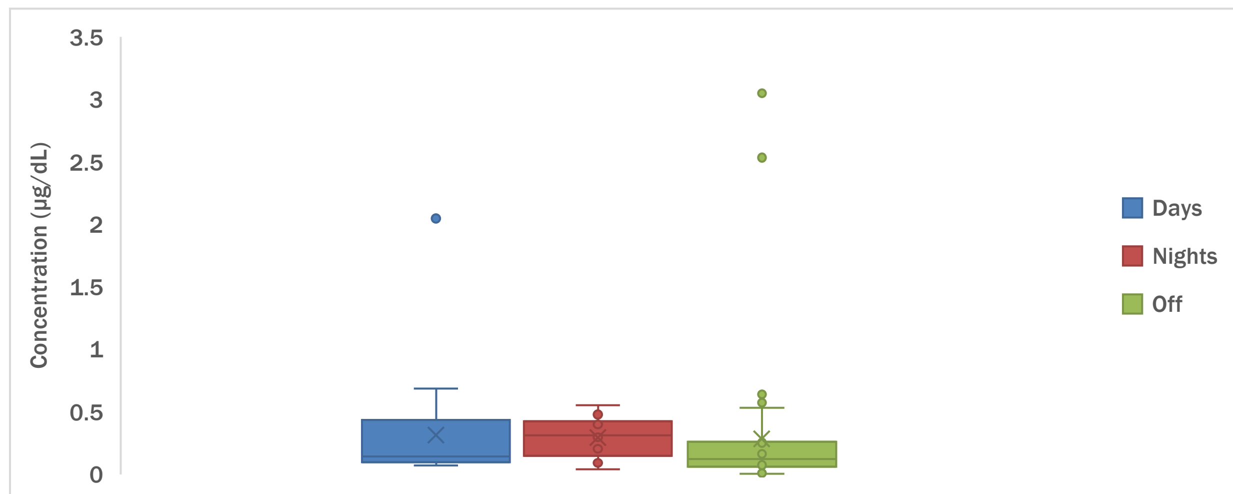
Mean Salivary Cortisol Concentrations (µg/dL) on Off Days in Period 1 and Period 2

	Off 1		Off 2		Off 3		Off 4	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Period 1	0.55	1.29	0.06	0.04	0.62	0.99	0.36	0.20
Period 2	0.05	0.03	0.06	0.03	0.23	0.20	0.34	0.23

A two-way repeated measures ANOVA was performed on morning salivary cortisol concentrations to examine if any differences existed between days of analyses (Days 1 and 2, Nights 1 and 2, Off 1-4). No significant difference was seen for the effect of day ($F(7, 35) = 0.779$, $p = 0.609$), period ($F(1, 5) = 1.069$, $p = 0.349$), or day*period interaction ($F(7, 35) = 1.061$, $p = 0.409$). This is seen below in Figure 4.2.2.1

Figure 4.2.2.1

Average (Period 1 and Period 2 Inclusive) Morning Cortisol Concentrations



The difference in cortisol concentrations on working days in Period 1 and Period 2 was calculated by subtracting the evening value from the morning value (Yang et al., 2001) (Table 4.2.2.3). This corresponded to [pre-shift]-[post-shift] for day shifts, and [post-shift]-[pre-shift] for night shifts. Shapiro-Wilk tests were conducted for each of the four days across both periods of collection, with only two violations (Day 2 and Night 1 in Period 1). The differences were then analyzed using a two-way repeated measures ANOVA. Mauchly's assumption of sphericity was satisfied in each case. There were no significant differences seen when analyzed for the effect of shift type ($F(3, 15) = 0.463$, $p = 0.712$), period ($F(1, 5) = 0.066$, $p = 0.807$), or the shift type*period interaction ($F(3, 15) = 0.974$, $p = 0.431$). This is visually depicted below in Figure 4.2.2.2.

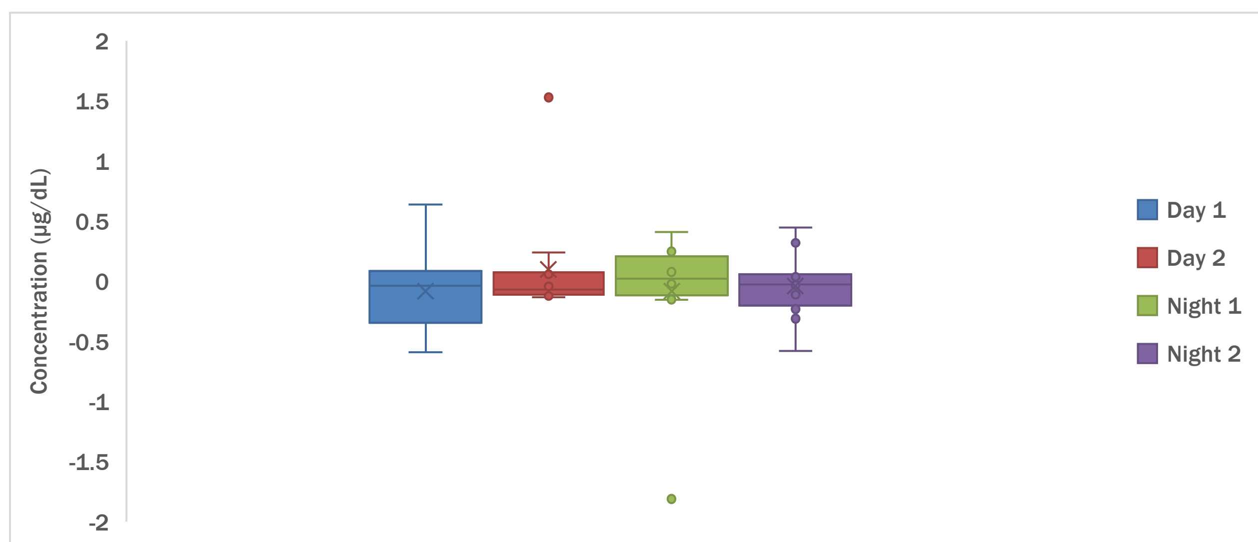
Table 4.2.2.3

Change in Cortisol Concentrations (μg/dL) from Morning to Evening on Working Days in Period 1 and Period 2

	Day 1		Day 2		Night 1		Night 2	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Period 1	-0.19	0.29	0.23	0.68	0.08	0.87	0.03	0.29
Period 2	0.03	0.37	-0.03	0.09	-0.01	0.11	-0.15	0.25

Figure 4.2.2.2

Difference Between Morning and Evening Cortisol Concentrations on Working Days in Period 1 and Period 2



4.2.3 Interleukin-6

Morning salivary IL-6 concentrations were obtained pre-shift on Days 1 and 2, post-shift on Nights 1 and 2, and upon waking or shortly thereafter on Off 1 and Off 4 (Table 4.2.3.1).

Table 4.2.3.1

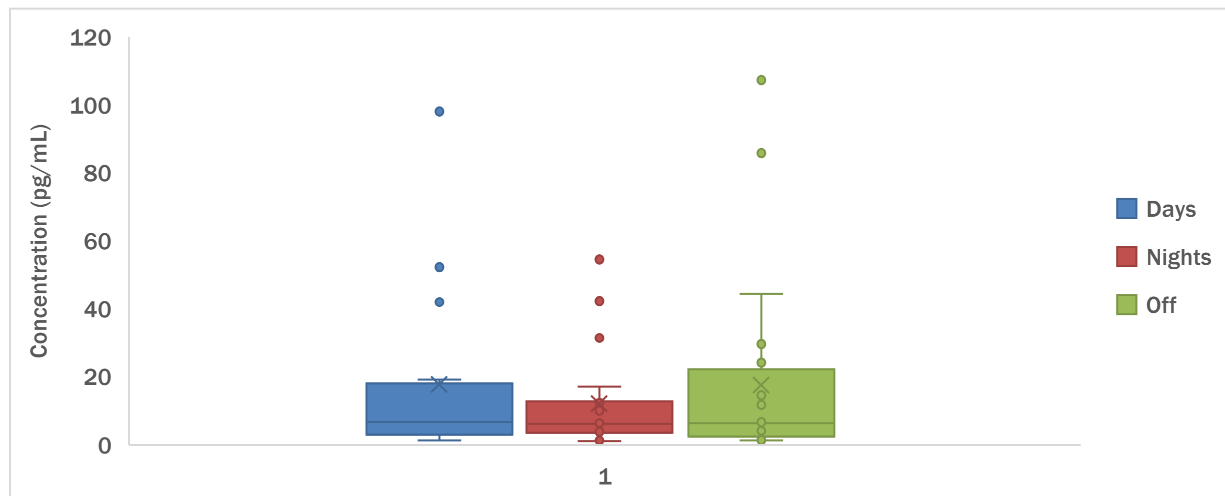
Mean Salivary Interleukin-6 Concentrations (pg/mL) in Period 1 and Period 2

	Day 1		Day 2		Night 1	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Period 1	12.62	21.00	12.86	20.38	11.28	12.16
Period 2	21.86	21.52	24.06	38.59	14.41	21.04
	Night 2		Off 1		Off 4	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Period 1	12.19	11.04	8.89	9.15	22.81	43.72
Period 2	10.91	16.70	16.34	11.93	23.09	36.87

Shapiro-Wilk tests were performed for the six days in question in both Period 1 and Period 2. Only four of the potential 12 days met the assumption of normality (Day 1 and Night 1 in Period 1, Day 1 and Off 1 in Period 2), with the rest having $p < 0.05$. This is likely due to the heightened appearance of outliers inherent within the available data. Regardless, a two-way repeated measures ANOVA was conducted to determine if any difference between days and periods of collection existed. Mauchly's assumption of sphericity was satisfied in each subgroup. No significant differences were existed for day ($F(5, 25) = 0.913$, $p = 0.488$), period ($F(1, 5) = 0.290$, $p = 0.614$), or the day*period interaction ($F(5, 25) = 0.215$, $p = 0.953$). Figure 4.2.3.1 displays the averages for each collection point in both Period 1 and 2.

Figure 4.2.3.1

Average (Period 1 and Period 2 Inclusive) Morning Interleukin-6 Concentrations



4.3 Expanded Nursing Stress Scale

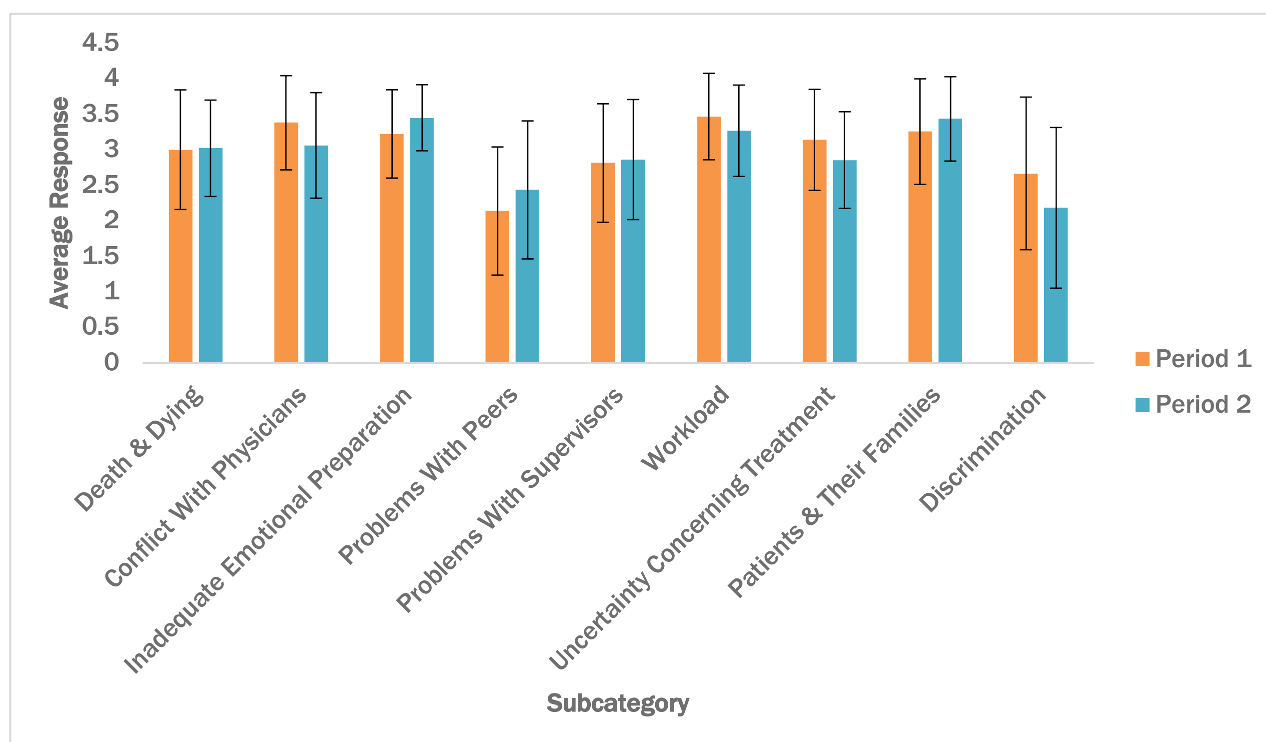
Participants completed the ENSS on an online platform in both Period 1 and Period 2 of data collection; the ENSS was completed following the second night shift (Night 2) of the eight-day collection schedule. 57 questions divided into nine subcategories were posed using a Likert scale following the scoring system developed by French et al. (2000). However, due to a high incidence of missing data sets and incomplete responses, statistical analyses were unable to be performed. Nonetheless, the visual depiction of overall trends within the data are available in Figures 4.3.1 and 4.3.2; missing data was either due to participants not answering questions or selecting *does not apply* options.

Figure 4.3.1 shows the average numerical response for the questions within the nine subcategories of the ENSS in both Period 1 and Period 2 of data collection. Higher scores represent higher perceived emotional stress (French et al., 2000). In Period 1, Workload had the highest average score for responses, whereas in Period 2 Inadequate Emotional Preparation had the highest average response score. Workload, Patients and Their Families, Inadequate

Emotional Preparation, and Conflict with Physicians remained within the top four in Periods 1 and 2, albeit with their positioning slightly altered.

Figure 4.3.1

Average Answer Scores for Expanded Nursing Stress Scale Responses



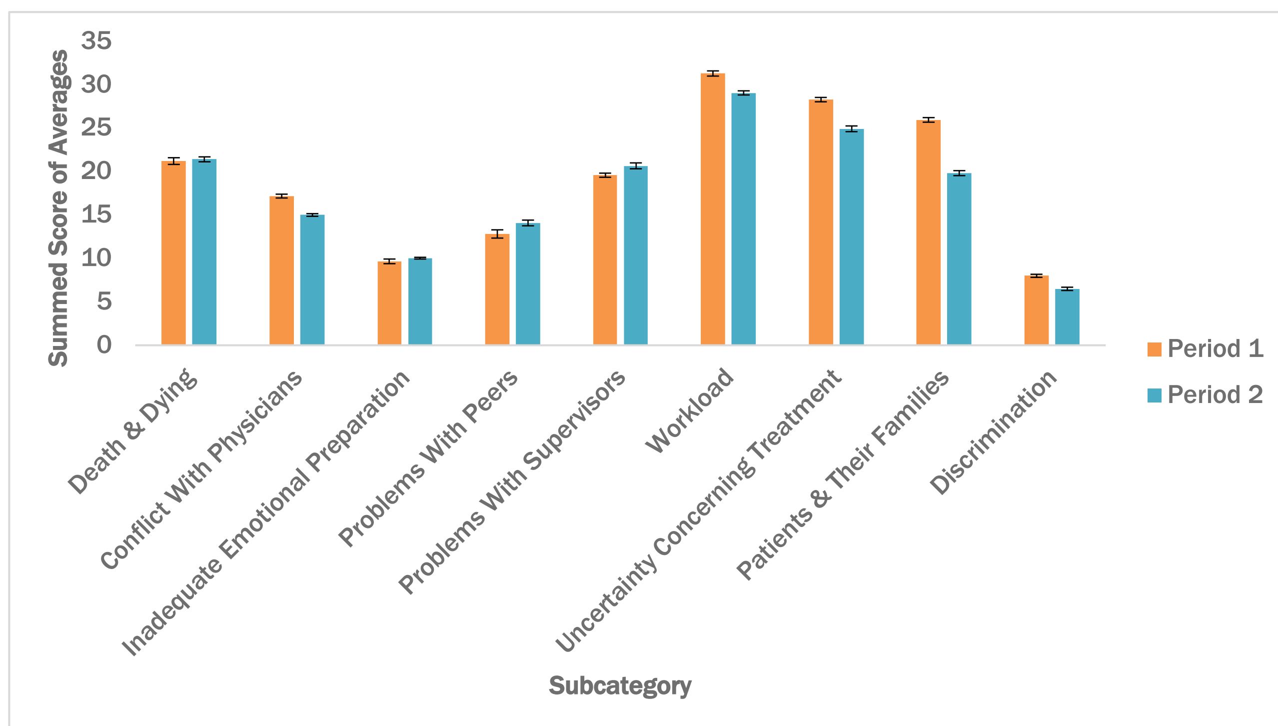
Note: Error bars represent 95% CI.

The ENSS sums the scores of responses in each subcategory that is ultimately used to provide a final score representative of perceived occupational stress (French et al., 2000). However, due to missing data within the current study, this was unable to be achieved. That said, a sum of the averages obtained for each response was tallied to allow for visualization of trends in data to observe how each subcategory changed between Period 1 and Period 2 (Figure 4.3.2). Death and Dying remained relatively constant, while Conflict with Physicians decreased in score from Period 1 to Period 2, representing lower associated perceived occupational stress.

Inadequate Emotional Preparation remained relatively consistent between periods, with a slight increase in Period 2, as did Problems with Peers and Problems with Supervisors. Workload decreased in the summed score of averages between Period 1 and Period 2; the same trend was seen with Uncertainty Concerning Treatment, Patients and Their Families, and Discrimination.

Figure 4.3.2

Summed Scores for Each Subcategory of the Expanded Nursing Stress Scale in Period 1 and Period 2



Note: Error bars represent 95% CI.

4.4 Heart Rate Variability

4.4.1 Period 1

To analyze HRV for Period 1 of collection, three days of data collection were chosen, which include the second day shift (Day 2), the second night shift (Night 2), and the second day off (Off 2). Complete sets of data from participants were only available on these three days. Data

was unavailable at other time points either due to participant error; files were either absent from participants (e.g., did not record on the appropriate day, forgot to start recording, etc.) or obtained only files containing data for heart rate rather than HRV due to not wearing the Polar H10 Chest Strap. All parameters related to frequency domain HRV recordings (i.e., $LFms^2$, $HFms^2$, LFnu, HFnu, LF/HF) were recorded and used for analyses Table 4.4.1.1 below shows the mean HRV and 95% confidence intervals (CI) for Period 1 of collection.

Table 4.4.1.1

Mean Heart Rate Variability Measurements in Period 1

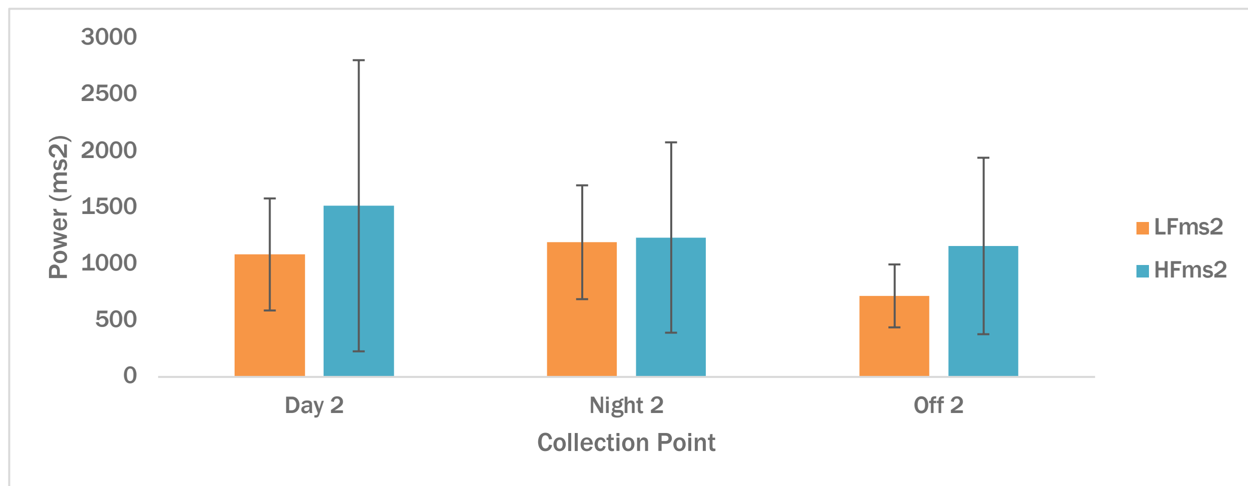
	Day 2		Night 2		Off 2	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
$LFms^2$	1084.42	495.99	1192.46	504.00	717.45	278.59
$HFms^2$	1515.51	1289.00	1234.31	842.76	1159.48	781.29
LFnu	47.72	14.96	53.16	12.14	45.93	19.41
HFnu	52.23	14.95	46.80	12.12	54.05	19.41
LF/HF	1.19	0.83	1.47	1.09	1.74	2.35

Shapiro-Wilk tests for normality were performed on each variable across the three days of interest. The assumption of normality was violated on the following: Day 2 (LF/HF), Night 2 ($LFms^2$, LFnu, HFnu, LF/HF), and Off 2 (LF/HF); this is likely due to presence of outliers within the data. Repeated measures ANOVAs were conducted for $LFms^2$, $HFms^2$, LFnu, and HFnu (LF/HF). Mauchly's assumption of sphericity was satisfied in each case. None of the ANOVAs determined significant differences between the three collection points for any of the variables of interest. Specifically, there was no significant difference between Day 2, Night 2, and Off 2 for $LFms^2$ (F(2, 14 = 1.862, $p = 0.192$), $HFms^2$ (F(2, 14 = 0936, $p = 0.415$), LFnu (F(2, 14 = 0.507, $p = 0.613$), HFnu (F(2, 14 = 0.512, $p = 0.610$). A repeated measures ANOVA with a Greenhouse-Geisser correction was used for LF/HF, and similarly no significant difference was

found between the three days ($F(2, 14 = 0.641, p = 0.641)$. Visual depiction is seen in Figure 4.4.1.1, Figure 4.4.1.2, and Figure 4.4.1.3.

Figure 4.4.1.1

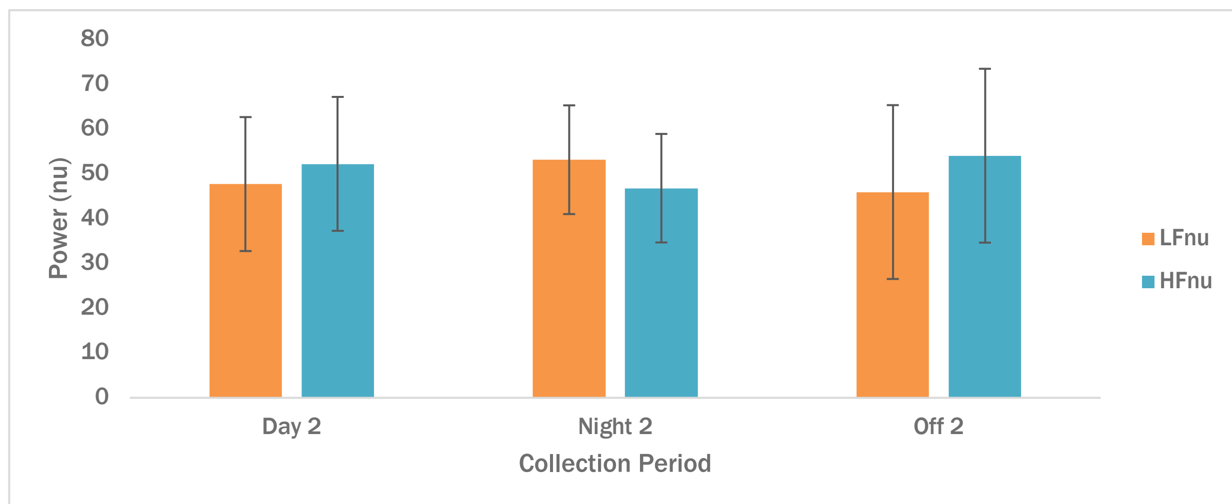
LFms² and HFms² Trends on Day 2, Night 2, and Off 2 in Period 1



Note: Error bars represent 95% CI.

Figure 4.4.1.2

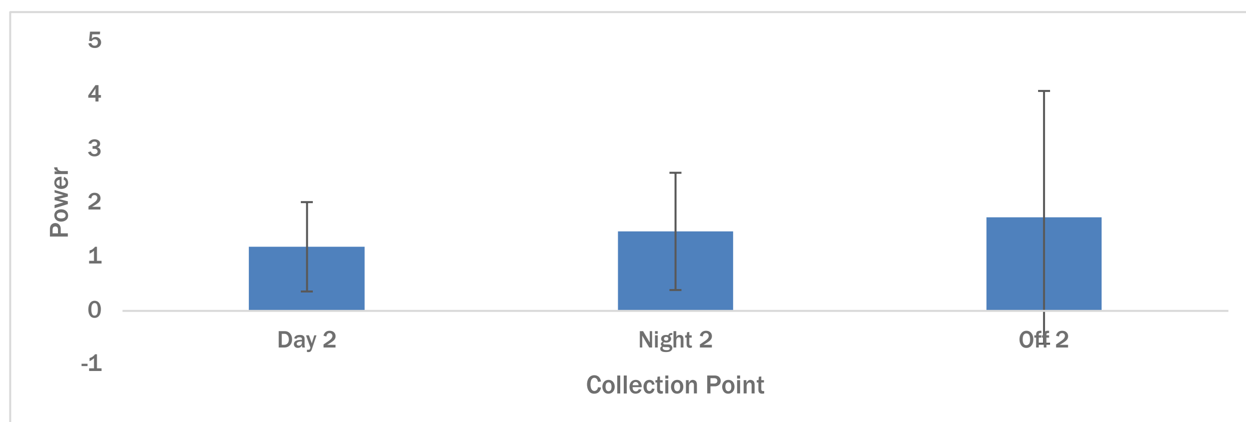
LFnu and HFnu Trends on Day 2, Night 2, and Off 2 in Period 1



Note: Error bars represent 95% CI.

Figure 4.4.1.3

LF/HF Trend on Day 2, Night 2, and Off 2 in Period 1



Note: Error bars represent 95% CI.

4.4.2 Period 2

In analyzing Period 2 of collection, six days of collection were selected due to availability of complete data sets from participants. These include the two day shifts (Day 1, Day 2), the two night shifts (Night 1, Night 2), and the last two days off (Off 3, Off 4). For this period, there sufficient data from six participants who were also in Period 1; two participants from Period 1 did not have available HRV recordings from this period, and one participant did not have data available for either period. Missing data was largely due to participant error (e.g., absent data on days of interest due to missed collection periods, forgot to start recordings, HR file instead of HRV file due to incorrect equipment usage, etc.). Table 4.4.2.1 displays the mean HRV and 95% Confidence Intervals for Period 2 of data collection.

Table 4.4.2.1

Mean Heart Rate Variability Measurements for Period 2 of Data Collection

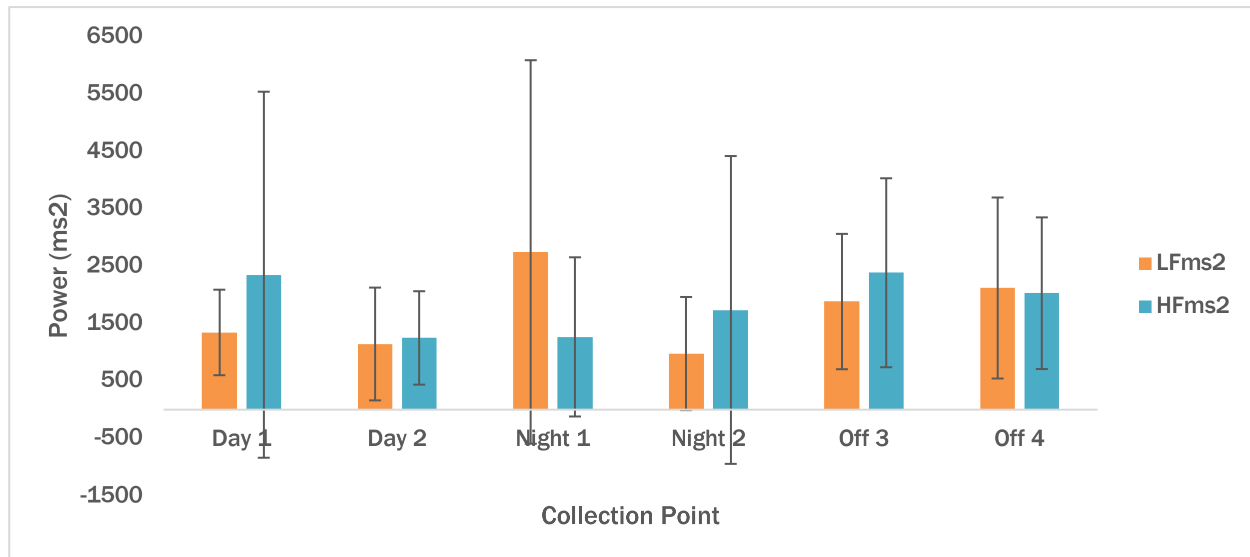
	Day 1		Day 2		Night 1	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
$LFms^2$	1344.28	744.91	1143.47	982.10	2745.69	3339.94
$HFms^2$	2349.77	3187.29	1249.17	813.32	1267.15	1386.12
LFnu	46.09	24.15	41.04	24.52	63.91	22.41
HFnu	53.90	24.15	58.94	24.52	36.07	22.40
LF/HF	1.17	0.95	1.09	1.37	2.59	1.83
	Night 2		Off 3		Off 4	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
$LFms^2$	976.85	985.39	1882.57	1178.66	2118.61	1576.18
$HFms^2$	1736.20	2680.53	2384.29	1645.41	2027.68	1320.79
LFnu	47.94	19.28	45.80	16.70	50.96	13.70
HFnu	52.00	19.38	54.15	16.68	49.02	13.71
LF/HF	1.20	1.04	1.01	0.73	1.18	0.69

Shapiro-Wilk tests were conducted on each variable of interest to determine if the assumption of normality was satisfied. Significance was determined on the following days, thus illustrating a rejection of this assumption: Day 1 ($HFms^2$), Day 2 (LF/HF), Night 2 ($LFms^2$, $HFms^2$). These violations are due to the increased presence of outliers within the data. However, parametric repeated-measures ANOVAs were still used for analyses.

Repeated measures ANOVAs were conducted across the six days for each variable of interest (i.e., $LFms^2$, $HFms^2$, LFnu, HFnu, LF/HF). Mauchly's test of sphericity was satisfied for each variable except for $HFms^2$, in which a Greenhouse-Geiser correction was employed for interpretation. There was no significant difference between Day 1, Day 2, Night 1, Night 2, Off 3, or Off 4 for $LFms^2$ ($F(5, 25) = 1.299$, $p = 0.296$), LFnu ($F(5, 25) = 1.804$, $p = 0.393$), HFnu ($F(5, 25) = 1.803$, $p = 0.394$), or LF/HF ($F(5, 25 = 2.026$, $p = 0.109$). The Greenhouse-Geisser correction for $HFms^2$ also revealed no significant difference between days ($F(1.642, 8.209 = 0.846$, $p = 0.442$). Visual trends are depicted in Figures 4.4.2.1 – 4.4.2.3.

Figure 4.4.2.1

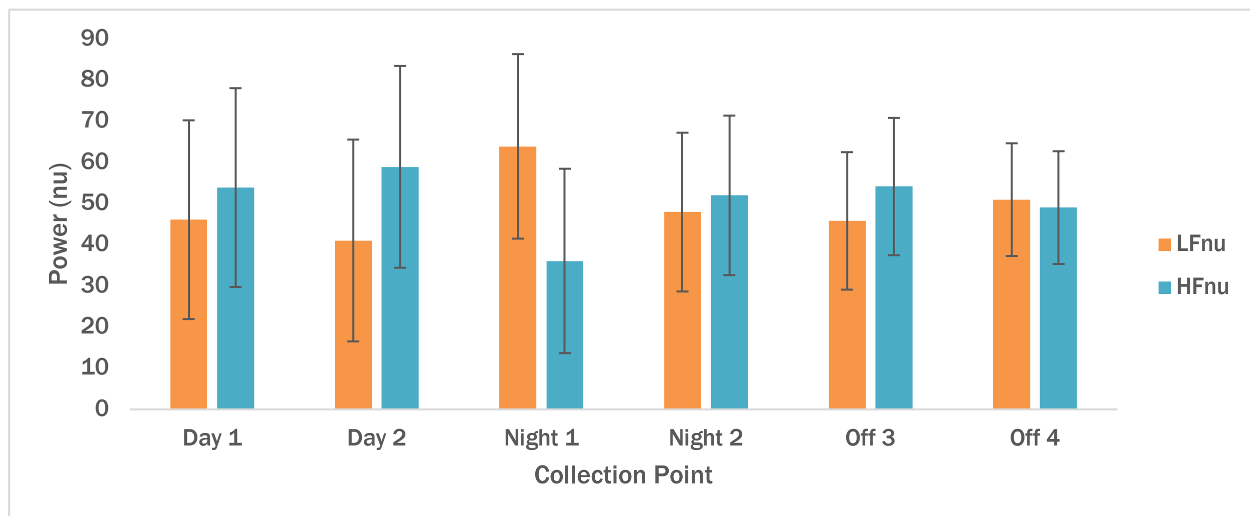
LFms² and HFms² Trends on Day 1, Day 2, Night 1, Night 2, Off 3, and Off 4 in Period 2



Note: Error bars represent 95% CI.

Figure 4.4.2.2

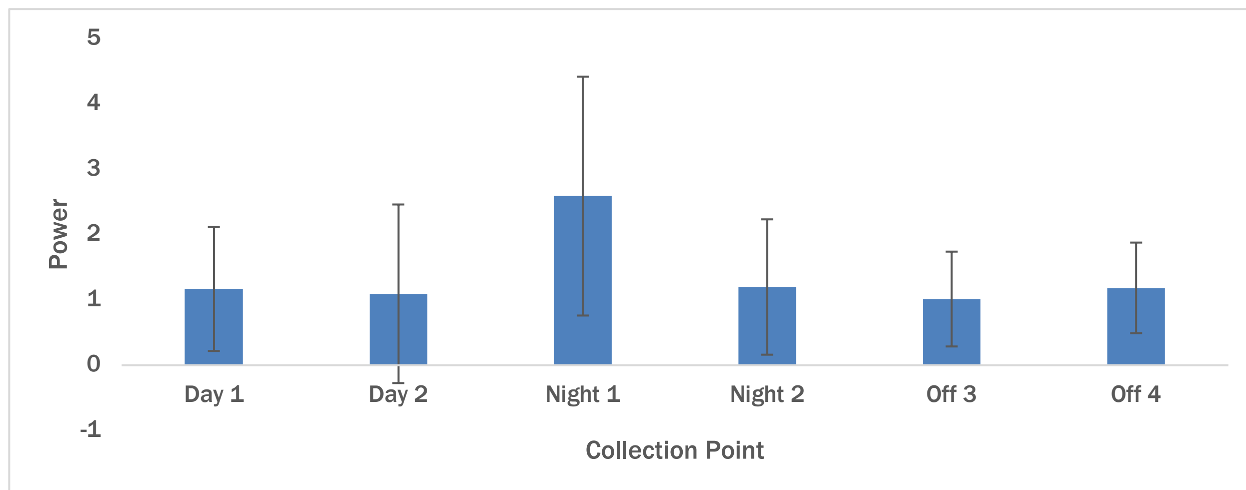
LFnu and HFnu Trends on Day 1, Day 2, Night 1, Night 2, Off 3, and Off 4 in Period 2



Note: Error bars represent 95% CI.

Figure 4.4.2.3

LF/HF Trend on Day 1, Day 2, Night 1, Night 2, Off 3, and Off 4 in Period 2



Note: Error bars represent 95% CI.

4.4.3 Comparison Between Period 1 and Period 2

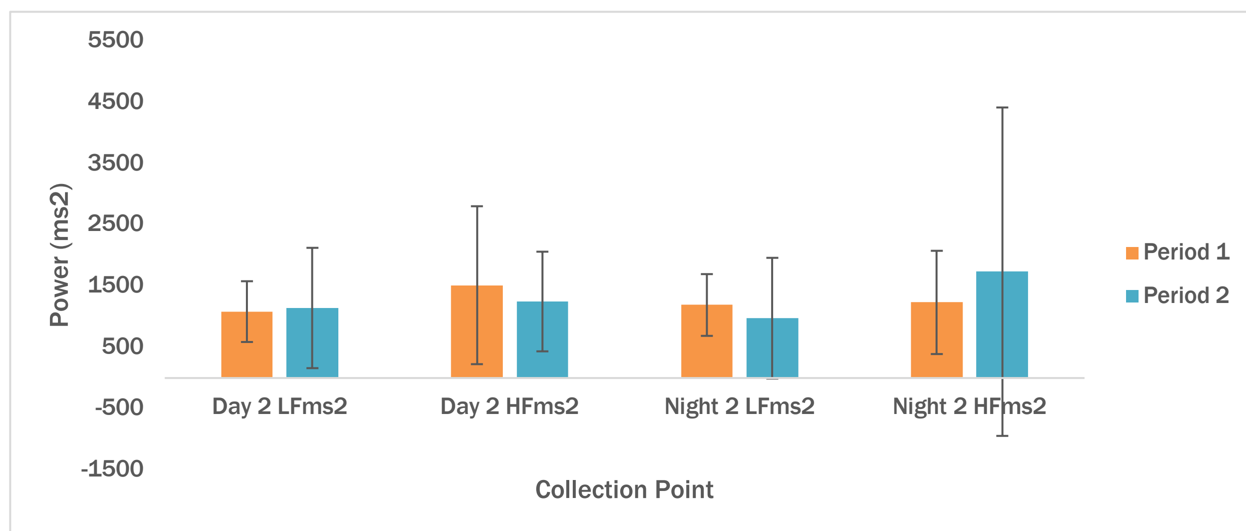
Frequency-domain HRV parameters in Period 1 and Period 2 were compared using two-way repeated measures ANOVAs. Only six subjects were included within this analysis due to similar issues as discussed previously regarding missing data due to participant error (e.g., data collected on incorrect days, inappropriate use of equipment). Constraints with missing data also meant that only two days of collection could be compared: Day 2 and Night 2, as full sets of data were only available on these days. This means that two-way repeated measures ANOVAs were conducted for each parameter of interest on Day 2 and Night 2 in Period 1 and Period 2.

When comparing the effects of period ($F(1, 5) = 0.060$, $p = 0.816$), shift type ($F(1, 5) = 0.112$, $p = 0.751$), and the interaction of period and shift type ($F(1, 5) = 0.719$, $p = 0.435$) for LFms^2, no significant differences were seen. This was also the case with HFms^2, as there were no significant differences for period ($F(1, 5) = 0.010$, $p = 0.925$), shift ($F(1, 5) = 0.025$, $p =$

0.882), or the period and shift interaction ($F(1, 5) = 0.557$, $p = 0.489$). This is further demonstrated in Figure 4.4.3.1.

Figure 4.4.3.1

$LFms^2$ and $HFms^2$ as seen on Day 2 and Night 2 in Period 1 and Period 2

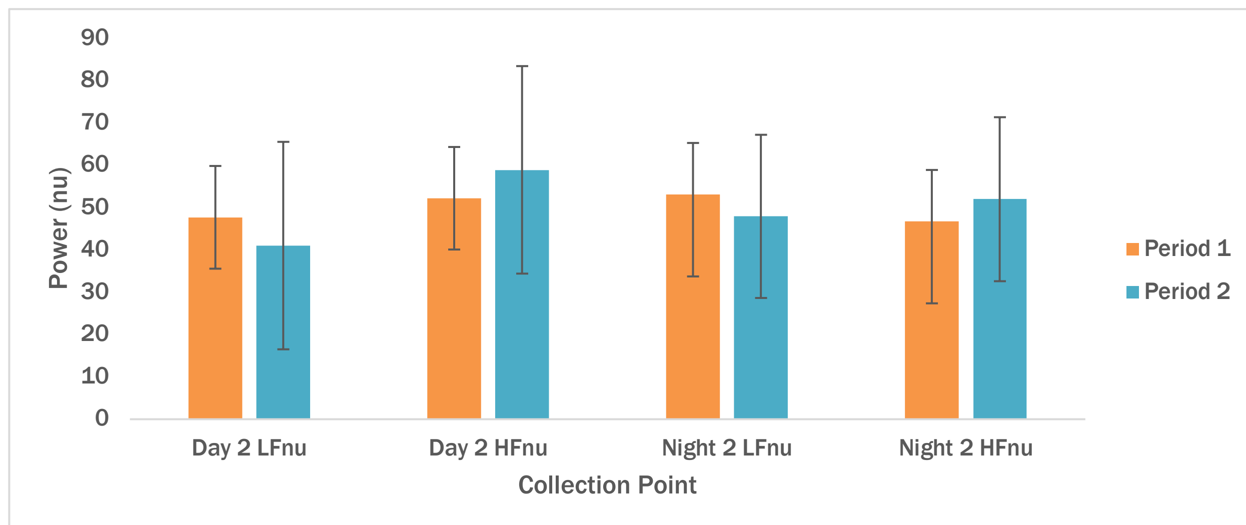


Note: Error bars represent 95% CI

There was no significant difference in LFnu in regard to period ($F(1, 5) = 0.247$, $p = 0.640$), shift type ($F(1, 5) = 5.210$, $p = 0.071$), or the period and shift interaction ($F(1, 5) = 0.076$ $p = 0.794$). Similarly, no significant difference existed for HFnu for period ($F(1, 5) = 0.249$, $p = 0.639$), shift type ($F(1, 5) = 5.225$ $p = 0.071$), or the period and shift interaction ($F(1, 5) = 0.075$, $p = 0.795$). This is further demonstrated in Figure 4.4.3.2.

Figure 4.4.3.2

LFnu and HFnu as seen on Day 2 and Night 2 in Period 1 and Period 2

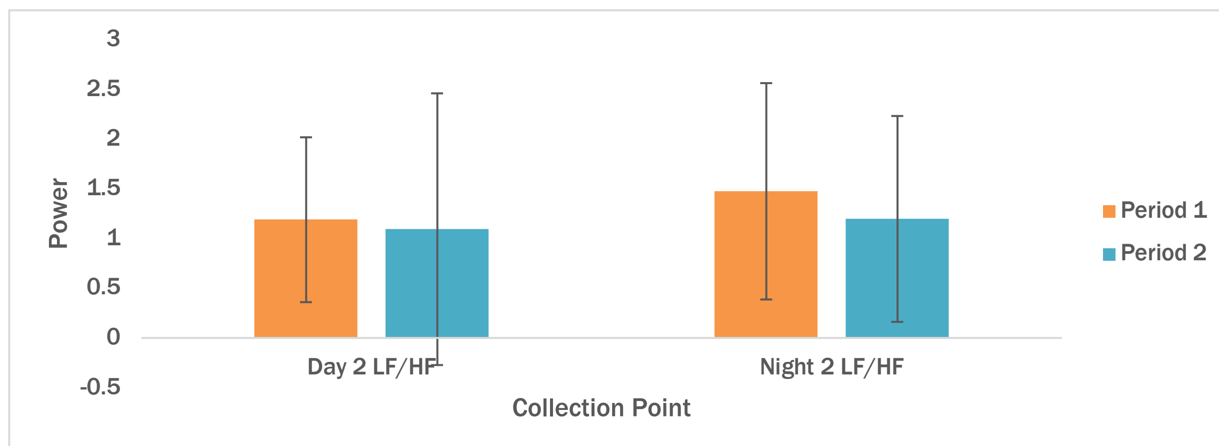


Note: Error bars represent 95% CI.

There was no significant difference in LF/HF for period ($F(1, 5) = 0.210$, $p = 0.666$), shift ($F(1, 5) = 2.644$, $p = 0.165$), or period and shift interaction ($F(1, 5) = 0.160$, $p = 0.706$). This can be seen in Figure 4.4.3.3 below.

Figure 4.4.3.3

LF/HF as seen on Day 2 and Night 2 in Period 1 and Period 2



Note: Error bars represent 95% CI.

Chapter 5: Discussion

The objective of this research was to examine the physiological responses of stress and health in nurses working rotating shift schedules during the COVID-19 pandemic in the Greater Victoria region. Specifically, three research questions were created to warrant further investigation; each subsection of the discussion addresses each research question in detail with the appropriate variables of interest.

5.1 Objective and Subjective Assessment of Stress

Question 1: Does working rotating shift work during the COVID-19 pandemic objectively result in greater physiological stress placed on female nurses at Greater Victoria hospitals as measured through cortisol and IL-6 levels, and does this align with heightened perceived stress?

5.1.1 Salivary Biomarkers

Cortisol concentrations did not exhibit any apparent difference between day and night shifts, as well as between Period 1 and Period 2. Period 1 did have elevated cortisol concentrations across the four shifts; this is due to the heightened presence of outliers towards the maximum, along with a larger range. For example, there were two cortisol concentrations above 2.0 μg/dL in Period 1 (2.05 μg/dL on Day 2 morning and 2.03 μg/dL on Night 1 evening), while no values above 0.7 μg/dL were seen in Period 2.

When comparing cortisol concentrations to Salimetrics standards (Appendix D) for women aged 21 – 30 years and 31 – 50 years, the present study had higher evening concentrations than what is seen in the manufacturer's reference ranges. These elevations in evening levels were also seen when compared to healthy individuals of both sexes (Laudat et al., 1988). The heightened evening concentrations can be evidence for alterations to the diurnal

nature of cortisol release through blunting the curve (Kalsbeek et al., 2012; M. Kumari et al., 2011; Yang et al., 2001). The effects of perceived stress are potentially responsible for this. Chronic stress has a negative effect on evening cortisol levels in workers across industries working shift work, (M. Kumari et al., 2011; Šušoliaková et al., 2018). Additionally, exposure to acute stressors can further increase the cortisol release pattern, and if a stressful event happened toward the end of the shift (e.g., code blue/medical emergency), this could temporarily heighten levels that could potentially be captured in the measurement (Morgan et al., 2002).

Comparing the difference in morning and evening cortisol concentrations allows for the examination of its diurnal nature (Adam et al., 2017). Cortisol release is typically greatest in the morning, before decreasing across the day and reading nadir at night (Kalsbeek et al., 2012). However, when examining the data within the present study, it is seen that in some cases that evening values were greater than or similar to morning values. Specifically, there were larger evening values seen in Period 1 on Day 1 and Night 1 (0.30 to 0.48 μg/dL, and 0.32 to 0.47 μg/dL respectively), and in Period 2 on Day 2 (0.21 to 0.36 μg/dL); similar results were seen in Period 2 on Night 1 (0.31 and 0.32 μg/dL). These could be due in part to the gargantuan inconsistency of stressors in the hospital; that is, each workday does not present a uniform patient flow and there is always the potential for adverse events to take place and cannot be controlled for.

Alterations to the circadian rhythmicity of cortisol's release pattern have been seen in previous shift work research. The general consensus is that cortisol's peak release remains in the morning and then decreases throughout shift, although the timing of events within the cycle can be shifted or even blunted (Amirian et al., 2015; Bracci et al., 2016; Hung et al., 2016; Jensen et al., 2016; Yang et al., 2001). However, the generalizability of these studies can be questioned

due to different populations and working environments; also, the studies were all conducted in a pre-pandemic workplace. One study that used a similar shift rostering schedule to the present study found that nurses in a Kingston, Ontario hospital experienced a second peak in cortisol release 17 hours post-awakening while on night shifts and that nurses had a flatter diurnal curve overall (Hung et al., 2016). The number of consecutive nights worked did not affect this curve, rather the hours worked per day did. This could explain some findings within the present study, as working hours were not consistently set at 12 hours, as some nurses worked overtime while others ended early; later end times can further result in increased post-shift measurements, thus affecting the observed diurnal response.

Previous nursing research has demonstrated that ward placement can affect cortisol response. Yang et al. (2001) found that nurses in general wards exhibited a greater range in morning and afternoon cortisol concentrations in comparison to those in the emergency ward while on day shifts. Furthermore, nurses in general wards had larger morning cortisol concentrations (when converted to µg/dL: 0.56 µg/dL compared to 0.33 µg/dL) (ScyMed, 2019; Yang et al., 2001). The data within the present study have averages that are more in line with the findings from emergency ward nurses, even though only two were included in the analyses. The present study had a diverse group of wards included, which again illustrates how there are many factors at play that can influence the cortisol release pattern and resultant concentrations.

IL-6 similarly exhibited no difference in mean concentrations across shift type and periods of collection. The mean values during working periods were greater in Period 2, and this can be in part to the greater range in mean concentrations seen within this period. That is, in Period 1 samples for day shifts, only three of the 12 collected samples were above 10 pg/mL. In Period 2, there were seven samples above 10 pg/mL on day shifts, with a high of 98.16 pg/mL

obtained on Day 2 but also a low of 2.5 pg/mL, thus exhibiting a larger range. Published norms show that IL-6 in saliva ranges between 4.4 pg/mL to 20.9 pg/mL, with a mean of 8.4 pg/mL (Izawa et al., 2013). 54% of working-day concentrations obtained within Period 1 and 58% obtained within Period 2 were outside of this reference range.

Previous nursing research has found that nurses on shift work rosters (though with a different distribution of days and nights) had 92% of measured serum IL-6 concentrations consistently below the level of detection of 4.7 pg/mL (Bjorvatn et al., 2020). While the present study used salivary IL-6 values, this is still well below the average values obtained. However, when comparing IL-6 concentrations in saliva and serum, research has found that salivary levels tend to be higher (Sjögren et al., 2006). Serum measurements account for IL-6 production from monocytes and macrophages, whereas salivary levels are comprised of IL-6 from muscle, endothelial, epithelial, and adipose cells (Van Snick, 1990). Furthermore, acinar and ductal cells within salivary glands themselves can produce IL-6 as well (Verstappen et al., 2021). Salivary sampling techniques were ultimately used to enhance the practicability of remote field research within the pandemic landscape through limiting invasiveness and ensuring optimal participant comfort. A 2015 review by Slavish et al. further states that salivary IL-6 levels share a similar response to psychosocial stress factors as serum measurements, which further lends credibility to the methods employed in the current study.

There are plenty of extraneous factors that could influence the observations, as IL-6 can be impacted by illness (e.g., colds, infections), menstrual cycle/menopausal status, as well as differences in perception of stress (Angstwurm et al., 1997; Szabo & Slavish, 2021). IL-6 is also related to joint pain, as it causes a pro-inflammatory cytokine response; if any participants had undisclosed injuries or undiagnosed rheumatoid arthritis, this could account for elevated

concentrations (Srirangan & Choy, 2010). The pro-inflammatory IL-6 response uses trans-signalling to enhance the acute immune phase response, particularly with the peripheral and central nervous systems (Scheller et al., 2011; Zhang & An, 2007). Contrarily, IL-6 can also exhibit anti-inflammatory properties in which it acts to control the pro-inflammatory response through interaction with cytokine inhibitors and receptors through classical cell signalling pathways (Xing et al., 1998).

The above-mentioned reasons can make it hard to differentiate whether observed IL-6 responses are strictly because of rotating shift work during the COVID-19 pandemic. This presents a future research opportunity to examine the interplay between various cytokine systems along with receptor-substrate complexes. Pairing IL-6 measurements with an additional pro-inflammatory cytokine, such as c-reactive protein (CRP) can enhance the ability to investigate causes of stress; if both IL-6 and CRP concentrations were correlated, this would represent an overall pro-inflammatory effect representative of the classical stress response (Izawa et al., 2013). Furthermore, measuring the IL-6/receptor complex can provide an even greater depth of measurement discrimination (N. Kumari et al., 2016; Scheller et al., 2011). This would best be obtained through serum measurements, which would further enhance the readability of the data.

Both cortisol and IL-6 demonstrated a high amount of inter-individual variability, resulting in many outliers within the current dataset that likely influenced statistical analyses. This variability is likely due to the lack of a shared collective experience; that is, it was not possible to control for nurses' exposures to stressors while on shift, as well as their individual activities of daily living/exposure to stressors while off-shift. This shows that responses to stress are wildly dependent on the individual themselves, particularly regarding IL-6 concentrations.

While cortisol samples did exhibit variability, the general trend shows an elevation in evening values in comparison to referenced norms, thus blunting the diurnal nature of cortisol release. Long-term blunting of the cortisol diurnal response can increase the risk for negative health outcomes. A flattened diurnal nature as seen through elevated evening concentrations has been linked to future development of cardiovascular disease and is also linked to increased risk for all-cause mortality (M. Kumari et al., 2011). Furthermore, if individuals also had chronic increased IL-6 concentrations, the proinflammatory state alongside the dampened cortisol response could lead to atherosclerosis and further heightening the risk for death (Nijm & Jonasson, 2009). This poses considerable risk to the current study population, as potential compounded increases in subjective stress alongside alterations to the physiological stress response can greatly affect health and functioning and place nurses at an increased risk for negative health outcomes later in life.

5.1.2 Expanded Nursing Stress Scale

The ENSS has been used in previous nursing research and has found an apparent link between perceived occupational stress and nurses' personal health and quality of care, and further has been used to indicate areas of concern amongst workplace structure and environment across various care settings (Alomari et al., 2021; McGilton et al., 2007; Said & El-Shafei, 2021; Sarafis et al., 2016). Higher scores on the ENSS are related to a higher incidence of mental health disorders in nurses; specifically, scores on the Mental Component Summary were negatively correlated with eight of the nine ENSS subscales, as the Discrimination subscale showed no association (Sarafis et al., 2016). However, the generalizability of such findings can be a challenge due to the vast differences in patient care systems around the world as well as the majority of research involving the use of the ENSS took place in a pre-COVID-19 world.

Perceived stress can be influenced by the physical and social work environments; the COVID-19 pandemic has both exacerbated current stressors within the medical system as well as introduced novel concerns that have impacted nurses across diverse care settings (Canadian Nurses Association, 2020a; Huang et al., 2020; Lai et al., 2020; Maben & Bridges, 2020; Neto et al., 2020; X. Shen et al., 2020).

While a lack of statistical analyses with the present study limits the ability to directly compare results, some trends amongst visual inspection of data remain apparent. When looking at ENSS scores in isolation from salivary biomarkers, Period 1 and Period 2 of collection consistently showed that higher scores were seen in the subscales of Inadequate Emotional Preparation, Workload, and Difficulties with Patients and Their Families. Nurses in the present study also had higher scores across all subcategories in comparison to studies conducted in pre-pandemic times. Specifically, Sarafis et al. (2016) consistently had ENSS average scores on the subscales ranging from 0.79 – 2.65, whereas the present study had much higher scores around 2.14 – 3.5 in Period 1 and 2.2 – 3.45 in Period 2. The categories that ranked highest in terms of perceived stress also aligned with findings from Alomari et al. (2021), who found that nurses working in emergency departments (pre-COVID-19) ranked Workload and Inadequate Emotional Preparation as the most stressful subcomponents, albeit to a lesser degree.

Working during the COVID-19 pandemic has drastically altered the medical landscape in a short period. The "new normal" of nursing has meant that both the physical and social occupational environments have adapted to new measures to best protect and limit potential exposure and transmission of COVID-19 (Canadian Nurses Association, 2020a, b; Maben & Bridges, 2020). These changes, in theory, could be reflected in nurses' responses to the ENSS. Only one study has been published during the COVID-19 pandemic that used the ENSS, which

took place in Egypt (Said & El-Shafei, 2021). It featured similar remote data collection as the present study, as responses were obtained from nurses in two different hospitals in Zagazig, Egypt; however, translation of these findings to the current study are muddled as Egypt and Canada have faced very different pandemic responses (World Health Organization, 2021). Nevertheless, Said & El-Safei (2021) found that factors related to Workload, Death and Dying, and Inadequate Emotional Preparation ranked highest in terms of perceived stress; both Workload and Inadequate Emotional Preparation were among the highest-scoring in terms of average scored response in Period 1 and Period 2 in the present study. This does show some similarity regardless of the working environment in terms of the burden that nurses have been working under since the pandemic began.

The COVID-19 pandemic has presented a multitude of challenges for nurses, and the emotional and psychological impact itself has been insurmountable (Neto et al., 2020). There are also logistical issues in nursing, such as changes to the physical and social work structure, PPE considerations, and social distancing protocols among others (Canadian Nurses Association, 2020 a,b; Maben & Bridges, 2020). Higher ENSS scores are further related to an increased prevalence of mental health issues; this could potentially lead to impaired coping strategies with nurses in dealing with the current global health crisis, as a link between heightened stress during this timeframe and poor mental health outcomes could become more apparent/widespread (Sarafis et al., 2016). This has been seen in recent research examining nurses in China, who have exhibited heightened bouts of mental health problems such as depression, anxiety, and even suicidal thoughts while working on the frontlines of the epicentre of the pandemic (Lai et al., 2020; X. Shen et al., 2020).

5.1.3 Subjective and Objective Lens Assessment

ENSS scores within the present study were consistently elevated when compared to pre-pandemic results. This is further captured in a physiological assessment of data; evening salivary cortisol scores, particularly on Night 2 (the designated time for ENSS completion) remained above normal reference range values (Appendix D). This also could have potentially influenced participants' responses to the questionnaire. Additionally, IL-6 salivary concentrations were wildly varied outside of previously published reference ranges, making it difficult to discern the extent of the effect that shift work exhibited on the current study population (Izawa et al., 2013).

When looking at ENSS scores, research has demonstrated that emergency department nurses (which comprised a small component of the current study population) experience an increase in perceived stress particularly with the subscales of Workload and Inadequate Emotional Preparation in non-COVID-19 working environments (Alomari et al., 2021). This is also captured in nurses working on the frontlines of the COVID-19 pandemic, albeit with higher average scored responses (Said & El-Shafei, 2021). Research has also demonstrated that emergency department nurses had a smaller difference in morning and afternoon cortisol samples, potentially illustrating a flattening of a diurnal response (Yang et al., 2001). The current study further exhibits a flattening of the cortisol diurnal curve, though with samples obtained at night rather than in the afternoon. This alludes to a potential interplay between the psychological and physiological components of stress and can have a great circumstance for future health risks such as cardiovascular disease and all-cause mortality (M. Kumari et al., 2011). Nurses are responsible for keeping patients healthy and safe but may ultimately be affecting their personal health in the process.

The impact of stress on IL-6 presents a less clear picture. Research has demonstrated that serum IL-6 levels are positively correlated with nurses' and emergency care residents' work stress (B. B. Arnetz et al., 2017; J. Arnetz et al., 2019). This has also been observed in older adult populations working as caregivers for an incapacitated family member over an extended period (Kiecolt-Glaser et al., 2003). However, a large cohort of workers in Japan from various industries found a lack of connection between serum IL-6 concentration and rotating shift work, rather only permanent night workers experienced elevations in IL-6; this was also observed when results were adjusted for occupational stress (Amano et al., 2018). The current study demonstrates a wide variation within IL-6 data and casts an issue as to whether work stress was directly responsible for observed results in the participant pool; instead, previously mentioned extraneous non-controlled influences on IL-6 likely had a greater contribution to results as well as the subsequent variability within them.

The ENSS encapsulates nursing-specific work stress, but does not account for other impacting factors, such as stress related to home, relationships, or socioeconomic status among others. The aspects of perceived occupational stress were captured within the ENSS but remained consistently elevated in both Period 1 and Period 2, but variation within both salivary cortisol and IL-6 concentrations make it hard to differentiate whether the responses were a direct result of working during the COVID-19 pandemic or from other factors at play.

5.2 Frequency Domain Heart Rate Variability

Question 2: Does rotating shift work during the COVID-19 pandemic affect autonomic function as measured with frequency domain HRV (i.e., LFms^2, HFms^2, LFnu, HFnu, LF/HF)?

There was no significant difference in resting frequency domain HRV profiles in Period 1 or Period 2. This lack of apparent difference between working days (i.e., day and night shifts) and days off has been observed in previous nursing research. Chung et al. (2011) found that nurses on rotating shift schedules exhibited an increase in HF power alongside a decrease in LF/HF following two days off. This could partially explain why no difference was apparent within the current study, as off day data was analyzed on either the second (Period 1) or third and fourth day off (Period 2) due to constraints associated with data retention; sensitivity of measurement likely played a role as well. Therefore, the immediate transition from working to days off was unable to be captured, meaning that a sort of cardiovascular recovery could have taken place to mitigate any potential alterations back to normal, healthy parameters. Similar results to the present study were also seen in nurses following consecutive 14 hour extended shifts in residential care settings, in that no difference was observed in working days and days off (Goffeng et al., 2018).

A 2019 systematic review conducted by Järvelin-Pasanen et al. revealed that occupational stress is typically associated with a decrease in HF power, which was not observed in the present study. Research examining populations of nurses has mixed findings concerning the impact of stress on HRV. Nurses experiencing prolonged high stress over one year had lower $HFms^2$ while on-shift in comparison to acute high stress and stable low-stress groups (Borchini et al., 2018). However, pairwise comparisons examining the difference between nurses experiencing prolonged high stress and those with sustained low stress revealed no significant difference (p = 0.08). Conversely, a sample of nurses in the Netherlands with a similar age demographic to the present study (35.9 years ± 8.5) found that job strain affected psychological variables such as anxiety and burnout but did not impact physiological assessments of blood pressure and time-

domain HRV parameters (Riese et al., 2004). That said, Reise et al. (2004) did not use the ENSS to evaluate stress, rather the Dutch Monitor on Stress and Physical Load was used.

There were no significant differences between Period 1 and Period 2 for any frequency domain HRV parameters. Period 1 and Period 2 HRV measurements were taken at two different time points of the pandemic within British Columbia; Period 1 occurred just as the "second wave" of infections was beginning and with no sweeping provincial restrictions, whereas Period 2 occurred amid the "second wave" with restrictions put in place indefinitely (Judd & Zussman, 2021; Linsday, 2020). Trends observed from the ENSS in the present study show that average responses consistently rated above those obtained in previous research conducted before the pandemic, showing that participants were experiencing heightened perceived occupational stress within both periods (Alomari et al., 2021; McGilton et al., 2007; Sarafis et al., 2016). Whether this translated into cardiovascular physiological stress is muddled, due to a lack of control data within the sample population. However, when compared to "normal" values presented by the Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology (1996), the data within the present study revealed that LF/HF for Day 2 and Night 2 in both Period 1 and Period 2 were consistently below the suggested norms of 1.5 – 2.0, thus revealing a shift toward parasympathetic dominance which goes against the suggested effects of stress on the cardiovascular system. It should be noted though, that recordings were obtained upon waking or shortly thereafter, which could have shifted variables toward HF/parasympathetic dominance due to circadian influence (Boudreau et al., 2012). Furthermore, it was not possible to compare HRV parameters to control data obtained in a pre-pandemic setting, so direct comparisons of the effects of stressful environments are unable to be made but do warrant cause for future investigation.

Perceived occupational stress can further impact the cardiovascular recovery in HRV on days off in nurses. Nurses experiencing chronic elevations in such stress were unable to reattain $LFms^2$ baselines while off work; additionally, greater perceived stress levels were linked to higher $LFms^2$ values seen on days off (Borchini et al., 2018). In contrast, no significant differences in $HFms^2$ were seen. The autonomic origin of LF HRV remains contested as to whether exclusive sympathetic control or a mix of sympathetic and parasympathetic balance is being obtained in the resultant signal (Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology, 1996). While the predominant system is debated, it is nevertheless apparent that a sort of sympathetic activation is apparent which places the body in an increased state of physiological stress that aligns with the psychological perception.

Additionally, this study analyzed a four-minute recording obtained upon waking or shortly thereafter; most research that has examined HRV within nurses has employed longer recordings of 24 or 36 hours (Goffeng et al., 2018; Ito et al., 2001; Järvelin-Pasanen et al., 2013; Riese et al., 2004). A five-minute recording is deemed sufficient length for frequency domain analysis; a five-minute recording period was used in the present study, but the first and last 30 seconds had to be removed due to washout effects (Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology, 1996). The shortened recording was preferable for nurses in the present study to minimize disruptions to an already extended workday of 12 hours. A 2001 study by Ito et al. had a similar sample size (n = 10) to the present study along with a similar average age (33 years ± 3 compared to 32.11 years ± 7.25 in the present) and had HRV recordings taken over two 24 hour periods with one day shift and one night shift respectively, and found that HRV showed similar patterns of circadian

rhythmicity regardless of shift type (i.e., LFnu and LF/HF were the largest during work and smallest during sleep, with HFnu following an opposite pattern). Similar findings have also been seen in a mixed-sex population of workers from multiple industries, including healthcare, with no significant difference between night shift workers and non-night shift workers regarding frequency-domain HRV parameters (Hulsegge et al., 2018). One study that did use a five-minute recording with nurses obtained the recording immediately following a shift; when compared to nurses working permanent day or night shift rosters, those on rotating cycles had a marked decrease in HFms^2 (S. H. Shen et al., 2016).

There are additional extraneous variables that must be considered with frequency domain HRV recordings that can influence the results. Posture is known to influence HRV; specifically, the supine position, which was used in this study, is associated with greater HF power (Fagard et al., 1999; Watanabe et al., 2007). Vagal function is enhanced in supine postures due to a decrease in demand placed on the cardiovascular system.

As mentioned previously, the duration of the recordings can also influence findings. The present study used a four-minute recording obtained upon waking or shortly thereafter (i.e., within 30 minutes of awakening), which could miss responses that previous research was able to encapsulate in longer timeframes (e.g., 24- or 36-hour recordings). Participant age and cumulative exposure to shift work could have also influenced the variability within the obtained results. Furthermore, the present study obtained recordings following a sleep period and represented waking HRV parameters. It is known that during sleep periods, HRV follows a circadian response to increase HF power due to heightened parasympathetic outflow, and concurrently has drops in LF power and LF/HF (Boudreau et al., 2012). This could mean that the sleep period itself may impact the resultant waking HRV recordings, as sleep does promote an

autonomic shift back to parasympathetic dominance. Sleep could also potentially mitigate potential effects as seen with stress; although, Borchini et al. (2018) reported that during sleep, $LFms^2$ was not different in nurses under varying loads of perceived occupational stress in both working and non-working days.

Ultimately, comparing research involving HRV in an occupational setting can be difficult due to the variety of methods employed, devices used, and environmental factors (Järvelin-Pasanen et al., 2019). Most of the research has been concerned with comparing responses of permanent day and night workers with rotating shift workers, which makes comparisons difficult with the current sample of workers as Island Health uses an exclusively rotating roster unless special circumstance (Health Employees Association of British Columbia, 2019). The present study is therefore unable to make such comparisons due to the homogenous sample of rotating shift nurses used.

5.3 Eight Day Shift Roster & Potential for Chronic Change

Q3: Is a circadian disruption or heightened stress response still present on days off as indicated through changes in concentrations of melatonin, cortisol, or IL-6?

5.3.1 Indicators of Stress

Cortisol concentration did not change across mornings of shift work and days off in both Period 1 and Period 2. The high variability between individuals likely plays a large factor in the resultant findings. A range of 1.99 μg/dL was seen in Period 1, and 0.68 μg/dL in Period 2; the larger range in Period 1 is due to three salivary concentrations above 2.00 μg/dL. All concentrations aside from the two outliers were within the normal reference range of Salimetrics values for morning cortisol concentrations in women aged 21 – 30 years and 31 – 50 years, with some of the off-day measurements below the threshold suggested by the manufacturer (Appendix

D). The evening values collected on day and night shifts were consistently above normal reference range values and previously published research, alluding to a flattening of the diurnal circadian curve rather than changes to the amplitude of the morning release itself (Laudat et al., 1988).

Previous research has demonstrated that a recovery of the cortisol cascade following shift work is possible across various industries and shift durations. The time required can differ though based on exposure to shift work (e.g., shift length, number of consecutive shifts worked) and industry type (Harris et al., 2010; Hung et al., 2016; Jensen et al., 2016). Contrarily, research has also demonstrated that sleep restrictions do not affect cortisol concentrations and that recovery sleeps actually can decrease cortisol concentrations to below baselines (Pejovic et al., 2013). This did not happen within the current research; instead, findings mirrored that of Eek et al. (2012), who found a lack of correlation between recovery sleep following an induced sleep restriction and salivary cortisol concentrations. That said, Pejovic et al. (2013) found that the circadian nature itself was affected within recovery from sleep restriction; cortisol's peak release shifted forward to 0600 when under sleep restriction conditions, and during recovery, it reached values similar to the baseline of 0700 – 0800. It is possible that this response could have occurred within the current sample population and was incidentally reflected within the results.

Morning cortisol concentrations obtained following night shift also could have been elevated due to the recent cessation of a 12-hour shift in comparison to coming off sleep as what was measured during day shifts and days off. However, the morning cortisol concentrations (combined Period 1 and Period 2 average) following night shift ranged between 0.21 μg/dL and 0.34 μg/dL; this is within the Salimetrics reference range for women between the ages of 21 – 30 years and 31 – 50 years (Appendix D). This heightens the possibility that evening levels were

affected and caused a resultant blunting of the diurnal curve, which can increase the risk for adverse future health outcomes and place added risk onto the current population (M. Kumari et al., 2011). Ultimately, only two salivary samples were collected from nurses (pre- and post-shift); these do not provide a full 24-hour canvas of the data but do provide some insight into the interplay of systems at hand. An additional lack of dual samples on off-days further illustrates the need for future research in this regard.

IL-6 also did not exhibit any significant changes across the eight-day monitoring phase within Period 1 and Period 2. This is likely due to the increased presence of outlier data, thus increasing variability between samples, and indicates that there was no discernable effect of rotating shift work during the COVID-19 pandemic on obtained concentrations. This was also seen in pre-pandemic results, as nurses did not exhibit any significant changes in serum concentrations of IL-6 from the sleep before a day shift, and immediately following a day shift and night shift (Bjorvatn et al., 2020).

The range in obtained morning salivary IL-6 concentrations is high. The maximum obtained concentration in Period 1 was 107.33 pg/mL (seen on Off 1), while the minimum was 1.44 pg/mL (on Day 1); in Period 2, there was a maximum of 98.16 pg/mL (from Day 2), and a minimum of 1.22 pg/mL (on Night 2). The maximum values are much higher than previously published normative data suggesting a range for IL-6 of 4.4 pg/mL to 20.9 pg/mL (Izawa et al., 2013). Izawa et al. similarly followed a passive-drool technique as the present study and recommend using this method for studies analyzing IL-6; other research has used cotton absorbent methods, which could affect the ELISA and IL-6 concentrations. Aforementioned variables such as unmentioned injury or infection, menstrual cycle status, undiagnosed joint disease (i.e., arthritis), or stress unaccounted for in the ENSS could elevate these readings

(Angstwurm et al., 1997; Srirangan & Choy, 2010; Szabo & Slavish, 2021). Furthermore, IL-6 has been theorized to show a diurnal variation, and the collection period within the present study would align with the trough which can explain the lower concentrations (Nilsonne et al., 2016).

Sleep restrictions akin to shift work can impact IL-6 concentrations (Faraut et al., 2015; Irwin et al., 2010). Irwin et al. (2010) found that in the morning immediately following sleep deprivation, both men and women had a stimulated increase in IL-6 and TNF-α, with women exhibiting a prolonged increase in comparison to men. Contrarily, research has also shown that sleep deprivation from shift work does not impact IL-6 concentrations (Amano et al., 2018; Bjorvatn et al., 2020; Van Mark et al., 2010). Van Mark et al. (2010) additionally suggest that long-term exposure to restricted sleep (e.g., similar to increase in years of shift exposure) could result in a compensation of proinflammatory immune mechanisms. Furthermore, the effects of sleep restrictions can be negated with recovery sleeps and naps for as little as 30 min (Faraut et al., 2015; Pejovic et al., 2013). Napping was not controlled for within the current study, which could have attributed to the vast variability seen in the results.

Night 1 and Night 2 IL-6 measurements were obtained immediately following a 12-hour night shift. During these shifts, nurses' heart rates fluctuated greatly and at times reached levels associated with moderate to intense exercise. The heightened heart rates could have mimicked the effects of IL-6 during exercise. IL-6 acts as a myokine during exercise that can induce an anti-inflammatory response, and if nurses had a stressful back end of a shift as evidenced through an elevated heart rate, it could impact the following saliva collection (Pedersen et al., 2001). Additionally, nurses' break activities were uncontrolled, so it is unknown whether nurses experienced some relaxation or even a quick nap during night shift which could further impact IL-6 concentrations (Faraut et al., 2015).

5.3.2 Indicators of Circadian Rhythmicity

Morning salivary melatonin values significantly differed by day ($p < 0.001$), but not by period. However, when Bonferroni post-hoc tests were conducted, no statistically significant difference was evident. This is likely due to the small statistical power resultant from the low sample size and outliers present within the dataset. There were also no differences between Period 1 or Period 2 or in the day*period interaction.

The average morning concentrations of salivary melatonin remained relatively constant between Day 1 and Day 2 before decreasing by 16.55 pg/mL on the morning following Night 1. This decrease is expected due to an increase in exposure to light at night associated with night shifts, thus affecting the entrained circadian rhythm inherent within the suprachiasmatic nucleus (SCN) (Tähkämö et al., 2019). This phenomenon has been observed in both laboratory and field research, as exposure to light during the entrained "dark" hours can suppress the amplitude of melatonin release or even delay its onset (Amirian et al., 2015; Burch et al., 2005; Daugaard et al., 2017; Davis et al., 2012; Dijk et al., 2012; Jensen et al., 2016; Papantoniou et al., 2014). However, studies examining nurses exclusively have also found contradictory findings (Bracci et al., 2016; Grundy et al., 2011). Unfortunately, the generalizability of such findings to the present study is hampered due to a mixture of shift systems that are not equivalent to what is employed at Island Health.

The mean salivary melatonin concentration increased from the first to the last day off by 10.24 pg/mL. This consequently illustrates that there was an apparent trend in circadian recovery, although the average on Off 4 was less than the average Day 1 morning concentration in Period 1 and Period 2. The decreased melatonin concentration on the first day off and the

progressive increase in morning salivary melatonin concentration seen on the fourth day off aligns with previous occupational research; average morning salivary melatonin concentrations increased from 9.03 pg/mL on Off 1 to 19.30 pg/mL on Off 4 (both averages are Period 1 and Period 2 inclusive) (Daugaard et al., 2017; Davis et al., 2012; Jensen et al., 2016; Stone et al., 2018). Stone et al. (2018) state that melatonin phase resetting can further be influenced by light exposure patterns and the relationship to an individual's baseline phase and chronotype; it should be noted that Stone et al. (2018) examined 6-sulfatoxymelatonin, a urinary metabolite of melatonin, rather than salivary concentrations.

The degree of light exposure experienced at night can also influence the degree of melatonin suppression (Benloucif et al., 2008; Papantoniou et al., 2014). Specifically, the amount of light present within key phase-shifting times in the rhythmic melatonin cycle is crucial to the degree of suppression observed (Stone et al., 2018). This raises concern with the generalizability of previous findings and the current research, as not every workplace has the same amount and quality of light, and seasonal effects (e.g., environment, longitude-latitude, etc.) can further affect results. The current study took place in Victoria, British Columbia, Canada during the fall and winter months; a group of nurses tested at a similar latitude and longitude in Seattle, Washington, USA, had a similar melatonin response, although a different scheduling roster was used (Davis et al., 2012).

A group of nurses in Kingston, Ontario, Canada that followed a similar schedule pattern (two 12-hour day shifts, two 12-hour night shifts, and five days off instead of four) had urinary and salivary melatonin concentrations collected across four testing periods (Grundy et al., 2011). Patterns of melatonin production were similar in both day and night shifts in each of the collection periods, which questions the effect of light exposure on secretion patterns. This was

also observed in earlier work by the same researchers and population group (Grundy et al., 2009). However, Grundy et al. did find that shift work history did impact melatonin production while on night shift work, alluding to a potential chronic circadian adaptation.

Additionally, shift workers' diurnal preference can further influence melatonin response (Stone et al., 2018). The present study featured diverse scores on the MEQ, which could have accounted for some variability observed in individual melatonin concentrations. Stone et al. (2018) suggest that individuals who are more of an "evening-type" may inadvertently seek increased light during the evening and night.

Salivary melatonin measurements were used in the present study to allow for optimal feasibility in remote data collection. Salivary melatonin concentrations do exhibit a high degree of variability due to various extraneous factors. Burgess & Fogg (2008) found that peak melatonin values in healthy men and women varied from 2 to 84 pg/mL, with onset ranging from 1813 – 0026 hours. Factors such as hormonal birth control, employment, sleep hygiene and schedule, body weight, age, vision status, and mental health can all impact salivary melatonin content and pose an interesting avenue for future research. That said, uncontrollable genetic factors are ultimately responsible for the largest amount of variability in results seen (Burgess & Fogg, 2008).

Serum measurements would have enhanced the viability of assays but impacted the comfort and ability of participants to collect their own data. While serum and salivary measurements are both able to illustrate the circadian response, serum measurements are three times greater than saliva (Benloucif et al., 2008; Kennaway, 2019). Furthermore, it is recommended to obtain samples at a greater frequency when investigating melatonin to best examine alterations to the circadian response through the magnitude and direction of potential

phase shifts (Benloucif et al., 2008; Kennaway, 2019; Tähkämö et al., 2019). However, constrictions related to remote data collection along with conducting research during the COVID-19 pandemic limited the scope of sampling in the present study.

Chapter 6: Conclusion

This research sought to evaluate the subjective and objective responses to stress in female nurses while working rotating shift schedules at two hospitals in the Greater Victoria region during the COVID-19 pandemic. Three research questions were created to address various aspects of health in response to working during the pandemic, with stress and circadian functioning profiled in various analytical captures.

The first research question examined the objective and subjective stress response related to working rotating shift work during the COVID-19 pandemic; this was assessed with an evaluation of salivary cortisol and IL-6 concentrations as well as ENSS scores obtained at two time points of collection. No difference was seen in morning salivary IL-6 concentrations during the four working days in the Island Health shift roster in either Periods 1 or 2; this is likely due to high variability observed within the current study population. There was also no statistically significant difference between morning and evening salivary cortisol concentrations for Period 1 and Period 2, and no significant difference was seen in the change from morning to evening concentrations while on day and night shifts. The lack of difference between morning and evening concentrations illustrates a blunted cortisol diurnal rhythm. If elevations in evening concentrations and resultant blunting were to occur long-term, the potential for adverse health outcomes would rise (M. Kumari et al., 2011; Laudat et al., 1988; Nijm & Jonasson, 2009). The dampened diurnal rhythm, due in part to evening cortisol concentrations, is evident of a prolonged stress response into the evening and is linked to an increased risk for cardiovascular disease and death (M. Kumari et al., 2011). Additionally, if individuals experience a heightened proinflammatory response (i.e., chronic elevations in IL-6) along with an altered cortisol rhythmicity it could promote the development of atherosclerosis (Nijm & Jonasson, 2009).

ENSS scores were obtained in both Period 1 and Period 2 of collection and while issues surrounding data retention made it difficult to conduct statistical analyses, visual inspection of trends revealed an elevation in responses in comparison to research published in a pre-pandemic environment (Alomari et al., 2021; Sarafis et al., 2016). Specifically, the factors of Inadequate Emotional Preparation, Workload, and Difficulties with Patients and Their Families consistently ranked amongst the top subcategories with the highest average scored responses, aligning with concurrent research performed in the COVID-19 pandemic (Said & El-Shafei, 2021).

The second research question was specifically concerned with frequency domain HRV parameters (i.e., LFms2, HFms2, LFnu, HFnu, LF/HF) over the eight-day shift roster. No differences were seen between day shifts, night shifts, and days off in either Period 1 or Period 2 for any parameter of interest; furthermore, no differences existed between the periods on Day 2 or Night 2. Like IL-6, there was vast variability present within the obtained data. That said, there is great potential for future research to address gaps and uncontrolled issues; these include (but are not limited to) using different equipment, improving sample retention through enhancing participant/researcher interaction, and elongating the recording itself.

The circadian and stress response into days off was the primary focus of the third research question. There were no statistically significant differences amongst the morning collections for cortisol or IL-6 in either Period 1 or Period 2. The lack of difference for cortisol across mornings further provides evidence of shunting of the diurnal response being affected during this time. There was a statistically significant difference seen across the days of analyses for salivary melatonin concentration across Periods 1 and 2. This aligns with previous occupational research showing that exposure to light at night with rotating shift work suppressed melatonin concentrations (Daugaard et al., 2017; Papantoniou et al., 2014). A recovery was

apparent on days off, although to varying degrees within the analyzed participants, and could have influenced the variability seen in results. Ultimately, long-term exposure to shift work is associated with chronic suppression of melatonin levels while at night and can further be associated with disease and injury (Schernhammer et al., 2001; Schernhammer & Hakinson, 2009; Ward et al., 2019).

The COVID-19 pandemic has widespread global effects, regardless of profession or geographic location. Nurses have undoubtedly faced a brunt of issues within their work environment while on the frontlines. The long-term effects of working during this time remain to be seen, but early analyses within the present study show alterations to both cortisol and melatonin circadian rhythms, along with heightened perceived occupational stress as seen with increased average scored responses to the ENSS. It should be noted that the nature of the present research makes it hard to discern whether these physiological and subjective responses were due in part to the pandemic, rotating shift work, or both is hard to do due to a lack of control/pre-pandemic data. That said, the blunted cortisol rhythm and melatonin suppression can both potentially affect nurses' future health; the elevated perceived stress, as captured with the ENSS, further compounds this risk, and can jeopardize long-term health outcomes. Ultimately, this provides evidence of the sacrifice these workers have undergone during the pandemic to keep society safe and healthy.

6.1 Limitations

Performing the current study during the COVID-19 pandemic made for the emergence of several unavoidable limitations. The fact that researchers and participants were unable to interact in person was a massive challenge; this meant that participants had to conduct their own data collection, leaving room for potential unintentional errors. The pandemic also limited the ability

to compare data to control or pre-pandemic data, making it hard to discern what factors were responsible for the observed physiological and subjective responses.

A small sample size was used for this study, which enhanced feasibility for remote data collection but also limited the ability to perform various statistical tests. Prospective interest may have also been hampered because the current research was needing nurses to participate during a pandemic; nurses were already required to work during a global health crisis and may not have wanted to add additional responsibilities at this time. Ultimately, the small sample size affected statistical power, and analyses were under greater influence from outlier data and could have affected the lack of observed significance in some cases.

The equipment for the present study was chosen with participant comfort and ease of use in mind. For example, an electrocardiogram (ECG) Holter monitor would have been best for HRV data retention and subsequent analyses but was deemed impractical due to the risks associated with independent data collection. Consequently, the Polar H10 Chest Strap and Polar Ignite Activity Tracker were used instead and could have impacted the captured datasets. The Polar H10 monitor is highly recommended for field research but proved to not be optimal for remote data capture as seen with issues surrounding data retention. Participants were instructed to wear both devices for the five-minute waking periods for HRV files to be available for synchronization and download to Polar Flow and Excel; if any of these settings were altered (even inadvertently), then data was unable to be downloaded for analysis, which was unfortunately seen in both Period 1 and Period 2. This also applies to salivary analyses; while serum measurements would have provided optimum viability for analyses, it was not possible to have nurses conduct their own venipuncture due to the remote nature of data collection.

There was also the issue of missing data, which was especially inherent within the ENSS and HRV datasets. With the ENSS, participants either did not answer questions or selected *does not apply,* which impacted the ability to conduct full statistical tests. For HRV, this, as previously discussed, was likely due to participant error itself; that is, either the Polar H10 was not worn or it lost synchronization with the Polar Ignite, or participants did not collect on the correct dates or times. This further emphasizes the challenges of conducting research remotely.

6.2 Directions for Future Research

There are plenty of potential avenues to build off the findings of the current study. For example, when examining HRV the recording time could be changed to encapsulate responses while on-shift. This would employ the use of time domain measures but could illustrate the autonomic cardiovascular control on-shift and further add to the overarching investigation of the stress response; frequency domain analyses could also still occur by selecting predetermined time bands (e.g., nurses could perform a recording for five minutes while on break) or collecting immediately after a shift as well. Sub-analyses could also be conducted for factors such as age, shift work exposure, or perceived stress level.

When examining salivary biomarkers (i.e., cortisol, IL-6, and melatonin), greater control for extraneous factors could be enforced to optimize analyses, or subgroup analyses could be performed for these factors. An example of this would be to examine the effect of hormonal contraceptives, which could potentially impact findings (Lovallo et al., 2019). Additionally, evaluating the cytokine nature of IL-6 can be further enhanced with a paired investigation of an additional biomarker like CRP. Ideally, future research would be able to encapsulate the full eight-day shift roster for analyses (instead of six, which was used for IL-6 and melatonin) as well as to collect from more participants to build off preliminary findings from the current study.

It would be beneficial to follow up with the current study population group throughout the latter stages of the COVID-19 pandemic while British Columbia continues progressing through its reopening phases, as well as once the pandemic is declared over. It would be interesting to see if the preliminary findings are still apparent within a post-pandemic landscape, and if so to what degree, as well as to follow-up with nurses to perform an overall health check to see if any other ailments have arisen. Additionally, this would allow for physiological and subjective responses to be further examined, which would help to discern the root cause of the observed phenomena to either rotating shift work, pandemic-related factors, or something else entirely.

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Appendix A: Recruitment Poster

Examination of Healthcare Workers' Response to Rotating Shift Work During the COVID-19 Pandemic in Greater Victoria Care Sites

Are you a female nurse between 20 and 60 years of age?
Do you currently work a rotating shift schedule?

We are seeking research participants for a study that will evaluate the physiological effects of working on the frontlines during the COVID-19 pandemic. There will be no physical contact with researchers, as participants will be provided with information and collection materials to use independently.

Your participation in this study would involve data collection for 2 morning shifts, 2 evening shifts, and 4 days off.

- Spit samples will be collected at the start and end of shifts. A heart rate monitor will be worn throughout the shift.
- Spit samples and heart rate will be collected at home upon waking on days off.
- Sleep quality will be assessed with a Garmin wristwatch.

If you are interested in participating or want more information, please contact:

Dr. Lynneth Stuart-Hill
School of Exercise Science, Physical and Health Education, University of Victoria

Marisa Harrington, MSc Kinesiology candidate
School of Exercise Science, Physical and Health Education, University of Victoria

V02 May 8, 2020

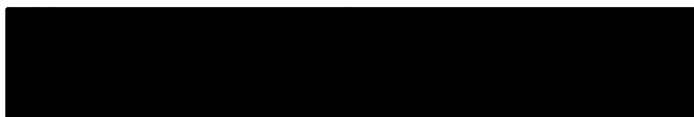
Appendix B: Informed Consent Package & Consent Log

Examination of Healthcare Workers' Response to Rotating Shift Work During the COVID-19 Pandemic in Greater Victoria Care Sites

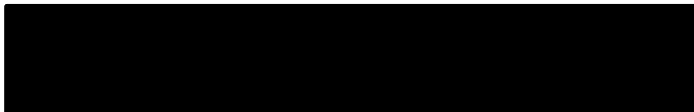
PARTICIPANT INFORMATION & CONSENT FORM

PRINCIPAL INVESTIGATOR AND STUDY TEAM:

Principal Investigator Name and Affiliation/Title: Dr. Lynneth Stuart-Hill, Faculty Member, School of Exercise Science, Physical and Health Education, University of Victoria



Sub-Investigator Name and Affiliation: Marisa Harrington, MSc Kinesiology Candidate, School of Exercise Science, Physical and Health Education, University of Victoria



Background and Purpose of the Study

You are invited to participate in a research study. Your participation must be free and voluntary. You are free to withdraw at any time. The purpose of this study is to conduct a comprehensive analysis measuring physiological indicators of stress and health in healthcare workers on rotating shift work schedules during the COVID-19 pandemic at care centres in the Greater Victoria region.

Working on the frontlines of the COVID-19 pandemic understandably presents its challenges, and further compounds physiological responses to stress which can place workers at increased risk for negative acute and long-term health outcomes. Nurses are already exposed to plenty of stressors in their line of work that can influence their health; some of these include exposure to shift work, subjective occupational

stress, patient care duties, and complexity in work environments. These can affect physiological markers of health and functioning, such as melatonin, cortisol, interleukin-6 (IL-6), and heart rate variability (HRV).

Anecdotal evidence has shown that nurses are facing novel stressors while working during the COVID-19 pandemic. These include the threat of contracting and/or transmitting the virus; self-isolation; considerations surrounding personal protective equipment; decreased socialization with coworkers, families, and friends; alterations to the pre-existing work environment and procedures among others. The aforementioned physiological markers can therefore be even more affected and can heighten future disease risk. Elevations in perceived occupational stress can increase cortisol and IL-6 levels, and also impact cardiovascular autonomic control as measured through HRV. This in turn elevates the risk for inflammatory disease, cardiovascular disease, and all-cause mortality. Being exposed to light at night through shift work suppresses natural melatonin release, which can affect cognition and mental performance and is inopportune for working on the frontlines of the pandemic. Chronic melatonin suppression through shift work has also been linked to breast cancer risk in women.

You are being asked to participate in this study because you are a female regulated nurse employed with Island Health between the ages of 20 and 60; are currently on a rotating shift roster; are not pregnant or nursing; do not have or are undergoing treatment for endocrine, autoimmune, or cardiac disorders; not diagnosed with a sleep disorder other than shift work sleep disorder; and do not currently use sleep medication, including melatonin supplementation.

Location of Research

This research study will be conducted in accordance with the School of Exercise Science, Physical and Health Education, at the University of Victoria. Participants will be from the Royal Jubilee Hospital and Victoria General Hospital. Data will be collected at the aforementioned hospitals as well as independently at participants’ homes. Investigators will perform data analysis at the University of Victoria.

Number of Participants

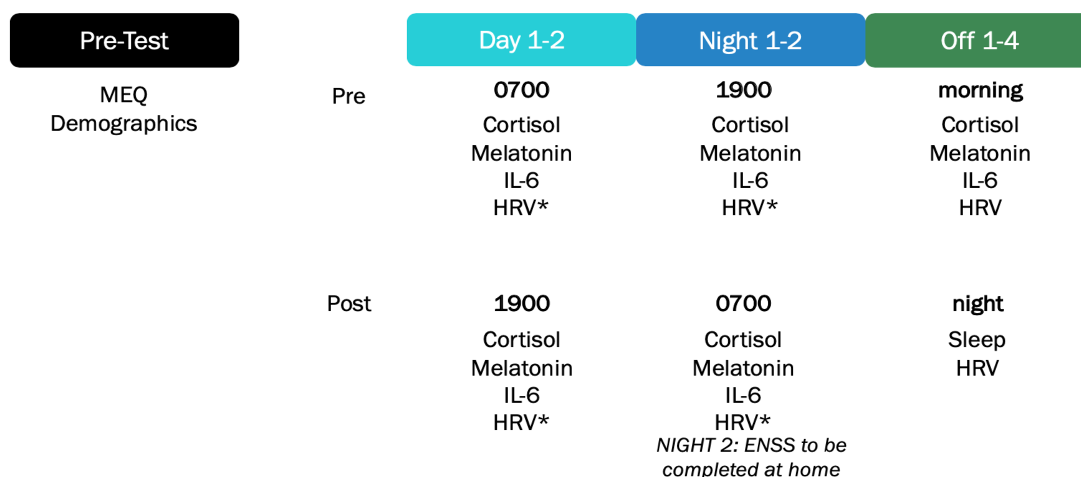
We are seeking a minimum of 20 participants to be included in this study including about 20 participants from this region.

Project Funding

This research is supported with funds from the WorkSafeBC research program.

What is Required if I Participate?
If you decide to participate in this study, you will be responsible for conducting your own data collection, as physical contact between participants and researchers will not occur to adhere to social distancing guidelines. All equipment and information required for participation will be provided from researchers; drop-off of sampling equipment will be coordinated between you and the researchers prior to beginning the study. Videos and additional study information can be found at a study website that will be sent to you prior to the first day of collection.

You will be asked to participate in two periods of data collection: the first being now, while the province is in Phase 2/3 of BC's Restart Plan, and another in the predicted Phase 3 in the fall. Overall, your involvement in the study should be around 110 minutes for each period (~220 minutes total). The data collection period and sampling timeframe is outlined in the diagram below:



Before you begin your first day of data collection, you will complete two online surveys. It is expected that these questionnaires are completed in full. You will receive a link to these studies through an email and they will also be available on the study website. The Morningness-Eveningness Questionnaire (MEQ) is a validated and widely used tool to evaluate your individual chronotype (your subjective preference for mornings or evenings and related hormone responses). Here are a couple of sample questions from the MEQ:

Approximately what time would you get up if you were entirely free to plan your day?

[5] 5:00 AM–6:30 AM (05:00–06:30 h)

[4] 6:30 AM–7:45 AM (06:30–07:45 h)

[3] 7:45 AM–9:45 AM (07:45–09:45 h)

[2] 9:45 AM–11:00 AM (09:45–11:00 h)

[1] 11:00 AM–12 noon (11:00–12:00 h)

You have two hours of hard physical work. You are entirely free to plan your day. Considering only your internal "clock," which of the following times would you choose?

[4] 8 AM–10 AM (08–10 h)

[3] 11 AM–1 PM (11–13 h)

[2] 3 PM–5 PM (15–17 h)

[1] 7 PM–9 PM (19–21 h)

A questionnaire created by the investigators to gain subject demographic information will also be completed online prior to testing. Questions are divided into the following categories: subject information, night shift exposure, and lifestyle factors. Included below are three questions (one from each aforementioned category):

Please list the ward(s) you work in:

____

____

How many total years have you worked shift work for (cumulative exposure – nursing and other work):

____`________

Do you consume alcohol? _____ YES _____NO

If yes, how many drinks per week? _____

Saliva samples will be collected at the beginning and end of each of your shifts for four consecutive working days (two day shifts and two night shifts). This should take 5 minutes at each time. An instructional video illustrating how to perform the saliva collection along with written instructions will be provided to you beforehand on the study website, along with all of the necessary equipment. The saliva will later be analyzed for melatonin, cortisol, and IL-6 content.

You will also wear a heart rate monitor and wristwatch for the entirety of your shift. The monitor is comprised of a small, lightweight sensor strapped to your chest by means of an elastic chest belt that is similar to a bra. You will also wear a watch to receive the data on your non-dominant hand. A video and written instructions will be provided on the study website to you to show how to put on and take off the monitor, which will be dropped off from the researchers along with the salivary sampling kits prior to the first shift.

Upon completion of your last shift, you will fill out one last online questionnaire. The Expanded Nursing Stress Scale (ENSS) will be sent to you via email for you to complete. This will be used to measure your subjective assessments of stress in the workplace through use of a Likert scale. It is expected that this will be completed in full. There are 57 questions divided into 9 subcategories, which include: death and dying, conflict with physicians, inadequate preparation, problems with peers, problems with supervisors, workload, uncertainty with treatment, patients and families, and discrimination. Some sample questions from this questionnaire are included below:

> Below are situations that commonly occur in a work setting. For each situation you have encountered in your **PRESENT WORK SETTING** indicate **HOW STRESSFUL** it has been for you using the following ranking system: 1 = never stressful, 2 = occasionally stressful, 3 = frequently stressful, 4 = always stressful, 5 = does not apply
>
> Feeling inadequately prepared to help with the emotional needs of a patient:
> 1 2 3 4 5
>
> Difficulty in working with a particular nurse (or nurses) in my immediate work setting:
> 1 2 3 4 5
>
> Being exposed to health and safety hazards:
> 1 2 3 4 5

You will be responsible for collecting data at your home for four consecutive off days following the last worked night shift. You will provide a passive drool salivary sample immediately upon waking; this will follow the same method as what is used on shift and should only take 5 minutes. You will also wear the chest strap heart rate monitor and watch for each off night sleep. This can be removed after obtaining the salivary sample. You are not required to participate in any additional activities throughout your off days.

To recap the above information pertaining to data collection, please see the following table below:

Variable of Interest	How it Will be Collected	When it Will be Collected
Subject demographics	Online questionnaire	Pre-test
Individual chronotype	Online questionnaire	Pre-test
Melatonin	Saliva sample	At work: immediately before and after shift Off work: immediately upon waking
Cortisol	Saliva sample	At work: immediately before and after shift Off work: immediately upon waking
IL-6	Saliva sample	At work: immediately before and after shift Off work: immediately upon waking
HRV	Chest strap monitor and wristwatch	At work: continuously throughout shift (monitor put on at start, taken off at end) Off work: throughout sleep (monitor put on before bed, taken off when done morning saliva sample)
Subjective perceived stress	Online questionnaire	Immediately after the last shift worked (last night shift in rotation)
Sleep composition	Wristwatch	Off night sleeps

Once you have completed the data collection cycle, a pickup location and time will be arranged with the researchers to obtain study equipment. This will be at a location and time of your convenience.

You will be contacted by the investigators when period 2 of data collection is set to begin. For information regarding consent, please see “On-Going Consent” on page 8 for more details.

Since your personal information and physiological indicators will be collected, your confidentiality of your indentity and your data are greatly important to us. Please see “Confidentiality and How my Personal Information will be Used” section on page 8 for more details.

What are the Possible Risks or Inconveniences of Participating?

You may be exposed to the following risks and inconveniences:

- Mild discomfort in wearing the heart rate monitor chest strap.
- Mild discomfort providing salivary samples.
- Some questions in questionnaires (particularly ENSS) may cause emotional discomfort.
- Time commitment.
- Arranging equipment collection with researchers.

To reduce these risks, the following steps will be taken:

- Providing thorough instruction for use of equipment through video demonstration by the principal investigator and sub investigator.
- Only the investigators will have access to your answers to the questionnaires. Additionally, no personal identifiers will be listed, rather they will be coded according to participant ID.
- You will not be asked to participate in any activity outside of what you would normally do while on shift, but you will be required to perform all duties within your scheduled work period.
- Using time efficient sampling methods and avoiding samples taking place during shift, rather only 5 minutes before and after work.
- Arranging an equipment collection time and location that best serves your convenience – the investigators will come to you.

What are the Possible Benefits of Participating?

The possible benefits of your participation include:

- Understanding how a global health emergency indirectly affects those responsible for keeping society healthy.

- Filling major gaps in occupational research, particularly with examining if physiological responses exist on days off and providing insight into the understudied Canadian cohort of healthcare workers.
- Gain insight into your own health and safety while on and off shift.
- Determine future occupational health and safety challenges and how to potentially mitigate/reduce these in terms of a future pandemic.

Do I Have to Take Part?

You are free to participate or not. If you decide not to participate, employment status will not be affected in any way. By consenting, you have not waived any rights to legal recourse connected to research-related harm. If you do decide to participate and then change your mind later, you can withdraw without any consequences or explanation. If you do withdraw from the study, we will ask you if we can still use your collected data.

Will I be Paid for Taking Part?

You will not be provided with any payments or coverage of costs for participating in this study.

On-Going Consent

If new information becomes available, or if this project takes place over a longer period of time, we will ask you to renew your consent to participate. Since this project is taking place over two testing periods, we will contact you again when the next stage of collection is set to begin to obtain your consent. It is anticipated that this should be in fall 2020. If you do not provide consent to provide data for the second period of data collection, your previous data will be discarded and not used in analysis.

Confidentiality & How my Personal Information will be Used

In order to protect your anonymity, no data will be recorded with your name on it. However, due to the nature of this research you will not be anonymous to the researchers involved. Your confidentiality and the confidentiality of your data will be protected by assigning a unique ID number to you that will be used to code all of your data. Your ID number will be assigned to you at the beginning of the study and will be used to code all sources of information (i.e. questionnaire responses, salivary samples, heart rate and sleep data). There will be no linkages being made between your information at Island Health or other sources of information about you.

Data will be stored on a password protected computer. The master list of participants, corresponding ID numbers, and the password protected computer will be locked in Office 132 in the McKinnon building at the University of Victoria. Only the principal investigator and sub investigator will have access to your personal information and data.

The information that will be collected from you includes the following:

- Individual responses to three questionnaires (MEQ, ENSS, Demographics).
- Salivary melatonin, cortisol, and IL-6 content.
- Heart rate and HRV.
- Sleep composition.

All physiological data will be analyzed at the University of Victoria. Questionnaire responses will be recorded on a reputable survey platform hosted in Canada (hostedincanadasurveys.ca) and will be assessed only by the investigators at the University of Victoria.

Your consent to collect your information for the purpose of this research project will expire when you complete the study.

Future Use of Data

The data collected for this study will not be used for any future research.

Disposal of Data

Your data from this study will be disposed of in the following manner:

Data will be securely stored on a password-protected computer in a locked faculty office at the University of Victoria for 5 years. Thereafer, identifiers and links to identifiers will be removed and the data will remain for a further 5 years before destruction, in which computer files will be deleted and any hard copies of data will be confidentially shredded.

Sharing of Study Results

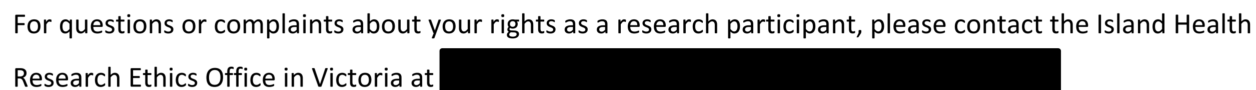
A summary of the study results will be provided to you upon request.

The study results will be published and presented to presentations at hospitals and scholarly conferences, as well as publication in a scholarly journal. This study will also be part of Ms. Harrington's graduate thesis.

Who Should I Contact if I Need More Information or Help?

The contact information for the Principal Investigator and Sub Investigator are provided on the first page of this Informed Consent Form.

For questions or complaints about your rights as a research participant, please contact the Island Health Research Ethics Office in Victoria at



CONSENT

Your signature below indicates that:

1. All sections of this Consent form have been explained to your satisfaction
2. You understand the requirements, risks, potential and responsibilities of participating in the research project, and;
3. You understand how your information will be accessed, collected and used.
4. All of your questions have been fully answered by the researchers.

____________________	____________________	____________
Name of Participant (print)	Signature	Date

____________________	____________________	____________
Name of Person Administering Informed Consent	Signature	Date

____

Role of Person Administering Informed Consent

A copy of this consent form will be given to you, and a copy will be kept by the researcher.

Consent Log:

Participant Name	Date	Verification of Verbal Consent (Period 1; Period 2)	Withdrawn from Study (date)

Appendix C: Ethics Certificate

Certificate of Ethical Approval: Amendments for Harmonized Minimal Risk Behavioural Study

Island Health Research Ethics Board
Queen Alexandra Centre
Main Building, Room 205
2400 Arbutus Road
Victoria, BC V8N 1V7
Tel: 250-370-8620

Also reviewed and approved by:

- **University of Victoria**

Principal Investigator:	**Primary Appointment:**	**Board of Record REB Number:**	**REB Number:**
Lynneth Stuart-Hill			H20-00575

Study Title:
Examination of Healthcare Workers' Response to Rotating Shift Work During COVID-19 Pandemic in Greater Victoria Care Sites

Approval Date: December 9, 2020 **Expiry Date: September 17, 2021**

Research Team Members: Marisa Harrington

Sponsoring Agencies: - WorkSafe BC

Documents included in this approval:

Document Name	Version	Date
Other Documents:		
Media Release	1.0	November 26, 2020

This ethics approval applies to research ethics issues only and does not include provision for any administrative approvals required from individual institutions before research activities can commence.

The Board of Record (as noted above) has reviewed and approved this study in accordance with the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS2, 2014).

The "Board of Record" is the Research Ethics Board delegated by the participating REBs involved in a harmonized study to facilitate the ethics review and approval process.

The application for ethical review and the document(s) listed above have been reviewed and the procedures were found to be acceptable on ethical grounds for research involving human subjects.

This study has been approved either by the Board of Record's full REB or by an authorized delegated reviewer.

Appendix D: Salimetrics ELISA – Manufacturer's Information

Salimetrics ELISA kits were used for all analyses. Detailed instructional manuals can be found at the links below:

Cortisol: https://salimetrics.com/wp-content/uploads/2018/03/salivary-cortisol-elisa-kit.pdf

Interleukin-6: https://salimetrics.com/wp-content/uploads/2018/03/il-6-saliva-elisa-kit.pdf

Melatonin: https://salimetrics.com/wp-content/uploads/2018/03/melatonin-saliva-elisa-kit.pdf

Appendix E: Heart Rate Log

For Researcher Use Only:
Participant ID: __________

Heart Rate Monitor Activity Log

Please fill out the following log throughout the monitoring period with the following instructions:

Time - use a 24hr clock to state times
Activity - list any activities performed during the monitoring period. These can include but are not limited to:

- Meals/snacks
- Exercise
- Urination/bowel movements
- Stress (physical or emotional)
- Significant events at work (i.e. something that could affect you personally/elicit an increased reading of stress etc.)
- Putting on or removing the device
- Anything else you find relevant

Details – please provide any pertinent details or symptoms related to activities performed (e.g. type of workout, experiencing any adverse events/symptoms such as dizziness, shortness of breath, etc.)

Date: _________________________

Time	Activity	Details

Appendix F: Morningness-Eveningness Questionnaire

*Please note that this questionnaire was available online for participants to complete. Included below is an adapted paper copy for the purposes of this thesis. The algorithms for scoring are built into the online software and participants do not have to calculate their chronotype by themselves, rather they are provided with their scores

MORNINGNESS-EVENINGNES05S QUESTIONNAIRE
Self-Assessment Version (MEQ-SA)[1]

Name: ___________________________ Date: ______________________

For each question, please select the answer that best describes you by circling the point value that best indicates how you have felt in recent weeks.

1. *Approximately* what time would you get up if you were entirely free to plan your day?

   [5] 5:00 AM–6:30 AM *(05:00–06:30 h)*
   [4] 6:30 AM–7:45 AM *(06:30–07:45 h)*
   [3] 7:45 AM–9:45 AM *(07:45–09:45 h)*
   [2] 9:45 AM–11:00 AM *(09:45–11:00 h)*
   [1] 11:00 AM–12 noon *(11:00–12:00 h)*

2. *Approximately* what time would you go to bed if you were entirely free to plan your evening?

   [5] 8:00 PM–9:00 PM *(20:00–21:00 h)*
   [4] 9:00 PM–10:15 PM *(21:00–22:15 h)*
   [3] 10:15 PM–12:30 AM *(22:15–00:30 h)*
   [2] 12:30 AM–1:45 AM *(00:30–01:45 h)*
   [1] 1:45 AM–3:00 AM *(01:45–03:00 h)*

3. If you usually have to get up at a specific time in the morning, how much do you depend on an alarm clock?

   [4] Not at all
   [3] Slightly
   [2] Somewhat
   [1] Very much

---

[1]Some stem questions and item choices have been rephrased from the original instrument (Horne and Östberg, 1976) to conform with spoken American English. Discrete item choices have been substituted for continuous graphic scales. Prepared by Terman M, Rifkin JB, Jacobs J, White TM (2001), New York State Psychiatric Institute, 1051 Riverside Drive, Unit 50, New York, NY, 10032. January 2008 version. Supported by National Institute of Health Grant MH42931. *See also:* automated English version (AutoMEQ) at www.cet.org.

Horne JA and Östberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. International Journal of Chronobiology, 1976: 4, 97-100.

MORNINGNESS-EVENINGNESS QUESTIONNAIRE
Page 2

4. How easy do you find it to get up in the morning (when you are not awakened unexpectedly)?

   [1] Very difficult
   [2] Somewhat difficult
   [3] Fairly easy
   [4] Very easy

5. How alert do you feel during the first half hour after you wake up in the morning?

   [1] Not at all alert
   [2] Slightly alert
   [3] Fairly alert
   [4] Very alert

6. How hungry do you feel during the first half hour after you wake up?

   [1] Not at all hungry
   [2] Slightly hungry
   [3] Fairly hungry
   [4] Very hungry

7. During the first half hour after you wake up in the morning, how do you feel?

   [1] Very tired
   [2] Fairly tired
   [3] Fairly refreshed
   [4] Very refreshed

8. If you had no commitments the next day, what time would you go to bed compared to your usual bedtime?

   [4] Seldom or never later
   [3] Less that 1 hour later
   [2] 1-2 hours later
   [1] More than 2 hours later

MORNINGNESS-EVENINGNESS QUESTIONNAIRE
Page 3

9. You have decided to do physical exercise. A friend suggests that you do this for one hour twice a week, and the best time for him is between 7-8 AM *(07-08 h)*. Bearing in mind nothing but your own internal "clock," how do you think you would perform?

[4] Would be in good form
[3] Would be in reasonable form
[2] Would find it difficult
[1] Would find it very difficult

10. At *approximately* what time in the evening do you feel tired, and, as a result, in need of sleep?

[5] 8:00 PM–9:00 PM *(20:00–21:00 h)*
[4] 9:00 PM–10:15 PM *(21:00–22:15 h)*
[3] 10:15 PM–12:45 AM *(22:15–00:45 h)*
[2] 12:45 AM–2:00 AM *(00:45–02:00 h)*
[1] 2:00 AM–3:00 AM *(02:00–03:00 h)*

11. You want to be at your peak performance for a test that you know is going to be mentally exhausting and will last two hours. You are entirely free to plan your day. Considering only your "internal clock," which one of the four testing times would you choose?

[6] 8 AM–10 AM *(08–10 h)*
[4] 11 AM–1 PM *(11–13 h)*
[2] 3 PM–5 PM *(15–17 h)*
[0] 7 PM–9 PM *(19–21 h)*

12. If you got into bed at 11 PM *(23 h)*, how tired would you be?

[0] Not at all tired
[2] A little tired
[3] Fairly tired
[5] Very tired

MORNINGNESS-EVENINGNESS QUESTIONNAIRE
Page 4

13. For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Which one of the following are you most likely to do?

[4] Will wake up at usual time, but will not fall back asleep
[3] Will wake up at usual time and will doze thereafter
[2] Will wake up at usual time, but will fall asleep again
[1] Will not wake up until later than usual

14. One night you have to remain awake between 4-6 AM *(04-06 h)* in order to carry out a night watch. You have no time commitments the next day. Which one of the alternatives would suit you best?

[1] Would not go to bed until the watch is over
[2] Would take a nap before and sleep after
[3] Would take a good sleep before and nap after
[4] Would sleep only before the watch

15. You have two hours of hard physical work. You are entirely free to plan your day. Considering only your internal "clock," which of the following times would you choose?

[4] 8 AM–10 AM *(08–10 h)*
[3] 11 AM–1 PM *(11–13 h)*
[2] 3 PM–5 PM *(15–17 h)*
[1] 7 PM–9 PM *(19–21 h)*

16. You have decided to do physical exercise. A friend suggests that you do this for one hour twice a week. The best time for her is between 10-11 PM *(22-23 h)*. Bearing in mind only your internal "clock," how well do you think you would perform?

[1] Would be in good form
[2] Would be in reasonable form
[3] Would find it difficult
[4] Would find it very difficult

MORNINGNESS-EVENINGNESS QUESTIONNAIRE
Page 5

17. Suppose you can choose your own work hours. Assume that you work a five-hour day (including breaks), your job is interesting, and you are paid based on your performance. At *approximately* what time would you choose to begin?

[5] 5 hours starting between 4–8 AM *(04–08 h)*
[4] 5 hours starting between 8–9 AM *(08–09 h)*
[3] 5 hours starting between 9 AM–2 PM *(09–14 h)*
[2] 5 hours starting between 2–5 PM *(14–17 h)*
[1] 5 hours starting between 5 PM–4 AM *(17–04 h)*

18. At *approximately* what time of day do you usually feel your best?

[5] 5–8 AM *(05–08 h)*
[4] 8–10 AM *(08–10 h)*
[3] 10 AM–5 PM *(10–17 h)*
[2] 5–10 PM *(17–22 h)*
[1] 10 PM–5 AM *(22–05 h)*

19. One hears about "morning types" and "evening types." Which one of these types do you consider yourself to be?

[6] Definitely a morning type
[4] Rather more a morning type than an evening type
[2] Rather more an evening type than a morning type
[1] Definitely an evening type

_____ **Total points for all 19 questions**

MORNINGNESS-EVENINGNESS QUESTIONNAIRE
Page 6

INTERPRETING AND USING YOUR MORNINGNESS-EVENINGNESS SCORE

This questionnaire has 19 questions, each with a number of points. First, add up the points you circled and enter your total morningness-eveningness score here:

Scores can range from 16-86. Scores of 41 and below indicate "evening types." Scores of 59 and above indicate "morning types." Scores between 42-58 indicate "intermediate types."

16-30	31-41	42-58	59-69	70-86
definite evening	moderate evening	intermediate	moderate morning	definite morning

Occasionally a person has trouble with the questionnaire. For example, some of the questions are difficult to answer if you have been on a shift work schedule, if you don't work, or if your bedtime is unusually late. Your answers may be influenced by an illness or medications you may be taking. *If you are not confident about your answers, you should also not be confident about the advice that follows.*

One way to check this is to ask whether your morningness-eveningness score approximately matches the sleep onset and wake-up times listed below:

Score	16-30	31-41	42-58	59-69	70-86
Sleep onset	2:00-3:00 AM *(02:00-03:00 h)*	12:45-2:00 AM *(00:45-02:00 h)*	10:45 PM-12:45 AM *(22:45-00:45 h)*	9:30-10:45 PM *(21:30-22:45 h)*	9:00-9:30 PM *(21:00-21:30 h)*
Wake-up	10:00-11:30 AM *(10:00-11:30 h)*	8:30-10:00 AM *(08:30-10:00 h)*	6:30-8:30 AM *(06:30-08:30 h)*	5:00-6:30 AM *(05:00-06:30 h)*	4:00-5:00 AM *(04:00-05:00 h)*

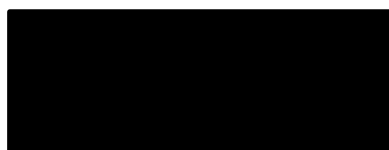
If your usual sleep onset is earlier than 9:00 PM *(21:00 h)* or later than 3:00 AM *(03:00 h)*, or your wake-up is earlier than 4:00 AM *(04:00 h)* or later than 11:30 AM *(11:30 h)*, you should seek the advice of a light therapy clinician in order to proceed effectively with treatment.

We use the morningness-eveningness score to improve the antidepressant effect of light therapy. Although most people experience good antidepressant response to light therapy when they take a regular morning session using a 10,000 lux white light device *(see www.cet.org for recommendations)* for 30 minutes, often this will not give the best possible response. If your internal clock is shifted relative to external time (as indirectly measured by your morningness-eveningness score), the timing of light therapy needs to be adjusted.

The table at the top of the next page shows the recommended start time for light therapy for a wide range of morningness-eveningness scores. If your score falls beyond this range (either very low or very high), you should seek the advice of a light therapy clinician in order to proceed effectively with treatment.
.

Appendix G: Demographics Questionnaire

*Please note that this questionnaire was available online for participants to complete. Included in this thesis is a paper copy for perusal.



Comparison of the response to shift work in pre- and postmenopausal nurses

Name: ____

Age: ____________

Height: __________ Weight: __________

Occupational Position (i.e. RN, NP, LNP): ______________________________________

____

Ward(s): ____

____

<u>Night Shift Work Exposure</u>:

Average number of night shifts worked per week: ________________________________

Average number of night shifts worked per month: _______________________________

Number of night shifts worked in the last week: _________________________________

Number of night shifts worked in the last month: ________________________________

How many total years have you worked shift work for (cumulative exposure – nursing and other work):

____

Version 1.0 Feb. 24, 2020

If you have worked night shift work somewhere else, please state where, for how long, and what the job entailed:

____

____

____

____

____

Lifestyle Factors:

Do you currently smoke cigarettes? ____ YES ____ NO

If yes, how many cigarettes per week? _____

Do you consume alcohol? ____ YES ____ NO

If yes, how many drinks per week? _____

Have you recently travelled across time zones? ____ YES ____ NO

If yes, how long ago and to where? ____

____

Have your menstrual periods ceased permanently? ____ YES ____ NO

If no, what was the date of your last period? ________________________________

Have you been pregnant in the past? _____ YES ____ NO

If yes, how many pregnancies have you had? ________________________________

Are you currently pregnant? ____

Are you currently diagnosed with a cardiovascular, neuroendocrine, or autonomic disease/disorder?

____ YES ____ NO

Do you have a sleep disorder (other than shift work sleep disorder)? ____ YES ____ NO

Are you currently taking medication to assist with sleep? _____ YES _____ NO

Appendix H: Expanded Nursing Stress Scale

*Please note that this questionnaire was available online for participants to complete. Included below is a paper copy in a similar format; questions are answered in a Likert Scale format.

	Below is a list of situations that commonly occur in a work setting. For each situation you have encountered in your **PRESENT WORK SETTING** indicate **HOW STRESSFUL** it has been for you:	Never Stressful	Occasionally	Frequently stress	Always Stressful	Does not apply
1	Performing procedures that patients experience as painful	1	2	3	4	5
2	Criticism by a physician	1	2	3	4	5
3	Feeling inadequately prepared to help with the emotional needs of a patient's family	1	2	3	4	5
4	Lack of opportunity to talk openly with other personnel about problems in the work setting	1	2	3	4	5
5	Conflict with a supervisor	1	2	3	4	5
6	Inadequate information from a physician regarding the medical condition of a patient	1	2	3	4	5
7	Patients making unreasonable demands	1	2	3	4	5
8	Being sexually harassed	1	2	3	4	5
9	Feeling helpless in the case of a patient who fails to improve	1	2	3	4	5
10	Conflict with a physician	1	2	3	4	5
11	Being asked a question by a patient for which I do not have a satisfactory answer	1	2	3	4	5
12	Lack of opportunity to share experiences and feelings with other personnel in the work setting	1	2	3	4	5
13	Unpredictable staffing and scheduling	1	2	3	4	5
14	A physician ordering what appears to be inappropriate treatment for a patient	1	2	3	4	5
15	Patients' families making unreasonable demands	1	2	3	4	5
16	Experiencing discrimination because of race or ethnicity	1	2	3	4	5
17	Listening or talking to a patient about his/her approaching death	1	2	3	4	5
18	Fear of making a mistake in treating a patient	1	2	3	4	5
19	Feeling inadequately prepared to help with the emotional needs of a patient	1	2	3	4	5
20	Lack of an opportunity to express to other personnel on the unit my negative feelings towards patients	1	2	3	4	5
21	Difficulty in working with a particular nurse (or nurses) in my immediate work setting	1	2	3	4	5
22	Difficulty in working with a particular nurse (or nurses) outside my immediate work setting	1	2	3	4	5
23	Not enough time to provide emotional support to the patient	1	2	3	4	5
24	A physician not being present in a medical emergency	1	2	3	4	5
25	Being blamed for anything that goes wrong	1	2	3	4	5

26	Experiencing discrimination on the basis of sex	1	2	3	4	5
27	The death of a patient	1	2	3	4	5
28	Disagreement concerning the treatment of a patient	1	2	3	4	5
29	Feeling inadequately trained for what I have to do	1	2	3	4	5
30	Lack of support of my immediate supervisor	1	2	3	4	5
31	Criticism by a supervisor	1	2	3	4	5
32	Not enough time to complete all of my nursing tasks	1	2	3	4	5
33	Not knowing what a patient or a patient's family ought to be told about the patient's condition and its treatment	1	2	3	4	5
34	Being the one that has to deal with the patients' families	1	2	3	4	5
35	Having to deal with violent patients	1	2	3	4	5
36	Being exposed to health and safety hazards	1	2	3	4	5
37	The death of a patient with whom you developed a close relationship	1	2	3	4	5
38	Making a decision concerning a patient when the physician is unavailable	1	2	3	4	5
39	Being in charge with inadequate experience	1	2	3	4	5
40	Lack of support by nursing administration	1	2	3	4	5
41	Too many non-nursing tasks required, such as clerical work	1	2	3	4	5
42	Not enough staff to adequately cover the unit	1	2	3	4	5
43	Uncertainty regarding the operation and functioning of specialized equipment	1	2	3	4	5
44	Having to deal with abusive patients	1	2	3	4	5
45	Not enough time to respond to the needs of patients' families	1	2	3	4	5
46	Being held accountable for things over which I have no control	1	2	3	4	5
47	Physician(s) not being present when a patient dies	1	2	3	4	5
48	Having to organize doctors' work	1	2	3	4	5
49	Lack of support from other health care administrators	1	2	3	4	5
50	Difficulty in working with nurses of the opposite sex	1	2	3	4	5
51	Demands of patient classification system	1	2	3	4	5
52	Having to deal with abuse from patients' families	1	2	3	4	5
53	Watching a patient suffer	1	2	3	4	5
54	Criticism from nursing administration	1	2	3	4	5
55	Having to work through breaks	1	2	3	4	5
56	Not knowing whether patients' families will report you for inadequate care	1	2	3	4	5
57	Having to make decisions under pressure	1	2	3	4	5

Appendix I: Study Website

In order to maintain social distance and adhere to government and university mandated protocols, this research was conducted remotely. A webpage was subsequently created that contains key information for this study and can be accessed using the URL below:

https://www.uvicnursesstudy.com/

Appendix J: COVID-19 Safety Plan

When at the University of Victoria:

- All researchers must pass a daily self-screening test to ensure health and safety standards are met prior to entering the building.
  - Anyone with symptoms of cold, flu, or COVID-19 will not be permitted to come onto the research site.
  - Researchers will also conduct temperature checks to ensure they are healthy and fit to work.
- Only approved researchers with appropriate biosafety certifications will be allowed to handle equipment and samples.
- Appropriate PPE (gloves, mask, goggles, or face shield) must be worn at all times when dealing with participants' samples.
- Any previously used equipment (i.e., heart rate monitor) will be disinfected and sterilized according to University of Victoria biosafety standards.
- All salivary sampling equipment will be new and in manufacturer packaging. It has been sterilized as per Salimetrics industrial standards. Collection boxes will be wiped down and sanitized prior to distribution to participants.
- Remote communication (e.g. FaceTime, email, phone, text message) will be prioritized for any activities that do not require on-campus equipment or service.
  - This includes researcher meetings, participant recruitment and communication, collaborative writing and editing of documentation.

When Transporting Equipment:

*All interactions between researchers and participants will be contactless

<u>Equipment Drop-Off</u>

- All equipment and instructions for data collection will be provided remotely from the research team.
- Drop-off will be arranged through remote communication between participants and researchers (i.e., email, phone call, text message).
  - A researcher will drop-off the equipment for the participant at a predetermined location and will wait to ensure the participant has successfully received the necessary materials.
  - No physical interaction between researcher and participant will occur.
- Any researcher who is involved with picking up or dropping off equipment will be wearing protective equipment and will have the appropriate biosafety certifications to handle biological samples.
- All researchers will wear gloves when collecting the samples from the participants and will deposit used gloves into biohazardous material bins.
- A vehicle will be used to deliver equipment packages (and later pick them up) from participants.
  - The supplies will be left in a bag, which will then be handled by the participant.

- Participants will have enough supplies to conduct 8 days of data collection remotely. Once the 8 days are completed, participants will notify a member of the research team to arrange for pickup of the packaging.
- All provided equipment will be sterilized and new.
    - Salimterics salivary samples come in individual sealed packets. Cryovials will be cleaned with a disinfectant wipe prior to being put in participants' storage boxes.
    - Garmin Forerunner watch and belt will be sterilized with disinfectant wipe as per standard Biosafety 2 level procedures at the University of Victoria.
    - Paper copies of a sleep log and heart rate log will be provided. These will be used to validate physiological data.
- The same contactless drop off instructions will be followed for both period 1 and period 2 of data collection.

<u>Equipment Pick-Up</u>

- The participant and researchers will also remotely interact to arrange a pick-up location for the samples akin to the drop-off process. Participants will be provided with equipment pick-up instructions on the website.
- Participants will place salivary samples into a bag with the designated biohazard labelling, and the Garmin Forerunner will go into a separate bag. Logs will also go in the bag with the Garmin.
- One researcher will drive the vehicle to the pick-up location, while the other will be responsible for handling the equipment.
- When the vehicle arrives at the designated location, the designated researcher will put on gloves outside of the vehicle to handle the package containing the participants' samples and bring them to the vehicle.
    - The driver will open the door for the samples to be placed into the vehicle.
    - Gloves will be removed outside the vehicle and placed into a biohazardous waste bag to be discarded appropriately at the University of Victoria.
- Once the materials are back at the University of Victoria, both the driver and collecting researcher will put on gloves outside of the vehicle for handling of materials. The collecting researcher will handle the bags with the sampling equipment, while the driver will open doors into the building and laboratory to allow for samples to be stored properly for future analysis. Both researchers will be wearing protective face masks
- The same contactless procedure will be followed for period 1 and period 2 of data collection