MELATONIN PATTERNS AMONG FEMALE HOSPITAL EMPLOYEES ON DAY AND NIGHT SHIFTS: ASSESSMENT BY DIFFERENT EXPOSURE METRICS

by

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Abstract

Objective:
To assess the associations of different shift work exposure metrics with circadian melatonin mesor, amplitude and acrophase.

Methods:
In a cross-sectional study of 328 female hospital personnel working fixed-day or rotation schedules, shift work exposure metrics were determined through self-report. 6-sulfatoxymelatonin concentrations were analyzed by cosinor analysis.

Results:
Shift workers working at night had lower mesors and earlier acrophases than day workers. Shift workers working three or more consecutive nights had lower mesors and smaller amplitudes than those working less than three consecutive nights. After adjusting for confounders, acrophases were no longer different, while smaller amplitudes were apparent among shift workers compared to day workers. Shift length or duration of shift work were not associated with melatonin pattern.

Conclusion:
Rotating patterns of shift work, specifically high intensity rotations, are associated with depressed melatonin rhythms, an indicator of circadian disruption.
Co-Authorship

This thesis presents the work of Michael Leung in collaboration with his supervisors, Kristan Aronson and Joan Tranmer. The cross-sectional study was designed and conducted by Joan E. Tranmer, Kristan Aronson, Ian Janssen, Linda McGillis Hall, Christine Collier and Andrew Day. Michael Leung was responsible for the conceptualization of this thesis project, along with the statistical analyses, interpretation of the results and writing of the manuscript with the supervision of Kristan Aronson and Joan Tranmer, and editorial feedback from Andrew Day and Eleanor Hung.
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<tr>
<td>aMT6s</td>
<td>6-sulfatoxymelatonin</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<td>CIHR</td>
<td>Canadian Institutes of Health Research</td>
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<td>CVD</td>
<td>Cardiovascular Disease</td>
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<td>DAG</td>
<td>Directed Acyclic Graph</td>
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<td>DM</td>
<td>Diabetes Mellitus</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<td>GPAQ</td>
<td>Global Physical Activity Questionnaire</td>
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<td>IARC</td>
<td>International Agency for Research on Cancer</td>
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<td>KGH</td>
<td>Kingston General Hospital</td>
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<td>LAN</td>
<td>Light at night</td>
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<td>MCF-7</td>
<td>Michigan Cancer Foundation-7</td>
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<td>MCTQ</td>
<td>Munich Chronotype Questionnaire</td>
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<tr>
<td>MSF</td>
<td>Mid-sleep time</td>
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<td>MVPA</td>
<td>Moderate-to-vigorous physical activity</td>
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<td>NAT</td>
<td>N-acetyltransferase</td>
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<tr>
<td>NC</td>
<td>North Carolina</td>
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<td>NHS</td>
<td>Nurses’ Health Study</td>
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<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drugs</td>
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<td>ON</td>
<td>Ontario</td>
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<tr>
<td>SCN</td>
<td>Suprachiasmatic nucleus</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<td>WSIB</td>
<td>Worker’s Safety and Insurance Board</td>
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Chapter 1

Introduction

1.1 Background and Rationale

In 2007, the International Agency for Research on Cancer (IARC) classified shift work, with circadian disruption, as a probable carcinogen (Group 2A)\(^1\). Current studies have suggested that shift work, including rotating shift work, is associated with an increased cancer risk at multiple sites, including breast, colon, endometrial and prostate\(^2\)–\(^12\). However, the majority of these studies have focused specifically on breast cancer, with three meta-analyses demonstrating significant increases in breast cancer risk associated with shift work\(^13\)–\(^15\). In addition to cancer, there is evidence to suggest that shift work increases the risk for other chronic diseases, where animal models have demonstrated that chronodisruption is an independent risk factor for weight gain and insulin resistance\(^16\). A few population studies have corroborated this finding, whereby shift work is associated with a cluster of different conditions, including hypertension, high blood sugar level and obesity, which may predispose individuals to heart disease, stroke and diabetes\(^17,18\).

A hypothesized biological mechanism for these relationships involves circadian disruption and the hormone melatonin, where increased light at night (LAN) exposure associated with shift work is thought to suppress melatonin levels. Laboratory studies on rodents have indicated that decreased melatonin levels are linked to increases in both cancer development and progression\(^19\)–\(^23\). In terms of metabolic disorders, melatonin suppression predisposed rats to insulin resistance and obesity\(^24,25\). However, the evidence from epidemiologic studies relating shift work, melatonin suppression and chronic disease has been inconsistent\(^11,26\)–\(^34\). This may be
related to methodological flaws, whereby exposure to shift work was inadequately classified (due to inconsistent definitions) or the relationship was confounded by circadian rhythm – where absolute melatonin measurements based on a single sample was used as the biomarker without taking circadian phase differences into account. Thus, mechanistic data from animal models in addition to circumstantial evidence from epidemiologic studies support a role for LAN and chronodisruption on both cancer risk and metabolic health.

Thus, the aim of this study was to address these methodological gaps, to further clarify the potential mechanistic link between occupational exposure to shift work and chronic diseases. Unlike previous studies, this project addresses the effect of different working time patterns associated with shift work. Without a clear definition of shift work in past studies, exposure to shift work was regarded as a variable that was categorical, meaning that one either worked shifts or did not, without consideration of the impact of rotational shifts, which lies in the spectrum between the two absolute entities. But several studies have used better definitions and also considered duration, where they determined an integrated lifetime exposure to shift work by classifying shift work by type of shift work, schedule of shift work, amount of shift work per year, number of years of shift work\textsuperscript{28,33}. By doing this, workers with different amounts of current and previous shift work were considered when comparing across exposure groups.

In addition to past history of shift work, other working time patterns including shift length, time of day and shift intensity have been recently identified to be different aspects of current shift work\textsuperscript{35}. With most previous studies using questionnaire data which provide only crude information on exposure to working hours\textsuperscript{7,8,12,15,34,36–38}, this project will also explore the effect of shift length, time of day and shift intensity within the context of current shift work in
order to better assess a shift worker’s working time pattern in relation to circadian melatonin patterns.

Moreover, to address potential confounding by circadian rhythm and phase shifting, there is a need for multiple biosamples over 24 hours, where cosinor analyses can be conducted to identify individual melatonin secretion patterns so that inter-individual variations will be captured. With environmental-occupational exposures being potentially modifiable, a better understanding of the effect of shift work on risk may help accelerate the understanding of and action around chronic disease prevention.

1.2 Overview of Study Design

To evaluate the associations of current shift work status and past shiftwork history with circadian melatonin patterns, a cross-sectional observational study, with a retrospective assessment of exposure to shift work rotations, was conducted. Data collection for each subject occurred over an eight-day period, and for rotating night shift workers included at least two night shifts. On day 1, the research coordinator conducted a physical examination and administered and/or distributed study questionnaires (Appendix B). Study participants completed the study questionnaire at the start of the study. The questionnaire collected personal information such as education, household income, number of children, health history, as well as work characteristics, including occupational position, status and length of employment. During the 8 day cycle there was a designated 2 day specimen collection period in which participants provided urine samples for each void. All urine samples were collected, aliquoted and stored in a -70 degree freezer. Urine samples were then analyzed to assess levels of the melatonin biomarker.
1.3 Objectives

The primary objectives of this thesis were to describe the circadian melatonin pattern according to current shift work status (day only, or rotating day and night) of female hospital personnel in terms of mesor (average concentration over a 24-hour period), amplitude (difference between peak and mesor) and acrophase (time of peak concentration) and to examine the associations of current shift work status and past shift work history with circadian melatonin patterns. Additional objectives are to explore the associations of other working time patterns within the context of current shift work – length of working hours, time of day and shift intensity – with circadian melatonin patterns. Several sensitivity analyses were also conducted to assess the effects of certain methodological decisions.

1.4 Thesis Organization

This is a manuscript based thesis that conforms to the regulations outlined by the Queen’s University School of Graduate Studies and Research. The second chapter of this thesis consists of a literature review describing the links between shift work and chronic diseases, as well as the relationship between light at night and melatonin levels. The third chapter provides detailed descriptions of the data collection methods and analysis strategies used in this thesis. The fourth chapter is a draft of a manuscript that addresses the relationship between shift work and urinary melatonin levels. The fifth chapter describes the additional results of further data analysis that was included in the thesis objectives but not in the manuscript; specifically, sensitivity analyses exploring possible bias from strong predictors of melatonin levels, and cosinor parameters generated from a linear mixed model. The final chapter of the thesis consists of general
discussion of findings in the fourth and fifth chapters, as well as overall conclusions and future research directions. Extra material may be found in appendices at the end.

1.5 Contribution

By collecting repeated measurements of melatonin levels from each study participant over 48 hours, this thesis will examine circadian melatonin patterns instead of comparing melatonin levels based on single urine samples at different functional times, which has been the convention in previous studies. Furthermore, given that there are few studies that actually study the impact of an integrated lifetime exposure to shift work, as well as other working time patterns such as shift length, time of day and shift intensity on circadian melatonin patterns, the findings from this study will help identify and clarify our understanding of this relationship through objectively assessing melatonin profiles by cosinor analysis. At the public health level, such an understanding will lead to the development of healthier workplace policy and disease prevention measures – as there may be a better way to structure and manage working conditions to minimize the risk of cancer and other chronic diseases.
1.6 References


Chapter 2

Literature Review

2.1 Shift Work

In 2007, long-term shift work involving circadian disruption was classified as a probable (Group 2A) carcinogen by the International Agency for Research on Cancer (IARC) on the basis of sufficient evidence in experimental models and limited evidence in humans\(^1\). Epidemiologic studies have suggested that working night shifts and rotating night shifts is associated with an increased cancer risk at multiple sites including breast, colon and prostate\(^2\)–\(^14\). In addition to cancer, there is also evidence to suggest that shift work increases the risk for a cluster of different conditions, including hypertension, high blood sugar and obesity\(^15\)–\(^17\), which may predispose individuals to heart disease, stroke and diabetes\(^18\)–\(^24\). With approximately one third of the Canadian labour force engaged in this work pattern\(^25\), the IARC classification of this exposure as a probable carcinogen is of paramount importance.

A limitation of existing studies is in the definition of ‘shift work,’ which generally refers to work schedules that differ from conventional daytime schedule of activity during the day and sleep at night – these include both fixed night shifts and rotational shift work. Since the definition is so broad and unrestrained with regards to specific work characteristics, its assessment in existing studies have been both imprecise and incomplete\(^26\). Most studies have used either current status or duration as exposures to shift work without considering other occupational domains relevant to the exposure, including shift length, time of day, shift intensity and social aspects of working hours\(^27\). For example, the exposure of ‘ever worked in night shifts,’ without controlling for shift length, intensity or duration may be erroneous as these aspects of shift work may actually be separate risk factors for disease. Thus, there is a gap in the
literature with regards to other occupational domains, where working time patterns such as irregularity of shifts, permanent/rotating shifts, shift length, shift intensity and speed of rotation are examples of further domains that need to be captured in order to improve the validity of future studies on shift work\textsuperscript{26–28}.

2.2 Shift Work and Cancer

2.2.1 Laboratory Studies

There have been several studies with laboratory animals examining the relationship between chronic alterations of light-dark environments on tumor progression and development. Studies that compare the impact of high intensity to low intensity light on tumor development showed not only an increase in the incidence of lung carcinomas, leukemia and lymphomas combined, but also an increase in the incidence and mortality from mammary tumors\textsuperscript{29–31}. Other studies have demonstrated that the major patterns of light-dark environments that influence cancer development and/or growth are circadian timing of constant light, dim light at night and simulated jetlag\textsuperscript{32–41}.

Mechanistically-oriented animal studies specifically aimed at investigating suppressed melatonin as a proxy for circadian disruption have almost consistently found there was an increase in tumor incidence\textsuperscript{42–46}. Furthermore, in the reverse experiment, direct inhibitory effects of melatonin on tumor cell proliferation have been shown in several animal models at both the pharmacological and physiological concentrations\textsuperscript{47,48}. However, one of the main problems with studying rodents is that they are mostly nocturnal, meaning that they have a photoperiodic response that differs from humans.
2.2.2 Breast Cancer

Previous studies examining the association between shift work and cancer have mainly focused on breast cancer, where results from a meta-analysis conducted in 2005 found evidence for a 48% increased breast cancer risk in shift workers\(^2\). The magnitude of this reported summary relative risk is further supported by a review published in 2008\(^3\). One major problem with evidence presented in these reviews is that past studies are not directly comparable due to the variety of methods used to assess shift work exposure\(^26\). Studies using job-exposure matrix-based methods or questionnaires may be prone to misclassification, which may reduce their ability to detect any significant associations. The evidence from these studies has generally been inconsistent, with some studies reporting a modest significant increase in risk of some cancers, while others have not. In addition to using definitions of the exposure that are not consistent, most of these studies are methodologically flawed in that the different aspects of shift work were not even considered. Although duration of shift work was often examined, other work characteristics such as shift length, intensity and social aspects of working hours were not considered.

More recent studies that have focused on duration of shift work, by using lifetime occupational history as an exposure, are generally of higher quality. By combining both long-term length and intensity of shift work, both rotating and permanent night shift patterns can be captured within a single definition of cumulative shift work. A systematic review summarizing the evidence regarding cumulative shift work has shown that there is a positive-dose response relationship between years of night shift work and breast cancer\(^5\). For every 5-year increase of exposure to night shift work would correspondingly enhance the risk of breast cancer by 3%, while an increase in 500-night shifts would result in a 13% increase in breast cancer risk. This
reported summary relative risk is further supported by a Canadian study, where a significant
association was reported for 30 or more years of shift work and breast cancer risk\textsuperscript{4}.

Only one study to date has attempted to capture the degree of shift intensity. They
reported that increased breast cancer risk was seen in nurses who worked longer than 5 years
with at least 6 consecutive night shifts in their schedule. However, there was no association when
4 consecutive night shifts was used instead of 6 consecutive night shifts, suggesting that the risk
may be related to number of consecutive night shifts\textsuperscript{49}.

Although there are a number of studies, some more methodologically sound than others,
with positive associations, there is still a gap in the literature with regard to specifics of exposure
assessment. In order to fill this gap, future studies need to collect information on other work
characteristics, such as shift length, intensity and social aspects, in order to better evaluate the
specifics of exposure to shift work\textsuperscript{26–28}.

\subsection*{2.2.3 Other Cancers}

Based on seven epidemiological studies, the evidence for an association between shift
work and prostate cancer is both limited and inconsistent. One prospective cohort study of
Japanese men reported that shift workers were three times more likely to develop prostate cancer
than day workers\textsuperscript{8}. Consistent with these findings, two Canadian case-control studies found that
ever having worked night shifts was associated with increased prostate cancer risk\textsuperscript{6,10}. A study in
Spain that looked at duration of shift work found that those exposed to shift work for more than
28 years or more had a higher prostate cancer risk than “never” night workers\textsuperscript{11}. In contrast, three
other studies looking at the association between shift work and prostate cancer risk did not find
an association\textsuperscript{7,9,12}.
Much like prostate cancer, evidence for a positive association between shift work and colorectal cancer is discordant. A Norwegian nested case-control study with female radio and telegraph operators found no association between shift work and colorectal cancer\textsuperscript{13}. This finding was further corroborated by a Swedish cohort study, where no association was found\textsuperscript{12}. However, findings from the Nurses’ Health Study (NHS) cohort reported that there was a significant increase in colorectal cancer risk among nurses who had worked 15 or more years rotating shifts with at least three working nights per month\textsuperscript{14}.

There are very few studies that have explored the relationship between shift work and risk of cancer at other sites other than those discussed above. One Finnish population-based cohort study investigated men and women who developed non-Hodgkin lymphoma in a nested case-control study; and they found that shift work was associated with a 10\% increased risk in men, but not women\textsuperscript{50}. One prospective cohort study of women in the United States reported that shift work was associated with a 47\% increase in risk of endometrial cancer for women who worked 20 or more years of rotating shifts\textsuperscript{51}. Another study found an association between shift work and lung cancer among current smokers, but not seen among non-smokers\textsuperscript{52}.

Studies examining the relationship between shift work and overall cancer risk, which includes both mortality and incidence, have not provided convincing evidence for a strong positive association. One study showed that the standardized incidence ratio for cancer at all sites was around unity in women and borderline significant in men for shift workers in comparison to day workers\textsuperscript{12}. However, this study did not assess shift work exposure individually, but rather used a registry of job titles to classify shift work status. Potential misclassification in such an approach may reduce the ability to detect a true association.
Overall, although animal models are convincing, the evidence from epidemiologic studies is only suggestive and not conclusive for a significant association between cancer and shift work, which includes both night and rotating shift work. This state of evidence is reflected in the IARC pronouncement of long-term shift work as a “probable” human carcinogen. Exposure assessment in the existing literature is inconsistent and has several gaps, where current exposure to shift work and duration of shift work are the main characteristics that have been examined. There are, in fact, four specific working time pattern domains – shift length, shift intensity, time of day and social aspects of shift work - that need to be considered in order to precisely define the multifaceted exposure of shift work.

2.3 Shift Work and Metabolic Health

2.3.1 Laboratory Studies

Several studies on laboratory animals have examined the relationship between LAN and metabolic health. One study on mice showed that LAN significantly increased body mass and reduced glucose tolerance compared with mice exposed to natural light-dark cycles\textsuperscript{53}. The same investigators found that LAN changes the timing of food consumption, where those exposed to LAN consume 55% of their food during the light phase, compared with 36% in control mice. Altered timing of food consumption has been associated with metabolic syndrome in other laboratory studies\textsuperscript{54,55}. Thus, these animal models provide evidence that mild changes in environmental lighting can alter both circadian and metabolic functions.
2.3.2 Metabolic Risk Profile

Cross-sectional studies examining characteristics of shift workers have suggested that working night shifts is associated with a cluster of conditions including hypertension, high blood sugar level, and obesity, and these predispose individuals to heart disease, stroke and diabetes. One study of over 40,000 women found that shift work was associated with a higher odds of smoking, obesity, and being in the lowest tertile of socioeconomic status – all potential risk factors related to cardio-metabolic risk\textsuperscript{15}. Furthermore, the only meta-analysis conducted to date on shift work and metabolic syndrome has shown that night shift work was associated with a 57% increased risk of metabolic syndrome\textsuperscript{16}. Risk increased to 103% when only considering cohort and nested-case control studies, and decreased to 39% when only considering case-control studies. This difference in risk ratios may be explained by the potential healthy worker effect, where comparisons of mainly healthy cases to the controls selected from the general population may bias the association towards the null. In terms of duration of shift work, the meta-analysis showed that a higher combined relative risk was associated with longer years of night shift work, where a threshold of 10 years of cumulative shift work was used\textsuperscript{16}. Consistent with these findings, a subsequent Brazilian cross-sectional study evaluating the association between lifetime exposure to shift work and blood pressure, fasting glucose, anthropometric variables, body composition and heart rate variability showed that shift work may promote unfavorable changes to cardiovascular health\textsuperscript{17}.

Although epidemiologic studies generally suggest that shift work is associated with a more adverse cardiovascular risk profile, interpretation of existing evidence is difficult due to a number of methodological flaws. There are discrepancies in the exposure assessment due to inconsistent definitions, and inadequate adjustment for other characteristics of shift work. Some
studies required night shift workers to work on night shifts for more than one night per week, where others required two or three rotating shifts, and a few did not have any requirements with regards to the pattern of shift work. Very few studies attempted to summarize evidence according to different working time patterns of shift work, where current exposure and cumulative years were the main aspects being examined. Furthermore, these studies were also inconsistent in their confounder adjustments, where some adjusted at different threshold values when confounding variables were categorized, while others did not adjust adequately.

2.3.3 Diabetes

A few epidemiological studies have assessed the association between shift work and risk of diabetes mellitus (DM), but the results are conflicting. A Japanese cross-sectional study of male factory workers conducted in 1983 reported a higher prevalence of diabetes among shift workers than day workers\textsuperscript{18}. Two more recent studies reported an increased risk of diabetes among rotating shift workers, but the results were not statistically significant\textsuperscript{19,20}. A recent meta-analysis of twelve studies showed that shift work is associated with an increased risk for diabetes\textsuperscript{21}. In the sensitivity analysis, the increase in risk was reported to be higher in men and the rotating shift group, whereas in women, the increase in risk was of only borderline significance.

2.3.4 Cardiovascular Disease

The epidemiological evidence regarding a causal link between shift work and cardiovascular disease is inconsistent, but overall is suggestive of an adverse association. A
review of 17 studies reported a 40% increase in cardiovascular disease risk when combining the more methodologically sound studies. In contrast, another review of 14 studies found that most estimates were around unity and concluded that was no convincing evidence for an association between shift work and cardiovascular disease. A more recent cross-sectional study has shown that light at night is associated with subclinical atherosclerosis in the general elderly population. Thus, a more cautious and detailed interpretation of the published data is warranted, especially since most of the studies reviewed include male workers and suffered from methodological flaws, including selection bias, quality of outcome and exposure measurements, as well as inadequate or ambiguous adjustment for confounders.

2.4 Melatonin and Chronic Diseases

A hypothesized biological mechanism for the relationship between shift work and chronic disease involves the hormone melatonin, where increased light at night associated with shift work is thought to suppress melatonin levels, which are then associated with increased cancer risk and poor metabolic health. Melatonin is a hormone produced in the pineal gland, where peak levels are produced at night in the absence of light. There is both animal and epidemiological evidence to support that melatonin levels have an impact on cancer development and/or progression, as well as weakening the cardiovascular protective response.

2.4.1 Melatonin and Circadian Rhythm

Understanding the mechanism for circadian time keeping is instrumental to understanding the harmful effects of shift work, which include night shifts and rotating night
shifts. The body’s circadian rhythm is controlled by cellular oscillators, via various clock genes\textsuperscript{63–65} (Figure 2.1).

![Diagram of circadian system hierarchy]

**Figure 2.1: Hierarchy of circadian system**

Within this hierarchical structure, the master oscillator is externally synchronized by zeitgebers – essentially, time cues provided by the natural light and dark cycle\textsuperscript{66}. Such external cues provide circadian time information, where the body uses a transcriptional-translational feedback loop to regulate the expression of clock genes, such as *CLOCK* and *Bmal*\textsuperscript{64,65}. Thus, in order to maintain internal biological order and efficiency, its circadian rhythms must be maintained within a certain phase with regard to one another in order to coordinate biological processes with the predictable 24 hour cycle of day and night\textsuperscript{67}.

In the pineal gland, melatonin is produced through a series of enzymatic reactions from the amino acid tryptophan\textsuperscript{68,69} (Figure 2.2). Specialized retinal photoreceptors transmit light information to the suprachiasmatic nucleus (SCN), which then regulates the production of melatonin\textsuperscript{56}. In the absence of light, the SCN activates the enzyme arylamine N-acetyltransferase (NAT), which catalyzes the conversion of serotonin to N-acetylserotonin, the rate-limiting step in this cascade\textsuperscript{68}. Therefore, since light inhibits melatonin production, peak levels are typically seen between 2:00 am and 4:00 am\textsuperscript{56}. 

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Zeitgebers are necessary to keep the period of the circadian oscillator system to 24 hours, because the genetically determined period in most individuals is either slightly longer or shorter\textsuperscript{70}. Individuals, who are normally synchronized to day time activity and night time sleep, must undergo phase adjustments in order to accommodate night shift work and rotational shift work. Such a phenomenon must occur in both central and peripheral oscillators; however; it has been observed that during this adjustment, some clock genes respond much faster than others, resulting in phase desynchronization within and between the oscillators\textsuperscript{71}. The system requires several 24-hour cycles to adjust, with the duration of adjustment being variable between different individuals. Therefore, phase desynchronization of shift workers then presents a major functional disturbance of the circadian organization.

Figure 2.2: Melatonin production from Tryptophan
2.4.3 Cancer

2.4.3.1 Mechanism

Experimental studies have demonstrated that melatonin is able to reduce both the incidence and progression of tumors. Studies using MCF-7 breast cancer cell lines have reported decreased cell proliferation and invasiveness with melatonin administration. Animal models have also found a decrease in both tumor incidence and growth after administering melatonin. Furthermore, in the reverse experiment, pinealectomy in rats has been shown to increase the incidence of mammary carcinomas. Consistent with these findings, melatonin-depleted blood from premenopausal women exposed to light at night stimulates the proliferation of human breast cancer xenografts in rats. Therefore, the accumulation of experimental evidence provides support for a protective role of melatonin on both tumor development and proliferation.

Mechanistically-oriented studies have suggested that melatonin inhibits cancer development through several different mechanisms. Melatonin is able to protect against DNA damage through its potent antioxidant effect, whereby it acts as a scavenger of free radicals. Melatonin also has anti-proliferative effects on cancer cells, as well as the ability to induce DNA repair. The hormone has also been shown to have immunomodulatory activity, where it can help promote immune functions protective against cancer development.

Furthermore, melatonin has been shown to influence the levels of several reproductive hormones, including estrogen. Melatonin production down-regulates the gonadal axis, by interfering with aromatases that converts androgens to estrogens, decreasing the circulating levels of estrogen. This is important in the etiology of breast cancer, and other hormone-
dependent cancers, where increased lifetime exposure to estrogen has been established as a risk factor.\textsuperscript{68,78}

Although there is a growing body of evidence that supports the association between melatonin suppression and cancer risk, there are other potential mechanisms that may be operating between shift work and cancer. These include phase shifting of circadian rhythm (which can be indicated by earlier or later peak melatonin), sleep disruption or poor sleep quality, lifestyle factors (such as diet, physical activity and BMI) and lower vitamin D.\textsuperscript{80} Different occupational domains of shift work may be related to the different sets of possible mechanisms, and thus it is important to be precise in exposure assessment in order to elucidate which aspects serve as risk factors for chronic diseases including cancer.

\textit{2.4.3.2 Epidemiologic Evidence}

There have only been few epidemiologic studies surrounding the relationship between melatonin levels and cancer development in human populations. Several studies have found that there is a decrease in both urinary and plasma melatonin levels in both breast and colon cancer cases compared to healthy controls.\textsuperscript{57–59} However, the lack of temporality due to the retrospective assessment of melatonin concentrations made it impossible to determine whether decreased melatonin production occurred prior to or as a consequence of cancer development. While prospective studies have been conducted, the evidence has been inconsistent. A nested case-control study within the NHS II found an inverse association between urinary melatonin levels measured from the first morning void and breast cancer development.\textsuperscript{60} In contrast, in another prospective cohort study, no association was found between urinary melatonin levels and incidence of breast cancer.\textsuperscript{61} However, it has been suggested that circadian amplitude of
melatonin production, which is not captured in these previous studies, may be an important factor for cancer development\textsuperscript{61}.

### 2.4.4 Metabolic Disorder

#### 2.4.4.1 Mechanism

Pinealectomized rodents showed glucose insensitivity which supports an effect of melatonin production on glucose homeostasis and metabolic health\textsuperscript{62,81,82}. Mice with a lesion of circadian clock gene \textit{Bmal1}, had disrupted locomotor rhythmicity and predisposed these rodents to insulin resistance and obesity, an effect that was reversed by transgenic \textit{Bmal1}\textsuperscript{83}. More recent studies have supported this phenomenon, where decreased melatonin was associated with the abnormalities of metabolic syndrome components\textsuperscript{84}, whereas artificial supply of melatonin can prevent the development of metabolic syndrome in rats\textsuperscript{85,86}. These findings provide strong evidence for the involvement of melatonin and circadian clock genes in the metabolic effects of chronodisruption.

#### 2.4.4.2 Epidemiologic Evidence

Only one study has been published on this issue, and it showed that reduced melatonin secretion was associated with increased risk of developing diabetes through disrupted glucose homeostasis in human patients\textsuperscript{62}. Thus, the mechanistic data from animal models, in addition to the limited number of epidemiologic studies is only suggestive of an association between disrupted rhythmicity on metabolic health.
2.5 Light at Night and Melatonin

2.5.1 Biomarkers of Melatonin

In both experimental and epidemiologic studies, there are three melatonin biomarkers that are commonly used to measure melatonin levels. Melatonin levels can be assessed from both blood and saliva samples, while levels of 6-sulfatoxymelatonin, the primary melatonin metabolite, are easily measured in the urine\textsuperscript{87}. Although assessment of plasma or serum melatonin levels is considered the most accurate method, its invasiveness is a barrier to capturing natural variations in hormone levels as multiple samples are required to characterize these rhythms. Therefore, less invasive techniques of measuring melatonin are preferred for observational research\textsuperscript{87,88}.

Measurements of melatonin from saliva and urine have been assessed in terms of their ability to accurately capture melatonin levels. Salivary melatonin assessment is generally thought to reflect the free plasma melatonin fraction, as plasma proteins are unable to pass through salivary glands into the saliva\textsuperscript{89,90}. Therefore, it would be considered unsuitable for the assessment of absolute melatonin levels.

On the other hand, urinary concentrations of 6-sulfatoxymelatonin in first morning void of urine have been shown to represent approximately 70\% of total nocturnal melatonin production\textsuperscript{91}. Several experimental studies have shown a strong correlation between urinary 6-sulfatoxymelatonin and plasma melatonin levels, with urinary melatonin levels considered a good estimate of absolute melatonin levels when corrected using creatinine levels as conversion factors\textsuperscript{87,89,91}.
2.5.2 Epidemiologic Evidence

Since melatonin production is sensitive to light, the synchronizing effects of light are commonly defined in terms of nighttime melatonin suppression. In animal models, it has been shown that shorter wavelengths and higher intensities suppresses melatonin production to a higher degree, where intensities as low as 90-180 lux are enough to suppress melatonin production\textsuperscript{92}.

As previously described, epidemiologic studies have suggested that shift work may be associated with an increased risk of breast cancer and metabolic disorder due to increased light at night. Although melatonin has been established as a hormone with protective properties in chronic diseases, its suppression in biomarker studies examining association between shift work and chronic disease has not been consistently observed\textsuperscript{93–95}. Studies using LAN exposure or shift work as a proxy show that the general pattern observed is decreased melatonin production with increased exposure to LAN\textsuperscript{96,97}; however, one major problem with these studies is that biomarker measurements may be confounded by circadian rhythm\textsuperscript{93}. By comparing functional instead of chronological time, investigators make the assumption that circadian melatonin secretion patterns have undergone a phase shift, where production occurring during the day while sleeping. If this assumption is not met, then the use of functional times does not account for differences in circadian stage between shift and non-shift workers, which may confound any observed associations between shift work and melatonin levels.

Only few studies have made the correct comparisons by taking circadian stage differences into account\textsuperscript{93,98,99}. A previous Canadian study found no difference in mean levels between day and night shifts for rotating shift workers\textsuperscript{93}. However, this particular study may be limited by inadequate study power. Due to the limited number of biosamples and the possibility
of confounding by circadian rhythm, the investigators were forced to stratify the population by shift group, therefore decreasing both the sample size and the variability in the exposure.

Another study from the USA reported a 62% reduction in mean melatonin levels during day time sleep and a similar reduction of 69% during night shifts\textsuperscript{99}. Finally, a study from Spain not only examined the differences in mean levels, but also other aspects of diurnal melatonin pattern\textsuperscript{98}. They reported that melatonin mesor decreased by 34% among night workers compared to day workers, melatonin amplitude decreased by a similar amount (results were not reported as mesor and amplitude were highly correlated), while a 3-hour delay of acrophase was reported among night workers compared to day workers. Since differences in circadian melatonin pattern were observed in the two studies where all participants were engaged in permanent fixed-night shifts as opposed to a rotating shift schedule, alterations of circadian rhythm and risk for chronic disease may be related to shift intensity and number of consecutive nights.

### 2.6 Potential Confounders

There are multiple factors that must be considered when assessing the relationship of light at night and melatonin levels (Figure 2.3). These factors include age, age of first birth, parity, education and diurnal chronotype.
Figure 2.3: Confounder assessment using a Directed Acyclic Graph (DAG). Confounders are ancestors of both exposure and outcome.

2.6.1 Age

A decline in melatonin levels with increasing age has been consistently observed in the epidemiologic literature\textsuperscript{68,100–102}. Age at which an individual performed shift work may also modulate the effects of shift work on health outcomes. Previous data from the NHS cohorts indicate that longer durations of shift work are associated with a higher cancer and CVD risk\textsuperscript{103}. 
2.6.2 Reproductive Factors

The etiology of breast cancer, as well as the pattern of melatonin secretion, varies between premenopausal and postmenopausal women, however the direction of this latter effect remains unclear\textsuperscript{61,88,104}. Parity has also been associated with melatonin levels: two studies have reported a positive association between parity and melatonin, where parous had higher urinary 6-sulfatoxymelatonin levels compared to nulliparous women\textsuperscript{58,88}. One study also found that age of first birth was significantly associated with melatonin, where women whose age at first birth was less than 25 years of age had lower melatonin levels compared to those who were older than 25 at the time of first birth\textsuperscript{101}.

2.6.3 Education

Highest level of education has also been associated with melatonin levels. One cross-sectional study has demonstrated a negative relationship between educational history and urinary melatonin levels, with lower 6-sulfatoxymelatonin levels significantly associated with lower socioeconomic neighborhoods\textsuperscript{105}.

2.6.4 Diurnal Chronotype

Diurnal chronotype, referring to the internal biological clock, may also confound or modify the relationship between shift work and melatonin levels\textsuperscript{106}. Diurnal chronotypes reflect the time of day functions are active\textsuperscript{107}. Such an attribute has been suggested to affect the extent to which an individual adapts to shift work\textsuperscript{106,108}. Those more active during the night, which coincides with later melatonin peaks, may adapt to shift work by phase delaying their circadian
rhythms, while those who are more active much earlier, may find shift work adaption much more difficult. Epidemiologic evidence is mixed for the association between diurnal preference and melatonin levels in shift workers and non-shift workers. Morning preference has been associated with an earlier melatonin peak time\textsuperscript{109-112}. Evening-types also reported better tolerance for night work compared to morning types\textsuperscript{113}. In contrast, other studies did not find an association between chronotype and peak melatonin times\textsuperscript{98}. To date, very little is known about the relationship between chronotype and chronic disease; however, it has been suggested that it serves as an effect modifier for the relationship between shift work and cancer\textsuperscript{114,115}.

2.7 Strong Predictors of Melatonin Levels

In addition to confounders, strong predictors of the outcome (Figure 2.3), which have not been identified as \textit{a priori} confounders in the literature but may be differentially distributed between the exposure groups, can bias the effect estimates\textsuperscript{116}. These include certain lifestyle factors such as alcohol consumption, BMI, caffeine consumption, physical activity, and smoking status, as well as the use of certain pharmacologic agents. The potential biasing effect of these predictors will be examined in a secondary analysis.

2.7.1 Lifestyle Factors

2.7.1.1 Alcohol

Studies that have investigated the association between alcohol consumption and melatonin have generally reported an inverse relationship. Laboratory studies have demonstrated that alcohol possesses an inhibitory effect on plasma melatonin levels, but did not show any
effect on urinary 6-sulfatoxymelatonin levels\textsuperscript{117,118}. A more recent observational study provided evidence to support this inverse association when alcohol consumption exceeded two drinks per day\textsuperscript{102}.

2.7.1.2 Smoking Status

Multiple studies have also investigated the relationship between smoking and melatonin levels. One study reported that current smokers had significantly lower melatonin levels compared to non-smokers, however past history of smoking did not appear to influence urinary melatonin production\textsuperscript{101}. Furthermore, another study reported that smoking status was also associated with lower plasma melatonin levels, providing further support for an inverse relationship between smoking status and melatonin production\textsuperscript{119}.

2.7.1.3 Body Mass Index

Body mass index (BMI) has also been consistently associated with melatonin levels. Several observation studies have reported an inverse relationship between BMI and urinary melatonin levels \textsuperscript{61,100–102}.

2.7.1.4 Caffeine Consumption

Several studies that have examined the impact of caffeine on melatonin levels have reported inconsistent results. Studies on both sexes have shown that caffeine possesses a negative effect on salivary melatonin levels, but this effect was shown to be weaker among women using oral contraceptives\textsuperscript{120,121}. In contrast, an experimental study found that caffeine consumption was
associated with an increase in melatonin levels\textsuperscript{122}. Although the direction of the effect remains unclear, these studies, combined with the ability of caffeine to influence sleep patterns, suggests that it may influence melatonin levels.

### 2.7.1.5 Physical Activity

Physical activity may play a possible role in altering circadian melatonin secretion patterns. Experimental studies have reported inconsistent results, where exercise has been shown to have positive, negative and null effects on melatonin levels\textsuperscript{123–127}. One review found that exercise produces only acute increases in melatonin levels, with hormone levels returning to baseline within an hour after exercise\textsuperscript{123}. Other studies have reported that melatonin secretion is influenced by the time of day, where exercise during the day had no acute effect on melatonin, but a decrease in hormone levels was observed during evening exercise\textsuperscript{128}. Despite conflicting results, there is a potential for physical activity to influence melatonin levels or affect the relationship between melatonin and other strong predictors.

### 2.7.1.6 Pharmacologic Agents

Pharmacologic agents such as antidepressants, beta-blockers, non-steroidal anti-inflammatory drugs (NSAIDs), hormone replacement therapy and sedatives have all been shown to inhibit melatonin production\textsuperscript{129–133}. On the other hand, melatonin supplements and oral contraceptives have been associated with increased melatonin levels\textsuperscript{134,135}. 
2.8 Summary of Rationale

Shift work is associated to some degree with both increased cancer risk\textsuperscript{2–14,50–52} and poor metabolic health\textsuperscript{15–24}. These associations are likely mediated by changes in melatonin levels as a result of increased light at night exposure through night shift work. Without a clear definition of shift work in some studies, exposure to shift work was often regarded as a categorical variable, meaning that one either worked shifts or did not, without the consideration of the impact of rotational shifts. However, several studies have used better definitions and also considered duration, where they determined an integrated lifetime exposure to shift work by classifying shift work by current and cumulative exposure\textsuperscript{4,17}. By doing this, workers with different amounts of current and previous shift work were considered when comparing across exposure groups.

In addition to duration of shift work, other working time patterns including shift length, time of day and shift intensity have been recently identified to be different aspects of shift work\textsuperscript{27}. While most previous studies using questionnaire data provide only crude information on exposure to working hours, this project will also explore the effect of shift length and shift intensity within the context of current shift work in order to better assess a shift worker’s working time pattern in relation to circadian melatonin rhythms.

Furthermore, while existing epidemiologic research has found associations between melatonin and increased risk of chronic disease, there has been very little methodologically sound research that specifically focuses on the association between shift work and melatonin levels. While existing studies of this specific relationship have demonstrated an inverse association between LAN and melatonin\textsuperscript{96,97}, most have compared functional as opposed to chronological times in day and night workers. By comparing functional instead of chronological time, their comparisons may be erroneous in that associations may be confounded by circadian
rhythm due to melatonin still peaking at night, despite working at night. Only very few studies use the correct comparisons, where they compared melatonin levels by chronological times and stratified the analysis by shift group in order to account for differences in circadian stage. Since differences in daily melatonin were observed in the two studies with more intense shift schedules, alterations of circadian rhythm and risk of chronic disease may be related to the number of consecutive nights.

The possibility of confounding by circadian rhythm only arises because most studies have had a limited number of biosamples due to logistic and cost constraints. To address this, there is a need for multiple biomarker measurements over 24 hours, where a cosinor analysis can be conducted to identify and better characterize individual melatonin secretion patterns for both day and night shifts. With environmental-occupational exposures being potentially modifiable, a better understanding of the effects of shift work on risk will accelerate the understanding of and action around chronic disease prevention.
2.9 References


Chapter 3

Methods

3.1 Objectives

The primary objectives of this thesis were to describe the circadian melatonin pattern according to current shift work status (day only, or rotating day and night) of female hospital personnel in terms of mesor, amplitude and acrophase and to examine the associations of current shift work status and past shift work history with circadian melatonin patterns. Additional objectives are to explore the associations of other working time patterns within the context of current shift work – length of working hours, time of day and shift intensity – with circadian melatonin patterns. Several sensitivity analyses were also conducted to assess the effects of certain methodological decisions.

3.2 Study Design

To evaluate the associations of current shift work and past shift work history with circadian melatonin patterns, a cross-sectional observational study, with a retrospective assessment of previous exposure to shift work was conducted. Because of the complexity of the health care issue, the study was designed by an interdisciplinary investigative team with population health, nursing human resources, physical activity and behavioral sciences, sleep and analytic modeling and health policy expertise. Team members include Joan E. Tranmer, Kristan Aronson, Ian Janssen, Linda McGillis Hall, Christine Collier and Andrew Day. The original study aimed to determine the influence of shift work exposure on indicators of CVD risk among adult working women and to elucidate the multifactorial mechanisms involved.
3.3 Study Population

The target population for this study was female personnel at Kingston General Hospital (KGH) working either fixed-day schedules or rotating shift schedules. The latter is typically characterized by two 12-hour days, two 12-hour nights, then five days off. Participants were recruited from inpatient units, laboratory, diagnostic and support services from the two affiliated hospital sites, Kingston General Hospital (n<sub>total</sub>=331). Advertisements on public bulletin boards within different units of the hospital described the study and asked potential participants to contact the project coordinator. Direct email communication to all nurses as well as notices in both the local intranet communication and unit communication books were also used for recruitment.

There were three data collection periods: September 2011 – May 2012, September 2012 – May 2013, and September 2013 – February 2014. The study participants were female personnel between the ages 22 and 71 working either fixed-day shift schedules or rotating shift schedules including nights. They were full-time or part-time employees with a history of regular employment in any setting for 1 year prior to the initiation of the study. This criterion ensured that participants had the opportunity to engage in various work patterns in order to capture the inter-individual variability in the exposure. Participants were asked to self-exclude if they were pregnant or had given birth in the last year.

The study protocol was approved by the Health Sciences Research Ethics Board at Queen’s University (Appendix A). All participants signed informed consent, while only three women did not complete the protocol due to a lack of urine provision.
3.4 Basic Data Collection Procedures

Three hundred and thirty one women were initially included in the study. All participants participated in a brief anthropometric and blood pressure assessment, and completed an interview and standard questionnaire package (Appendix B). This was followed by a scheduled data collection period that was 8 days in length and for rotating shift workers this included at least two night shifts. During the 8 day cycle, there was a designated 2 day specimen collection period. All urine samples were collected, aliquoted and stored in a -70 degree freezer. Urine samples were then analyzed to assess levels of the melatonin biomarker.

3.5 Shift Work Exposure Assessment

The two primary exposures to shift work are current shift work status and past shift work history (Table 3.1). Current shift work status was determined through self-report (i.e. shift or non-shift worker), where a shift worker was defined as an individual who works in a rotational pattern that includes a night shift. Shift work duration was determined through interview, where number of years an individual worked in a rotation was estimated by the following formula: cumulative shift work = full-time years + 0.5*part-time years.

To analyze the primary exposure of current shift work more precisely, three different exposure metrics of current status were used: shift length, time of day and shift intensity (Table 3.2). Shift length was determined by calculating the average length in hours of all shifts in the current shift schedule. Time of day was determined by time and type of shift. Shift intensity was determined by calculating the average number of consecutive night shifts in the current shift schedule. Due to the limited variability in shift length and shift intensity, they were not treated as continuous variables and were dichotomized in the analysis. Cut-offs of 12-hours for shift length
and four consecutive night shifts for intensity were identified in previous studies and existing European legislation\textsuperscript{2–6}, and six nights in a row has been associated with increased breast cancer risk\textsuperscript{7}. However, because most people worked 2 or 3 consecutive nights, a cut off of 3 consecutive nights was chosen as the threshold for shift intensity in order to retain an adequate sample size in the intensity exposure groups.

Table 3.1: Assessment of primary shift work exposures

<table>
<thead>
<tr>
<th>Primary Exposures</th>
<th>Measurement tool</th>
<th>Presentation of variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current shift work</td>
<td>Self-reported status</td>
<td>Categorical</td>
</tr>
<tr>
<td>Cumulative shift work</td>
<td>Lifetime exposure in years</td>
<td>Continuous</td>
</tr>
</tbody>
</table>

Table 3.2: Assessment of different exposure metrics of current shift work

<table>
<thead>
<tr>
<th>Current Shift Work Domains</th>
<th>Measurement tool</th>
<th>Presentation of variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shift length</td>
<td>Length of shift in hours</td>
<td>Categorical</td>
</tr>
<tr>
<td>Time of day</td>
<td>Day or night shift</td>
<td>Categorical</td>
</tr>
<tr>
<td>Shift intensity</td>
<td>Number of consecutive nights</td>
<td>Categorical</td>
</tr>
</tbody>
</table>

3.6 Melatonin Assessment

3.6.1 Urine Collection Procedures

Participants who were rotating shift workers completed data collection on their last night of the schedule followed by the next day; fixed day shift workers completed any two consecutive work days. Melatonin levels were assessed from up to 20 urine samples that were provided by study participants over two 24-hour periods. On average, participants gave their first sample
within 1.6 hours of waking up, and ended their sample collection at the end of the 24 hour period, in accordance with the protocol. It should be noted that for the night shift worker, participants started urine collection on awakening after their first night shift, that is sometime in the afternoon. Average time between urine samples was 3.6-3.8 hours (Table 3.3).

Table 3.3: Times of sampling

<table>
<thead>
<tr>
<th>Current shift work status</th>
<th>Time of first sample (hours since waking)</th>
<th>Total time (hours since waking)</th>
<th>Average time between samples (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day worker</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day shift 1</td>
<td>0.8 ± 2.2</td>
<td>24.1 ± 2.1</td>
<td>3.6 ± 2.8</td>
</tr>
<tr>
<td>Day shift 2</td>
<td>0.8 ± 2.2</td>
<td>23.7 ± 3.5</td>
<td>3.7 ± 2.9</td>
</tr>
<tr>
<td>Shift worker</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day shift</td>
<td>1.0 ± 2.4</td>
<td>24.1 ± 3.4</td>
<td>3.8 ± 2.5</td>
</tr>
<tr>
<td>Night shift</td>
<td>1.6 ± 2.8</td>
<td>22.4 ± 4.3</td>
<td>3.7 ± 2.5</td>
</tr>
</tbody>
</table>

The frequent collection of urine samples was designed to capture the circadian melatonin pattern during either day or night shifts depending on current shift work status. Random urine samples have drawbacks in that urinary output is highly variable throughout the day. Much of this variability can be compensated for by adjustment of the concentration of the measured analyte based on the level of creatinine in the urine. Thus, to control for urine production and output, creatinine levels were measured and applied as conversion factors.

3.6.2 Laboratory Analysis

Levels of the primary urinary metabolite, 6-sulfatoxymelatonin, were assessed using the Buhlmann 6-sulfatoxymelatonin ELISA kit. The melatonin analysis kit is a competitive
immunoassay that uses the capture antibody technique. In the urine assay, 6-sulfatoxymelatonin present in the samples competes with biotinylated 6-sulfatoxymelatonin for a highly specific rabbit anti-6-sulfatoxymelatonin antibody. During the first 3-hour incubation period, the biotinylated 6-sulfatoxymelatonin-antibody complexes are captured by the second antibody coated on the wells of a microtiter plate. During the 30-minute second incubation step, an enzyme label, streptavidin conjugated to horseradish peroxidase, is added, which binds to the biotinylated 6-sulfatoxymelatonin-antibody complexes captured in the wells. Unbound enzyme label is then removed by washing the wells, and a substrate tetramethylbenzidine is added. During the 30 minute final incubation step, a colored product is formed inversely proportional to the amount of melatonin present in the original sample. The color turns from blue to yellow after addition of an acidic stop solution, and the absorbance of the plate can then be measured at 450 nm; where the concentration of melatonin is determined from a standard curve provided with the urine assay.

3.6.3 Quality Control

3.6.3.1 Assays

For the analytical performance of the assays, internal and external quality controls were conducted. The reproducibility of standard curve parameters and control values were checked against the established performance characteristics of the assays for melatonin and creatinine (Table 3.4). The measurements were also compared to the reference interval of standard kits (Table 3.5).
Table 3.4: Assay performance characteristics for 6-sulfatoxymelatonin and creatinine

<table>
<thead>
<tr>
<th>Performance Characteristics</th>
<th>6-sulfatoxymelatonin</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-Assay Precision</td>
<td>≤ 7.1%</td>
<td>3.2 – 3.5%</td>
</tr>
<tr>
<td>Inter-Assay Precision</td>
<td>≤ 11.9%</td>
<td>4.0 – 5.5%</td>
</tr>
<tr>
<td>Functional Sensitivity</td>
<td>1.5 ng/ml</td>
<td>0.01 – 0.07 mg/dL</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>None specified</td>
</tr>
</tbody>
</table>

Table 3.5: Reference intervals for 6-sulfatoxymelatonin and creatinine

<table>
<thead>
<tr>
<th>Source</th>
<th>Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-sulfatoxymelatonin (ng/ml)</td>
<td></td>
</tr>
<tr>
<td>Buhlmann ELISA</td>
<td>0.8 – 40</td>
</tr>
<tr>
<td>IBL ELISA</td>
<td>1.7 - 420</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td></td>
</tr>
<tr>
<td>Quest Diagnostics</td>
<td>20 – 320</td>
</tr>
<tr>
<td>Parameter Assay</td>
<td>0.31 - 20</td>
</tr>
</tbody>
</table>

3.6.3.2 Missing Data

Three participants were excluded in the subsequent analyses due to a lack of urine provision. In the remaining 328 participants with two days of observations, three were excluded on day 1 while six were excluded on day 2 due to missing times for their biosamples. The final sample consisted of 325 participants on day 1 and 322 on day 2 (Figure 3.1).
3.6.3.3 Outliers and Elevated Observations

Each participant had multiple biosamples of melatonin and creatinine. Outliers were excluded manually after screening for biologically implausible values (>1000 ng/ml). On day 1, six women had one improbable aMT6s concentration in their time series; while on day 2, eight women had one improbable aMT6s concentration in their time series. After excluding outliers, melatonin levels were still observed to be higher than the reference intervals. These were not considered outliers because usage of melatonin supplements and other sleep aids was not an exclusionary criterion at the study’s inception. These elevated values were controlled for in the statistical analysis by adding another variable for usage of sleep aid to adjust for in the regression analysis.
3.7 Potential Confounders

Information about potential confounders identified in the literature review using a DAG (Figure 2.3) was obtained from the study questionnaire for inclusion in the statistical analysis. These variables included personal and reproductive characteristics, as well as several drugs.

3.7.1 Personal Characteristics

The study questionnaire assessed several personal and health history characteristics. Age at the time of study participation was determined from self-reported date of birth and was treated as a continuous variable. Highest level of education was self-reported and was treated as a categorical variable with a threshold of post-secondary education. Subjects also reported several reproductive characteristics including their menstrual status, parity (number of full-time births) and the age at which they gave birth to their first child.

Diurnal chronotype, which refers to their internal biological clock, may also confound or modify the relationship between shift work and melatonin levels\(^8,9\). Chronotype was assessed by the Munich Chronotype Questionnaire (MCTQ), which characterizes people using the mid-sleep time (MSF) of their sleep phase. For analysis purposes, chronotype was kept as a continuous variable. Because chronotype depends on light-dark cycles and the geographical location someone is living in, categorization, without correcting for longitude and latitude will lead to either an over- or under-estimation of the percentage within the sample. Keeping chronotype continuous reflects more appropriately the nature of the variable.

Although menopausal status is an etiologic factor for breast cancer, it is not an established interacting factor for shift work and melatonin\(^10-12\). Therefore, potential effect
modification by menopausal status was be explored as an additional analysis. Women were classified as postmenopausal if they satisfied one of the following criteria:

1. They had stopped menstruating natural for at least one year at time of study entry
2. They had stopped menstruating natural and were over 50 years of age
3. They had stopped menstruating and had had a bilateral oophorectomy
4. They had had a bilateral oophorectomy and were over 55 years of age

3.8 Data Management

All data was entered into a secure system maintained by the Clinical Research Centre at Kingston General Hospital. This data was converted into a SAS database where missing values were identified and their logical consistency was verified. Errors were reported to the study team on a periodic basis using an automated data checking system.

3.9 Statistical Analysis

3.9.1 Main Objectives

3.9.1.1 Descriptive Statistics

Descriptive statistics were used to describe the work and health characteristics of the study population. Means and standard deviations were calculated for continuous variables. Comparisons were made between the shift work exposure groups using the Wilcoxon rank sum tests for continuous variables and the Chi-square or Fischer’s exact tests for categorical
variables. All statistical analyses were performed using SAS Version 9.4 (SAS Institute, Cary, NC).

3.9.1.2 Cosinor Analysis

Individual melatonin profiles on each observation day were examined by cosinor analysis, where the parameters, mesor (circadian mean), amplitude (difference between peak concentration and mean) and acrophase (peak time of production) can be obtained (Figure 3.2). Each study participant should have two different circadian melatonin patterns; women with fixed-day schedules should have patterns for two day shifts, while women with rotating night schedules should have patterns for one day shift and one night shift.

Descriptive statistics of the cosine function were used to describe the sample. Crude geometric means and 95% confidence intervals (which represent the inter-individual variation within each shift work exposure group) were calculated for acrophase, mesor and amplitude for both groups. For a visual comparison, the cosinor parameters were also used to graph individual melatonin profiles, as well as the different shift work group profiles.
3.9.1.3 Shift Work and Melatonin Analysis

Multivariate least-squares regression analysis was used to evaluate the association between the log-transformed cosinor parameters and the different shift work exposure domains (Table 3.6 and Table 3.7). Comparisons on the first observation day, which included day shifts for both fixed-day and rotating night workers, were aimed to capture the effect of current shift work exposure. Comparisons on the second observation day, which included day shifts for fixed-day workers and night shifts for rotating night workers, were aimed to capture the effect of time of day as a proxy for light at night. Both observation days were used to examine the effect of cumulative shift work in both day and shift workers, and the effects of shift intensity and shift length which are assessed among shift workers only.

The cosinor parameters were log-transformed for normality, and the regression estimates were then back-transformed so that they could be presented as a geometric mean percentage change. Phase shifts, a measure of internal desynchronization, were estimated by calculating the
geometric mean difference of the predicted acrophases across the different shift work exposure groups. Potential confounders such as age, parity, age at which they gave birth to their first child, education and chronotype were kept in the model. Because these variables in the hypothesized causal model (DAG) are well-established causal antecedents of the outcome, it is essential to keep them in the model without regard to strength or statistical significance of their associations in order to establish face validity\textsuperscript{13}.

Table 3.6: Comparison groups of primary shift work exposures

<table>
<thead>
<tr>
<th>Primary Exposures</th>
<th>Exposure Group</th>
<th>Reference Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current shift work</td>
<td>Shift worker</td>
<td>Day worker</td>
</tr>
<tr>
<td>Cumulative shift work</td>
<td>Per Year</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.7: Comparisons groups of different shift work exposure metrics of current shift work

<table>
<thead>
<tr>
<th>Current Shift Work Domains</th>
<th>Exposure Group</th>
<th>Reference Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shift length</td>
<td>12+ hour shifts</td>
<td>&lt;12 hour shifts</td>
</tr>
<tr>
<td>Time of Day</td>
<td>Night shift</td>
<td>Day shift</td>
</tr>
<tr>
<td>Shift intensity</td>
<td>3+ consecutive night shifts</td>
<td>&lt;3 consecutive night shifts</td>
</tr>
</tbody>
</table>

3.9.2 Sensitivity Analyses

3.9.2.1 Strong Predictors

In addition to confounders, strong predictors of melatonin levels (Figure 2.3) which are not a \textit{priori} confounders in the literature, but may be differentially distributed between the
comparison groups can bias the effect estimates. A sensitivity analysis, selecting for strong predictors using backwards elimination and adjusting for these baseline imbalances, was conducted in order to demonstrate that the inference from the primary analysis is not quantitatively affected by any imbalances of these strong determinants.

Body mass index (BMI) was calculated from height and weight measured by the study nurse. For analysis purposes, BMI was kept as a continuous variable. Furthermore, the literature review identified antidepressants, melatonin supplements, beta-blockers, non-steroidal anti-inflammatory drugs (NSAIDS), hormone replacement therapy, sedatives and oral contraceptives as drugs with the potential to impact melatonin levels\textsuperscript{14-20}. Information regarding the use of these pharmacologic agents was collected in the study questionnaire. Participants using these drugs were asked to report the brand name, medication type, date started and duration of use in years.

Both caffeine and alcohol were assessed in a questionnaire about nutrition. Participants were asked to report if they on average had more than 3 caffeinated beverages and 1-2 alcoholic drinks a day. Smoking status was assessed in the study questionnaire where subjects were asked to identify whether or not they were a current smoker. These lifestyle factors were treated as categorical variables. Physical activity was measured using the Global Physical Activity Questionnaire (GPAQ) developed by the World Health Organization (WHO). The GPAQ quantifies physical activity at work, during leisure time, and recreational activity while accounting for intensity. Participants were classified as having met or not met the following WHO guidelines:

- \( \geq150 \) minutes of moderate intensity aerobic activity (increases heart rate or breathing) per week for 10 continuous minutes OR
• ≥75 minutes of vigorous-intensity aerobic physical activity per week for 10 continuous minutes OR
• An equivalent combination

For analysis purposes, physical activity was assessed categorically as either yes or no to meeting the WHO recommendations.

3.9.2.2 Linear Mixed Model

This thesis also explored cosinor parameters generated from a linear mixed model, using the Mixed Models procedure in SAS 9.4 (PROC MIXED). These were compared to those derived from the standard linear regression. The type of data assumed is multiple time series, which consists of the repeated measurements of melatonin levels on a single subject.

Two key extensions to the usual cosinor analysis are the inclusion of the autoregressive error structures to model the strong serial correlation often seen in biorhythm data\textsuperscript{21}, and the use of mixed models to account for normal subject-to-subject biological variation\textsuperscript{22,23}. Since samples are usually closely spaced, and are probably serially correlated, treating them as independent in a regression model can be perceived as inappropriate. PROC MIXED can accommodate a first order autoregressive covariance structure, which is likely to provide acceptable modeling of the serial correlation. The mixed model can also take into account subject-to-subject biological variation, which is a random effect. By modeling a random intercept, each subject has an individual level for his or her circadian melatonin pattern.
3.10 Additional Analyses

3.10.1 Menopausal Status as an Effect Modifier

Although menopausal status is not an established interacting factor for shift work and melatonin, it is an etiologic factor for breast cancer. Thus, as an additional analysis, associations between the cosinor parameters and the primary exposure of current shift work were analyzed after stratifying the participants by menopausal status.

Interactions with specific aspects of current shift work – shift length, time of day, shift intensity – could not be analyzed due to inadequate power as stratification, first by shift work status then by menopausal status, reduced the sample size. For example, when stratifying the shift length exposure metric twice, there were only two postmenopausal participants who worked on average <12 hour shifts.

Furthermore, interactions with cumulative shift work also could not be analyzed, as we were interested in current menopausal status. If the etiology is indeed different by menopausal status, then analytically, stratifying menopausal status when looking at cumulative shift work is not logical, as the timing and occurrence of menopause needs to be taken into account otherwise the comparison of each individual past year of shift work is not consistent. For example, if two postmenopausal women who have a cumulative shift work exposure of 10 years, where menopause occurred two years ago for one shift worker and it occurred five years ago for the other, then past duration of shift work cannot be compared as they both spent a different amount of time in pre and postmenopausal states.
3.11 Validity of Exposure and Covariate Measures

Several questions used in the questionnaire in this study have not been validated; however, they either have been used in previous questionnaires or are in the process of being validated. For the main analysis which only included *a priori* confounders selected from a DAG, questions on current shift work, age, number of full-term births, age at first birth as well as education have been judged to be valid as they require obvious self-reported information on personal and reproductive factors. For cumulative shift work, although there may be some random misclassification due to poor memory, based on the previous experience of our research group collecting similar information in other studies, women were able to accurately recall number of years they worked either full-time and/or part-time. For the measurement of chronotype, the mid-sleep time is a valid measure of chronotype\textsuperscript{24,25}.

For the sensitivity analyses of the determinants of melatonin, although body mass index (BMI) is not a highly accurate measure of obesity, it has been shown to be strongly correlated with body fat levels. For physical activity, since validity studies on the Global Physical Activity Questionnaire (GPAQ) have indicated that it is a valid measure of moderate-to-vigorous physical activity (MVPA) and change in MVPA\textsuperscript{26}, the GPAQ appears to be an appropriate measure for assessing physical activity. For other determinants, such as pharmacological agents, alcohol and caffeine consumption, questions ascertained only average use and consumption, while the more pertinent measurement would be assessment 24 hours prior to bio-sampling. Thus, our research group recognizes that these are only crude representations of what was intended to be measured.
3.12 References


Chapter 4

Melatonin patterns among female hospital employees on day and night shifts:

Assessment by shift work exposure metrics
4.1 Abstract

Objective:
To determine the associations of shift work exposure metrics with circadian melatonin mesor, amplitude and acrophase.

Methods:
In a cross-sectional study of 328 female hospital personnel working fixed-day or rotation schedules, shift work exposure metrics were determined through self-report. Six-sulfatoxymelatonin concentrations were analyzed by cosinor analysis.

Results:
Nurses working at night had lower mesors and earlier acrophases than day workers. Those working three or more consecutive nights had lower mesors and smaller amplitudes than those working less than three consecutive nights. After adjusting for confounders, acrophases were no longer different, while smaller amplitudes were apparent among shift workers compared to day workers. Shift length or duration of shift work were not associated with melatonin pattern.

Conclusion:
Rotating patterns of shift work, specifically higher intensity of 3+ nights, are associated with depressed melatonin rhythms, an indicator of circadian disruption.
4.2 Introduction

In 2007, long-term shift work involving circadian disruption was classified as a probable (Group 2A) carcinogen by the International Agency for Research on Cancer (IARC) on the basis of sufficient evidence in experimental models and limited evidence in humans\textsuperscript{1}. Epidemiologic studies have suggested that working night shifts and rotating night shifts are associated with not only an increased cancer risk at multiple sites including breast, colon and prostate\textsuperscript{2-14} but also an increased risk for metabolic syndrome\textsuperscript{15-17} which may predispose individuals to heart disease, stroke and diabetes\textsuperscript{18-24}. Results from meta-analyses demonstrate that long-term night shift work is associated with an increased risk of 40-50\% for breast cancer\textsuperscript{2,3} and 50-60\% for metabolic syndrome\textsuperscript{16}. The main hypothesized mechanism linking night shift work and chronic disease involves the hormone melatonin\textsuperscript{25}. Increased light at night associated with shift work is thought to suppress the production of melatonin, a hormone which has been shown to possess both cancer and cardiovascular protective properties\textsuperscript{26-31}. Although there is a growing body of evidence to support this mechanism, other potential mechanisms may operate between shift work and chronic disease\textsuperscript{32}.

In most studies using shift work as a proxy for light at night, absolute urinary 6-sulfatoxymelatonin (aMT\textsubscript{6}s) levels have been assessed and not peak timing of production. The general pattern observed is decreased melatonin production with increased exposure to light at night\textsuperscript{33,34}. However, one major problem with these studies is that biomarker measurements may be confounded by circadian rhythm, where comparisons using functional times do not account for circadian stage\textsuperscript{35}. The possibility of confounding by circadian rhythm arises because most studies have had a limited number of biosamples due to logistics and cost constraints. Another limitation is in the definition of ‘shift work’, which generally refers to work schedules that differ from conventional daytime schedule of activity during the day and sleep at night. Since the
definition of shift work involving nights has been so broad, exposure assessment has been both
imprecise and incomplete\textsuperscript{36,37}. Most studies have used either current status or duration of shift
work without considering other occupational domains relevant to the exposure such as shift
length, time of day, shift intensity and social aspects working hours\textsuperscript{38}. For example, the exposure
of ‘ever worked in night shifts’ is likely too broad, whereas precision is gained by assessing shift
length (average number of hours worked), intensity (number of consecutive nights, for example)
and cumulative duration of shift work in years.

Since shift work is a necessary component of many occupations, precise exposure and
outcome assessment is needed in order to improve the validity of research and increase impact
on workplace health policies. The objective of this study is to evaluate the association between
shift work exposure metrics and circadian melatonin patterns in terms of mesor, amplitude and
acrophase.

4.3 Materials and Methods

4.3.1 Study Population

The target population was female personnel at Kingston General Hospital. Participants
were recruited from inpatient units, laboratory, diagnostic and support services from one hospital
site in Kingston, Ontario, Canada ($n_{\text{total}}=331$). Advertisements on bulletin boards within hospital
units described the study and asked potential participants to contact the project coordinator, and
direct email communication to all nurses as well as notices in both the local intranet
communication and unit communication books were also used for recruitment.

All participants worked either fixed-day schedules or rotating shift schedules, with the
latter characterized by two 12-hour days, two 12-hour nights, and five days off. Participants were
full-time or part-time employees with a history of regular employment in for one year prior to the study. Participants were asked to self-exclude if they were pregnant or had given birth in the last year. This research was approved by the Health Sciences Research Ethics Board at Queen’s University, and all participants signed informed consent.

4.3.2 Data collection

Each data collection period was eight days, and for rotating shift workers this included at least two night shifts. Before the initiation of the data collection period, the coordinator conducted an assessment measuring weight, height and waist circumference, and administered a questionnaire including information on number of children, health history, as well as complete job history including occupational title, status and length of employment.

The two primary exposures of shift work were current shift work status and past shift work history. Current shift work status was determined through self-report, where a shift worker was defined as an individual who works in a rotational pattern that includes nights. Cumulative shift work duration was determined through interview, where number of years an individual worked in a rotation was estimated as full-time years + 0.5*part-time years.

To analyze the primary exposure of current shift work more precisely, three exposure metrics of current status were used: shift length, time of day and shift intensity. Shift length was determined by calculating the average length in hours of all shifts in the current shift schedule. Time of day was determined by time and type of shift. Shift intensity was the average number of consecutive night shifts in the current shift schedule. Due to the limited variability in shift length and shift intensity, they cannot be treated as continuous variables and were dichotomized in the analysis. Cut-offs of 12-hours for shift length and four consecutive night
shifts for intensity have been identified in previous studies and existing European legislation\textsuperscript{37,39–42}, and six nights in a row has been associated with increased breast cancer risk\textsuperscript{43}. However, because most participants worked 2 or 3 consecutive nights, a cut off of 3+ consecutive nights was chosen.

4.3.3 Melatonin

During the eight day data collection period, there was a designated 48-hour specimen collection period where all urine was collected. All urine samples were time-stamped in diaries, aliquoted and stored in a -70 degree freezer. To control for urine output, creatinine levels were determined and applied as correction factors for the melatonin levels. Three participants were excluded because they had missing biomarker data, while outliers were excluded after screening for biologically implausible values: on the first day, six women had one improbable aMT6s concentration, while on the second day, eight women had one improbable aMT6s concentration.

Levels of the primary urinary metabolite of melatonin, 6-sulfatoxymelatonin, were assessed in urine using the Buhlmann ELISA kit, a competitive immunoassay that uses the capture antibody technique. Although assessment of plasma or serum melatonin levels is considered the most accurate method, its invasiveness is a barrier in observational research; therefore, collection of urine samples was used. For the analytical performance of assays, internal and external quality controls were conducted. The reproducibility of standard curve parameters and control values were checked against the established performance characteristics of the assay for melatonin and creatinine, and measurements were also compared to the reference interval of standard kits.
4.3.4 Statistical Analysis

Comparisons of work and health characteristics were made between groups using the Wilcoxon Rank Sum test for continuous variables and Chi Square and Fischer’s Exact test for categorical variables. Individual melatonin profiles on each observation day were examined by cosinor analysis, where the parameters mesor (circadian mean), amplitude (difference between peak concentration and mean) and acrophase (peak time of production) were obtained. Each rotational shift worker had two circadian melatonin patterns: one pattern for their day shift and one for their night shift, while day workers had one pattern derived from the average of the parameters over two day shifts.

Geometric means and 95% confidence intervals (which represent the inter-individual variation within each shift work exposure group) were calculated for acrophase, mesor and amplitude. Multivariable least-squares regression analysis was used to evaluate the association between the log-transformed cosinor parameters and the shift work exposure domains. The cosinor parameters were log-transformed for normality, and regression estimates were then back-transformed to represent a geometric mean percentage change. Phase shift, a measure of internal desynchronization, was estimated by calculating the geometric mean difference of the predicted acrophases across the shift work exposure groups, where a positive value means later peak and a negative value means earlier peak. Potential confounders such as age, parity, age at first birth, education and chronotype were retained in all models in accordance with our hypothesized causal model (directed acyclic graph (DAG) seen in Figure 1). All statistical analyses were performed using SAS Version 9.4.
4.4 Results

4.4.1 Characteristics of the study population

Three hundred and thirty one women were initially included in the study, with three were excluded because as they did not provide adequate urine samples. A total of 328 were included in the analysis with 160 working regular day schedules and 168 working rotating schedules including nights. As described in Table 1, average age was 42 years, and about 66% were pre-menopausal; however, women working days only were older, had a slightly higher number of full-term births, were more likely to smoke, and reported lower use of oral contraceptives than women working rotating shifts. These two groups were similar in: age at first birth, distribution of BMI, mid-sleep time, physical activity level, and reported use of sleep aid and hormone replacement therapy. While both groups had similar proportions of full-time and part-time workers, there were far more nurses among those working rotating shifts (85% versus 47%) than among those working days. Furthermore, rotating shift workers had a longer past shift work history (12 years versus 8 years) compared to women working days.

4.4.2 Melatonin

Summary measures for melatonin patterns according to work exposure metrics are reported in Table 2. Rotating shift workers on their night shift had a lower mesor, smaller amplitude and an earlier acrophase by about an hour compared to day-only workers (Figure 2). In terms of shift intensity, rotating shift workers working nights in a rotation with 3+ consecutive nights had a lower mesor and smaller amplitude than rotating shift workers with <3 consecutive nights (Figure 3). In terms of shift length, there was no difference in any comparison made for those working <12 hours and ≥12 hours.
4.4.3 Multivariable associations with melatonin

Associations between the primary exposures – current shift work and cumulative shift work - and 6-sulfatoxymelatonin cosinor parameters are shown in Table 2. After adjusting for a priori confounders according to our DAG, rotating shift workers on their day schedule had 25% lower mesor and 29% smaller amplitude compared to day workers. No difference between shift and day workers was seen in acrophase. Rotating shift workers on their night schedule had 37% lower mesor and 36% smaller amplitude compared to day workers, and there was no difference in acrophase. Melatonin pattern parameters were not associated with duration of past shift work.

Associations between specific exposure metrics of current shift work and 6-sulfatoxymelatonin cosinor parameters are shown in Table 2. For shift intensity, rotating shift workers working days in a rotation schedule with 3+ consecutive nights had 23% lower mesor and 26% smaller amplitude than those working <3 consecutive nights. No difference in acrophase was seen. Shift workers working nights in a rotation schedule with 3+ consecutive nights had a 32% smaller amplitude than those working <3 consecutive nights, while no difference in mesor or acrophase was seen between the two groups. For time of day and shift length, these exposure metrics of current shift work were not associated with melatonin pattern parameters.

Sensitivity analyses (not shown) were conducted by controlling for known determinants of melatonin and using a linear mixed model to account for serial correlation and normal subject-to-subject variation, and results were very similar to those of the main analysis. As an additional analysis (not shown), associations between current shift work and melatonin parameters were analyzed after stratifying by menopausal status. The effects of the main analysis were observed...
primarily among postmenopausal women, while a 30 minute phase delay was apparent among premenopausal rotating shift workers when they were working days compared to day-only workers. This may suggest that pre-menopausal women may be able to adapt to new sleep-wake patterns, by not only keeping their melatonin production elevated to normal levels, but also by being able to delay acrophase closer to sleep onset. The converse is seen among post-menopausal shift workers, as they have lower mesors and smaller amplitudes with no observable phase delay. These results are indicative that the protective role of melatonin against chronic disease may be diminished in post-menopausal women due to light-at-night effects and night work that disrupts circadian rhythm.

4.5 Discussion

Current exposure to rotating shift work was associated with 25% and 37% reduction in 6-sulfatoxymelatonin mesor and 29% and 36% reduction in 6-sulfatoxymelatonin amplitude among rotating shift workers, on a day and night shift, respectively, compared to day workers. These results are in accordance with some recent studies such as the Nurse’s Health Study that reported a reduction of melatonin of 69% during night shifts\textsuperscript{44}, while a study from Spain observed a decrease of 34% in mesor among permanent night workers compared to day workers\textsuperscript{45}. For amplitude, one previous study found that rotating shift workers had a reduction in amplitude (45 versus 80 ng aMT6s/mg creatinine)\textsuperscript{46}; however, the investigators did not adjust for confounders or statistically assess the difference. This is in accordance with experimental evidence, where more light at night exposure can lead to decreases in the mesor and amplitude of the melatonin rhythm\textsuperscript{47,48}. Since both circadian parameters may play an important role in cancer
development, future studies should consider identifying and quantifying both these parameters. Thus, our study provides evidence for light-at-night effects and night work related circadian disruption among current rotating shift workers.

Our study found no difference in acrophase between rotating shift workers and day workers, and very few other studies have assessed this. Previous work from our research group found that rotating shift schedules did not change the timing of peak melatonin production, although bio-sampling was more limited over a 24 hour period\textsuperscript{35}. In contrast, a study in Spain found that rotating shift workers had a later acrophase (08:31 versus 07:13) compared to day workers\textsuperscript{46}; however, the investigators did not adjust for any confounder or statistically assess the difference in timing of peaks. In another study, a 3-hour delay in peak time was reported among night workers compared to day workers\textsuperscript{45}; however, all participants were engaged in permanent fixed night shifts, the most extreme group in the shift work exposure spectrum, making it more likely for phase shifts to occur. These results are in accordance with current evidence on circadian adaptation, where more night shifts delay the acrophase closer to sleep onset in order to better align circadian rhythms to a new sleep-wake pattern\textsuperscript{49,50}. Although, full adaptation is unlikely to occur given that these workers with extreme shift schedules still only experience partial adaptations, where their acrophase was still earlier than sleep onset. Our results provide additional evidence for this concept of adaptation. This finding is of paramount importance, as it has been suggested that the adverse effects of shift work stems from internal desynchrony and the lack of adaptation to new sleep-wake cycles\textsuperscript{51,52}.

Domains of current shift work, such as time of day (only for rotational shift work), shift length and shift intensity may have important biologic impacts\textsuperscript{38}. Although these exposure metrics have been examined in the context of disease outcomes, they have rarely been analyzed
at an earlier physiological stage such as through biomarkers. In this study, no apparent difference in 6-sulfatoxymelatonin patterns was observed between day or night shifts (time of day) when controlling for shift length and intensity. These results are in accordance with previous work done by our research group, where no difference in mean levels between day and night shifts for rotating shift workers was observed\textsuperscript{35}. However, their study may be limited by inadequate study power: the possibility of confounding by circadian rhythm due to a limited number of biosamples forced the investigators to stratify the study population by shift group, therefore reducing the sample size as well as decreasing the variability in light exposure. Furthermore, shift length was not associated with melatonin pattern parameters. Possible reasons for the lack of associations are the rotating pattern of shift work and limited variability in shift lengths, where 83% of rotational shift workers worked 12-hour shifts, reducing statistical power. Finally, shift intensity was inversely associated with 6-sulfatoxymelatonin mesor and amplitude where working 3+ consecutive nights resulted in lower mesors and smaller amplitudes. These results for shift intensity, in conjunction with the non-association with time of day, provide new evidence that the presence of light at night itself in a rotation is not enough to alter the circadian rhythm of melatonin, but rather it is the exposure to light at night with higher intensity (longer sequence of consecutive night shifts) which can affect circadian melatonin patterns. This observation helps support the evidence in the existing breast cancer literature by demonstrating at a biomarker level the mechanism suggested from experimental research at the population level: breast cancer risk increases by only 3% for every 5-years of shift work, while the increase in risk elevates to 80% for every 5 years of shift work with 6+ consecutive nights in a rotation\textsuperscript{43}. Therefore, in light of this evidence, we can identify shift intensity as an aspect of current rotational shift work that alters circadian melatonin patterns. Since this is the first study to show an association between
shift intensity and depressed melatonin rhythms, future studies should try examine and replicate this association, especially since melatonin has been shown to possess both cancer and cardiovascular protective properties\textsuperscript{26–31}.

In terms of past shift work history, cumulative exposure to shift work was not associated with any 6-sulfatoxymelatonin parameters among both day only workers and rotating shift workers. One possible reason for the lack of an association among current shift workers is that long-term rotational schedules may not be enough to change melatonin production patterns. Most rotational patterns consist of two 12-hour days, two 12-hour nights, followed by five days off. By working only two nights within a nine-day period, there is adequate time to readjust to conventional daytime schedules of activity during the day and sleep at night.

One of the strengths of this study is the collection of all urine samples over two 24-hour working days that enabled the characterization of circadian melatonin patterns using cosinor analyses. Since we can describe the patterns of melatonin, we can see where the peaks occur regardless of shift schedule, and we can avoid making comparisons that are confounded by circadian rhythm. Studies that have assumed that melatonin peaks during the day when night workers are sleeping during the day may be wrong in this assumption, and their comparisons of levels upon waking after night sleep would be confounded. A second key strength of our study is its observational nature, where circadian melatonin patterns can be captured during working hours of both day and night shifts to demonstrate at the population level a potential mechanism suggested from experimental research. Another strength is the use of several exposure metrics of shift work for a more precise examination of the exposure to elucidate which aspects of shift work are related to circadian misalignment and risk for chronic disease, where its assessment in previous studies has been both inconsistent and incomplete. Further, \textit{a priori} confounders such
as age, parity, age of first birth, education and chronotype were selected carefully using a Directed Acyclic Graph. Adjustment for confounding is extremely important, and has been inadequately addressed in several previous studies.

Four aspects of shift work characterize the different facets of current exposure: shift length, time of day, shift intensity and social aspects of working hours; and, therefore a limitation of this study is that we were not able to examine the social aspects of working hours. Information regarding the distribution of free days, irregularity and predictability of working hours was not collected. Since there is evidence to show that control of working time is associated with health, future studies should consider quantifying this exposure metric in addition to time of day, shift length and shift intensity in order to better evaluate the specifics of exposure to shift work. Another limitation of this study is the potential lack of generalizability since our study population was restricted to female health care workers who worked either rotational or day only schedules – no participant was a permanent night worker. Although individual light exposure measures were not included in this study, our previous studies in these hospitals have shown that night lighting is quite dim, at 40-50 lumens/m². These values are much lower than experimental conditions, and this may help explain the attenuated effect estimates and lack of phase shifting for some of exposure metrics. Since intensity and timing of light exposures can impact circadian melatonin patterns, individual light assessment should be incorporated in future studies.

In conclusion, this study evaluated the association of various exposure metrics of shift work with circadian melatonin patterns. Rotating patterns of shift work, specifically high intensity rotations, are associated with depressed melatonin rhythms. To our knowledge, this is the first study to assess the impact of these shift work exposure metrics, including duration of
past shift work among current day workers, on circadian melatonin patterns. Although there is a growing body of evidence to support the role of melatonin in chronic disease, more research is needed to elucidate which specific aspects of shift work are related to higher risks for chronic diseases, to guide intervention strategies and healthy workplace policies.

4.6 Acknowledgements

The Canadian Institutes for Health Research (CIHR) and the Workers Safety and Insurance Board (Ontario) supported the main study from which this project originates. The investigative team (A. Day, L. McGillis Hall, I. Jansenn, C. Collier); research manager (R. Corbin); statistical support (X. Sun) and Kingston General Hospital.
4.7 References


TABLE 4.1. Description of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day Workers (N=160)</th>
<th>Shift Workers (N=168)</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
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<td>Menopausal status</td>
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<tr>
<td>Pre</td>
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<td>115 (68.5)</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>59 (36.9)</td>
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</tr>
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<td>Age at first birth</td>
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<tr>
<td>Chronotype as measured by Mid Sleep Time (MSF)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<td>Physical Activity Recommendation&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>21 (13.21)</td>
<td>9 (5.39)</td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>138 (86.79)</td>
<td>158 (94.61)</td>
<td>0.02</td>
</tr>
<tr>
<td>Current Oral Contraceptive Use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21 (13.13)</td>
<td>40 (24.10)</td>
<td>0.01</td>
</tr>
<tr>
<td>No</td>
<td>139 (86.88)</td>
<td>126 (75.90)</td>
<td></td>
</tr>
<tr>
<td>Current Sleep Aid Use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>23 (14.38)</td>
<td>33 (19.64)</td>
<td>0.21</td>
</tr>
<tr>
<td>No</td>
<td>137 (85.63)</td>
<td>135 (80.36)</td>
<td></td>
</tr>
<tr>
<td>Any Hormone Replacement Therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14 (8.81)</td>
<td>9 (5.49)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>145 (91.19)</td>
<td>155 (94.51)</td>
<td>0.25</td>
</tr>
<tr>
<td>Employment Category</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nursing</td>
<td>75 (46.9)</td>
<td>143 (85.1)</td>
<td></td>
</tr>
<tr>
<td>Management/Administration</td>
<td>23 (14.4)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>62 (38.7)</td>
<td>25 (14.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Full-time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full-time</td>
<td>130 (81.2)</td>
<td>135 (80.4)</td>
<td>0.84</td>
</tr>
<tr>
<td>Part-time</td>
<td>30 (18.8)</td>
<td>33 (19.6)</td>
<td></td>
</tr>
<tr>
<td>Duration of cumulative shift work (years)</td>
<td>7.79 ± 8.42</td>
<td>11.74 ± 9.56</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

<sup>a</sup>p-value for differences between day and rotating shift workers using the Wilcoxon Rank Sum Test for continuous variables, and Chi-Square or Fisher’s Exact test for categorical variables

<sup>b</sup>Mid sleep time (MSF) using the Munich Chronotype Questionnaire

<sup>c</sup>WHO Guidelines: 150 minutes of moderate-intensity aerobic physical activity/75 minutes of vigorous-intensity aerobic physical activity/ equivalent combination of moderate- and vigorous-intensity activities
TABLE 4.2. 6-sulfatoxymelatonin cosinor parameters according to different shift work exposure metrics

<table>
<thead>
<tr>
<th>Exposure Metrics</th>
<th>N</th>
<th>Cosinor Parameters</th>
<th>Acrophase (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crude Geometric Mean (ng/mg)</td>
<td>Adjusted Mean % Change</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amplitude (95% CI)</td>
<td>Crude Geometric Mean (ng/mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acrophase (95% CI)</td>
<td>Crude Geometric Mean (hh:mm)</td>
</tr>
<tr>
<td><strong>Primary Exposures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Current Shift Work Status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day worker</td>
<td>159</td>
<td>36.8 (16.0, 57.6)</td>
<td>Ref</td>
</tr>
<tr>
<td>Shift worker on day rotation</td>
<td>166</td>
<td>19.5 (14.6, 24.4)</td>
<td>-25.1 (-41.2, -4.5)</td>
</tr>
<tr>
<td>Shift worker on night rotation</td>
<td>163</td>
<td>22.3 (11.7, 32.8)</td>
<td>-37.3 (-51.8, -18.4)</td>
</tr>
<tr>
<td><strong>Cumulative Shift Work</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day worker</td>
<td>159</td>
<td>-</td>
<td>0.9 (-1.3, 3.2)</td>
</tr>
<tr>
<td>Shift worker on day rotation</td>
<td>166</td>
<td>-</td>
<td>0.8 (-1.3, 2.8)</td>
</tr>
<tr>
<td>Shift worker on night rotation</td>
<td>163</td>
<td>1.3 (-1.3, 4.1)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Current Shift Work Domains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Time of Day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>159</td>
<td>36.8 (16.0, 57.6)</td>
<td>Ref</td>
</tr>
<tr>
<td>Night</td>
<td>163</td>
<td>22.3 (11.7, 32.8)</td>
<td>-25.2 (-53.9, 21.1)</td>
</tr>
<tr>
<td><strong>Shift Length</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shift worker on day rotation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12 hours</td>
<td>27</td>
<td>14.4 (11.4, 17.5)</td>
<td>Ref</td>
</tr>
<tr>
<td>≥12 hours</td>
<td>138</td>
<td>20.3 (14.4, 26.2)</td>
<td>7.3 (-21.1, 45.9)</td>
</tr>
<tr>
<td>Shift worker on night rotation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12 hours</td>
<td>26</td>
<td>19.7 (1.7, 37.8)</td>
<td>Ref</td>
</tr>
<tr>
<td>≥12 hours</td>
<td>136</td>
<td>22.8 (10.6, 35.0)</td>
<td>-2.4 (-34.3, 45.0)</td>
</tr>
<tr>
<td><strong>Shift Intensity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shift worker on day rotation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3 nights</td>
<td>97</td>
<td>22.6 (14.0, 31.2)</td>
<td>Ref</td>
</tr>
<tr>
<td>3+ nights</td>
<td>69</td>
<td>15.6 (12.8, 18.5)</td>
<td>-23.2 (-39.3, -2.8)</td>
</tr>
<tr>
<td>Shift worker on night rotation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3 nights</td>
<td>94</td>
<td>28.0 (9.4, 46.6)</td>
<td>Ref</td>
</tr>
<tr>
<td>3+ nights</td>
<td>69</td>
<td>15.4 (8.8, 22.0)</td>
<td>-25.1 (-44.7, 1.5)</td>
</tr>
</tbody>
</table>

a Adjusted models were controlled for a priori confounders selected by the DAG, use of sleep aid, as well as the other primary exposure
b Adjusted models were controlled for a priori confounders selected by the DAG, use of sleep aid, as well as the other current shift work domains
c Shift workers only
1 p<0.05 using the Wilcoxon rank-sum test
2 p<0.05 using the Wald test
FIGURE 4.1. Confounder assessment using a Directed Acyclic Graph (DAG) using DAGitty (http://www.dagitty.net). Confounders are ancestors of both shift work and melatonin.
FIGURE 4.2. Circadian melatonin pattern of 6-sulfatoxymelatonin by current shift work status

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FIGURE 4.3. Circadian melatonin pattern of 6-sulfatoxymelatonin in shift workers working nights by shift intensity
Chapter 5

Additional Results

This chapter presents additional results that are too detailed for inclusion in the manuscript, as well as results that refer to issues that arose in the analysis.

5.1 Sensitivity Analysis

5.1.1 Adjusting for Strong Predictors

Determinants of melatonin levels that are not *a priori* confounders may be differentially distributed between the comparison groups can bias the effect estimates. At baseline, women working day shifts had a higher proportion of current smokers (13% versus 5%) than women working rotating shifts. Both groups had similar BMI, physical activity levels, caffeine and alcohol consumption. In terms of medication, women working day shifts had a lower proportion oral contraceptive use (13% versus 24%) than women working rotating shifts. Both groups had similar uses of sleep aid and hormone replacement therapy. A sensitivity analysis was conducted in order to demonstrate that the regression estimates were not quantitatively affected by any imbalances of these strong predictors of melatonin levels. Strong predictors were eliminated in a backwards stepwise process using a liberal p-value of 0.20, and none were retained in the final models. The results of this sensitivity analysis are identical to the main analysis, as any imbalances in these strong predictors did not appear to bias any of the effect estimates.
5.1.2 Linear Mixed Model

Since a linear regression does not take into account serial correlation of samples or normal subject-to-subject biological variation, a sensitivity analysis was conducted using a linear mixed model with a first order auto-regressive covariance structure\(^1-^3\). Results were similar to the main analysis.

Melatonin parameters generated from the mixed model can be seen in Table 5.1. Rotating shift workers on their night shift had a lower mesor, smaller amplitude and an earlier acrophase by about an hour compared to day only workers. There was no difference in any comparison made between rotating shift workers working days and day only workers. In terms of shift intensity, rotating shift workers working days in a rotation with 3+ consecutive nights had a lower mesor and smaller amplitude than rotating shift workers with <3 consecutive nights. In terms of shift length, there was no difference in any comparison made for those working <12 and \(\geq12\) hours.

After adjusting for *a priori* confounders according to our DAG, rotational shift workers working both days and nights had a 24% and 33% reduction in mesor compared to day only workers. Rotational shift workers working days had a 27% smaller amplitude compared to day only workers. However, one difference lies in the effect estimate of the melatonin production amplitude for shift workers working nights. In the main analysis, shift workers working nights had smaller amplitudes compared to day only workers, but in this sensitivity analysis, no difference was observed in amplitude. For cumulative shift work, effect estimates are similar to the main analysis, where melatonin pattern parameters were not associated with duration of past shift work.
Associations between specific exposure metrics of current shift work and 6-sulfatoxymelatonin were similar to the main analysis. For time of day and shift length, melatonin pattern parameters were not associated with these exposure metrics of current shift work. For shift intensity, rotating shift workers working days in a rotation schedule with 3+ consecutive nights had a 26% lower mesor and a 29% smaller amplitude compared to those working <3 consecutive nights. Another difference from the main analysis is seen in shift workers working nights in a rotation schedule with 3+ consecutive nights. In the main analysis, those working nights with 3+ consecutive nights had a smaller amplitude than those working <3 consecutive nights; but, in this sensitivity analysis, no difference was observed in amplitude.

To conclude, the results were similar, but there were two differences in the adjusted models between the main analysis and this sensitivity analysis. In the main analysis, shift workers working nights and those working nights with higher intensity had lower amplitudes; however, in this sensitivity analysis no associations were found between these shift workers working nights (p=0.06) and those working nights with higher intensity (p=0.07) with melatonin amplitude. These effect estimates were borderline not significant, suggesting that the difference may be due to serial correlation and random effects. It also must be noted that using a mixed model resulted in a slightly smaller sample as some models did not converge. Therefore, there was also slightly less power to detect an association. However, given that mesor and amplitude are still both aspects of magnitude, shift work can still be perceived to affect melatonin production magnitude.
Table 5.1: 6-sulfatoxymelatonin cosinor parameters according to different shift work exposure metrics using a linear mixed model

<table>
<thead>
<tr>
<th>Exposure Metrics</th>
<th>N</th>
<th>Mesor (95% CI)</th>
<th>Amplitude (95% CI)</th>
<th>Acrophase (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crude Geometric Mean (ng/mg)</td>
<td>Adjusted Mean % Change</td>
<td>Crude Geometric Mean (ng/mg)</td>
</tr>
<tr>
<td><strong>Primary Exposures</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current Shift Work Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day worker</td>
<td>156</td>
<td>36.4 (16.4, 56.4)</td>
<td>Ref</td>
<td>58.5 (12.1, 104.9)</td>
</tr>
<tr>
<td>Shift worker on day rotation</td>
<td>159</td>
<td>18.6 (12.7, 24.6)</td>
<td>-24.0 (-40.5, -3.0)&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>25.0 (17.8, 32.2)</td>
</tr>
<tr>
<td>Shift worker on night rotation</td>
<td>154</td>
<td>23.7 (11.6, 35.8)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>-32.5 (-48.1, -12.4)&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>26.2 (15.4, 36.9)&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Cumulative Shift Work</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day worker</td>
<td>156</td>
<td>-</td>
<td>1.0 (-1.2, 3.2)</td>
<td>-</td>
</tr>
<tr>
<td>Shift worker on day rotation</td>
<td>159</td>
<td>-</td>
<td>0.5 (-1.5, 2.7)</td>
<td>-</td>
</tr>
<tr>
<td>Shift worker on night rotation</td>
<td>154</td>
<td>-</td>
<td>1.6 (-1.1, 4.4)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Current Shift Work Domains</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of Day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>156</td>
<td>36.4 (16.4, 56.4)</td>
<td>Ref</td>
<td>58.5 (12.1, 104.9)</td>
</tr>
<tr>
<td>Night</td>
<td>154</td>
<td>23.7 (11.6, 35.8)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>-25.6 (-53.5, 19.0)</td>
<td>26.2 (15.4, 36.9)&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>Shift Length&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shift worker on day rotation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12 hours</td>
<td>26</td>
<td>14.7 (11.6, 17.8)</td>
<td>Ref</td>
<td>18.2 (14.1, 22.4)</td>
</tr>
<tr>
<td>≥12 hours</td>
<td>132</td>
<td>19.2 (12.1, 26.3)</td>
<td>6.7 (-24.1, 49.9)</td>
<td>26.3 (17.7, 35.0)</td>
</tr>
<tr>
<td>Shift worker on night rotation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12 hours</td>
<td>26</td>
<td>19.6 (2.0, 37.2)</td>
<td>Ref</td>
<td>19.4 (7.4, 31.4)</td>
</tr>
<tr>
<td>≥12 hours</td>
<td>127</td>
<td>24.6 (10.3, 38.9)</td>
<td>1.3 (-35.8, 59.9)</td>
<td>27.6 (14.8, 40.4)</td>
</tr>
<tr>
<td>Shift Intensity&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shift worker on day rotation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3 nights</td>
<td>92</td>
<td>23.8 (14.7, 33.0)</td>
<td>Ref</td>
<td>29.0 (17.7, 40.3)</td>
</tr>
<tr>
<td>3+ nights</td>
<td>67</td>
<td>11.5 (5.3, 17.8)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>-26.1 (-42.2, -5.6)&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>19.5 (12.3, 26.7)&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>Shift worker on night rotation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3 nights</td>
<td>88</td>
<td>29.2 (8.7, 49.7)</td>
<td>Ref</td>
<td>33.2 (14.8, 51.6)</td>
</tr>
<tr>
<td>3+ nights</td>
<td>66</td>
<td>16.4 (8.8, 24.0)</td>
<td>-21.1 (-43.3, 9.6)</td>
<td>16.8 (11.5, 22.0)&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Models were adjusted for <i>a priori</i> confounders selected by the DAG, use of sleep aid, as well as the other primary exposure

<sup>b</sup>Models were adjusted for <i>a priori</i> confounders selected by the DAG, use of sleep aid, as well as the other current shift work domains

<sup>c</sup>Shift workers only

<sup>†</sup><i>p<0.05</i> using the Wilcoxon rank-sum test

<sup>‡</sup><i>p<0.05</i> using the Wald test
5.2 Additional Analysis

5.2.1 Menopausal Status as an Effect Modifier

Although menopausal status is not an established interacting factor for shift work and melatonin, it is an etiologic factor for breast cancer. That is, most breast cancer studies stratify by menopausal status. Thus, as an additional analysis, associations between the cosinor parameters and exposure to current shift work were analyzed after stratifying the participants by menopausal status (Table 5.2). Women were classified as postmenopausal if they satisfied one of the following criteria:

1. They had stopped menstruating naturally for at least one year at time of study entry
2. They had stopped menstruating naturally and were over 50 years of age
3. They had stopped menstruating and had had a bilateral oophorectomy
4. They had had a bilateral oophorectomy and were over 55 years of age

For current exposure to shift work, premenopausal shift workers working days had a 30 minute phase delay compared to day only workers, while no difference in mesor and amplitude was seen between the two groups. No difference in any cosinor parameters was observed between shift workers working nights and day only workers among premenopausal women. For postmenopausal women, rotational shift workers working days had a 41.8% lower mesor and 49.2% smaller amplitude, while those working nights had a 54.9% lower mesor and 57.1% smaller amplitude compared to day only workers. No difference in acrophases was seen among shift workers working days or nights and day only workers.

To conclude, these results may suggest that premenopausal women may be able to adapt to new sleep-wake patterns, by not only keeping their melatonin production elevated to normal
levels, but also by being able to delay acrophase closer to sleep onset\textsuperscript{4,5}. The converse is seen in postmenopausal shift workers, as they have lower mesors and smaller amplitudes with no observable phase delay. These results are indicative that the protective role of melatonin against chronic disease may be diminished in postmenopausal women due to hormonal influences on light-at-night effects and night work related circadian disruption.
Table 5.2: Multivariable associations with 6-sulfatoxymelatonin parameters according to current shift work status stratified by menopausal status

<table>
<thead>
<tr>
<th>Current Shift Work</th>
<th>N</th>
<th>Pre</th>
<th>Post</th>
<th>Pre (%)</th>
<th>Post (%)</th>
<th>Pre (%)</th>
<th>Post (%)</th>
<th>Pre (h)</th>
<th>Post (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day worker</td>
<td>78</td>
<td>46</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Shift worker on day rotation</td>
<td>80</td>
<td>41</td>
<td>-9.2 (-31.7, 20.5)</td>
<td><strong>-41.8 (-63.6, -6.9)</strong>‡</td>
<td>-8.4 (-33.6, 26.5)</td>
<td><strong>-49.2 (-69.9, -14.4)</strong>‡</td>
<td>0.5 (0.1, 0.9)‡</td>
<td>-0.1 (-0.9, 0.6)</td>
<td></td>
</tr>
<tr>
<td>Shift worker on night rotation</td>
<td>76</td>
<td>41</td>
<td>-24.3 (-44.0, 2.4)</td>
<td><strong>-54.9 (-72.2, -26.8)</strong>‡</td>
<td>-19.1 (-41.4, 11.6)</td>
<td><strong>-57.1 (-75.1, -26.2)</strong>‡</td>
<td>0.002 (-0.6, 0.6)</td>
<td>-0.8 (-1.8, 0.2)</td>
<td></td>
</tr>
</tbody>
</table>

*Models were adjusted for *a priori* confounders selected by the DAG, use of sleep aid, as well as cumulative shift work exposure

‡p<0.05 using the Wald test
5.3 References


Chapter 6

General Discussion

6.1 Summary of Main Findings

The primary objectives of this thesis were to describe the circadian melatonin pattern in terms of mesor, amplitude and acrophase among female hospital personnel, and to examine associations between current shift work status and past shift work history and circadian melatonin patterns. Additional objectives were to explore the associations of other working time patterns within the context of current shift work – time of day, length of working hours and shift intensity – with circadian melatonin patterns.

6.1.1 Current Shift Work and Circadian Melatonin Patterns

This study found that current rotational shift work was associated with lower mesors and smaller amplitudes when either working days or nights. Sensitivity analyses also showed similar results; however, one difference was that there was no association between shift workers working nights and melatonin amplitude after taking into account serial correlation and normal subject-to-subject variation using a linear mixed model. However, given that mesor and amplitude are still both different aspects of magnitude, rotating shift work was associated with decreased 6-sulfatoxymelatonin production.

For melatonin mesor, our results are consistent with most existing studies, where shift work including nights had been associated with decreased 6-sulfoxymelatonin levels. However, many of these previous studies are confounded by individual circadian rhythms, where comparisons of melatonin levels were made without taking into account differences in circadian...
stage at the time of melatonin assessment. To account for this source of confounding, cosinor analyses were conducted to characterize circadian melatonin patterns based on repeated biosamples of 24-hour working days in order to account for circadian stage in the analysis. In comparison to the few studies that correctly account for differences in circadian stage, our results are similar\(^1,2\). One previous study from Spain observed a similar decrease of 34% in mesor among night workers compared to day workers\(^1\), and one from the USA reported a reduction of 69% during night shifts\(^2\). In our study, although the effect current shift work appears to be more attenuated, this difference may stem from the less intense shift schedule among our participants, as none of them were engaged in permanent night shifts.

Melatonin production amplitudes have not been adequately examined in the literature. One study found that rotating shift workers had a reduction in amplitude (45 versus 80 ng aMT6s/mg creatinine); however, the investigators did not adjust for confounders or statistically assess the difference. Another study looking at 6-sulfatoxymelatonin cosinor parameters did not present the results for 6-sulfatoxymelatonin amplitude; their rational was that amplitude is highly correlated with mesor. In this study, a 29% and 26% reduction of amplitude was found among rotating shift workers on days and nights, respectively, compared to day only workers. This is in accordance with experimental evidence, where exposure to light at night can lead to a decrease in amplitude of the melatonin rhythm.

Few studies have assessed the association between current shift work and 6-sulfatoxymelatonin acrophase. Our study found no difference in acrophase between rotating shift workers and day workers. These results are similar to a previous study from our research group in the same setting that found that rotating shift schedules did not change the timing of peak melatonin production\(^3\). On the other hand, a study from Spain found that rotating shift workers
had a later acrophase (08:31 versus 07:13) compared to day workers; however, the investigators did not adjust for any confounders or statistically assess the difference in timing of peaks\textsuperscript{4}. Also, in this study, a 3-hour delay in peak time was reported among night workers compared to day workers\textsuperscript{1}. However, all participants were engaged in permanent fixed night shifts making it more likely for internal desynchrony of circadian rhythms to occur. These results coincide with current theories on circadian adaptation, where more night shifts, and therefore more exposure to light at night, delay the acrophase closer to sleep onset in order to better align circadian rhythms to a new sleep-wake pattern\textsuperscript{5,6}. However, full adaptation is unlikely to ever occur given that these workers with extreme shift schedules still only experience partial adaptations, where their acrophase was still earlier than sleep onset. Our results provide additional evidence for this concept of adaptation, where no phase shift was seen in timing of peak melatonin production among rotating shift workers, since most of them were only exposed to two consecutive nights. This finding is of paramount importance, as it has been suggested that the adverse effects of shift work stems from the lack of adaptation to new sleep-wake cycles\textsuperscript{7,8}.

6.1.1.1 Current Shift Work Domains and Circadian Melatonin Patterns

This study found that among the three different exposure metrics of current shift work – shift length, time of day and shift intensity – only shift intensity was associated with 6-sulfatoxymelatonin pattern parameters, where working 3+ consecutive nights resulted in lower mesors and smaller amplitudes. The other two domains, time of day and shift length, were not associated with melatonin pattern parameters.

Since current shift work is associated with lower mesor and smaller amplitudes, these results seem to suggest that the change in melatonin pattern does not stem from either the time or
length aspects of current shift work, but instead from shift intensity. These results for shift intensity, in conjunction with the non-association with time of day, provide new evidence that the presence of light at night itself in a rotation is not enough to alter the circadian rhythm of melatonin, but rather it is the exposure to light at night with higher intensity (longer sequence of consecutive night shifts) that likely affects circadian melatonin patterns. This observation helps support the evidence in the existing breast cancer literature by demonstrating at a biomarker level the mechanism suggested from experimental research at the population level: breast cancer risk increases by 3% for every 5-years of shift work, while the increase in risk is 80% for every 5 years of shift work with 6+ consecutive nights in a rotation. Therefore, we can identify shift intensity as an aspect of current rotational shift work that alters circadian melatonin patterns.

6.1.2 Cumulative Shift Work and Circadian Melatonin Patterns

In terms of past shift work history, cumulative exposure to shift work was not associated with any 6-sulfatoxymelatonin parameters among both day only workers and rotating shift workers. Both sensitivity analyses showed similar results. One possible reason for the lack of an association among current shift workers is that long-term rotational schedules may not be enough to change melatonin production patterns. Most rotational patterns consist of two 12-hour days, two 12-hour nights, followed by five days off. By working only two nights within a nine day period, there is plenty of time to readjust to conventional daytime schedules of activity during the day and sleep at night.
6.1.3 Interaction by Menopausal Status

For exposure to current shift work, no difference in 6-sulfatoxymelatonin mesor and amplitude was observed between premenopausal shift workers and day only workers, suggesting that the premenopausal state may be able to resist the direct effects of light at night and therefore, reduce its adverse effects by keeping melatonin production elevated to a normal level. In addition, premenopausal shift workers working days had a 30 minute phase delay compared to day only workers. This may suggest that premenopausal women may be able to adapt to new sleep-wake patterns\textsuperscript{5,6}. The ability to delay acrophase closer to sleep onset has been suggested to be a form of adaption to shift work, in order to better align circadian rhythms and reduce the adverse effects of internal desynchrony\textsuperscript{7,8}. However, in premenopausal women working nights, this phase shift was not observed. One possible reason may be inadequate power to detect an association due to a reduced sample size after stratification by menopausal status. The converse is seen in postmenopausal shift workers, as they have lower mesors and smaller amplitudes with no observable phase delays. With melatonin possessing cancer and cardiovascular protective effects\textsuperscript{10–15}, its protective role against chronic disease is diminished in postmenopausal women. In addition to lower production, no phase shift was observed in shift workers working either days or nights, suggesting that postmenopausal women are not as able to adapt to their new work schedule unlike their premenopausal counterparts. By not being able to phase delay their peak production time closer to their sleep onset, these women have circadian rhythms that are out of sync. This misalignment of circadian rhythms has been suggested to be the cause of many adverse effects which may predispose individuals to chronic disease\textsuperscript{7,8}.

These results show that premenopausal hormones may play a protective role against shift work and/or light at night exposure, as melatonin production does not change, and the ability to
partially adapt can be seen through phase delays. Thus, menopausal status at exposure to shift work may play an important role in the etiology of chronic disease. This notion has already been suggested in the breast cancer literature, where age at exposure (not menopausal status per se) may be associated with risk. If this is indeed the case, then future studies should then try and confirm these findings.

6.2 Strengths

The main strength of this study is the observational nature of this research: data collection occurred while women were working or were at home in routine conditions, and not in a controlled experimental setting that does not reflect reality. Another major strength is collection of all biosamples over two 24-hour working days that enabled detailed characterization of circadian melatonin patterns. Analysis with cosinor functions captures the richness of these data. This takes into account the role of circadian variations of melatonin production in rotating shift workers when making comparisons between those working days and those working nights, an idea that has not been emphasized in previous work. Since we can describe the daily patterns of melatonin, we can see time of peak production regardless of shift schedule, and therefore comparisons were not confounded by an individual’s circadian rhythm. Another strength is the use of many different exposure metrics of shift work for a more precise examination of the exposure. A limitation of existing studies is in the definition of shift work, which generally refers to work schedules that differ from conventional daytime schedule of activity during the day and sleep at night. Since this definition is so broad and unrestrained with regards to specific work characteristics, its assessment in existing studies have been both imprecise and incomplete. Most studies have used either current status or duration as exposures to shift work without
considering other occupational domains relevant to the exposure, including shift length, time of
day, shift intensity and social aspects of working hours. This may be erroneous as these aspects
of shift work may actually be separate risk factors for disease. Thus, there is a gap in the
literature with regards to other occupational domains. In this study, due to the observation nature
of biosample collection as well as the ascertainment of a complete lifetime occupational history,
we were able to analyze specific exposure metrics of current shift work – shift length, time of
day and shift intensity – for a more precise and complete assessment of the exposure.

This study also contributes a more careful and complete assessment of confounders
regarding the relationship between shift work and melatonin. Confounding is generally
considered an important factor in studies on shift work and was one of the reasons why the
International Agency for Research on Cancer (IARC) working group classified the
epidemiological findings available at the time as only limited\textsuperscript{17}. It should be noted that in most
studies looking at this relationship, control for confounding was either absent, inadequate or
unsystematic across studies. Adjustment for confounding is extremely important and only one
paper to date has discussed the selection of confounders using Directed Acyclic Graph (DAG)\textsuperscript{1} –
which was the method implemented in this study. \textit{A priori} confounders such as age, parity, age of
first birth, education and chronotype were carefully selected using a DAG.

Finally, the use of mid-sleep time (MSF) from the Munich Chronotype Questionnaire
(MCTQ) to measure chronotype is an improvement over the Horne-Ostberg questionnaire as
MSF more appropriately reflects the nature of the variable. Because chronotype depends on
light-dark cycles and the geographical location someone is living in using MSF reflects more
accurately the nature of the variable. The MCTQ has been validated, where the mid-sleep time
has been shown to be a valid measure of chronotype\textsuperscript{18,19}. 

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6.3 Limitations

Despite the strengths, this study also has several limitations. Four different aspects of current shift work have been outlined to be different facets of current exposure: shift length, time of day, shift intensity and social aspects of working hours\textsuperscript{16}. Due to the way the study was designed, we analyzed three out of the four exposure metrics, but we were not able to examine the social aspects of working hours. Information regarding the distribution of free days, irregularity and predictability of working hours was not collected. Since there is evidence to show that control of working time is associated with health\textsuperscript{20}, future studies should consider quantifying this exposure metric in addition to the other three in order to better evaluate the specifics of exposure to shift work.

Another limitation is the inadequate sample size to analyze interactions. Despite this limitation, an exploratory analysis for effect modification by menopausal status was attempted for exposure to current shift work as it is an etiologic factor for breast cancer. This analysis was conducted only for current exposure for shift work as this was the only exposure metric to use the full sample. This analysis could not be done for any of the specific exposure metrics of current shift work – shift length, time of day, shift intensity – as the sample had to be stratified first by shift work status, then stratified again by menopausal status. Double stratification produced a reduced sample size, which limited the power of the study to detect any associations and/or effect modification.

In this thesis, we are looking at shift work as a proxy for light at night, but in fact, it is more accurately a reflection of shift work itself as a cause of circadian disruption. Thus, one potential limitation is that we were not able to analyze the direct effects of light at night on melatonin rhythms. However, our research group has collected objective light measurements
using accelerometers. These have not yet been analyzed in the context of shift work and melatonin, but they will be in the near future to improve both internal and external validity. Its incorporation into future studies is the next step in the natural progression of this project.

6.4 Future Directions

The results of this thesis suggest that a rotational pattern of shift work, specifically the intensity of the rotation, is associated with depressed melatonin rhythms. Future studies should collect and incorporate information on the irregularity and predictability of working hours at the design stage, where it has been suggested that the number of annual leave days, number of weekend work and number of single free days may be appropriate variables to reflect this exposure metric\(^{16}\). Future studies should also try and recruit shift workers with more extreme rotational schedules, although it may not be ethically feasible as employers are also trying to develop healthier shift schedules. The typical schedule in Canada is two 12-hour nights, two 12-hour days and then five days off; thus reducing the variability in the exposure metrics of shift intensity and shift length. In the breast cancer literature, one study has shown that shift workers who work \(\geq 5\) years with \(\geq 6\) consecutive night shifts have an increased risk of breast cancer\(^9\); however such an intensity could not be analyzed in this study population as most people worked either 2 or 3 consecutive nights. Much like shift intensity, there was limited variability in shift length, where most shifts were 12 hours long. Therefore, due to the limited variability in exposure this thesis could not fully examine the effects of shift intensity and shift length, as these results cannot be directly applied to other shift work schedules such as those with permanent night shifts or longer rotation patterns – and it is possible that the effects of these types of schedules could be different.
In the additional results chapter, there is suggestive evidence that there may be potential effect modification by menopausal status, in that age at exposure to shift work may play an important role in the etiology of chronic disease. Premenopausal shift workers were able to keep their melatonin production elevated to normal levels while also showing signs of partial adaptation to new sleep-wake cycles by phase delaying their circadian rhythm. On the other hand, postmenopausal women showed decreased melatonin production, while also showing no observable ability to adapt to new sleep-wake patterns as there was no change in acrophase. Interactions with specific aspects of current shift work – shift length, time of day, shift intensity – could not be analyzed due to inadequate power as stratification, first by shift work status then by menopausal status, reduced the sample size. For example, when stratifying the shift length exposure metric twice, there were only two postmenopausal participants who worked on average <12 hour shifts. So in addition to recruiting shift workers with more extreme rotational schedules, future studies should also try to recruit more postmenopausal shift workers in order to examine potential interaction with other exposure metrics of current shift work.

Finally, even though the melatonin pathway is the main hypothesis linking shift work with chronic disease, there may be several other pathways operating between exposure and disease\textsuperscript{21}. Future studies should therefore try collect and incorporate information of other pathways beyond melatonin, such as sleep quality and vitamin D levels. It will be useful to take these pathways into account in the analysis as it could improve understanding of shift work characteristics that might lead to chronic disease. Since shift work is a necessary component of many occupations – about one third of the Canadian labor force is engaged in this work pattern\textsuperscript{22} - understanding which shift pattern or what it is about shift work that may increase cancer risk is important to the development of healthy workplace policy.
6.5 Contribution of Research and Conclusions

The overall aim of this thesis was to describe the circadian melatonin pattern according to different shift work exposure metrics as well as to evaluate the associations of these metrics with these patterns. The 48 hour observational urine collection allowed for the characterization of circadian pattern through cosinor analyses, which improves upon many previous studies which may have been confounded by circadian rhythm. This novel and precise method of outcome assessment allowed the study to specifically investigate different pattern parameters: mesor, amplitude and acrophase which may be different risk factors for chronic disease – an improvement over many previous studies that compared only mean levels.

The utilization of different exposure metrics of current shift work is also an improvement over previous studies, as it helps clarify which aspects of shift work may increase the risk of chronic disease. In this study, we found that it was the intensity of rotation, rather than the time of day or length of working hours, that actually affects circadian melatonin patterns. This is new evidence in the context of biomarker studies, as it helps strengthen the evidence for the role of melatonin in chronic disease. From the perspective of public health, by demonstrating at a biomarker level the mechanisms suggested from experimental research at the population level, these results can help guide intervention strategies and healthy workplace policies.
6.6 References


Appendix A

Ethics

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD-DELEGATED REVIEW

October 17, 2014

Mr. Michael Leung
Department of Public Health Sciences
Queen’s University

Dear Mr. Leung

Study Title: EPID-486-14 The influence of shiftwork on melatonin profiles in female hospital workers
File # 6013962
Co-Investigators: Dr. K. Aronson, Dr. J. Tranmer

I am writing to acknowledge receipt of your recent ethics submission. We have examined the protocol for your project (as stated above) and consider it to be ethically acceptable. This approval is valid for one year from the date of the Chair’s signature below. This approval will be reported to the Research Ethics Board. Please attend carefully to the following listing of ethics requirements you must fulfill over the course of your study:

**Reporting of Amendments:** If there are any changes to your study (e.g., consent, protocol, study procedures, etc.), you must submit an amendment to the Research Ethics Board for approval. Please use event form: HSREB Multi-Use Amendment/Full Board Renewal Form associated with your post review file # 6013962 in your Researcher Portal (https://eservices.queensu.ca/romeo_researcher/)

**Reporting of Serious Adverse Events:** Any unexpected serious adverse event occurring locally must be reported within 2 working days or earlier if required by the study sponsor. All other serious adverse events must be reported within 15 days after becoming aware of the information. Serious Adverse Event forms are located with your post-review file 6013962 in your Researcher Portal (https://eservices.queensu.ca/romeo_researcher/)

**Reporting of Complaints:** Any complaints made by participants or persons acting on behalf of participants must be reported to the Research Ethics Board within 7 days of becoming aware of the complaint. Note: All documents supplied to participants must have the contact information for the Research Ethics Board.

**Annual Renewal:** Prior to the expiration of your approval (which is one year from the date of the Chair’s signature below), you will be reminded to submit your renewal form along with any new changes or amendments you wish to make to your study. If there have been no major changes to your protocol, your approval may be renewed for another year.

Yours sincerely,

[Signature]

Chair, Health Sciences Research Ethics Board
October 17, 2014

Investigators please note that if your trial is registered by the sponsor, you must take responsibility to ensure that the registration information is accurate and complete
QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD

The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards and operates in compliance with the Tri-Council Policy Statement; Part C Division 5 of the Food and Drug Regulations, OHRP, and U.S DHHS Code of Federal Regulations Title 45, Part 46 and carries out its functions in a manner consistent with Good Clinical Practices.

Federalwide Assurance Number: #FWA00004184, #IRB00001173

Current 2014 membership of the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board:

Dr. A.F. Clark, Emeritus Professor, Department of Biomedical and Molecular Sciences, Queen's University (Chair)

Dr. H. Abdollah, Professor, Department of Medicine, Queen's University

Dr. R. Brison, Professor, Department of Emergency Medicine, Queen's University

Dr. M. Evans, Community Member

Ms. J. Hudacin, Community Member

Mr. D. McNaughton, Community Member

Ms. S. Rohland, Privacy Officer, ICES-Queen's Health Services Research Facility, Research Associate, Division of Cancer Care and Epidemiology, Queen's Cancer Research Institute

Dr. M. Sawhney, Assistant Professor, School of Nursing, Queen's University

Dr. A. Singh, Professor, Department of Psychiatry, Queen's University

Dr. J. Walia, Assistant Professor and Clinical Geneticist, Department of Paediatrics, Queen's University and Kingston General Hospital

Ms. K. Weisbaum, LL.B. and Adjunct Instructor, Department of Family Medicine (Bioethics)

Dr. J. Whiteley, Community Member
Appendix B

Questionnaire Shift

Study # ____________

Date ________________

Sponsored by:

CIHR IRSC

Shift work and cardiovascular risk in working women

Project Manager
Carly Kelly
Phone: 613-549-6666, ext 2869
carly.kelly@queensu.ca

Kingston General Hospital
Nursing Research Unit
76 Stuart St.
Kingston, ON, K7L 2V7

Queen's University
BACKGROUND INFORMATION

1. What is your marital status?
   - Married
   - Living common-law
   - Widowed
   - Separated
   - Divorced
   - Single, never married

2. How many children do you have? ____________

3. What is the highest level of education you have obtained?
   - High School
   - Post Secondary (Certificate/Diploma)
   - University Undergraduate Degree
   - Graduate Degree (Master, PhD, etc)
   - Physician or Resident
   - Other ________________

GENERAL HEALTH
The next few questions relate to your general health and well-being.

1. Do you currently use any form of oral contraception?
   a. Yes
   b. No

2. Have your natural menstrual periods ceased?
   a. Yes
   b. No

3. If Yes for question 3: For what reason has your periods ceased?
   a. Natural menopause (Periods have ceased for 12 consecutive months)
   b. Surgical
   c. Other (radiation/condition): ____________________________

4. Are you currently taking Hormone Replacement Therapy to treat menopausal symptoms?
   a. Yes
   b. No

5. How many times have you been pregnant? (This would include any losses as well)
   ____________
6. How many full-term births have you had? _________

7. How many pre-term births have you had? _________

8. How many spontaneous or therapeutic losses have you experienced? _________

9. Please fill in the below table regarding any pregnancy related conditions you may have experienced during all of your pregnancies.

Please state the date of birth (DOB) as Year/Month/Date (i.e. 75/08/26)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preeclampsia</td>
<td>1  2  3  4</td>
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<tr>
<td>Gestational Hypertension (hypertension during pregnancy)</td>
<td>DOB  DOB  DOB  DOB</td>
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<tr>
<td>Gestational Diabetes or Gestational Impaired Glucose Tolerance</td>
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<tr>
<td>Placental Abruption</td>
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<td>Pre-term Birth (&lt; 37 weeks)</td>
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<tr>
<td>Intrauterine growth restriction (your baby weighed much less than expected for their gestational age, i.e., &lt; 2500 gms for a term birth)</td>
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</tbody>
</table>
10. Please fill in the below table indicating any of the long term conditions listed that have lasted or been expected to last 6 months or more and were diagnosed by a Health Care Professional.

Please indicate the date of first diagnosis (Year/Month/Date - 75/08/26)

<table>
<thead>
<tr>
<th>Long Term Conditions</th>
<th>Date of Diagnosis</th>
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<tbody>
<tr>
<td>Allergies</td>
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<tr>
<td>Asthma</td>
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<tr>
<td>Fibromyalgia</td>
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<tr>
<td>Arthritis or osteoporosis (excluding fibromyalgia)</td>
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</tr>
<tr>
<td>Back problems, excluding fibromyalgia and arthritis</td>
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<tr>
<td>Migraine headaches</td>
<td></td>
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<tr>
<td>Cancer</td>
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<tr>
<td>Stomach or intestinal ulcers</td>
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<tr>
<td>A sleep disorder (such as sleep apnea)</td>
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<tr>
<td>A bowel disorder (such as Crohn’s Disease or colitis)</td>
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<tr>
<td>A thyroid condition</td>
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<td>Chronic fatigue syndrome</td>
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<td>Neurological disease</td>
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<td>Depression</td>
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<td>Anxiety or Panic Disorder</td>
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<td>Lung Disease</td>
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<td>Other</td>
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11. Do you have a history of and/or take medication for any of the following? Please indicate the date of diagnosis on the space provided.
   a. High blood pressure ________________________________
   b. High cholesterol levels ___________________________
   c. Diabetes __________________________________________
   d. Dyslipidemia _______________________________________

12. Do you have a family history of premature heart disease or any of the following conditions?
   a. High blood pressure    Yes  No
   b. High cholesterol levels Yes  No
   c. Diabetes               Yes  No
   d. Dyslipidemia            Yes  No

13. Did either of your parents have a heart attack before the age of 60?
   a. Yes
   b. No
14. What is your smoking status?
   a. Currently smoke
   b. Quit recently (4 weeks to 5 years ago)
   c. Quit remotely (over 5 years ago)
   d. Never smoked.

15. Does anyone in your household smoke regularly inside the house?
   a. Yes
   b. No

16. During the past 12 months, how often did you drink alcoholic beverages?
   a. Never / Do not Drink
   b. Less than once a month
   c. Once a month
   d. 2-3 times a month
   e. Once a week
   f. 2-3 times a week
   g. 4-6 times a week
   h. Everyday

17. How often in the past 12 months have you had 5 or more drinks on one occasion?
   a. Never
   b. Less than once a month
   c. Once a month
   d. 2-3 times a month
   e. Once a week
   f. More than once a week

18. List below the medications you are taking, the dose, and how often you take the medication.
    Please copy this information from your pill bottle label. If you need more space you may need to use the back of the page.

<table>
<thead>
<tr>
<th>Name of Medication</th>
<th>Dose</th>
<th>How many times a day do you take this medication?</th>
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Version: October 14, 2011
### NUTRITION

In this section we would like to get an idea of your eating habits. Please check the box that best describes your habits in an average week.

<table>
<thead>
<tr>
<th>Topic</th>
<th>In an average week, how often do you:</th>
<th>Usually/Often</th>
<th>Sometimes</th>
<th>Rarely/Never</th>
</tr>
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<tbody>
<tr>
<td><strong>Meals</strong></td>
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<tr>
<td>1. Skip breakfast?</td>
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<tr>
<td>2. Eat 4 or more meals from sit-down or take out restaurants?</td>
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<tr>
<td><strong>Grains</strong></td>
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<tr>
<td>3. Eat less than 6 servings of whole grain products a day? Serving = 1 slice of 100% whole grain bread; 1 cup whole grain cereal like Shredded Wheat, Wheaties, Grape Nuts, high fiber cereals, oatmeal, 3-4 whole grain crackers, 1/2 cup brown rice or whole wheat pasta, 1 sm. muffin, 1 1/2 bagel or pita.</td>
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<tr>
<td><strong>Fruits &amp; Vegetables</strong></td>
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<td>4. Eat or drink less than 7 servings of fruits and/or vegetables a day? Serving= 1/2 cup or 1 med fruit or 4 oz 100% juice, 1/2 cup vegetables, or 1 cup raw leafy vegetables.</td>
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<td><strong>Milk &amp; Alternatives</strong></td>
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<td>5. Eat or drink less than 2-3 servings of milk yogurt or cheese a day? Serving= 1 cup milk or 3/4 cup yogurt; 1.5-2 oz. Cheese.</td>
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<tr>
<td>6. Use 2% (reduced fat) or whole milk instead of skim (non-fat) or 1% (low-fat) milk?</td>
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<td>7. Use regular cheese (American, cheddar, Swiss, Monterey Jack) instead of low fat or part skim cheeses as a snack, on sandwiches pizza, etc.?</td>
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<td><strong>Meats &amp; Alternatives</strong></td>
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<tr>
<td>8. Eat beef, pork, or dark meat chicken more than 2 times a week?</td>
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</tr>
<tr>
<td>9. Eat more than 6 oz. (50-100g) of meat, chicken, turkey or fish per day? Note: 3 oz of meat or chicken is the size of a deck of cards or one of the following: 1 regular hamburger, 1 chicken breast or leg (thigh &amp; drumstick), or one pork chop, 1/3 cup tofu, 1-2 eggs, 2 tbsp Peanut Butter.</td>
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<tr>
<td>10. Choose higher fat red meats like prime rib, T-bone steak, hamburger, ribs, etc. Instead of lean red meats.</td>
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<tr>
<td>11. Eat the skin on the chicken and turkey or the fat on meat?</td>
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</tr>
<tr>
<td>Topic</td>
<td>In an average week, how often do you:</td>
<td>Usually/ Often</td>
<td>Sometimes</td>
<td>Rarely/ Never</td>
</tr>
<tr>
<td>-----------------------</td>
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</tr>
<tr>
<td>Meats &amp; Alternatives</td>
<td>12. Use <strong>regular processed meats</strong> (bologna, salami, corned beef, hotdogs, sausage or bacon) instead of low fat processed meats (roast beef, turkey, lean ham, low-fat cold cuts/hotdogs)?</td>
<td></td>
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<tr>
<td>Fried Foods</td>
<td>13. Eat <strong>fried foods</strong> such as fried chicken, fried fish or french fries?</td>
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<td></td>
<td>14. Eat <strong>regular potato chips, nacho chips, corn chips, crackers, regular popcorn, nuts</strong> instead of pretzels, low-fat chips or low-fat crackers, air-popped popcorn?</td>
<td></td>
<td></td>
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<tr>
<td>Fats and Oils</td>
<td>15. Use <strong>regular salad dressing &amp; mayonnaise</strong> instead of low-fat or fat-free salad dressing and mayonnaise?</td>
<td></td>
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<tr>
<td></td>
<td>16. Add <strong>butter, margarine or oil</strong> to bread, potatoes, rice or vegetables on the table?</td>
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<tr>
<td></td>
<td>17. <strong>Cook with oil, butter, or margarine</strong> instead of using non-stick sprays like Pam or cooking without fat?</td>
<td></td>
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<tr>
<td>Sweets</td>
<td>18. Eat <strong>regular sweets</strong> like cake, cookies, pastries, donuts, muffins, and chocolate instead of low fat or fat-free sweets?</td>
<td></td>
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<tr>
<td></td>
<td>19. Eat <strong>regular ice cream</strong> instead of sherbet, sorbet, low-fat or fat-free ice cream, frozen yogurt, etc.?</td>
<td></td>
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<tr>
<td></td>
<td>20. Eat <strong>sweets</strong> like cake, cookies, pastries, donuts, muffins, chocolate and candies more than 2 times per day.</td>
<td></td>
<td></td>
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<tr>
<td>Soft drinks</td>
<td>21. Drink <strong>16 oz or more of non-diet soda, fruit drink/punch or Kool-Aid a day?</strong></td>
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<tr>
<td></td>
<td><strong>Serving = 1 can of soda = 12 oz.</strong></td>
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<tr>
<td>Sodium</td>
<td>22. Eat high sodium processed foods like canned soup or pasta, frozen packaged meals (TV dinners, etc.) chips?</td>
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<td></td>
<td>23. Add salt to foods during cooking or at the table?</td>
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<tr>
<td>Caffeine</td>
<td>24. Drink <strong>more than 3 caffeinated beverages a day?</strong></td>
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<tr>
<td></td>
<td><strong>Serving = 100 mg caffeine = 1 cup (8oz.) of coffee.</strong></td>
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<td></td>
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<tr>
<td>Topic</td>
<td>Question</td>
<td>Usually/Often</td>
<td>Sometimes</td>
<td>Rarely/Never</td>
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<td>--------------------------------------------------------------------------</td>
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<tr>
<td>Alcohol</td>
<td>25. Drink more than 1-2 alcoholic drinks a day? <strong>One drink = 12 oz beer, 5 oz wine, one shot of hard liquor or mixed drink with one shot.</strong></td>
<td></td>
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<tr>
<td>Activity</td>
<td>26. Do less than 30 minutes of physical activity 3 days a week or more? (Examples: walking briskly, gardening, golf, jogging, swimming, biking, dancing, etc.)</td>
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<td></td>
<td>27. Watch more than 2 hours of television or videos a day?</td>
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</tr>
</tbody>
</table>

28. Do you usually shop and prepare your own food?
   a. Yes
   b. No

29. Due to time constraints for responsibilities do you ever have trouble being able to shop or cook?
   a. Yes
   b. No

30. Do you follow a special diet, eat or limit certain foods for health or other reasons
   a. Yes
   b. No

31. How able are you to make changes in what, how or how much you eat in order to eat healthier? (Circle the number that best describes how you feel)

<table>
<thead>
<tr>
<th>Very able</th>
<th>Not at all able</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
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<tr>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

32. Are you currently enrolled in any programs involving food or weight management?
   a. Yes
   b. No

If so, describe ________________________________
CHRONOTYPE INFORMATION
In this section, we are interested in your “chronotype”
(i.e. when you go to bed and when you wake up)

I have a regular schedule (this includes being for example, a housewife):
☐ Yes…I work on… 1□ 2□ 3□ 4□ 5□ 6□ 7□ days/per week.
☐ No

(If your answer is “Yes, on 7 days” or “No”, please again think, if your sleep times may
differ between regular “Workdays” and “Weekend days”, anyhow and fill out the MCTQ
in this in this respect)

Please use a 24-hour time scale (e.g. 23:00 instead of 11pm)

Workdays
I go to bed at ________ o’clock.
(Note that some people stay awake for some time while in bed)

I actually get ready to fall asleep at ________ o’clock

I need ________ minutes to fall asleep

I wake up at ________ o’clock

After ________ minutes, I actually get up.
I use an alarm clock on workdays
☐ Yes
☐ No

If “yes”, I regularly wake up BEFORE the alarm rings
☐ Yes
☐ No

Free Days
I go to bed at ________ o’clock.
(Note that some people stay awake for some time while in bed)

I actually get ready to fall asleep at ________ o’clock

I need ________ minutes to fall asleep

I wake up at ________ o’clock

After ________ minutes, I actually get up.

My wake-up time is due to the use of an alarm clock
☐ Yes
☐ No
Study # ____________________

There are particular reasons why I CANNOT freely choose my sleep times on free days
☐ Yes
☐ No

If "yes", is it:
☐ Children/pets
☐ Hobbies
☐ Others, for example ______________________

**Time Spent Outdoors**

On average, I spend the following amount of time outdoors in daylight (without a roof above my head):

*In the spring/summer:*

On Workdays ________ hours ________ minutes
On Free Days ________ hours ________ minutes

*In the fall/winter:*

On Workdays ________ hours ________ minutes
On Free Days ________ hours ________ minutes

**Work Details**

In the last 3 months, I worked as a shift worker.
☐ Yes (please continue at "My work schedules are...")
☐ No

My usual work schedule...
...starts at ________ o'clock
...ends at ________ o'clock

My work schedules are...
☐ very flexible
☐ a little flexible
☐ rather inflexible
☐ very inflexible

I travel to work...
☐ Within an enclosed vehicle (e.g. car, bus)
☐ NOT within an enclosed vehicle (e.g. on foot, by bike)
☐ I work at home

10
Version: October 14, 2011
For the commute to work I need _______ hours and _______ minutes
For the commute home I need _______ hours and _______ minutes

**Stimulants**
*Please give approximate/average amounts!*

<table>
<thead>
<tr>
<th>per</th>
<th>Day</th>
<th>Week</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>I smoke _______ cigarettes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I drink _______ glasses of beer…</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I drink _______ glasses of wine…</td>
<td></td>
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<tr>
<td>I drink _______ glasses of liquor…</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I drink _______ cups of coffee…</td>
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<tr>
<td>I drink _______ cans of caffeinated drinks (soda)…</td>
<td></td>
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<tr>
<td>I drink _______ cups of black tea…</td>
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<tr>
<td>I take sleep medication _______ times…</td>
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</tbody>
</table>

**WORK ENVIRONMENT**
*In this section, we are interested in the characteristics of your employment at KGH or HDH.*

1. What is your current position? ________________________

2. In what year did you start working in your current position? _______

3. Is this position permanent, temporary, casual, etc?
   a. Permanent
   b. Temporary
   c. Casual / on call
   d. Other: ____________________________

4. Do you currently do shift work or night work?
   [ ] Yes
   [ ] No

5. Are you working full-time or part-time?
   a. Full-time
   b. Part-time
6. If working part-time, what is/are the reason(s) for working part-time? (Circle all that apply)
   a. Prefer fewer hours
   b. Full-time positions are not available
   c. Could not find full-time work in area of specialization
   d. Flexible work hours
   e. Have another position outside of the hospital
   f. Under-qualified for a full-time position
   g. Full-time positions are too demanding
   h. Do not want to work shift work
   i. Own illness or disability
   j. Caring for own children
   k. Caring for elderly relative
   l. Going to school
   m. Other (please specify): ________________________________

7. How many paid hours do you usually work per week? ____________

8. How many hours of paid overtime do you usually work per week? ____________

9. How many hours of unpaid overtime/extra time do you usually work per week? _____

10. Do you usually work?
    a. 8 hour shifts
    b. 12 hour shifts
    c. Various shifts
    d. Some other shift (please describe): ________________________________

11. Do you usually work days, evenings, or nights?
    a. Days
    b. Evenings
    c. Nights
    d. Mixed

12. What level of skill is REQUIRED on your job in terms of years of formal training? (Not necessarily the same as your education)
    a. Elementary education only
    b. High school graduate
    c. Post secondary education (certificate/diploma)
    d. University/undergraduate degree
    e. Graduate degree (Masters/PhD, etc)
    f. MD or resident
    g. Other (please specify): ________________________________
13. Please mark your pre-tax household income for the past year:
- [ ] less than 15,000
- [ ] 15,000 to 19,999
- [ ] 20,000 to 29,999
- [ ] 30,000 to 39,000
- [ ] 40,000 to 49,999
- [ ] 50,000 to 74,000
- [ ] 75,000 to 99,999
- [ ] 100,000 to 150,000
- [ ] > 150,000

**REGARDING YOUR CURRENT POSITION, PLEASE CHECK THE BOX IN THE MOST APPROPRIATE CATEGORY FOR THE STATEMENTS LISTED IN TABLES 1-8**

**TABLE 1.**

<table>
<thead>
<tr>
<th>Statement</th>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. My job requires that I learn new things.</td>
<td></td>
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<tr>
<td>b. My job involves a lot of repetitive work.</td>
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<td>c. My job requires me to be creative.</td>
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<tr>
<td>d. My job requires a high level of skill.</td>
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<tr>
<td>e. I get to do a variety of different things in my job.</td>
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<tr>
<td>f. I have an opportunity to develop my own special abilities.</td>
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</tbody>
</table>
### TABLE 2.

<table>
<thead>
<tr>
<th>Statement</th>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. My job allows me to make a lot of decisions on my own.</td>
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<tr>
<td>b. On my job I have very little freedom to decide how I do my work.</td>
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<tr>
<td>c. I have a lot to say about what happens on my job.</td>
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<tr>
<td>d. I have a significant influence over decisions in my work group or unit.</td>
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<tr>
<td>e. My work group or unit makes decisions democratically.</td>
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<tr>
<td>f. I have at least some chance that my ideas will be considered about company policy (e.g., hiring, firing, wage levels, plants closing, new machinery, etc.).</td>
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<tr>
<td>g. My union or employee association is influential in affecting company policy.</td>
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<tr>
<td>h. Have influence over the policies of the union or employee association.</td>
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</tbody>
</table>

13. How many people are in your work group or unit?
   a. I work alone
   b. 2-5 people
   c. 6-10 people
   d. 10-20 people
   e. >20 people

14. I supervise people as a part of my job.
   a. No
   b. Yes, 1-4 people
   c. Yes, 5-10 people
   d. Yes, 11-20 people
   e. Yes, more than 20 people

15. I am a member of a union or employee association.
   a. Yes
   b. No
### TABLE 3.

<table>
<thead>
<tr>
<th></th>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>My job requires working very fast.</td>
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<tr>
<td>b.</td>
<td>My job requires working very hard.</td>
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<tr>
<td>c.</td>
<td>I am not asked to do an excessive amount of work.</td>
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<tr>
<td>d.</td>
<td>I have enough time to get the job done.</td>
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<tr>
<td>e.</td>
<td>I am free from conflicting demands that others make.</td>
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<tr>
<td>f.</td>
<td>My job requires long periods of intense concentration on the task.</td>
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<tr>
<td>g.</td>
<td>My tasks are often interrupted before they can be completed, requiring attention at a later time.</td>
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<tr>
<td>h.</td>
<td>My job is very hectic.</td>
<td></td>
<td></td>
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<tr>
<td>i.</td>
<td>Waiting on work from other people or departments often slows me down on the job.</td>
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</tbody>
</table>

### TABLE 4.

<table>
<thead>
<tr>
<th></th>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>My job requires lots of physical effort.</td>
<td></td>
<td></td>
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<tr>
<td>b.</td>
<td>I am often required to move or lift very heavy loads on my job.</td>
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<tr>
<td>c.</td>
<td>My work requires rapid and continuous physical activity.</td>
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<tr>
<td>d.</td>
<td>I am often required to work for long periods with my body in physically awkward positions.</td>
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<td></td>
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<tr>
<td>e.</td>
<td>I am required to work for long periods with my head or arms in physically awkward positions.</td>
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</tbody>
</table>
### TABLE 5.

<table>
<thead>
<tr>
<th></th>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. My job security is good.</td>
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<tr>
<td>b. My prospects for career development and promotions are good.</td>
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<tr>
<td>c. In five years my skills will still be valuable.</td>
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</tbody>
</table>

16. How steady is your work? (check one ONLY)
   a. Regular and steady
   b. Seasonal
   c. Frequent layoffs
   d. Both seasonal and frequent layoffs
   e. Other: ___________________________

17. During the past year, how often were you in a situation where you faced job loss or layoff?
   a. Never
   b. Faced the possibility once
   c. Faced the possibility more than once
   d. Constantly
   e. Laid off

18. Sometimes people permanently lose jobs they want to keep. How likely is it that during the next couple of years you will lose your present job with your employer?
   a. Not likely at all
   b. Not too likely
   c. Somewhat likely
   d. Very likely

19. In the next 12 months, do you plan to leave your current position?
   a. Yes
   b. No
### TABLE 6.

<table>
<thead>
<tr>
<th></th>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. My supervisor is concerned about the welfare of those under him/her.</td>
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<tr>
<td>b. My supervisor pays attention to what I am saying.</td>
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<tr>
<td>c. I am exposed to hostility or conflict from my supervisor.</td>
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<tr>
<td>d. My supervisor is helpful in getting the job done.</td>
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<tr>
<td>e. My supervisor is successful in getting people to work together.</td>
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</table>

### TABLE 7.

<table>
<thead>
<tr>
<th></th>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. People I work with are competent in doing their jobs.</td>
<td></td>
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<tr>
<td>b. People I work with take a personal interest in me.</td>
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<td></td>
</tr>
<tr>
<td>c. I am exposed to hostility or conflict from the people I work with.</td>
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<tr>
<td>d. People I work with are friendly.</td>
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<tr>
<td>e. The people I work with encourage each other to work together.</td>
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</tr>
<tr>
<td>f. People I work with are helpful in getting the job done.</td>
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</tbody>
</table>
TABLE 8.

<table>
<thead>
<tr>
<th></th>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. I often have to arrive early or stay late to get my work done.</td>
<td></td>
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<tr>
<td>b. I often have to work through my breaks to complete my assigned workload.</td>
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<tr>
<td>c. It often seems like I have too much work for one person to do.</td>
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<tr>
<td>d. I have too much to do, to do everything well.</td>
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</tbody>
</table>

20. If you are planning on leaving, why are you planning to leave? (circle all that apply)
   a. Retirement
   b. Career advancement
   c. Career change
   d. More time with family
   e. Health problems
   f. Physical demands of your position
   g. Too much responsibility
   h. Inability to provide safe, competent care
   i. Burnout
   j. Poor salary
   k. Workload
   l. Management practices
   m. Conflict with management
   n. Lack of respect
   o. Other: ____________________________

21. Do you have another paid position outside of your current position?
   a. Yes
   b. No

22. Is this other position full-time or part-time?
   a. Full-time
   b. Part-time
### Emotional Health

In this section we are trying to determine how you generally felt last week. Below is a list of the ways you might have felt or behaved. Please indicate how often you have felt this way during the past week.

<table>
<thead>
<tr>
<th></th>
<th>Rarely or none (less than 1 day)</th>
<th>Some or a little (1-2 days)</th>
<th>Occasionally or moderately (3-4 days)</th>
<th>Most or all (5-7 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I was bothered by things that usually don’t bother me.</td>
<td></td>
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<tr>
<td>2. I did not feel like eating; my appetite was poor.</td>
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<tr>
<td>3. I felt that I could not shake off the blues even with help from my family or friends.</td>
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<td>4. I felt I was just as good as other people.</td>
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<tr>
<td>5. I had trouble keeping my mind on what I was doing.</td>
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<tr>
<td>6. I felt depressed.</td>
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<td>7. I felt that everything I did was an effort.</td>
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<td>8. I felt hopeful about the future.</td>
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<td>9. I thought my life had been a failure.</td>
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<tr>
<td>10. I felt fearful.</td>
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<tr>
<td>11. My sleep was restless.</td>
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<tr>
<td>12. I was happy.</td>
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<tr>
<td>13. I talked less than usual.</td>
<td></td>
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<tr>
<td>15. People were unfriendly.</td>
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<tr>
<td>16. I enjoyed life.</td>
<td></td>
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<tr>
<td>17. I had crying spells.</td>
<td></td>
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<tr>
<td>18. I felt sad.</td>
<td></td>
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<tr>
<td>19. I felt that people disliked me.</td>
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<tr>
<td>20. I could not get “going.”</td>
<td></td>
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</tbody>
</table>
Physical Activity

We are interested in finding out about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be physically active.

ACTIVITY AT WORK

1. Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like [carrying or lifting heavy loads, digging or construction work] for at least 10 minutes continuously?
   
   Yes
   No           If no, go to Question 4

2. In a typical week, on how many days do you do vigorous-intensity activities as part of your work?

   Number of days ____________

3. How much time do you spend doing vigorous-intensity activities at work on a typical day? (Think of one day you can recall easily. Consider only those activities undertaken continuously for 10 minutes or more.)

   Hours ______ Minutes ______

4. Does your work involve moderate-intensity activity, that causes small increases in breathing or heart rate such as brisk walking [or carrying light loads] for at least 10 minutes continuously?

   Yes
   No           If no, go to Question 7

5. In a typical week, on how many days do you do moderate-intensity activities as part of your work?

   Number of days ____________

6. How much time do you spend doing moderate-intensity activities at work on a typical day? (Think of one day you can recall easily. Consider only those activities undertaken continuously for 10 minutes or more.)

   Hours ______ Minutes ______
TRAVEL TO AND FROM PLACES

The next questions exclude the physical activities at work that you have already mentioned. Think about the usual way you travel to and from places. For example, to work, for shopping, to market, to place of worship.

7. Do you walk or use a bicycle (pedal cycle) for at least 10 minutes continuously to get to and from places? (Please circle the appropriate response)

   Yes
   No     If no, go to Question 10

8. In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?

   Number of days ____________

9. How much time do you spend walking or bicycling for travel on a typical day? (Think of one day you can recall easily. Consider the total amount of time walking or bicycling for trips of 10 minutes or more.)

   Hours _______ Minutes _______

RECREATIONAL ACTIVITIES

The next questions exclude the work and transport activities that you have already mentioned. Think about sports, fitness and recreational activities and leisure activities you participate in on a regular basis (not occasionally). It is important to focus on only recreational activities and not to include any activities already mentioned.

10. Do you do any vigorous-intensity sports, fitness or recreational (leisure) activities that cause large increases in breathing or heart rate like running or football for at least 10 minutes continuously?

    Yes
    No     If no, go to Question 13

11. In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational (leisure) activities?

    Number of days ____________
12. How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day? (Think of one day you can recall easily. Consider the total amount of time doing vigorous recreational activities for periods of 10 minutes or more.)

   Hours ______  Minutes ______

13. Do you do any moderate-intensity sports, fitness or recreational (leisure) activities that causes a small increase in breathing or heart rate such as brisk walking, cycling, swimming, or volleyball for at least 10 minutes continuously?
   Yes
   No    If no, go to Question 16

14. In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational (leisure) activities?

   Number of days ____________

15. How much time do you spend doing moderate-intensity sports, fitness or recreational (leisure) activities on a typical day? (Think of one day you can recall easily. Consider the total amount of time doing moderate recreational activities for periods of 10 minutes or more.)

   Hours ______  Minutes ______

SEDENTARY BEHAVIOUR

The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent sitting at a desk, sitting with friends, travelling in car, bus, train, reading, playing cards or watching television. DO NOT include the time you spend sleeping.

16. How much time do you usually spend sitting or reclining on a typical day? (Consider total time spent at work sitting, in an office, reading, watching television, using a computer, doing hand craft like knitting, resting etc. Do not include time spent sleeping.)

   Hours ______  Minutes ______
Sleep Quality
The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month.

1. Have you been told by a doctor or other health professional that you have a sleep disorder, for example insomnia, obstructive sleep apnea, restless legs or narcolepsy?
   □ Yes, what disorder? ________________________________
   □ No

2. Are you aware, or have you been told by a family member or bed partner that you snore most nights when sleeping?
   □ Yes
   □ No

3. How many hours of sleep on work days do you usually get? ________________

4. During the past month, what time have you usually gone to bed at night?
   Bed time ____________

5. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?
   Number of minutes ____________

6. During the past month, what time have you usually gotten up in the morning?
   Getting up time ____________

7. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.)
   Hours of sleep per night ____________
Study #

For each of the remaining questions, check the ONE best response. Please answer all questions.

8. During the past month, how often have you had trouble sleeping because you . . .

   a) Cannot get to sleep within 30 minutes

      Not during the past month _______ Less than once a week _______ Once or twice a week _______ Times or more times a week _______

   b) Wake up in the middle of the night or early morning

      Not during the past month _______ Less than once a week _______ Once or twice a week _______ Times or more times a week _______

   c) Have to get up to use the bathroom

      Not during the past month _______ Less than once a week _______ Once or twice a week _______ Times or more times a week _______

   d) Cannot breathe comfortably

      Not during the past month _______ Less than once a week _______ Once or twice a week _______ Times or more times a week _______

   e) Cough or snore loudly

      Not during the past month _______ Less than once a week _______ Once or twice a week _______ Times or more times a week _______

   f) Feel too cold

      Not during the past month _______ Less than once a week _______ Once or twice a week _______ Times or more times a week _______

   g) Feel too hot

      Not during the past month _______ Less than once a week _______ Once or twice a week _______ Times or more times a week _______

   h) Had bad dreams

      Not during the past month _______ Less than once a week _______ Once or twice a week _______ Times or more times a week _______

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i) Have pain

<table>
<thead>
<tr>
<th>Not during the past month</th>
<th>Less than once a week</th>
<th>Once or twice a week</th>
<th>Three or more times a week</th>
</tr>
</thead>
</table>

j) Other reason(s), please describe____________________________________________________

9. How often during the past month have you had trouble sleeping because of this?

<table>
<thead>
<tr>
<th>Not during the past month</th>
<th>Less than once a week</th>
<th>Once or twice a week</th>
<th>Three or more times a week</th>
</tr>
</thead>
</table>

10. During the past month, how would you rate your sleep quality overall?

☐ Very good
☐ Fairly good
☐ Fairly bad
☐ Very bad

11. During the past month, how often have you taken medicine to help you sleep (prescribed or "over the counter")?

<table>
<thead>
<tr>
<th>Not during the past month</th>
<th>Less than once a week</th>
<th>Once or twice a week</th>
<th>Three or more times a week</th>
</tr>
</thead>
</table>

12. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

<table>
<thead>
<tr>
<th>Not during the past month</th>
<th>Less than once a week</th>
<th>Once or twice a week</th>
<th>Three or more times a week</th>
</tr>
</thead>
</table>

13. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

☐ No problem at all
☐ Only a very slight problem
☐ Somewhat of a problem
☐ A very big problem
Overall Health and Quality of Life

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Answer every question by selecting the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1. In general, would you say your health is:
   - Excellent
   - Very good
   - Good
   - Fair
   - Poor

2. Compared to one year ago, how would you rate your health in general now?
   - Much better now than one year ago
   - Somewhat better now than one year ago
   - About the same as one year ago
   - Somewhat worse now than one year ago
   - Much worse now than one year ago

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

   a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports
   b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf
   c. Lifting or carrying groceries
   d. Climbing several flights of stairs
   e. Climbing one flight of stairs

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Study # __________________

f  Bending, kneeling, or stooping

g  Walking more than a mile

h  Walking several blocks

i  Walking one block

j  Bathing or dressing yourself

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

   Yes  No

   a  Cut down on the amount of time you spent on work or other activities

   b  Accomplished less than you would like

   c  Were limited in the kind of work or other activities

   d  Had difficulty performing the work or other activities (for example, it took extra effort)

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

   Yes  No

   a  Cut down on the amount of time you spent on work or other activities

   b  Accomplished less than you would like

   c  Did work or other activities less carefully than usual

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6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

Not at all  Slightly  Moderately  Quite a bit  Extremely

7. How much bodily pain have you had during the past 4 weeks?

None  Very mild  Mild  Moderate  Severe  Very severe

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all  A little bit  Moderately  Quite a bit  Extremely

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the past 4 weeks...

<table>
<thead>
<tr>
<th></th>
<th>All of the time</th>
<th>Most of the time</th>
<th>A good bit of the time</th>
<th>Some of the time</th>
<th>A little of the time</th>
<th>None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>a  Did you feel full of pep?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b  Have you been a very nervous person?</td>
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<tr>
<td>c  Have you felt so down in the dumps that nothing could cheer you up?</td>
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<td>d  Have you felt calm and peaceful?</td>
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<tr>
<td>e  Did you have a lot of energy?</td>
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</tbody>
</table>
10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

<table>
<thead>
<tr>
<th>All of the time</th>
<th>Most of the time</th>
<th>Some of the time</th>
<th>A little of the time</th>
<th>None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="radio-buttons.png" alt="Radio buttons" /></td>
<td><img src="radio-buttons.png" alt="Radio buttons" /></td>
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</tbody>
</table>

11. How TRUE or FALSE is each of the following statements for you?

<table>
<thead>
<tr>
<th>Statement</th>
<th>Definitely true</th>
<th>Mostly true</th>
<th>Don't know</th>
<th>Mostly false</th>
<th>Definitely false</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. I seem to get sick a little easier than other people</td>
<td><img src="radio-buttons.png" alt="Radio buttons" /></td>
<td><img src="radio-buttons.png" alt="Radio buttons" /></td>
<td><img src="radio-buttons.png" alt="Radio buttons" /></td>
<td><img src="radio-buttons.png" alt="Radio buttons" /></td>
<td><img src="radio-buttons.png" alt="Radio buttons" /></td>
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<tr>
<td>b. I am as healthy as anybody I know</td>
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<td><img src="radio-buttons.png" alt="Radio buttons" /></td>
<td><img src="radio-buttons.png" alt="Radio buttons" /></td>
<td><img src="radio-buttons.png" alt="Radio buttons" /></td>
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<tr>
<td>c. I expect my health to get worse</td>
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<td><img src="radio-buttons.png" alt="Radio buttons" /></td>
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<td>d. My health is excellent</td>
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<td><img src="radio-buttons.png" alt="Radio buttons" /></td>
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THANK YOU SO MUCH FOR COMPLETING THIS QUESTIONNAIRE. YOUR FEEDBACK IS VERY IMPORTANT TO US.