DETERMINANTS AND METHODS OF ASSESSMENT OF MELATONIN LEVELS
AMONG ROTATING SHIFT NURSES

by

Anne Louise Grundy

A thesis submitted to the Department of Community Health and Epidemiology in
conformity with the requirements for the degree Master of Science

Queen’s University
Kingston, Ontario, Canada
June, 2008

Copyright © Anne Louise Grundy, 2008
Abstract

Background: Long-term night shift work has been associated with multiple cancer sites, including breast, prostate, colon and endometrial. The mechanism for this effect is hypothesized to include the hormone melatonin; where increased light at night exposure during shift work reduces melatonin production and decreased melatonin levels are associated with increased cancer risk. In addition, physical activity has been shown to reduce cancer risk and existing laboratory studies indicate it has the potential to influence melatonin levels.

Methods: A cross-sectional study of light intensity exposure, physical activity and melatonin levels was conducted among 61 rotating shift nurses at Kingston General Hospital. Light intensity exposure was measured using a light intensity data logger and melatonin concentrations were measured from urine and saliva samples, collected over a 24-hour period. Physical activity was assessed from a study questionnaire and one-day diary.

Results: A statistically significant inverse association between light exposure and urinary melatonin levels was observed; however, the relationship was no longer significant when stratified by shift group. Analysis of salivary melatonin levels demonstrated that circadian rhythms of melatonin production in night workers were not altered in timing, such that peak melatonin production occurred at night. No association between light exposure and the magnitude of salivary melatonin variation was observed. The
relationship between recent physical activity and melatonin differed by shift group, with a positive association seen among day workers, while an inverse relationship was seen among night workers. There was no association between usual physical activity and melatonin in either shift group. Finally, no significant correlation was observed between sleep duration and melatonin among either day or night workers.

**Conclusions:** While this study demonstrated an inverse relationship between light intensity and melatonin, the comparison of functional time points between day and night workers meant that differences in urinary melatonin levels between shift groups could be attributed to differences in the time of day when urine samples were collected. No consistent relationship between recent or usual physical activity and melatonin levels was observed in either shift group. Sleep duration was not correlated with urinary melatonin levels, suggesting it cannot be used as a proxy for melatonin production.
Co-Authorship Statement

This thesis presents the work of Anne Grundy in collaboration with her supervisors, Kristan Aronson and Charles Graham. The cross-sectional study was designed and conducted by Kristan Aronson, Maria Sanchez, Charles Graham, Harriet Richardson and Joan Tranmer with funding from Breast Cancer Action Kingston. The statistical analysis that included light exposure, urinary and salivary melatonin, and physical activity was designed and performed by Anne Grundy with supervision by Kristan Aronson. Interpretation of results and writing of the manuscript was performed by Anne Grundy with supervision by Kristan Aronson and editorial feedback from Charles Graham, Maria Sanchez, Harriet Richardson, Joan Tranmer, and Marilyn Borugian.
Acknowledgements

I am extremely grateful to my supervisors for their assistance and guidance with this project. To Dr. Kristan Aronson, thank you for providing me with the opportunity to be involved with this project and your patience with my many questions. Your support, encouragement and belief in my abilities were instrumental in the completion of this thesis. Thank you also to Dr. Charles Graham for your help and thoughtful advice.

I would like to acknowledge the faculty and staff in the Department of Community Health and Epidemiology for providing a friendly and supportive learning environment. Thank you also to my classmates; I feel privileged to have been a part of such an intelligent and supportive group of people who made coming to class every day so rewarding and enjoyable. I would particularly like to acknowledge the ladies in the “happy pod” for making the office such a cheerful and positive place to be. Thank you to Meghan Hamel for your friendship, smiles and seemingly endless supply of knowledge about everything from statistics to minor formatting problems, I would truly have been lost without you. Thank you also to Amy Schneeberg for your help with even the most minor of problems, your positive attitude put a smile on my face every day.

I would like to acknowledge funding from the CIHR Transdisciplinary Training Program in Cancer Research for this project. Funding for the pilot study was generously provided by Breast Cancer Action Kingston. Study participants are thanked for generously providing information and completing the study protocol.

Finally, I would like to thank my parents and my brothers Rob and Ian for their love and support throughout this entire project. Your constant desire to understand what I
was doing and patience as you heard more than you ever wanted to know about melatonin was very much appreciated.
Table of Contents:

Abstract ............................................................................................................................... i
Co-Authorship Statement ............................................................................................... iii
Acknowledgements .......................................................................................................... iv
Table of Contents ............................................................................................................. vi
List of Tables .................................................................................................................... ix
List of Figures .....................................................................................................................x

Chapter 1: Introduction ....................................................................................................1
  1.1 Background and Rationale .........................................................................................1
  1.2 Overview of Study Design .........................................................................................2
  1.3 Objectives ..................................................................................................................3
  1.4 Thesis Organization .................................................................................................3
  References .........................................................................................................................5

Chapter 2: Literature Review ...........................................................................................7
  2.1 Shift Work and Cancer .............................................................................................7
  2.2 Melatonin and Cancer ............................................................................................9
    2.2.1 Melatonin .........................................................................................................9
    2.2.2 Mechanisms of Cancer Inhibition ..................................................................10
    2.2.3 Epidemiologic Evidence .................................................................................11
  2.3 Light at Night and Melatonin ..................................................................................13
  2.4 Physical Activity ......................................................................................................14
    2.4.1 Physical Activity and Cancer ..........................................................................14
    2.4.2 Physical Activity and Melatonin .....................................................................15
  2.5 Biomarkers of Melatonin .......................................................................................16
  2.6 Circadian Rhythms of Melatonin Variation ............................................................18
  2.7 Potential Confounders ............................................................................................20
    2.7.1 Lifestyle Factors .............................................................................................20
    2.7.2 Reproductive Factors ......................................................................................21
    2.7.3 Pharmacologic Agents ....................................................................................22
  2.8 Sleep Duration and Melatonin .................................................................................22
  2.9 Summary of Rationale .............................................................................................24
  References .........................................................................................................................26

Chapter 3: Methods ..........................................................................................................34
  3.1 Objectives ..................................................................................................................34
  3.2 Study Design .............................................................................................................34
  3.3 Study Population .......................................................................................................34
  3.4 Basic Data Collection Procedures ..........................................................................36
  3.5 Light Intensity Assessment .......................................................................................36
  3.6 Physical Activity Assessment ....................................................................................37
  3.7 Melatonin Assessment .............................................................................................38
    3.7.1 Urine and Saliva Collection Procedures .........................................................39
    3.7.2 Laboratory Analysis .........................................................................................40
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8 Study Diary and Questionnaire</td>
<td>41</td>
</tr>
<tr>
<td>3.9 Potential Confounders</td>
<td>41</td>
</tr>
<tr>
<td>3.9.1 Personal Characteristics</td>
<td>42</td>
</tr>
<tr>
<td>3.9.2 Pharmacologic Agents</td>
<td>42</td>
</tr>
<tr>
<td>3.9.3 Lifestyle Factors</td>
<td>43</td>
</tr>
<tr>
<td>3.10 Statistical Analysis</td>
<td>44</td>
</tr>
<tr>
<td>3.9.4 Descriptive Statistics</td>
<td>44</td>
</tr>
<tr>
<td>3.9.5 Light and Melatonin Analysis</td>
<td>45</td>
</tr>
<tr>
<td>3.9.6 Physical Activity and Melatonin Analysis</td>
<td>47</td>
</tr>
<tr>
<td>3.9.7 Sleep Duration and Melatonin</td>
<td>47</td>
</tr>
<tr>
<td>References</td>
<td>48</td>
</tr>
<tr>
<td>Chapter 4: Manuscript</td>
<td>51</td>
</tr>
<tr>
<td>Abstract</td>
<td>52</td>
</tr>
<tr>
<td>Introduction</td>
<td>53</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>54</td>
</tr>
<tr>
<td>Results</td>
<td>59</td>
</tr>
<tr>
<td>Discussion</td>
<td>61</td>
</tr>
<tr>
<td>References</td>
<td>67</td>
</tr>
<tr>
<td>Chapter 5: Additional Results</td>
<td>80</td>
</tr>
<tr>
<td>5.1 Associations Between Potential Confounders and Melatonin</td>
<td>80</td>
</tr>
<tr>
<td>5.2 Spearman Correlation Between Urine and Saliva</td>
<td>81</td>
</tr>
<tr>
<td>5.3 Light Intensity Exposure and Salivary Melatonin</td>
<td>82</td>
</tr>
<tr>
<td>5.3.1 Salivary Melatonin</td>
<td>82</td>
</tr>
<tr>
<td>5.3.2 Conceptualization of light exposure variable for salivary melatonin analysis</td>
<td>84</td>
</tr>
<tr>
<td>5.3.3 Associations between light intensity exposure and salivary melatonin</td>
<td>87</td>
</tr>
<tr>
<td>5.4 Recent Physical Activity and Urinary Melatonin Levels</td>
<td>88</td>
</tr>
<tr>
<td>5.4.1 Recent Physical Activity</td>
<td>88</td>
</tr>
<tr>
<td>5.4.2 Recent Physical Activity and Melatonin</td>
<td>90</td>
</tr>
<tr>
<td>5.5 Usual Physical Activity and Urinary Melatonin Levels</td>
<td>93</td>
</tr>
<tr>
<td>5.5.1 Usual Physical Activity</td>
<td>93</td>
</tr>
<tr>
<td>5.5.2 Usual Physical Activity and Melatonin</td>
<td>95</td>
</tr>
<tr>
<td>5.6 Sleep Duration and Melatonin</td>
<td>97</td>
</tr>
<tr>
<td>References</td>
<td>99</td>
</tr>
<tr>
<td>Chapter 6: General Discussion</td>
<td>100</td>
</tr>
<tr>
<td>6.1 Summary of Main Findings</td>
<td>100</td>
</tr>
<tr>
<td>6.1.1 Light intensity exposure and urinary melatonin</td>
<td>100</td>
</tr>
<tr>
<td>6.1.2 Saliva as a measure of absolute melatonin levels</td>
<td>101</td>
</tr>
<tr>
<td>6.1.3 Light intensity exposure and salivary melatonin levels</td>
<td>102</td>
</tr>
<tr>
<td>6.1.4 Physical Activity and Urinary Melatonin</td>
<td>103</td>
</tr>
<tr>
<td>6.1.5 Sleep Duration and Melatonin</td>
<td>105</td>
</tr>
<tr>
<td>6.2 Strengths and Limitations</td>
<td>106</td>
</tr>
</tbody>
</table>
6.3 Future Directions ...........................................................................................109
6.4 Contribution of Research and Conclusions.................................................112
References............................................................................................................115

Appendix A: Ethics Approval.......................................................................................118
Appendix B: Study Diary ..............................................................................................119
Appendix C: Study Questionnaire ...............................................................................128
List of Tables:

Chapter 4:

**Table 1:** Characteristics of study population

**Table 2:** Light intensity by shift group

**Table 3:** Urinary 6-sulfatoxymelatonin values by shift group

**Table 4:** Multivariate association between light intensity and 6-sulfatoxymelatonin

**Table 5:** Mean salivary melatonin level by shift group comparing chronological time

Chapter 5:

**Table 5.1:** Bivariate associations between log-transformed urinary 6-sulfatoxymelatonin levels and potential melatonin determinants

**Table 5.2:** Mean magnitude of salivary melatonin variation

**Table 5.3:** Mean light intensity exposure between 12AM and 5AM

**Table 5.4:** Mean urinary melatonin level by recent physical activity group for day and night workers

**Table 5.5:** Mean urinary melatonin levels by usual physical activity group among day and night workers

**Table 5.6:** Correlation between urinary melatonin and sleep duration among day and night workers
List of Figures:

Chapter 2:

Figure 2.1: Production of melatonin from tryptophan ................................................10

Figure 2.2: Conceptual model of associations between light at night, physical activity, melatonin and cancer .................................................................23

Chapter 3:

Figure 3.1: Timeline of urine and saliva sample collection ........................................40

Chapter 4:

Figure 1: Urine and saliva sample collection timeline ................................................75

Figure 2: Association between log-transformed light intensity during sleep and log-transformed urinary melatonin levels (all subjects) .........................76

Figure 3: Salivary melatonin secretion patterns comparing chronological time points ............................................................................................................77

Figure 4a: Association between log-transformed light intensity during sleep and log-transformed urinary melatonin levels in model that accounts for age and alcohol consumption in 24-hours of melatonin collection (Day workers only) ........................................................................78

Figure 4b: Association between log-transformed light intensity and log-transformed urinary melatonin levels in model controlling for age and use of NSAIDs in both the 24-hours of melatonin collection and the previous two weeks (Night workers only) ........................................................................79

Chapter 5:

Figure 5.1: Distribution of the magnitude of salivary melatonin variation ..............83

Figure 5.2: Distribution of light intensity exposure between 12AM and 5AM .......85

Figure 5.3: Distribution of log-transformed light intensity between 12AM and 5AM ........................................................................................................86

Figure 5.4: Multivariate association between log-transformed light intensity exposure and the log-transformed magnitude of salivary melatonin variation ......88

Figure 5.5: Distribution of recent physical activity as a continuous variable ........89
Figure 5.6: Categorical recent physical activity distribution

Figure 5.7: Multivariate association between log-transformed urinary melatonin level and recent physical activity group for day and night workers

Figure 5.8: Distribution of total hours of usual physical activity

Figure 5.9: Categorical usual physical activity distribution

Figure 5.10: Multivariate association between log-transformed urinary melatonin level and usual physical activity group for day and night workers

Chapter 6:

Figure 6.1: Revised urine and saliva collection timeline
Chapter 1: Introduction

1.1 Background and Rationale:

Epidemiologic studies have found links between shift work and cancers of the breast (1-5), prostate (6,7), endometrium (8) and colon (9). The majority of these studies have focused specifically on breast cancer, with a meta-analysis demonstrating an approximately 40 – 50% increase in risk associated with shift work (10). Shift work involving circadian disruption was recently classified as a probable (Group 2A) carcinogen by the International Agency for Research on Cancer (11).

A hypothesized biological mechanism for the relationship between shift work and cancer involves the hormone melatonin, where increased light at night exposure associated with night shift work is thought to produce decreases in melatonin levels, which are then associated with increased cancer risk (12). Laboratory studies have indicated that decreased melatonin levels are linked to increases in both cancer development and progression (13-16) and epidemiologic studies have indicated an inverse association between melatonin and cancer development at the population level (17). Furthermore, studies using multiple methods of light exposure including job classifications and the number of years of shift work have demonstrated an inverse association between light at night and melatonin levels, providing further scientific support for this relationship (2,3,18-20). However, few studies to date incorporate objective measures of light exposure, thus more research with direct measures of both light exposure and melatonin levels is needed to clarify the relationship between these two variables.
In addition, physical activity, which has been shown to reduce the risk of several cancers including breast and colon (21), has also been associated with melatonin levels in laboratory-based studies where exercise has been shown to increase melatonin levels (22). There have been few observational studies of this relationship at a population level, although one recent study did find a positive association between exercise duration and urinary melatonin levels (23). Thus, more observational evidence regarding the relationship between physical activity and melatonin that incorporates aspects of physical activity other than simply exercise, such as household and occupational activity, is needed to clarify the extent to which physical activity is able to influence melatonin levels.

1.2 Overview of Study Design:

To evaluate the associations of both light at night exposure and physical activity with melatonin levels, a cross-sectional study of a group of female rotating shift nurses at Kingston General Hospital was conducted. Data collection for each subject occurred over a three-day period during which subjects wore a light intensity data logger, to collect objective measures of light exposure, and collected one urine and four saliva samples over a 24-hour period from which biomarkers of melatonin were measured. Subjects also completed a study questionnaire and one-day diary during the period of melatonin assessment where physical activity and other personal characteristics were assessed.
1.3 Objectives:

The primary objectives of this thesis were to examine the association of light at night exposure with urinary and salivary melatonin levels and to examine the associations of both usual and recent physical activity with melatonin levels. This thesis also describes the pattern of salivary melatonin secretion and compares this with urinary melatonin measurements to determine the extent to which salivary melatonin measures absolute melatonin levels. Finally, from a methodological perspective this thesis investigates the extent to which sleep duration may be used as a proxy for melatonin levels, where increased sleep duration was expected to be associated with increased melatonin.

1.4 Thesis Organization:

The second chapter of this thesis consists of a literature review describing the links between shift work, melatonin and cancer, as well as the relationships of both light at night and physical activity with melatonin levels. The third chapter provides description 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 light exposure 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, chapter 5 addresses the relationships between salivary and urinary melatonin levels, associations between light at night and salivary melatonin, as well as those of both usual and recent physical activity with urinary melatonin levels, and finally, describes the relationship between sleep duration and melatonin levels. The final
chapter of the thesis consists of a general discussion of findings in the fourth and fifth chapters, as well as overall conclusions and future research directions.
References


Chapter 2: Literature Review

2.1 Shift Work and Cancer:

In 2007, shift work involving circadian disruption was classified as a probable (Group 2A) carcinogen by the International Agency for Research on Cancer (1). The relationship between shift work and cancer has been studied in an epidemiologic context with respect to several different cancer sites, including breast, colon, endometrial and prostate (2-10).

Most studies of this relationship to date have examined the association between shift work and breast cancer, and these have found that shift work of long duration over the career is associated with increased cancer risk. Assessment of shift work has occurred using a variety of methods in different studies. A case-control study of nurses in Norway obtained shift work history from a registry where nurses working in a hospital were classified as shift workers and those working outside the hospital were not (9). This study found a significantly increased risk of breast cancer among nurses who had spent 30 or more years working nights (9). Another study of Norwegian radio and telegraph operators, again using a registry of job histories to classify shift work, also found an increased risk of breast cancer with shift work for women over 50 years of age (11). Other studies have used questionnaire-based methods to obtain direct individual shift work measures for study participants. A case-control study by Davis et al. found that women who worked “the graveyard shift” (defined as work that began after 7:00PM and ending before 9:00AM) at least once had a 60% increased risk of breast cancer, and that risk increased with the number of years working the graveyard shift (2). Schernhammer et al. found that among nurses enrolled in the Nurses Health Study, increasing duration of
shift work was associated with increased breast cancer risk, with a statistically significant 36% increase in risk among nurses had worked 30 or more years of rotating shifts (3). In a group of premenopausal nurses, the same investigators found that those who had worked 20 or more years of rotating shifts had a significant 79% increased risk of breast cancer (8). A meta-analysis of studies of shift work and breast cancer conducted in 2005 found a stable accumulation of evidence for an approximately 50% increased risk of breast cancer with shift work (12), a finding supported by a more recent systematic review (13).

In addition to an association with breast cancer, recent studies have begun to show a relationship between shift work and other cancer sites. A cohort study of Japanese men found a significant increase in risk of prostate cancer among rotating shift workers compared to day workers (7). A recent case-control study of Canadian men found that ever having worked a rotating shift was associated with increased prostate cancer risk and that risk was highest among those who had worked less than 7 years of full-time rotating shift work (5). Colon cancer has also been associated with shift work, with a significant increase in risk seen among nurses enrolled in the Nurses Health Study who had worked 15 or more years of rotating shifts (10). Finally, endometrial cancer has also been associated with shift work with a 47% increase in risk observed for women who worked 20 or more years of rotating shifts (4).

While the majority of studies have found an increased risk of cancer associated with shift work, a recent register-based cohort in Sweden found an overall pattern of no association with prostate, breast or colon cancer (6). However, this study did not include individual assessments of shift work among subjects and used registry-based job
classifications to define shift work exposure (6). This suggests the possibility of exposure misclassification, which may have reduced the study’s ability to detect associations.

2.2 Melatonin and Cancer:

The association between shift work and cancer is thought to be mediated by the impact of light at night exposure on levels of the hormone melatonin (14). Melatonin is a hormone produced by the pineal gland in a pattern that follows circadian rhythms, with peak levels produced at night when light is absent (15). There is both experimental and epidemiologic evidence that melatonin levels have an impact on cancer development and progression (15-18).

2.2.1 Melatonin:

Melatonin is the main biologically active substance secreted by the pineal gland, where it is produced through a series of enzymatic reactions from the amino acid tryptophan (Figure 2.1) (17,19). Melatonin production is regulated by input from the suprachiasmatic nucleus (SCN) of the hypothalamus (15). Specialized retinal photoreceptors transmit light information from the eyes to the SCN and output from these photoreceptors is inhibited by light (15). The SCN then controls the activity of arylamine N-acetyltransferase (NAT), which catalyzes the conversion of serotonin to N-acetylserotonin, the rate-limiting step in the conversion of tryptophan to melatonin (17). In the absence of light, this enzyme is activated by the SCN, leading to the production of melatonin (17). Since light inhibits melatonin production, peak levels are usually seen at night between 2AM and 4AM (15).
2.2.2 Mechanisms of Cancer Inhibition

Experimental studies, in both in vitro and in vivo models, have demonstrated that melatonin is able to reduce both the incidence and growth of tumours (19). Studies using MCF-7 breast cancer cell lines have reported decreased cell proliferation and invasiveness, as well as changes in cell structure with the administration of physiological concentrations of melatonin (20,21). In animal models, multiple studies in rats have found a decrease in both tumour incidence and proliferation after melatonin administration with several different tumour types (22-25). Furthermore, pinealectomy (removal of the pineal gland) has been shown increase incidence of mammary carcinomas in rats, suggesting a protective role for melatonin in tumour development (23). Finally, both rat hepatomas and human breast cancer xenografts displayed decreased proliferation in situ after perfusion with melatonin-rich blood collected from healthy premenopausal female volunteers, an effect that was removed when melatonin-
depleted blood from volunteers exposed to bright light was used (24). These observations, taken together, provide strong experimental evidence of a protective role for melatonin in tumour development and proliferation.

Laboratory work has suggested that melatonin inhibits cancer development through several different mechanisms. Melatonin is able to protect against DNA damage by acting as a potent scavenger of both oxygen and nitrogen based free radicals (16). The hormone also has antiproliferative effects on cancer cells, causing cell-cycle specific delays at the G1 – S transition and decreasing DNA synthesis in cells that have already progressed to the S phase (17). Melatonin has been shown to have immunomodulatory activity, where increased melatonin promotes several immune functions and this mechanism is also thought to be protective against cancer development (15).

Finally, melatonin can also have an impact on the levels of several reproductive hormones, including estrogen (15). Melatonin interferes with aromatases that convert androgens to estrogens, such that high levels of melatonin are associated with lower hormone levels. This is important in the etiology of hormone-dependent cancers such as breast cancer, where increased estrogen levels are thought to increase breast cancer risk (16,17).

2.2.3 Epidemiologic Evidence:

Despite extensive experimental work surrounding the relationship between melatonin and cancer development, there have been few epidemiologic studies of this association in human populations. Several studies of melatonin and cancer have compared melatonin levels in cancer cases with those of healthy controls (26-29). These
studies have found a decrease in both urinary and plasma melatonin levels in both breast and colon cancer cases compared to controls (27-29). A study by Danforth et al. found that while overall there was no difference in plasma melatonin levels in breast cancer cases compared to healthy controls, there was a significant inverse relationship between estrogen receptor positive breast cancer and melatonin production (26). However, the retrospective nature of these studies prevented them from establishing a temporal relationship between changes in melatonin levels and cancer development, such that it was impossible to determine if decreased melatonin production occurred prior to cancer development or as a result of it (18,30).

While prospective studies of melatonin and cancer have been conducted, conflicting results have been obtained (18,30). A case-control study nested within the Nurses Health Study II cohort looking at the relationship between urinary melatonin levels and breast cancer development found a significant inverse association between peak urinary 6-sulfatoxymelatonin levels measured in first morning void and breast cancer development (18). Conversely, in a prospective cohort study, no significant association was observed between 24-hour urinary melatonin levels and subsequent development of breast cancer (30). However, previous work has suggested the circadian amplitude of melatonin variation, which is not captured in 24-hour urinary melatonin levels, may be the important factor in cancer development (31). If this is indeed the case, the inability of this study to capture peak melatonin levels may have explained the observed lack of association between melatonin and cancer development (31).
2.3 Light at Night and Melatonin:

The release of melatonin is stimulated by darkness and inhibited by light (15). As previously described, epidemiologic studies have suggested that shift work may be associated with an increased risk of breast cancer due to increased light at night exposure (2,3,32,33). Experimental studies in humans have demonstrated a dose-response relationship between light exposure and melatonin levels with greater light intensities associated with greater decreases in melatonin levels (34,35). While full spectrum bright light is usually used in these experiments, blue light with wavelengths between 446 and 477 nm has the strongest inhibitory effect on melatonin production (36).

Studies in shift workers have been used to examine the association between light at night and melatonin with multiple methods including questionnaires regarding shift work history and bedroom lighting conditions, as well as direct light intensity measurements, used to assess light exposure (2,3,37,38). Among studies using shift work as a proxy for light at night exposure, the general pattern observed is decreased melatonin production with increased night work (38,39). The Nurses Health Study found an inverse relationship between the number of nights worked and melatonin secretion, with a 56% decrease in 6-sulfatoxymelatonin, the primary metabolite of melatonin found in urine, associated with night work in the previous two weeks (38). Another study demonstrated that those working nights had significantly lower melatonin levels on work days compared to days off and that melatonin secretion on work days was lower in night workers compared to day workers (39).

Other studies have used more objective measures of light exposure, with direct light intensity readings. A study of permanent shift workers found that those on the
evening shift had higher 24-hour ambient light exposure and night workers had altered melatonin secretion patterns, where post-work melatonin levels were higher and post-sleep melatonin levels lower than among day workers (40). Another study found that 7-day patterns of light exposure were different between rotating shift workers and those working days only, with average light exposure on the night shift almost double that seen on days off or on the day shift (37). Melatonin levels were different among rotating shift workers compared to day-only workers, as those on the night shift had higher melatonin levels during work and lower levels during sleep compared to those working days (37). The results of these studies suggest that the hypothesized inverse relationship between light at night exposure and reduced melatonin levels does exist at the population level.

2.4 Physical Activity:

2.4.1 Physical Activity and Cancer:

Physical activity is defined as any bodily movement produced by skeletal muscles that results in caloric expenditure (41), while exercise is considered a subcategory of physical activity defined as an activity that is “planned, structured, repetitive and results in the improvement or maintenance of one or more facets of physical fitness” (41). Physical activity is thought to reduce the risk of multiple cancers, where the level of evidence ranges in strength from convincing (colon and breast) to probable (prostate) and possible for endometrial cancer (42). The relationship between physical activity and colon cancer appears strongest with studies showing an average 40 – 50% reduction in risk when both recreational and occupational physical activity are considered (42). Most studies of physical activity and breast cancer show a risk reduction of approximately
30%, which is generally confined to postmenopausal subjects (42-44). However one study of premenopausal women found a 39% reduced risk of breast cancer among women who engaged in both recreational and occupational physical activity (45).

The exact mechanism through which physical activity reduces cancer risk remains unknown; however, several possible mechanisms have been proposed. These include a decrease in estrogen exposure due to delayed menarche through activity during adolescence, decreased estrogen exposure from adipose tissue after menopause, effects on body fat distribution, and possible immunologic pathways (42,46).

2.4.2 Physical Activity and Melatonin:

While the mechanism through which physical activity impacts cancer risk remains unclear, there exists a possible role for melatonin in this relationship. The majority of work investigating the relationship between physical activity and melatonin in humans has been experimental, in controlled laboratory conditions, and has focused mainly on the impact of exercise on melatonin, with very few observational studies.

Results from experimental work have been inconsistent and have shown exercise to have positive, negative and null effects on melatonin levels (47-51). One review article noted that several studies have found that exercise produces acute increases in melatonin levels, but that these increases are short lived, with hormone levels returning to normal within one hour after exercise (47). Studies have also found that melatonin secretion is affected differently at different times of day. Buxton et al. found that exercise near the offset of melatonin secretion (during the day) had no acute effect on melatonin; however, a decrease in melatonin was observed with evening exercise, and an increase observed
with nighttime exercise (52). One recent observational study of the relationship between moderate and vigorous exercise and melatonin levels supported these experimental findings, where melatonin levels increased with increasing duration of exercise (53). The role of time of day in the relationship was also supported with evening and night exercise more strongly associated with elevated melatonin levels (53).

Most studies of physical activity and melatonin have focused on the acute effects of exercise; however, the chronic effects of exercise and other forms of activity have not been extensively investigated. One study of elite athletes found a general decline in melatonin levels over several weeks, but the sample size was very small and the results may not be generalizable to a broader population (54). However, another study of female runners found that chronic training had no effect on melatonin levels (51). Therefore, there appears to be an acute increase in melatonin following exercise but the effects of chronic activity on melatonin levels are not known.

2.5 Biomarkers of Melatonin:

There are three melatonin biomarkers that have been used in both experimental and epidemiologic research to measure melatonin levels. Melatonin concentrations can be assessed directly from blood and saliva samples, while concentrations of 6-sulfatoxymelatonin, the primary melatonin metabolite, are easily measured in urine (55). Assessment of plasma or serum melatonin levels is considered the most accurate method for measurement of absolute melatonin levels in the body, as it is used as the reference method when determining the accuracy of other melatonin assessment strategies (55-59). However, many studies involving melatonin wish to account for natural circadian
variations in hormone levels, and multiple melatonin samples are often required to characterize these rhythms. Under such circumstances, the blood sampling required to assess plasma or serum melatonin levels is generally not feasible for observational research, as a laboratory setting is usually required to obtain multiple blood samples, and other methods of measuring melatonin using less invasive techniques are preferred (55,59).

Measurements of melatonin from urine and saliva have been assessed in terms of their ability to accurately describe melatonin levels. Urinary concentrations of 6-sulfatoxymelatonin in first morning void of urine have been shown to represent approximately 70% of total nocturnal melatonin production (58). Several laboratory 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 (55,58,59). However, while these samples give a good estimate of total nighttime production of melatonin, they are unable to describe circadian melatonin variations (37).

The majority of endogenous melatonin in the blood is found bound to high molecular weight serum proteins, particularly albumin (60,61). While a range of results has been seen, previous studies have found that approximately 70% of plasma melatonin is found bound to serum proteins (60-62). Salivary melatonin assessment has been found to represent approximately 30% of plasma melatonin levels and is generally thought to reflect the free plasma melatonin fraction, with plasma proteins unable to pass through salivary glands into saliva (60,63). While generally considered unsuitable for assessment
of absolute melatonin levels, salivary samples are thought to be a good measure of melatonin variability (55,57,63).

2.6 Circadian Rhythms of Melatonin Variation:

As previously described, melatonin levels vary according to circadian rhythms with peak hormone levels occurring at night in the absence of light (15). The ability to synthesize melatonin differs among individuals, with multiple studies showing wide inter-individual variations in peak melatonin levels (19,36). However, studies of circadian variations have found that in terms of phasing and amplitude, melatonin rhythms within an individual are relatively constant (19,36). Therefore, due to these natural circadian variations, it is important to consider and account for time of day when measuring melatonin.

The question of whether the timing of melatonin secretion is altered by rotating shift work has been examined in previous studies. Borugian \textit{et al.} found that melatonin secretion patterns differed for rotating shift workers depending on whether they were on the day or night shift (37). However, these observations may have been a result of the time of day of melatonin sampling, since functional and not chronological time points were compared between shift types. Consequently, rotating shift workers sleeping during the day may have had low melatonin levels during sleep compared to those sleeping at night due to natural circadian variations and not to light at night exposure \textit{per se}.

The ability of permanent night work to alter circadian phase of melatonin production has been studied more extensively, but has produced conflicting results. While some studies have shown a shift in the timing of melatonin production (64,65),
others have found that melatonin secretion patterns among permanent night workers are no different from those of individuals with a day-oriented schedule (66-68). For example, a study by Dumont et al. found that 22 out of 30 nurses on permanent night shift had melatonin exposure patterns that were typical of day-oriented people, suggesting that circadian rhythms of melatonin production were not altered in night shift workers (66). Several studies that report a shift in melatonin production among permanent night workers compare functional time points with day workers (39,40) and therefore, as in the Borugian et al. study (37), differences in melatonin levels could be due to comparisons between different times of day and not due to a true circadian shift.

The observation that night work does not shift the circadian rhythm of melatonin production has been supported by studies of simulated night work with a study by Santhi et al. showing that although simulated night work did produce a shift in the timing of melatonin secretion, peak secretion still occurred at night (69). Another simulation study by Roach et al. showed that at least 3 days on the night shift combined with fixed sleeping times were required in order for melatonin levels to return to those found prior to beginning the night shift, with decreased melatonin levels seen in unadjusted night workers (70). These observations suggest that the two nights spent on night shift work among rotating shift nurses at Kingston General Hospital would not be sufficient to alter the timing of melatonin secretion, and thus time of melatonin sampling must be considered when comparisons are made between day and night rotating shift workers.
2.7 Potential Confounders:

There are multiple additional factors that must be considered when assessing the relationships of light at night and physical activity with melatonin levels (see conceptual model in Figure 2.2). These factors include lifestyle and reproductive factors as well as several drugs.

2.7.1 Lifestyle Factors:

Multiple studies have investigated the relationship between alcohol consumption and melatonin levels. Laboratory-based studies have demonstrated an inhibitory effect of alcohol on plasma melatonin levels but did not show any effect on urinary melatonin production (71,72). A more recent observational study did indicate an inverse association between alcohol consumption and urinary 6-sulfatoxymelatonin levels with alcohol consumption greater than two drinks per day (73). Smoking has also been associated with decreased melatonin levels where current smokers had significantly lower urinary 6-sulfatoxymelatonin levels compared to non-smokers, however past smoking did not appear to impact on melatonin production (74). A similar effect of smoking on plasma melatonin levels has been observed, suggesting a negative effect on melatonin (75). Body mass index (BMI) has also been associated with melatonin levels (74). Several observational studies have demonstrated a negative relationship between BMI and urinary melatonin levels, with increased BMI associated with reduced levels of melatonin (30,73,74,76).

Several laboratory-based studies have examined the effects of caffeine on melatonin levels, with conflicting results. Studies in both males and females have shown
caffeine to have an inhibitory effect on salivary melatonin levels, but this effect was somewhat blunted among women when using oral contraceptives (77,78). Another experimental study found that caffeine ingestion was associated with an increase in serum melatonin levels (79). Despite conflicting results, these studies, combined with the ability of caffeine to influence sleep patterns, suggest there is potential for this compound to influence melatonin levels or affect the relationship between melatonin and other determinants.

2.7.2 Reproductive Factors:

The observation of a decline in melatonin levels with increasing age is fairly consistent in the epidemiologic literature (59,73,74,76). There have been suggestions that menopausal status specifically may impact melatonin levels, but the direction of this effect remains unclear (30,59,80). One study observed that nocturnal melatonin serum melatonin levels increased transiently during menopause (80), while other studies have found no significant difference in melatonin levels between pre- and post-menopausal women (27,59).

In addition to menopausal status, parity has been associated with melatonin levels. Multiple studies have found a positive relationship between parity and melatonin, with urinary 6-sulfatoxymelatonin levels higher among parous women than among nulliparous subjects (27,59). One study also found that the relationship appeared to differ by age at first birth, with lower melatonin levels in women whose age at first birth was < 25 compared to those who were older than 25 at the time of first birth (74).
2.7.3 Pharmacological Agents:

Pharmacological agents such as antidepressants, beta-blockers, non-steroidal anti-inflammatory drugs (NSAIDs), hormone replacement therapy and sedatives have all been shown to inhibit melatonin synthesis (81-86). Additionally, oral contraceptive use has been associated with increases in melatonin levels (87,88).

2.8 Sleep Duration and Melatonin:

Sleep duration has been associated with melatonin production and there have been several studies investigating the possible association between sleep duration and breast cancer risk (89-92). Studies of sleep duration and breast cancer have not found significant associations between these two factors (91-93). Despite the lack of significance, a study by Verksalo et al. did find that risk estimates for breast cancer were consistently lower for longer sleepers, suggesting that a protective effect could exist (91). Studies of the association between sleep duration and melatonin levels have shown potential for an association between these factors (89,90). A study by Aeschbach et al. demonstrated that the duration of nocturnal melatonin secretion was of longer duration in long (>9 hours) compared with short (<6 hours) sleepers; however, peak plasma melatonin levels did not differ between groups (89). Another study found sleep duration was correlated with both average and peak melatonin levels, where longer sleep was correlated with reduced melatonin production, a result opposite to that which the investigators had predicted (90). However, the authors of this study suggested that the results could have been influenced by the observation that black subjects had systematically shorter sleep duration but higher
Figure 2.2: Conceptual model of associations between light at night, physical activity, melatonin and cancer.
melatonin levels compared to other subjects in the study (90). Despite these conflicting results, it has been suggested that cumulative time at sleep would correspond positively with melatonin levels, and that it could be possible to use sleep duration as a substitute for melatonin measurement in studies where melatonin levels were not available (94).

2.9 Summary of Rationale:

Shift work has been associated with increased cancer risk at multiple sites, including breast, colon, endometrial and prostate (2-10). This association is likely mediated by changes in melatonin levels as a result of increased light at night exposure through night shift work (14).

While existing epidemiologic research has found associations between shift work and melatonin (38,39), and laboratory studies have demonstrated a relationship between melatonin and cancer risk (19-25), there has been little work that specifically focuses on the association between light at night exposure and melatonin levels (37,40). While existing studies of this specific relationship have demonstrated an inverse association between light and melatonin in shift workers, they have compared functional as opposed to chronological times in day and night workers (37). This method of comparison makes the assumption that the circadian rhythms of night workers have shifted, such that peak melatonin production occurs during the day while sleeping and not at night while working. If this assumption is not met and the circadian rhythms of rotating night workers did not shift, the use of functional time points does not account for differences in circadian stage between day and night workers at the time of melatonin assessment, which may confound any observed associations between light and melatonin levels.
Thus, more research concerning the direct relationship between light exposure and melatonin levels that considers the influence of circadian rhythms of melatonin production is needed.

In addition to the role of light exposure, physical activity is known to reduce cancer risk (42) and has the potential to influence melatonin levels (47). However, existing studies of this relationship have been primarily laboratory based concerning the association of exercise and melatonin and have had conflicting results (47-50). Furthermore, the one observational study of physical activity and melatonin assessed only recreational activity (53). Additional observational studies that use a more complete exposure assessment of physical activity, including recreational, occupational and household activity, are needed to clarify the relationship between physical activity and melatonin. If physical activity is found to influence melatonin levels, it represents a modifiable risk factor that could be altered to reduce any impact of light at night on melatonin levels, and possibly cancer risk.

Finally, the influence of sleep duration on both melatonin levels and cancer risk has been explored in several studies (89-92). While existing studies have found both negative and positive relationships between sleep duration and melatonin, it has been suggested that sleep duration could be used as a proxy for melatonin production (89,90,94). More work is needed to clarify the relationship between these two variables and to assess whether sleep duration is an appropriate approximation of melatonin levels.
References


25. Anisimov VN, Popovich IG, Zabezhinski MA. Melatonin and colon carcinogenesis: I. Inhibitory effect of melatonin on development of intestinal


85. Murphy PJ, Myers BL, Badia P. Nonsteroidal anti-inflammatory drugs alter body temperature and suppress melatonin in humans. Physiol Behav 1996; 59(1)133-139.


Chapter 3: Methods

3.1 Objectives:

The primary objectives of this thesis are to examine the association of light at night exposure with urinary and salivary melatonin levels and to examine the associations of both usual and recent physical activity with melatonin levels. This thesis will also describe the pattern of salivary melatonin secretion and compare this with urinary melatonin measurements to determine the extent to which salivary melatonin measures absolute melatonin levels. Finally, from a methodological perspective, this thesis will investigate the extent to which sleep duration may be used as a proxy for melatonin levels, where increased sleep duration is expected to be associated with increased melatonin.

3.2 Study Design:

To evaluate both the independent and combined effects of light at night exposure and physical activity on urinary and salivary melatonin levels among rotating shift nurses, a cross-sectional study was conducted. This study was designed by Dr. Aronson and her team that included a previous MSc student, Maria Sanchez. Data collection methods and preliminary data analysis were reported in a previous thesis (1).

3.3 Study Population:

The target population for this study was all nurses employed at Kingston General Hospital (KGH) between the ages of 30 and 65 working a rotating shift schedule. To recruit subjects for the study, five individual units within the hospital (ICU, NICU,
Davies 4, Kidd 4 and Connell 9/10) were targeted, with nurses here representing approximately 30% of the hospital nursing staff. The standard rotating shift pattern at KGH is two 12-hour days, followed by two 12-hour nights, followed by five days off. Individuals using hormone therapy, beta-blockers, antidepressants, fertility drugs or melatonin supplements self-excluded from the study. Exclusion criteria were advertised so that subjects could self-select out of the study without having to disclose this information. Study participants were volunteers and the study was advertised to units using posters, pamphlets and seminars that were held as “in-service” clinical education sessions to provide information about the study. Consent to contact forms were enclosed in pamphlets and provided at the seminars. These forms were completed by potential participants, giving the study team permission to contact them. Data collection for this project took place between April 2006 and August 2006.

Seventy-six women asked to be contacted to learn more about the study. Of these women, 8 were ineligible, 4 declined to participate once the details of the study were disclosed, and 3 did not contact us to participate. A total of 61 participants who provided informed consent were enrolled in the study and age-frequency matched (within 2 years) to be followed on their day shift or night shift. Twenty-nine women were assigned to be followed on their day shift and 32 women were followed on their night shift. At completion, participants were provided with a $50 honorarium. The study was approved for ethical compliance by the Queen’s University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board (Appendix A).
3.4 Basic Data Collection Procedures:

Nurses who consented to participate were followed for a 3-day period covering either their day or night shifts, depending on whether they were classified as day or night workers. They were asked to wear a light data logger for this entire period that was used to assess ambient light intensity. Participants also provided one urine sample and four saliva samples within a 24-hour period covering either the second day or second night shift. Urine and saliva samples were then analyzed to assess levels of melatonin biomarkers. Participants also completed a study questionnaire at the beginning of the study period and a one-day diary over the 24-hour period of melatonin assessment.

3.5 Light Intensity Assessment:

Light intensity exposure was measured by StowAway light intensity data loggers (Hoskin Scientific Ltd) worn by study participants for the entire 3-day study period. These loggers record ambient light intensity levels in lumens per square metre or per square foot and are able to detect light intensities from 0.01 lumens/sq ft to 1,000 lumens/sq ft. Lumens are a measure of total light output and most indoor lamps range from 50 to 10,000 lumens (2). Light intensity, which is measured by the light data loggers, usually ranges from 10 to 1,000 lux (lumens per square metre) indoors (2). The loggers were programmed to begin recording at midnight on the first day of study participation and took readings every five minutes for the duration of study participation. Nurses wore the loggers as close to eye level as possible, usually around their neck, for the full three days of study participation and placed the logger on the bedside table while sleeping. Participants were asked to remove the loggers while showering, bathing or
swimming and to protect the logger if it was at risk of getting wet, such as if it was raining outside.

### 3.6 Physical Activity Assessment:

Study participants provided information regarding both recent and usual physical activity. All physical activity measures were assessed via information collected by self-report from the study questionnaire and one-day diary. For each activity recorded, nurses provided information about the type of activity, time of day it was completed, intensity, duration and whether the recorded level of activity was typical for that particular subject. The intensity of the activity was classified into one of four categories: sedentary, light, moderate or heavy. Sedentary activities were those that involved sitting only, with minimal walking. Light activities required minimal physical effort such as standing or walking. Moderate activities were those that were not exhausting, but slightly increased the heart rate and caused light perspiration. Activities of heavy intensity increased the heart rate and caused heavy sweating. Physical activity questions were based on questions from the Lifetime Physical Activity Questionnaire (3).

Recent physical activity was defined as activity performed during the 24-hours of melatonin assessment covered by the one-day diary and was used to examine the acute association between physical activity and melatonin levels. The diary collected information on recreational, household and occupational activity (Appendix B). For each participant these data were used to create variables for total recreational, occupational and household activity separately. Only activities reported as either moderate or heavy intensity were included in order to ensure that activity and not inactivity was assessed and
the total number of hours spent on each type of activity was summed. An estimate of total recent physical activity, which combined recreational, occupational and household activity, was created by adding the number of hours of moderate and heavy intensity activity in each category together for each subject.

Activity during the previous two weeks was used as an estimate of usual physical activity. This period was chosen as it incorporated both work days and days off since activity patterns were expected to be different on days when subjects were not working. Usual physical activity was assessed in the study questionnaire, which collected information about recreational and household activities performed over the previous two weeks (Appendix C). Activity variables for usual activity were constructed in the same way as for recent activity, with the number of hours of moderate or heavy intensity activity for the recreational and household activity categories summed for each participant. As with recent activity, total usual activity was assessed by combining the recreational and household activity variables.

3.7 Melatonin Assessment:

Melatonin levels were assessed from one urine and four saliva samples that were provided by study participants over a 24-hour period that covered either their second day or second night shift, depending on whether they were classified as a day or a night worker.
3.7.1 Urine and Saliva Collection Procedures:

For assessment of salivary melatonin levels each subject collected four saliva samples using the Bühlmann saliva collection device, which consisted of a cotton plug that subjects were asked to chew until it became saturated. Samples were collected at four identical functional time points for day and night workers: upon arising, mid-shift, before bed and again upon awakening (Figure 3.1). When providing saliva samples participants were asked not to drink alcohol, coffee, tea or other coloured beverages for one hour prior to sample collection. The primary purpose of salivary melatonin assessment in this study was to provide a marker of circadian melatonin variation. While previous research has indicated that salivary melatonin is not a good estimate of absolute melatonin levels, saliva has been shown to accurately reflect melatonin variations (4-6).

To assess levels of urinary 6-sulfatoxymelatonin subjects provided one urine sample at the end of the 24-hour melatonin assessment period upon awakening at the same time as the fourth saliva sample (Figure 3.1). Participants were instructed that this urine sample should be the first morning void and were instructed to collect the entire void. The timing of urine sample collection was designed to capture peak melatonin production during sleep, where previous studies have indicated that levels of 6-sulfatoxymelatonin in the first morning void of urine is a good estimate of peak plasma melatonin levels (7).
3.7.2: Laboratory Analysis:

Two biomarkers were used to assess melatonin levels in this study. Levels of the primary urinary melatonin metabolite, 6-sulfatoxymelatonin, were assessed using the Bühlmann 6-sulfatoxymelatonin ELISA kit (American Laboratory Products Company) from the single morning void urine sample. The Bühlmann Direct Saliva Melatonin EIA kit (American Laboratory Products Company) was used assess melatonin levels directly from saliva samples. Both the salivary and urinary melatonin analysis kits are competitive immunoassays that use the capture second antibody technique. All laboratory analyses were conducted by Maria Sanchez in the lab of Dr. Charles Graham.

In both the urine and saliva assays, melatonin present in the samples competes with biotinylated 6-sulfatoxymelatonin (urine samples) or melatonin (saliva samples) for a highly specific melatonin antibody. The biotinylated 6-sulfatoxymelatonin or melatonin antibody complexes then bind to a second polyclonal antibody coated onto the well of a microtiter plate provided with the assay, during the second incubation period for
the saliva assay and the first incubation period for urine. The plates are then washed with an enzyme label, streptavidin conjugated to horseradish peroxidase, which binds to the melatonin-biotin-antibody complexes bound to the wells. Unbound enzyme is then washed from the wells and a substrate tetramethylbenzidine is added. During a final incubation step a coloured product is formed in inverse proportion to the amount of melatonin present in the original sample. The absorbance of the plate can then be measured using a spectrophotometer at 450 nm and the concentration of melatonin determined from a standard curve provided with the assay.

3.8 Study Diary and Questionnaire:

Subjects completed the study questionnaire at the start of study participation and the one-day diary during the 24-hours of melatonin assessment. In addition to physical activity information, the questionnaire collected personal information, health history and for the preceding two weeks medication use, lifestyle habits including smoking, alcohol and caffeine consumption, sleep duration and lighting conditions. The diary collected information about lighting conditions, sleep duration, smoking, alcohol and caffeine consumption, and use of medications in addition to information regarding recreational, household and occupational physical activity previously described.

3.9 Potential Confounders:

Information about variables identified in the literature review as potential confounders was obtained from the study questionnaire and one-day diary for inclusion in
the statistical analysis. These variables included personal characteristics, lifestyle factors and several drugs.

3.9.1 *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. Subjects also reported several reproductive characteristics including their menstrual status, parity (number of pregnancies) and the age at which they gave birth to their first child. Subjects also self-reported their height in centimetres or inches and their weight in pounds or kilograms. This information was then used to calculate body mass index (BMI) for each subject as weight in kilograms divided by the square of height in metres. When imperial units were reported, they were converted to metric units for BMI calculations. Previous research indicates that while self-reported height and weight correlate well with measured values, errors associated with self-report tend to underestimate weight and overestimate height, leading to underestimations of BMI, particularly among women (8,9). Since information used to calculate BMI in this study was self-reported, BMI values may represent an underestimation of the truth.

3.9.2 *Pharmacologic Agents:*

The literature review identified antidepressants, beta-blockers, non-steroidal anti-inflammatory drugs (NSAIDs), hormone replacement therapy, sedatives and oral contraceptives as drugs with the potential to impact on melatonin levels (10-17). The
influence of antidepressants, beta-blockers and hormone replacement therapy was controlled at the design stage by having use of these drugs in the exclusion criteria. For the remaining drugs, information regarding use was collected in the study questionnaire and diary to allow potential confounding to be controlled in the statistical analysis. Use of oral contraceptives was assessed in the questionnaire and subjects who reported using these drugs were asked to report the brand name, medication type, date started and duration of use in years. Use of NSAIDs and muscle relaxants in the previous two weeks was also assessed in the questionnaire, with subjects who reported use of these drugs asked to report the brand name, dose, frequency of use and whether they had taken the drug in the previous 24-hours. Use of NSAIDs, sedatives or muscle relaxants during the 24-hours of melatonin assessment was assessed in the one-day diary and again, subjects who reported use of any of these drugs were asked to report the brand name and the number of tablets taken.

3.9.3 Lifestyle Factors:

Smoking status, along with alcohol and caffeine consumption were lifestyle characteristics that were identified with the potential to influence melatonin levels (18-25). Smoking status was assessed in the study questionnaire where subjects were asked to identify whether or not they were a current smoker. Alcohol consumption in the previous two weeks was assessed in the questionnaire where participants reported whether they had consumed more than twice in the preceding two weeks any of beer, wine or spirits. For analysis purposes, alcohol types were combined and alcohol consumption was assessed categorically as either yes or no to consumption of any type of alcohol. Caffeine
consumption in the preceding two weeks was assessed similarly to alcohol with subjects reporting if they had consumed tea, coffee or other caffeinated beverages more than twice in the previous two weeks. Again, drink types were combined for analysis and evaluated categorically as either yes or no to caffeine consumption in the previous two weeks. For both alcohol and caffeine, subjects who answered yes were asked to specify the number of drinks per day or per week and for caffeinated beverages, the size of the drink.

Alcohol and caffeine consumption during the 24-hours of melatonin assessment were evaluated in the one-day diary. The question format was similar to the study questionnaire with subjects reporting consumption of wine, beer or spirits to assess alcohol use and consumption of tea, coffee or other caffeinated beverages to assess caffeine intake. As in the questionnaire, subjects who answered yes to any of the above were asked to report the number of drinks of each beverage type that were consumed. Alcohol and caffeine consumption were again assessed categorically with the three beverage types combined for both variables. The study diary also assessed whether subjects smoked during the 24-hours of melatonin assessment.

3.10 Statistical Analysis:

3.10.1 Descriptive Statistics:

Descriptive statistics were used to describe the characteristics of the study population. Means and standard deviations were calculated for continuous variables, and frequency tables were created for categorical variables. Comparisons were made between shift groups using Wilcoxon rank sum tests for continuous variables and Chi Square and
Fischer’s exact tests for categorical variables. All statistical analyses were performed using SAS (Version 9.1, SAS Institute, Cary, NC)

3.10.2 Light and Melatonin Analysis:

To assess the association between light intensity and urinary 6-sulfatoxymelatonin levels, light intensity was measured as the average light intensity during sleeping time. These values were not normally distributed and were therefore log-transformed to create a more normal distribution. Average light intensity values were compared between shift groups using Wilcoxon rank sum tests. Peak urinary 6-sulfatoxymelatonin levels measured from urine samples were also compared between shift groups using Wilcoxon rank sum tests. Urinary 6-sulfatoxymelatonin levels were also not normally distributed and thus were log-transformed to create a more normal distribution prior to regression analysis.

The association between light intensity during sleeping time and urinary 6-sulfatoxymelatonin levels was assessed by multiple linear regression. The multivariate model was built using an all-possible models manual backwards selection procedure (26). The bivariate association with urinary 6-sulfatoxymelatonin of all covariates assessed in the diary and questionnaire that were identified a priori as potential confounders was examined and those associated with urinary 6-sulfatoxymelatonin levels at p < 0.25 were included in the backwards selection procedure. Potential confounders were retained in the final model if they changed the parameter estimate for light intensity by more than 10% upon deletion (26). Analysis was also stratified by shift group to assess if the relationship between light intensity and melatonin levels differed according to shift worked. The all-
possible models backwards selection process was repeated in the stratified analysis to identify variables that confound relationships in each specific shift group.

Since the urine samples were taken at identical functional time points (Figure 1), mean salivary melatonin levels at similar times of day (chronological time points) were compared between day and night workers using Wilcoxon rank sum tests to assess if the circadian patterns of melatonin production differed between these groups. If there did not appear to be a shift in the circadian pattern of melatonin production in night workers, Spearman rank correlation was performed to assess the extent to which salivary melatonin levels from the fourth saliva sample captured the pattern of absolute melatonin levels measured by urine samples. Since these two samples were collected at the same time, they should represent the same absolute melatonin concentrations. To account for the fact that salivary and urinary melatonin levels were measured in different units, urinary levels were converted to salivary units prior to correlation analysis.

In the absence of a strong correlation between urinary and salivary melatonin levels, the magnitude of variation in salivary melatonin was used as an outcome measure to look at the associations with light intensity levels while controlling for differences in circadian stage at the time of urinary melatonin assessment. For this analysis, the average light intensity between the hours of 12AM and 5AM (the time of peak melatonin production) was used as the main exposure variable (13,27). This association was assessed using multiple linear regression with models built using the same all-possible models manual backwards selection procedure as in the urinary melatonin analysis (26).
3.10.3 Physical Activity and Melatonin Analysis:

The association of both recent and usual physical activity with urinary melatonin levels was assessed by multiple linear regression and by comparing mean urinary melatonin levels between categories of physical activity. Due to the absence of a shift in the circadian pattern of melatonin production in night workers and the potential confounding of the relationship between physical activity and melatonin by differences in circadian stage at the time of urinary melatonin assessment, these analyses were stratified by shift group. Means and 95% confidence intervals were calculated for each category of physical activity for both recent and usual activity in both the day and night shift groups. Multivariate models were created for the day and night shift groups separately using the same all-possible models manual backwards selection procedure used for the light and melatonin analysis (26).

3.10.4 Sleep Duration and Melatonin:

Correlation analysis was used to assess the relationship between sleep duration and urinary melatonin levels. Average sleep duration values were compared between shift groups using Wilcoxon rank sum tests. As with the physical activity analysis, this analysis was stratified by shift group due to confounding by circadian rhythm at the time of urinary melatonin assessment. Spearman rank correlation was used as sleep duration and log transformed urinary melatonin levels were not normally distributed when stratified by shift group.
References


Chapter 4: Manuscript

This manuscript is written according specifications for submission to the peer-reviewed journal *Journal of Occupational and Environmental Medicine*.

**Title:** Light intensity exposure and biomarkers of melatonin among rotating shift nurses

Anne Grundy, BSc<sup>a</sup>

Maria Sanchez, MSc<sup>a</sup>

Harriet Richardson, PhD<sup>a</sup>

Joan Tranmer, PhD<sup>a,b</sup>

Marilyn Borugian, PhD<sup>c</sup>

Charles Graham, PhD<sup>d</sup>

Kristan Aronson, PhD<sup>a</sup>

<sup>a</sup> Community Health and Epidemiology, and Cancer Research Institute, Queen’s University; <sup>b</sup> School of Nursing, Queen’s University; <sup>c</sup> British Columbia Cancer Agency, Vancouver, British Columbia, Canada; <sup>d</sup> Anatomy and Cell Biology, Queen’s University, Kingston, Ontario, Canada

Running Title: Light intensity and biomarkers of melatonin

Support: Breast Cancer Action Kingston; CIHR Transdisciplinary Cancer Training Program; Programme of Research in Environmental Etiology of Cancer, NCIC

Correspondence: Dr. Kristan Aronson, Division of Cancer Care and Epidemiology, Queen’s Cancer Research Institute, 10 Stuart Street second floor, Queen’s University, Kingston, ON, K7L 3N6 Canada

E-mail: aronson@queensu.ca

Fax: (613) 533-6794; Telephone: (613) 533-6000 ext. 78522
ABSTRACT

Objective: To assess the association between light intensity exposure and melatonin production in rotating shift nurses.

Methods: A cross-sectional study was conducted among 61 female rotating shift nurses (work schedule: two 12 hour days, two 12-hour nights, 5 days off). Light intensity was measured using a light intensity data logger and melatonin concentrations were measured from urine and saliva samples.

Results: An inverse association (p = 0.002) between light exposure and urinary melatonin levels was observed; however, this was no longer significant when stratified by shift group. Analysis of salivary melatonin levels indicated the circadian rhythms of night workers were not shifted, such that peak melatonin production occurred at night during work.

Conclusions: This study indicates that a rotating pattern of shift work may not change the timing of peak melatonin production to the day among those working at night.
INTRODUCTION

The International Agency for Research on Cancer (IARC) recently classified shift work that involves circadian disruption as a probable (Group 2A) carcinogen (1). Epidemiologic studies of shift work and cancer have found evidence of associations with breast (2-8), colon (9), prostate (10-11) and endometrial cancer (12), often with cancer risk increasing with the number of years of shift work (4,5,8). The majority of studies of this relationship to date have focused specifically on breast cancer, with a recent meta-analysis demonstrating that existing studies of female shift workers consistently found a 40 – 50% increased risk of breast cancer associated with shift work (13). The biological mechanism for this relationship is thought to involve the hormone melatonin, which is produced by the pineal gland in a pattern that follows circadian rhythms with peak levels observed at night when light is absent (14). Melatonin production is inhibited by light and increased light at night exposure during night shifts is thought to decrease melatonin levels (2,15). Laboratory studies have demonstrated several anticarcinogenic properties of melatonin where increased melatonin levels can help inhibit tumour development (16-19) and decrease production of reproductive hormones such as estrogen, which play a role in the development of hormone-dependent cancers (14,20,21).

Studies of shift workers have examined the association between light at night exposure and melatonin with multiple methods including questionnaires regarding shift work history and bedroom lighting conditions, as well as with direct light intensity measurements (4,5,22-24). One study of shift work and melatonin levels found an inverse association between the number of nights worked and melatonin secretion, with recent night work associated with a 56% decrease in levels of 6-sulfatoxymelatonin, the primary
The melatonin metabolite found in urine (23). Another study using objective measures of light intensity and salivary melatonin levels found that 7-day patterns of light exposure and melatonin were different in rotating shift workers compared to day-only workers (22). Burch et al., in a population of non-rotating shift workers, found higher 24-hour ambient light levels in evening shift workers and altered melatonin secretion patterns in night workers, with post-work melatonin levels higher and post-sleep levels lower compared to day workers (25).

Since shift work is a necessary component of many occupations, studies with precise assessment of both light exposure and melatonin levels are needed to help clarify the relationship between these two factors. If changes in melatonin levels do impact cancer risk, then a more complete understanding of how light exposure in rotating shift workers affects melatonin levels is required. To evaluate the association between light at night exposure and melatonin levels, a cross-sectional study among rotating-shift nurses at Kingston General Hospital was conducted. The objective of this study was to evaluate the association between light intensity, measured using a light intensity data logger, and melatonin levels measured in urine and saliva samples.

**MATERIALS AND METHODS**

**Study Population**

Rotating shift nurses between the ages of 30 and 65 were the target population. All nurses from 5 inpatient units at Kingston General Hospital (KGH), representing approximately 30% of the hospital clinical nursing staff, were approached. The standard rotating shift pattern at KGH is two 12-hour days, followed by two 12-hour nights,
followed by five days off. Individuals using hormone therapy, beta-blockers, antidepressants, fertility drugs or melatonin supplements were excluded from the study. Potential participants were informed about the study through poster advertisements, pamphlets and “in-service” seminars held during clinical education sessions. Data collection for this project took place between April 2006 and August 2006.

Seventy-six women asked to be contacted to learn more about the study. Of these women, 8 were ineligible, 4 declined to participate once the details of the study were disclosed and 3 did not follow-up after initial contact. All participants in this study were rotating shift workers and were assigned to participate in the study on either their day or night shift. Nurses who participated on their day shift were called “day workers” and those participating on their night shift were known as “night workers”. A total of 61 participants were enrolled in the study and were age-frequency matched (within 2 years) to be included in either the day or night shift group. Participants were designated to shift groups depending on their schedule, the availability of light loggers and the need for participation in certain age-frequency strata. Twenty-nine women were assigned to data collection on their day shift and 32 women were followed on their night shift. At the completion of the study participants were provided with a $50 honorarium. The study was approved for ethical compliance by the Queen’s University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board.

**Procedures**

Nurses participated in the study for a 3-day period during which they were asked to wear a light data logger, provide one urine sample and four saliva samples and complete a study diary and questionnaire.
Study participants were asked to wear a StowAway light intensity data logger for the entire 3-day study period to measure light exposure. The loggers record ambient light intensity levels in lumens per metres$^2$ and were programmed to begin recording at midnight on the first day of study participation. Nurses wore the loggers as close to eye level as possible, usually around their neck, for the duration of the study period and placed the logger on the bedside table while sleeping. Participants were asked to remove the loggers while showering, bathing or swimming and to protect the logger if it was at risk of getting wet, such as if it was raining outside.

Melatonin levels were assessed from one urine and four saliva samples that were provided by study participants over a 24-hour period that covered either their second day or second night shift, depending on whether they were classified as a day or a night worker. Saliva samples were collected using the Bühlmann saliva collection device at four identical functional time points (Figure 1) for both day and night workers: upon arising, mid-shift, before bed and again upon awakening. Urine samples were taken at the same time as the fourth saliva sample.

Participants were also asked to complete a one-day diary during the 24-hours of melatonin assessment and a study questionnaire prior to study participation. The diary collected information about lighting conditions, sleep duration, smoking, alcohol and caffeine consumption and use of medications. The study questionnaire collected personal information, health history and for the preceding two weeks medication use, lifestyle habits including smoking, alcohol and caffeine consumption, sleep duration, lighting conditions and shift patterns.
Melatonin Laboratory Analysis:

Two biomarkers were used to assess melatonin levels in this study. Levels of the primary urinary melatonin metabolite, 6-sulfatoxymelatonin, were assessed using the Bühlmann 6-sulfatoxymelatonin ELISA kit (Bühlmann Laboratories) from a single waking void urine sample collected from each participant. Urinary 6-sulfatoxymelatonin levels in the first morning void represent approximately 70% of plasma levels, the gold standard for melatonin assessment, and have been found to correlate well with plasma melatonin levels (26-28). Salivary melatonin levels were directly assessed from four saliva samples collected in a time pattern designed to capture circadian melatonin variations using the Bühlmann saliva collection device and assessed using the Bühlmann Direct Saliva Melatonin EIA kit (Bühlmann Laboratories). Salivary melatonin levels represent approximately 30% of plasma levels and are not considered to be a good estimate of absolute melatonin levels; however, saliva is considered a good marker of melatonin variability (29-31). Both laboratory analysis kits used are competitive immunoassays that use the capture second antibody technique.

Statistical Analysis:

Descriptive statistics were used to characterize the study population. Means and standard deviations were calculated for continuous variables and frequency tables were created for categorical variables. Comparisons were made between shift groups using Wilcoxon rank sum tests for continuous variables and Chi Square and Fischer’s exact tests for categorical variables.

To assess the association between light intensity and urinary 6-sulfatoxymelatonin levels light intensity was measured as the average light intensity during sleeping time.
These values were not normally distributed and were therefore log-transformed to create a more normal distribution. Average light intensity values were compared between shift groups using Wilcoxon rank sum tests. Peak urinary 6-sulfatoxymelatonin levels measured from urine samples taken at the same time as the fourth saliva sample were also compared between shift groups using Wilcoxon rank sum tests. Urinary 6-sulfatoxymelatonin levels were also not normally distributed and thus were log-transformed to create a more normal distribution prior to regression analysis.

The association between light intensity during sleeping time and urinary 6-sulfatoxymelatonin levels was assessed by multiple linear regression. The multivariate model was built using an all possible models manual backwards selection procedure (32) that included covariates assessed in the diary and questionnaire that were associated with urinary 6-sulfatoxymelatonin levels at p < 0.25 (age, NSAID use in previous two weeks, caffeine consumption during the period of melatonin collection and parity). Potential confounders were retained in the final model if they changed the parameter estimate for light intensity by more than 10% upon deletion (32).

Since the urine samples were taken at identical functional time points (Figure 1), mean salivary melatonin levels at similar times of day (chronological time points) were compared between day and night workers using Wilcoxon rank sum tests to assess if the circadian patterns of melatonin production differed between these groups. If there did not appear to be a change in the circadian pattern of melatonin production among night workers, then regression analysis was stratified by shift group to assess if the relationship between light intensity and melatonin levels differed according to shift worked. Separate multivariate models for day and night workers were constructed using the same methods
as those used for the full study population. All statistical analyses were performed using SAS (Version 9.1, SAS Institute, Cary NC).

RESULTS

Characteristics of the study population are described in Table 1. Overall, day and night workers had similar characteristics and there were few significant differences between the study groups. Nurses on the night shift had significantly shorter sleep duration (p < 0.0001), higher BMI (p = 0.02) and consumed less alcohol in the 24 hours covered by the diary (p = 0.02) compared to those working the day shift. The shorter sleep duration and reduced alcohol consumption among night workers is likely explained by the fact that this group was working at night and sleeping during the day, providing less opportunity for both sleep and alcohol consumption. The elevated BMI among night workers was not expected and simply occurred by chance, since all participants worked both day and night shifts.

Mean light intensity levels during both sleep time (p < 0.0001) and during the night between the hours of 12AM and 5AM (p < 0.0001) were significantly higher for night workers compared to day workers (Table 2). Urinary 6-sulfatoxymelatonin levels assessed from urine samples taken upon arising at the end of the 24-hour period covered by the one-day diary were significantly lower among night workers (p = 0.0003) compared to those working the day shift (Table 3). One participant (night shift) was excluded from further analysis due to missing light exposure data.

Multivariate linear regression was performed to assess the association between light intensity while sleeping and urinary melatonin levels. When all subjects (both shifts)
were considered together, a significant inverse relationship between urinary 6-sulfatoxymelatonin levels and light intensity during sleeping time was observed (Table 4 and Figure 2). Confounder assessment revealed that no variable was a confounder in this relationship.

Comparison of salivary melatonin levels between day and night workers at similar times of day and night (chronological time points) found no major change in the timing of melatonin production among those working at night (Figure 3). Peak salivary melatonin levels were seen at night in both shift groups, indicating that the circadian rhythms of melatonin production among rotating shift nurses did not change to the day when they were working at night. Urinary 6-sulfatoxymelatonin levels were assessed at identical functional time points (ie. after sleeping) among those working during the day and those working at night. However, this assessment strategy made the assumption that circadian rhythms of melatonin production in night workers would be altered, such that peak melatonin production would occur during the day while sleeping, and not at night while working. When comparing chronological time points, there was no significant difference in salivary melatonin levels between those working during the day or night (Table 5). Although it appears that the peak level of melatonin is lower among those working at night, the amplitude of melatonin variation between day and night shift groups does no differ statistically (Wilcoxon rank sum test, p = 0.44). Therefore, differences in circadian stage between shift groups at the time of urinary melatonin assessment could confound associations between light intensity and melatonin levels when both shift groups are considered together.
To account for differences in circadian stage at the time of urinary melatonin assessment, the relationship between light intensity during sleeping time and urinary 6-sulfatoxymelatonin levels was assessed among day and night shift groups separately. The inverse relationship between light intensity and melatonin levels seen in the full study population remained for day workers (Figure 4a), but was not statistically significant. There was no relationship between light intensity during sleeping time and urinary melatonin levels in night workers (Figure 4b). In sensitivity analysis, the influence of one extreme observation was assessed, and upon removal the inverse relationship between light intensity and melatonin in the day shift group disappeared.

**DISCUSSION**

This study examined the association between light intensity and urinary and salivary melatonin levels among rotating shift nurses. In the full study population, a significant inverse association between light intensity during sleeping time and urinary 6-sulfatoxymelatonin levels was observed. This result is consistent with previous studies of night work and melatonin where night shift work has been associated with decreased 6-sulfatoxymelatonin levels (23,24). Previous work has demonstrated that first morning void urine samples are able to accurately assess levels of peak melatonin production during sleep (27,28). In this study, the timing of urine sample collection was designed such that 6-sulfatoxymelatonin levels upon arising would be able to approximate melatonin production during sleep. Urinary 6-sulfatoxymelatonin levels were significantly lower during sleep in night workers compared to those working days, a
result consistent with those from the study by Borugian et al., one of the few previous studies employing direct measures of both light intensity and melatonin (22).

The use of a sampling pattern that compared functional (upon awakening etc.) and not chronological (time of day) time points made the assumption that the circadian rhythms of those working nights were shifted such that peak melatonin secretion occurred in the daytime hours during sleep, and not in the nighttime hours during work. The question of whether the timing of melatonin secretion is altered by rotating shift work has been examined in previous studies. Borugian et al. found that melatonin secretion patterns differed for rotating shift workers depending on whether they were on the day or night shift (22). However, these observations may have been a result of the time of day of melatonin sampling as functional and not chronological time points were compared. Consequently, rotating shift workers sleeping during the day may have had low melatonin levels during sleep compared to those sleeping at night due to natural circadian variations and not light at night exposure.

While some studies of permanent night workers have shown a shift in the timing of melatonin production (33,34), others have found that melatonin secretion patterns in permanent night workers are no different from those of individuals with a day-oriented schedule (35-37). For example, a study by Dumont et al. observed that 22 out of 30 permanent night shift nurses had melatonin profiles that were typical of day-oriented people, such that peak melatonin production still occurred at night (35). Studies of simulated night work have shown that while it is possible to produce a change in the timing of melatonin secretion, at least three days of simulated night work combined with fixed sleeping times are required (38,39). These observations suggest that the two night
shifts involved in the rotating shift pattern at our hospital would not be sufficient to produce a shift in circadian rhythms. From the perspective of workplace policy, this may suggest that if it is the change in timing of melatonin production that impacts on cancer risk then rotating shift patterns that do not allow these changes to occur may have a smaller impact on cancer risk among shift workers.

Comparison of salivary melatonin levels between those working day and night shifts at similar chronological times revealed that melatonin secretion patterns among night workers were not altered and that the peak melatonin levels measured from salivary melatonin samples were seen at the mid-shift (11PM – 1AM) time point (Figure 3). This observation suggests that differences in urinary melatonin levels between those working days and nights may have been the result of differences in circadian stage at the time of melatonin assessment. Therefore, the time of day of melatonin assessment is independently associated with melatonin levels, with higher melatonin observed at night compared to during the day in both the day and night shift groups. The time of day of melatonin assessment was also independently associated with shift group, where urinary melatonin levels were assessed at different times of day in the day and night shift groups. Thus, since time of day is independently associated with both shift group and melatonin, the observed differences in urinary melatonin levels between shift groups are likely confounded by differences in circadian stage at the time of melatonin assessment. To account for the potential for confounding by circadian rhythms in urinary melatonin levels, we stratified by shift group and examined associations between light intensity and 6-sulfatoxymelatonin levels in day and night workers separately.
After stratification of the regression analysis by shift group, there was no relationship between light intensity during sleep and urinary 6-sulfatoxymelatonin levels in night workers (Figure 4b). While an inverse association among day workers was suggested, this relationship was no longer statistically significant (Figure 4a).

One possible explanation for the absence of an association between light and melatonin among those working at night is that while urine sampling was designed to measure peak melatonin levels while sleeping, peak melatonin production among night workers actually occurred at night while they were awake and not during the day while they slept. Therefore, if there was an association between light exposure and peak melatonin levels among those working at night, the melatonin assessment strategy employed in this study would not be able to capture it. Although the inverse association between light intensity and melatonin seen among day workers was not statistically significant, with only 29 participants in the day shift group, it is likely that this study did not possess adequate statistical power to detect associations in the stratified sample. Additionally, the variation in light exposure between day and night workers is lost in the stratified analysis, which would further reduce the ability of this study to detect associations between light exposure and melatonin. Therefore, since urinary melatonin levels in day workers would be expected to reflect peak melatonin levels, unlike the urine samples from those working nights, the suggested inverse association between light exposure and melatonin seen in this group may represent a true relationship.

The main strengths of this study lie in its objective measurement of both light intensity and urinary and salivary melatonin levels. Few previous studies have used an objective measure of light intensity, with the majority using one or both of frequency and
duration of night shift work as a proxy for light exposure (4,5,22-24). Our precise measurement of both exposure and outcome provided sufficient power in the full study population to detect associations with a fairly small sample size. Using multiple saliva samples over a 24-hour period, our study was also able to characterize the melatonin secretion patterns of rotating shift workers on their day and night shifts, with comparisons of chronological time points revealing that circadian rhythms of melatonin secretion were not altered on the night shift of a rotating shift pattern. This observation highlights the importance of accounting for the role of circadian rhythms of melatonin production when making comparisons between day and night workers in a population working rotating shifts, a factor that has not been emphasized in previous work (22,24,25).

This study is limited by the presence of confounding by circadian stage at the time of melatonin assessment in urine samples, which meant that results had to be stratified by shift group when assessing the relationship between light intensity and melatonin. Stratification by shift group produced a reduced sample size, as well as decreased variability in light exposure, and limited the power of the study to detect associations between light exposure and melatonin levels.

While this study suggests an inverse relationship between light intensity exposure and melatonin levels, the presence of confounding by circadian stage in urinary melatonin levels means the results should be interpreted with caution. Future studies among rotating shift workers must incorporate the role of circadian melatonin variation at the design stage to minimize this source of confounding. This could be done by collecting urine samples at similar chronological as well as functional time points among day and
night workers to allow the effect of circadian variation to be controlled when assessing melatonin.
REFERENCES


19. Anisimov VN, Popovich IG, Zabezhinski MA. Melatonin and colon carcinogenesis: I. Inhibitory effect of melatonin on development of intestinal


<table>
<thead>
<tr>
<th>Variable</th>
<th>All Workers</th>
<th>Day Workers</th>
<th>Night Workers</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Age (years) - Mean (SD)</td>
<td>42.62 (7.50)</td>
<td>42.76 (7.91)</td>
<td>42.48 (7.23)</td>
<td>0.89</td>
</tr>
<tr>
<td>Sleep Duration (hours) – Mean (SD)</td>
<td>6.47 (2.29)</td>
<td>8.27 (1.24)</td>
<td>4.78 (1.67)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Height (cm) – Mean (SD)</td>
<td>164.16 (6.68)</td>
<td>165.77 (6.96)</td>
<td>162.65 (6.14)</td>
<td>0.06</td>
</tr>
<tr>
<td>Weight (kg) – Mean (SD)</td>
<td>71.00 (16.35)</td>
<td>68.03 (14.02)</td>
<td>73.77 (18.05)</td>
<td>0.26</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²) – Mean (SD)</td>
<td>26.25 (5.43)</td>
<td>24.64 (4.32)</td>
<td>27.76 (5.98)</td>
<td>0.02</td>
</tr>
<tr>
<td>Number of pregnancies – Mean (SD)</td>
<td>2.3 (1.71)</td>
<td>2.17 (1.89)</td>
<td>2.42 (1.54)</td>
<td>0.44</td>
</tr>
<tr>
<td>Menstrual Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-menopausal</td>
<td>50</td>
<td>83.33</td>
<td>25</td>
<td>86.21</td>
</tr>
<tr>
<td>Post-menopausal</td>
<td>10</td>
<td>16.67</td>
<td>4</td>
<td>13.79</td>
</tr>
<tr>
<td>Smoking Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>6</td>
<td>10.00</td>
<td>1</td>
<td>3.45</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>54</td>
<td>90.00</td>
<td>28</td>
<td>96.55</td>
</tr>
<tr>
<td>Used NSAIDs in 24-hours of melatonin assessment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14</td>
<td>23.33</td>
<td>4</td>
<td>13.79</td>
</tr>
<tr>
<td>No</td>
<td>46</td>
<td>76.67</td>
<td>25</td>
<td>86.21</td>
</tr>
<tr>
<td>Used NSAIDs in previous two weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>44</td>
<td>73.33</td>
<td>21</td>
<td>72.41</td>
</tr>
<tr>
<td>No</td>
<td>16</td>
<td>26.67</td>
<td>8</td>
<td>27.59</td>
</tr>
<tr>
<td>Used Sedatives or Muscle Relaxants in 24-hours of melatonin assessment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>5.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>No</td>
<td>57</td>
<td>95.00</td>
<td>29</td>
<td>100.00</td>
</tr>
<tr>
<td>Used Sedatives or Muscle Relaxants in previous two weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>8.33</td>
<td>1</td>
<td>3.45</td>
</tr>
<tr>
<td>No</td>
<td>55</td>
<td>91.67</td>
<td>28</td>
<td>96.55</td>
</tr>
<tr>
<td>Currently Use of Oral Contraceptives</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>81.67</td>
<td>5</td>
<td>17.24</td>
</tr>
<tr>
<td>No</td>
<td>49</td>
<td>18.33</td>
<td>24</td>
<td>82.76</td>
</tr>
<tr>
<td>Consumed Alcohol in 24-hours of melatonin assessment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>13.33</td>
<td>7</td>
<td>24.14</td>
</tr>
<tr>
<td>Beer</td>
<td>2</td>
<td>3.33</td>
<td>2</td>
<td>6.90</td>
</tr>
<tr>
<td>Wine</td>
<td>6</td>
<td>10.00</td>
<td>5</td>
<td>17.24</td>
</tr>
<tr>
<td>Consumed &gt;2 Alcoholic Beverages in previous two weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>32</td>
<td>53.33</td>
<td>18</td>
<td>62.07</td>
</tr>
<tr>
<td>Beer</td>
<td>13</td>
<td>21.67</td>
<td>8</td>
<td>27.59</td>
</tr>
<tr>
<td>Wine</td>
<td>25</td>
<td>41.67</td>
<td>13</td>
<td>44.83</td>
</tr>
<tr>
<td>Spirits</td>
<td>8</td>
<td>13.33</td>
<td>5</td>
<td>17.24</td>
</tr>
<tr>
<td>Consumed Caffeine in 24-hours of melatonin assessment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>55</td>
<td>90.00</td>
<td>24</td>
<td>82.76</td>
</tr>
<tr>
<td>Coffee</td>
<td>43</td>
<td>71.67</td>
<td>21</td>
<td>72.41</td>
</tr>
<tr>
<td>Tea</td>
<td>21</td>
<td>35.00</td>
<td>10</td>
<td>34.48</td>
</tr>
<tr>
<td>Other</td>
<td>21</td>
<td>33.33</td>
<td>8</td>
<td>27.59</td>
</tr>
<tr>
<td>Consumed &gt;2 Caffeinated Beverages in previous two weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>60</td>
<td>100.00</td>
<td>29</td>
<td>100.00</td>
</tr>
<tr>
<td>Coffee</td>
<td>47</td>
<td>78.33</td>
<td>23</td>
<td>79.31</td>
</tr>
<tr>
<td>Tea</td>
<td>36</td>
<td>60.00</td>
<td>18</td>
<td>62.07</td>
</tr>
<tr>
<td>Other</td>
<td>26</td>
<td>43.33</td>
<td>12</td>
<td>41.38</td>
</tr>
</tbody>
</table>

*Differences between day and night workers compared using the Wilcoxon Rank Sum test for continuous variables and Chi-Square and Fischer’s Exact test for categorical variables.

**All subjects consumed more than two caffeinated beverages in previous two weeks.
### Table 2: Light intensity by shift group

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Workers Mean (SD) (n = 60)</th>
<th>Day Shift Mean (SD) (n = 29)</th>
<th>Night Shift Mean (SD) (n = 31)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean light intensity during sleeping time (lumens/m²)</td>
<td>26.53 (52.53)</td>
<td>6.26 (10.67)</td>
<td>45.49 (72.03)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Log-transformed mean light intensity during sleeping time (log lumens/m²)</td>
<td>-0.31 (1.38)</td>
<td>-1.49 (0.76)</td>
<td>0.79 (0.79)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Light intensity exposure between 12AM and 5AM (lumens/m²)</td>
<td>4.82 (9.39)</td>
<td>2.48 (11.23)</td>
<td>7.02 (6.73)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Log-transformed light intensity exposure between 12AM and 5AM (log lumens/m²)</td>
<td>-1.04 (1.30)</td>
<td>-2.01 (0.73)</td>
<td>-0.13 (1.01)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

*Differences between day and night workers compared using the Wilcoxon Rank Sum test.

### Table 3: Urinary 6-sulfatoxymelatonin values by shift group

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Workers Mean (SD) (n = 61)</th>
<th>Day Shift Mean (SD) (n = 29)</th>
<th>Night Shift Mean (SD) (n = 32)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-sulfatoxymelatonin (ng/mL)</td>
<td>14.09 (16.13)</td>
<td>20.98 (17.15)</td>
<td>7.64 (12.16)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Log-transformed 6-sulfatoxymelatonin (ng/mL)</td>
<td>1.89 (1.43)</td>
<td>2.51 (1.39)</td>
<td>1.31 (1.22)</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

*Differences between day and night workers compared using the Wilcoxon Rank Sum test.

### Table 4: Multivariate association between light intensity and 6-sulfatoxymelatonin

<table>
<thead>
<tr>
<th>Model*</th>
<th>Regression Coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All workers (n = 61)</td>
<td>-0.40210</td>
<td>0.002</td>
</tr>
<tr>
<td>Day workers only (n = 29)</td>
<td>-0.22984</td>
<td>0.19</td>
</tr>
<tr>
<td>Night workers only (n = 32)</td>
<td>0.08857</td>
<td>0.10</td>
</tr>
</tbody>
</table>

*Primary exposure variable in all models was mean log transformed light intensity levels during sleep (lumens/m²). All models were adjusted for confounders that changed the parameter estimate by more than 10%. Variables considered were age, use of NSAIDs in previous two weeks, caffeine consumption in 24-hours of melatonin collection and parity for the full study population, however none were retained in the final model. In the day worker only analysis, age and alcohol consumption in 24-hours of melatonin consumption were considered and both were retained in the final model. In the night worker only analysis age, NSAID use in both the 24-hours of melatonin collection and previous two weeks and the number of day shifts in the previous two weeks were considered, but only age and NSAID use in the previous two weeks were retained in the final model.
<table>
<thead>
<tr>
<th>Collection Time(^a)</th>
<th>All Workers(^b)</th>
<th>Day Shift(^b)</th>
<th>Night Shift(^b)</th>
<th>p-value(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Time 1 (5 – 7AM)</td>
<td>17.71 (14.68)</td>
<td>17.71 (14.68)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(n = 24)</td>
<td>(n = 24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time 2 (3 – 5PM)</td>
<td>5.25 (8.60)</td>
<td>4.40 (5.26)</td>
<td>6.13 (11.10)</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>(n = 55)</td>
<td>(n = 28)</td>
<td>(n = 27)</td>
<td></td>
</tr>
<tr>
<td>Time 3 (11PM – 1AM)</td>
<td>12.58 (11.58)</td>
<td>13.43 (11.05)</td>
<td>11.70 (12.26)</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>(n = 55)</td>
<td>(n = 28)</td>
<td>(n = 27)</td>
<td></td>
</tr>
<tr>
<td>Time 4 (5 – 7AM)</td>
<td>13.12 (15.33)</td>
<td>17.13 (20.39)</td>
<td>9.69 (7.96)</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>(n = 52)</td>
<td>(n = 24)</td>
<td>(n = 28)</td>
<td></td>
</tr>
<tr>
<td>Time 5 (3 – 5PM)</td>
<td>5.19 (5.31)</td>
<td>N/A</td>
<td>5.19 (5.31)</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(n = 27)</td>
<td></td>
<td>(n = 27)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Represents comparison of chronological not functional time points
\(^b\) Salivary melatonin levels measured in pg/mL.
\(^c\) Comparison between day and night workers
<table>
<thead>
<tr>
<th>Day Workers:</th>
<th>Functional</th>
<th>Time Point</th>
<th>Upon Awakening</th>
<th>Mid-Shift</th>
<th>Before Sleep</th>
<th>Upon Awakening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of Day</td>
<td>5 - 7 AM</td>
<td>3 - 5 PM</td>
<td>11PM - 1AM</td>
<td>5 - 7AM</td>
<td>Urine Sample</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saliva Sample 1</td>
<td>Saliva Sample 2</td>
<td>Saliva Sample 3</td>
<td>Saliva Sample 4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Night Workers:</th>
<th>Functional</th>
<th>Time Point</th>
<th>Upon Awakening</th>
<th>Mid-Shift</th>
<th>Before Sleep</th>
<th>Upon Awakening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of Day</td>
<td>3 - 5 PM</td>
<td>11PM - 1AM</td>
<td>5 - 7 AM</td>
<td>3 - 5PM</td>
<td>Urine Sample</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saliva Sample 1</td>
<td>Saliva Sample 2</td>
<td>Saliva Sample 3</td>
<td>Saliva Sample 4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2: Association between log-transformed light intensity during sleep and log-transformed urinary melatonin levels (all subjects). Day workers are shown with squares and night workers are shown with circles. 
(Parameter Estimate = -0.40, p = 0.002)
Figure 3: Salivary melatonin secretion patterns comparing chronological time points. (1 = 5 – 7AM, 2 = 3 – 5PM, 3 = 11PM – 1AM, 4 = 5 – 7AM, 5 = 3 – 5PM)
Figure 4a: Association between log-transformed light intensity during sleep and log-transformed urinary melatonin levels in model that accounts for age and alcohol consumption in 24-hours of melatonin collection (Day workers only)
(Parameter Estimate: -0.23, p = 0.19)
Figure 4b: Association between log-transformed light intensity and log-transformed urinary melatonin levels in model controlling for age and use of NSAIDs in both the 24-hours of melatonin collection and the previous two weeks. (Night workers only) (Parameter Estimate = 0.09, p = 0.10)
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 the other thesis objectives not addressed in the manuscript.

5.1 Associations Between Potential Confounders and Melatonin:

The bivariate associations between potential melatonin determinants and log-transformed 6-sulfatoxymelatonin were assessed to determine what, if any, variables were determinants of melatonin in the study population, in addition to light intensity and physical activity (Table 5.1). In the full study population only shift worked (night vs day) had a statistically significant (p < 0.05) association with melatonin levels. Among day workers, no variable had a significant association with melatonin, while among night workers use of non-steroidal anti-inflammatory medications (NSAIDs) during the two weeks prior to study participation was significantly associated with urinary melatonin levels (p = 0.04)

Table 5.1: Bivariate associations between log-transformed urinary 6-sulfatoxymelatonin levels and potential melatonin determinants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All workers Parameter estimate (p-value)</th>
<th>Day Shift Parameter estimate (p-value)</th>
<th>Night Shift Parameter estimate (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.05 (0.05)</td>
<td>-0.05 (0.17)</td>
<td>-0.05 (0.08)</td>
</tr>
<tr>
<td>Shift Worked</td>
<td>-1.21 (0.001)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Body Mass Index (BMI)</td>
<td>-0.03 (0.42)</td>
<td>0.04 (0.56)</td>
<td>-0.01 (0.75)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>-0.01 (0.33)</td>
<td>0.002 (0.94)</td>
<td>-0.004 (0.76)</td>
</tr>
<tr>
<td>Use of NSAIDs in 24-hours of melatonin assessment</td>
<td>0.42 (0.34)</td>
<td>0.63 (0.41)</td>
<td>0.81 (0.08)</td>
</tr>
<tr>
<td>Smoked during 24-hours of melatonin assessment</td>
<td>0.10 (0.86)</td>
<td>0.64 (0.54)</td>
<td>0.24 (0.68)</td>
</tr>
<tr>
<td>Menstrual Status</td>
<td>0.39 (0.43)</td>
<td>0.09 (0.91)</td>
<td>0.43 (0.44)</td>
</tr>
<tr>
<td>Measure</td>
<td>Spearman Correlation Coefficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------------------</td>
<td>----------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of muscle relaxants during 24-hours of melatonin assessment</td>
<td>-0.48 (0.47)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.99 (0.49)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.12 (0.86)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of NSAIDs in previous two weeks</td>
<td>0.49 (0.24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.03 (0.96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.02 (0.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current oral contraceptive (OC) use</td>
<td>-0.01 (0.98)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25 (0.72)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.17 (0.76)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure to second hand smoke</td>
<td>-0.07 (0.53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.19 (0.44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.03 (0.78)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td>-0.12 (0.84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.56 (0.70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.24 (0.68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption during 24-hours of melatonin assessment</td>
<td>-0.17 (0.76)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-1.00 (0.10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.28 (0.83)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption during previous two weeks</td>
<td>-0.29 (0.43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.60 (0.27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.47 (0.28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine consumption during 24-hours of melatonin assessment</td>
<td>-0.75 (0.23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.39 (0.57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.20 (0.87)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at first birth</td>
<td>0.01 (0.68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.05 (0.49)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.52 (0.52)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>-0.16 (0.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.11 (0.43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.15 (0.28)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 5.2 Spearman Correlation Between Urine and Saliva:

Spearman rank correlation was used to assess the extent to which salivary melatonin levels in this study captured the melatonin levels as measured by urine samples. A correlation coefficient of 0.7 was chosen *a priori* as the level at which salivary melatonin would be considered a good approximation of melatonin levels measured in the urine samples. Salivary melatonin levels from the fourth saliva sample were compared with urinary melatonin levels, as these samples were taken at the same time and thus should represent the same absolute melatonin levels. With a Spearman rank correlation coefficient of 0.34 (p = 0.01), salivary melatonin levels were not able to capture the pattern of absolute melatonin levels seen in urine. When stratified by shift group, the correlation became weaker in both the day and night groups, with a correlation of 0.17 (p = 0.42) among day workers and 0.27 (p = 0.17) among night workers.
Therefore, salivary melatonin was not considered representative of urinary melatonin levels and was not used as a proxy for absolute melatonin in this study.

5.3 Light Intensity Exposure and Salivary Melatonin:

5.3.1 Salivary Melatonin:

While salivary melatonin was considered inappropriate as a proxy for absolute melatonin levels in this study, saliva is considered a good measure of melatonin variability (1-3). Therefore, the magnitude of variation in salivary melatonin levels was used as an outcome measure to allow for assessment of the effects of light intensity on the variability of melatonin levels when shift groups are combined, while accounting for differences in circadian stage at the time of urinary melatonin assessment. The magnitude of salivary melatonin variation was calculated as the difference in salivary melatonin levels between saliva samples 2 and 4 for day workers and saliva samples 1 and 3 for night workers, which represent similar chronological times (times of day) for these two groups. The magnitude of salivary melatonin variation was somewhat normal in the full study population and in both the day and night shift groups (Figure 5.1). While the mean magnitude of the salivary melatonin variation was much lower among those on their night shift, there was no statistical difference in the magnitude of salivary melatonin variation between shift groups (p = 0.44) (Table 5.2). This variable was log-transformed prior to regression analysis to create a more normal distribution. Due to missing salivary melatonin levels, an estimate of the magnitude of salivary melatonin variation could not be calculated for 12 subjects (5 day and 7 night workers).
Figure 5.1 Distribution of the magnitude of salivary melatonin variation

a) All workers (n = 49)

b) Day workers only (n = 24)

c) Night workers only (n = 25)
### Table 5.2: Mean magnitude of salivary melatonin variation

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Workers Mean (SD) (n = 49)</th>
<th>Day Shift Mean (SD) (n = 24)</th>
<th>Night Shift Mean (SD) (n = 25)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnitude of salivary melatonin variation (pg/mL)</td>
<td>8.50 (19.14)</td>
<td>12.51 (21.87)</td>
<td>4.66 (15.58)</td>
<td>0.44</td>
</tr>
<tr>
<td>Log-transformed magnitude of salivary melatonin variation (log pg/mL)</td>
<td>0.91 (1.41)</td>
<td>1.02 (1.51)</td>
<td>0.80 (1.33)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

*Differences between day and night workers compared using the Wilcoxon Rank Sum test

#### 5.3.2 Conceptualization of light exposure variable for salivary melatonin analysis:

The mean light intensity level between the hours of 12AM and 5AM, the time of peak melatonin production, during the 24-hours covered by the one-day diary, was used as the main light exposure variable for the analysis assessing the impact of light exposure on salivary melatonin levels (3,4). Mean light intensity was not normally distributed in either the full study population or when stratified by shift group (Figure 5.2), and was therefore log-transformed prior to regression analysis to create a more normal distribution (Figure 5.3). There was a significant difference in light intensity exposure in this time period between day and night workers (p < 0.0001) (Table 5.3). Due to missing light logger data, light intensity exposure between 12AM and 5AM could not be calculated for one subject.

### Table 5.3: Mean light intensity exposure between 12AM and 5AM

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Workers Mean (SD) (n = 60)</th>
<th>Day Shift Mean (SD) (n = 29)</th>
<th>Night Shift Mean (SD) (n = 31)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light intensity exposure (lumens/m²)</td>
<td>4.82 (9.39)</td>
<td>2.48 (11.23)</td>
<td>7.02 (6.73)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Log-transformed light intensity exposure (log lumens/m²)</td>
<td>-1.04 (1.30)</td>
<td>-2.01 (0.73)</td>
<td>-0.13 (1.01)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

*Differences between day and night workers compared using Wilcoxon Rank Sum tests
Figure 5.2: Distribution of light intensity exposure between 12AM and 5AM

a) All workers (n = 60)

b) Day workers only (n = 29)

c) Night workers (n = 31)
Figure 5.3: Distribution of log-transformed light intensity between 12AM and 5AM

a) All workers (n = 60)

b) Day workers only (n = 29)

c) Night workers only (n = 31)
5.3.3 Association between light intensity exposure and salivary melatonin:

The magnitude of salivary melatonin variation was used as an outcome measure to examine the association between light intensity exposure and melatonin levels in order to account for confounding by differences in circadian stage at the time of urinary melatonin assessment. Since neither the magnitude of salivary melatonin variation nor light intensity exposure between 12AM and 5AM were normally distributed, these were log-transformed prior to regression analysis to create a more normal distribution. The association between light intensity exposure and the magnitude of salivary melatonin variation was assessed using multiple linear regression.

The multivariate model was built using an all-possible models manual backwards selection procedure (5). The bivariate association of covariates measured in the one-day diary and study questionnaire that were identified in the literature review as potential confounders with the log-transformed magnitude of salivary melatonin variation was assessed. All variables that were associated with the outcome variable with \( p < 0.25 \) were included in the backwards selection procedure and were retained in the model if they changed the parameter estimate for log-transformed light intensity by more than 10%. Smoking status, use of NSAIDs and having smoked during the 24-hours of melatonin assessment, and the number of night shifts worked in the previous two weeks were considered as potential confounders and all except having smoked during the 24-hours of melatonin assessment were retained in the final model. The final model did not suggest any relationship between light intensity exposure and the log-transformed magnitude of salivary melatonin variation, with a regression coefficient of 0.09 (\( p = 0.07 \)) (Figure 5.4).
Figure 5.4: Multivariate association between log-transformed light intensity exposure and the log-transformed magnitude of salivary melatonin variation. This model is controlled for the effects of smoking status, use of NSAIDs during the 24-hours of melatonin assessment and the number of night shifts worked during the previous two-weeks. The line of best fit and 95% confidence intervals are superimposed.

5.4 Recent Physical Activity and Urinary Melatonin Levels:

5.4.1 Recent Physical Activity:

Recent physical activity was assessed from the one-day diary covering the 24-hours of melatonin assessment and included recreational, household and occupational activity of either moderate or heavy intensity. The number of hours of activity of each type was combined to create a summary of total recent physical activity. When treated as a continuous variable, the distribution of total recent activity was positively skewed (Figure 5.5). To create a more even distribution, total recent activity was divided into three categories: 0 hours, 1 – 3 hours and > 3 hours of recent activity of moderate or heavy intensity (Figure 5.6). Overall, day workers were more active when working
compared to night workers, with 14% of day workers reporting 0 hours of moderate or heavy intensity physical activity compared to 41% of night workers; however this difference was not statistically significant ($p = 0.06$).

**Figure 5.5 Distribution of recent physical activity as a continuous variable.**

**Figure 5.6 Categorical recent physical activity distribution.** Physical activity categorized such that 0 represents 0 hours of activity, 1 represents 1 – 3 hours of activity and 2 represents > 3 hours of activity.

a) Day workers only (n = 29)
b) Night workers only (n = 32)

5.4.2 Recent Physical Activity and Melatonin:

The relationship between recent physical activity and urinary melatonin levels was assessed for the day and night shift work groups separately due to the presence of confounding by circadian stage at the time of melatonin assessment in urine samples. The mean urinary melatonin levels and associated 95% confidence intervals for each of the three categories of physical activity (0 hours, 1 – 3 hours and > 3 hours of moderate or heavy intensity physical activity during the 24-hours of melatonin assessment) were calculated for day and night workers (Table 5.4 and Figure 5.7). Qualitatively, while an increasing trend in urinary melatonin levels with increasing physical activity was observed among day workers, this pattern was not seen in the night shift group, where the highest urinary melatonin levels were seen among nurses who reported 0 hours of moderate or heavy intensity physical activity. There was no statistically significant difference in mean urinary melatonin levels between physical activity groups for either day or night workers, as seen by the overlap in the 95% confidence intervals between physical activity groups. Multiple linear regression was used to quantify the association
between recent physical activity and urinary melatonin levels. A positive relationship between physical activity performed during the 24-hours of melatonin assessment and urinary 6-sulfatoxymelatonin levels was suggested among day workers (Figure 5.7a), although it was not statistically significant (p = 0.13). Among night workers a statistically significant negative association (p = 0.03) was seen between recent physical activity and melatonin levels (Figure 5.7b).

Table 5.4: Mean urinary melatonin level by recent physical activity group for day and night workers.

<table>
<thead>
<tr>
<th>Total Recent Activity</th>
<th>Urinary melatonin level (ng/mL)</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>95% Confidence Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day Workers:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 hours</td>
<td></td>
<td>4</td>
<td>10.59</td>
<td>8.03</td>
<td>-2.19 - 23.37</td>
</tr>
<tr>
<td>1 – 3 hours</td>
<td></td>
<td>15</td>
<td>17.09</td>
<td>16.92</td>
<td>7.72 - 26.46</td>
</tr>
<tr>
<td>&gt;3 hours</td>
<td></td>
<td>10</td>
<td>30.97</td>
<td>16.26</td>
<td>19.34 - 42.61</td>
</tr>
<tr>
<td>Night Workers:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 hours</td>
<td></td>
<td>13</td>
<td>11.11</td>
<td>16.04</td>
<td>1.42 - 20.81</td>
</tr>
<tr>
<td>1 – 3 hours</td>
<td></td>
<td>12</td>
<td>3.93</td>
<td>2.63</td>
<td>2.26 - 5.60</td>
</tr>
<tr>
<td>&gt;3 hours</td>
<td></td>
<td>7</td>
<td>6.79</td>
<td>12.73</td>
<td>-4.99 - 18.56</td>
</tr>
</tbody>
</table>
Figure 5.7: Multivariate association between log-transformed urinary melatonin levels and recent physical activity group for day and night workers. *Physical activity categorized such that 0 represents 0 hours of activity, 1 represents 1 – 3 hours of activity and 2 represents > 3 hours of activity.*

a) Association between physical activity performed during the 24-hours of melatonin assessment and log-transformed urinary melatonin levels in a model that accounts for age and alcohol consumption during the 24-hours of melatonin collection (Day workers only) (Parameter estimate: 0.46, p = 0.13)

![Graph a)

b) Association between physical activity performed during the 24-hours of melatonin assessment and log-transformed urinary melatonin levels in a model that accounts for use of NSAIDs during the 24-hours of melatonin collection and the number of day shifts worked during the previous two weeks. (Night workers only) (Parameter estimate: -0.32, p = 0.03)

![Graph b)
5.5 Usual Physical Activity and Urinary Melatonin Levels:

5.5.1 Usual Physical Activity:

Usual physical activity was assessed in the study questionnaire and included recreational and household activities performed over the preceding two weeks. This time frame was chosen to incorporate both work days and days off, as activity patterns were expected to be different on days when subjects were not working. Total usual physical activity was calculated as the total number of hours reported by study subjects of activity of either moderate or heavy intensity over the two-week period prior to study participation. One subject (night worker) was excluded from this analysis due to missing usual physical activity data. When analyzed as a continuous variable, usual physical activity was also positively skewed (Figure 5.8) and was therefore analyzed categorically with activity divided into four categories: 0 hours, 1 – 10 hours, 10 – 20 hours and > 20 hours of moderate or heavy intensity physical activity over the previous two weeks (Figure 5.9). No day worker reported 0 hours of moderate or heavy physical activity in the two weeks prior to study participation compared to 9.7% of night workers. However, the overall distribution of usual physical activity between the day and night shift groups was quite similar, with no statistically significant difference (p = 0.41).
Figure 5.8: Distribution of total hours of usual physical activity. Only hours of moderate and heavy intensity physical activity were included.

Figure 5.9: Categorical usual physical activity distribution. Categories are defined in terms of hours of moderate and heavy intensity physical activity and are coded such that 0 = 0 hours activity, 1 = 1 – 10 hours of activity, 2 = 10 – 20 hours of activity and 3 = >20 hours of activity over the previous two weeks.

a) Day workers only (n = 29):

b) Night workers only (n = 31):
5.5.2 Usual Physical Activity and Melatonin:

The relationship between usual physical activity and urinary melatonin levels was assessed separately for day and night workers, similar to the recent physical activity analysis. This association was assessed by comparing mean urinary melatonin levels between the four physical activity categories: 0 hours, 1 – 10 hours, 10 – 20 hours and > 20 hours of physical activity of moderate or heavy intensity over a two week period. There was no statistically significant difference in urinary melatonin levels between physical activity groups among either day or night workers, as seen by the overlap of the 95% confidence intervals (Table 5.5). Among day workers, there did not appear to be a pattern with respect to physical activity and melatonin production, with the highest average urinary melatonin levels seen in the group with 10 – 20 hours of moderate or heavy intensity physical activity in the two weeks prior to study participation. Among night workers, the highest average urinary melatonin level was seen in the group reporting 1 – 10 hours of moderate or heavy intensity physical activity. After this peak, as physical activity increased urinary melatonin levels decreased, suggesting the possibility of an inverse relationship between usual physical activity and urinary melatonin levels among night workers. As with the recent physical activity analysis, multiple linear regression was used to quantify the association between usual physical activity and urinary melatonin levels in each shift group. Among day workers, no association between usual physical activity and melatonin was observed (Figure 5.10a), while among night workers a small inverse association was seen (Figure 5.10b).
Table 5.5: Mean urinary melatonin levels by usual physical activity group among day and night workers.

<table>
<thead>
<tr>
<th>Total Usual Activity (hours)</th>
<th>Urinary melatonin level (ng/mL)</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day Workers:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>1 – 10</td>
<td>15</td>
<td>17.83</td>
<td>15.85</td>
<td>9.05 - 26.61</td>
<td></td>
</tr>
<tr>
<td>10 – 20</td>
<td>7</td>
<td>27.23</td>
<td>22.00</td>
<td>6.88 - 47.58</td>
<td></td>
</tr>
<tr>
<td>&gt;20</td>
<td>7</td>
<td>21.49</td>
<td>15.23</td>
<td>7.40 - 35.57</td>
<td></td>
</tr>
<tr>
<td><strong>Night Workers:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>3</td>
<td>2.81</td>
<td>2.99</td>
<td>-4.61 - 10.22</td>
<td></td>
</tr>
<tr>
<td>1 – 10</td>
<td>17</td>
<td>10.70</td>
<td>15.76</td>
<td>2.59 - 18.80</td>
<td></td>
</tr>
<tr>
<td>10 – 20</td>
<td>6</td>
<td>4.93</td>
<td>3.27</td>
<td>1.50 - 8.36</td>
<td></td>
</tr>
<tr>
<td>&gt;20</td>
<td>5</td>
<td>2.69</td>
<td>2.04</td>
<td>0.16 - 5.23</td>
<td></td>
</tr>
</tbody>
</table>

Figure 5.10: Multivariate association between log-transformed urinary melatonin levels and usual physical activity group for day and night workers. Categories are defined in terms of hours of moderate and heavy intensity physical activity and are coded such that 0 = 0 hours of activity, 1 = 1 – 10 hours of activity, 2 = 10 – 20 hours of activity and 3 = >20 hours of activity.

a) Association between usual physical activity and log-transformed urinary melatonin levels in a model that accounts for age and alcohol consumption during the 24-hours of melatonin assessment. (Day workers only) (Parameter estimate: -0.03, p = 0.23)
b) Association between usual physical activity and log-transformed urinary melatonin levels in a model that accounts for age, use of NSAIDs in both the 24-hours of melatonin assessment and during the two weeks prior to study participation and the number of day shifts during the two weeks prior to the study. (Night workers only) (Parameter estimate: -0.14, p = 0.05)

5.6 Sleep Duration and Melatonin:

Sleep duration was defined as the total number of hours of sleep during the 24-hours of melatonin assessment and was measured in the one-day diary from self-reported sleep and wake times for each subject. Overall, sleep duration was longer among those working days, with an average duration of 8.27 hours, compared to 4.85 hours among those working nights. This difference was statistically significant (p < 0.0001). Sleep duration information was missing for one subject (day worker).

The association between sleep duration and urinary melatonin levels was assessed by correlation analysis in order to determine the extent to which sleep duration could act as a proxy for melatonin levels, as has been suggested in previous literature (6). To
account for the potentially confounding influence of shift worked, the analysis was stratified by shift group since shift was independently associated with both sleep duration and urinary melatonin levels. The distributions of both sleep duration and urinary melatonin levels were not normal, so Spearman rank correlation analysis was used. There was no significant correlation between sleep duration and melatonin among either the day or night groups (Table 5.6), although it came close among day workers, in the direction of increased sleep duration associated with higher melatonin levels. Among night workers, the sign of the coefficient indicates an inverse relationship, the magnitude is very weak.

Table 5.6: Correlation between urinary melatonin and sleep duration among day and night workers.

<table>
<thead>
<tr>
<th>Shift Group</th>
<th>Correlation Coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>0.35782</td>
<td>0.06</td>
</tr>
<tr>
<td>Night</td>
<td>-0.10791</td>
<td>0.56</td>
</tr>
</tbody>
</table>
References


Chapter 6: General Discussion

6.1 Summary of Main Findings:

The primary objectives of this thesis were to examine the associations of light at night and physical activity exposure with melatonin levels among rotating shift nurses. This thesis also described the extent to which saliva can measure absolute melatonin levels, and whether sleep duration can act as a proxy for melatonin production.

6.1.1 Light intensity exposure and urinary melatonin:

This study found a significant inverse association between light intensity exposure and urinary 6-sulfatoxymelatonin levels in the full study population. While this result was consistent with previous studies where night work had been associated with decreased 6-sulfatoxymelatonin levels (1,2), analysis of salivary melatonin secretion patterns demonstrated that melatonin production was not altered to the daytime hours among those working at night. Urinary melatonin sampling was designed to assess peak melatonin levels after sleeping among both day and night workers, a method that made the assumption that peak melatonin production among night workers would occur during the day while they slept and not at night while working. Since this assumption was not met, it is likely that differences in melatonin levels between day and night workers are confounded by differences in circadian stage at the time of melatonin assessment.

To account for this source of confounding, the analysis of light intensity and urinary melatonin levels was stratified by shift group and associations were examined among day and night workers separately. After stratification, no relationship was observed between light exposure during sleep and urinary melatonin levels among night
workers. While the day worker analysis suggested an inverse relationship, it was not statistically significant. The lack of an association between light intensity and melatonin among those working at night may have been due to the fact that peak melatonin production in this group actually occurred at night during work, meaning that urine samples taken after sleeping during the day would not be able to capture peak melatonin production. Conversely, urine samples taken from day workers upon awakening after sleeping at night would be expected to capture peak melatonin production, and the lack of statistical significance for this group may have been due to the reduced sample size (only 29 subjects).

6.1.2 Saliva as a measure of absolute melatonin levels:

The extent to which both urine and saliva are able to describe absolute melatonin levels measured in serum has been assessed in several previous studies (3-7). While urine, which captures approximately 70% of serum melatonin levels, is considered a good measure of absolute melatonin (3-5), saliva captures only 30% of serum melatonin levels and is not generally considered a good representation of absolute levels (5-7). Since urine measurements in this study were confounded by differences in circadian stage between day and night workers at the time of melatonin assessment, the extent to which saliva was able to capture the pattern of absolute melatonin levels seen in urine was assessed. If saliva was able to accurately describe the absolute melatonin levels seen in urine, it could be used as a proxy melatonin outcome measure in this study. Since multiple saliva samples were collected at similar times of day, this method could be used to account for the confounding by circadian rhythms seen in the urine samples.
Spearman rank correlation analysis revealed a correlation of 0.34 (p = 0.01) between urine and saliva. We interpret this to mean that saliva was not able to capture the absolute melatonin levels measured in urine. Given the known relationships of both salivary and urinary melatonin with absolute melatonin levels measured from serum, the observed correlation between urinary and salivary melatonin seen in this study was expected (3-7). Additionally, existing validation studies have indicated that salivary melatonin is not a good measure of absolute melatonin levels and as such, consistent with previous research, saliva was not considered an accurate measure of absolute melatonin levels in this study (5-7).

6.1.3 Light intensity exposure and salivary melatonin levels:

Previous research has demonstrated that although saliva is not a good measure of absolute melatonin levels, salivary melatonin is able to accurately describe melatonin variability (6-8). In order to examine the association between light intensity and melatonin levels while controlling for the confounding by circadian rhythm seen in the urine samples, the magnitude of salivary melatonin variation was used as a measure of melatonin outcome.

However, unlike when urinary melatonin levels were used as an outcome measure, no association was seen between light intensity exposure between 12AM and 5AM, the time of peak melatonin production (8,9), and the magnitude of salivary melatonin variation. This lack of association could indicate that although there were significant differences in nighttime light exposure between shift groups (p < 0.0001), these differences did not impact melatonin production. This would suggest that, contrary
to the results of previous research, light exposure during rotating night shifts does not impact on melatonin levels (10). However, 12 subjects (20%) were excluded from the salivary melatonin analysis due to missing saliva data. This, coupled with the weak correlation between salivary and urinary melatonin levels, suggests that salivary melatonin measures are not a valid outcome measure in this study and thus cannot be used to accurately describe the relationship between light intensity and melatonin.

6.1.4 Physical Activity and Urinary Melatonin:

Urinary melatonin levels were used as the outcome measure for this analysis, as salivary melatonin levels were unable to accurately describe absolute melatonin levels. Due to the presence of confounding by differences in circadian stage at the time of urinary melatonin assessment between day and night workers, this analysis was stratified by shift group in order to compare melatonin levels between physical activity groups. No significant difference in mean urinary melatonin levels was seen between physical activity groups for either recent or usual activity in either shift group.

Among day workers, while it was not statistically significant, a positive relationship between recent physical activity and urinary melatonin levels was suggested. However, a similar trend was not observed in the night shift group, where a negative relationship between recent physical activity and urinary melatonin levels was seen. There have been few previous observational studies of physical activity and melatonin levels; however, one recent study found that duration of moderate and vigorous exercise was positively associated urinary melatonin levels (11). The direction of the relationship between recent physical activity and urinary melatonin among day workers in our study
is consistent with this work. While laboratory based studies of exercise and melatonin have been somewhat inconsistent, those that demonstrate an increase in melatonin levels after exercise have indicated that these increases are short-lived, with melatonin levels returning to normal within one hour after exercise (12-16). This suggests that with only one melatonin measurement after sleeping there exists the possibility that, depending on the time of day at which physical activity was performed, some potential effects of activity on melatonin levels may not have been captured. This could have lead to non-differential misclassification of melatonin levels, making melatonin production in different physical activity groups more similar, which would bias effect estimates towards the null such that small associations could have been missed. Finally, among night workers salivary melatonin analysis revealed that peak melatonin levels occurred at night while working, suggesting that assessment of urinary melatonin after sleeping during the day was not able to capture peak melatonin production. It is likely that if a relationship between physical activity and urinary melatonin levels exists, measurement of peak melatonin levels is necessary in order to observe it. This would mean that differences in the apparent effect of recent physical activity on melatonin levels between shift groups could be a result of the failure of urinary melatonin levels among night workers to accurately measure peak melatonin production, and not due to a true difference in the relationship between activity and melatonin between shift groups.

Among both day and night workers, there was no statistically significant difference in mean urinary melatonin levels between levels of usual physical activity. Regression analysis demonstrated no association between usual physical activity and melatonin among day workers; however, in the night shift group, a small inverse
association between urinary melatonin levels and usual physical activity was observed. There is little existing research regarding the relationship between usual activity and melatonin, with one study of elite athletes demonstrating a decline in melatonin levels over several weeks, while another found no relationship between chronic exercise and melatonin levels (16,17).

6.1.5 Sleep Duration and Melatonin:

This study found no significant correlation between sleep duration and melatonin among either day or night workers. Previous studies of sleep duration and melatonin production have had conflicting results, with one study demonstrating a positive relationship between sleep time and duration of melatonin production, while another study found that longer sleep duration was associated with decreased melatonin production (18,19). Despite these conflicting results, it has been hypothesized that sleep duration would be positively associated with melatonin production such that sleep duration could be used as a proxy for melatonin in studies where direct measures of melatonin were not available (20). While a positive correlation consistent with this hypothesis was observed among day workers, among night workers there appeared to be an inverse relationship between sleep duration and melatonin. Among both day and night workers, the correlations between sleep duration and urinary melatonin levels were weak (0.36 for day workers, and -0.11 for night workers), suggesting that sleep duration was not a good proxy for melatonin levels in this population.
6.2 Strengths and Limitations:

The main strength of this study is its objective measurement of light intensity exposure and urinary and salivary melatonin levels. The majority of previous research used night shift work as a proxy for light exposure (1,2,21,22), with few studies incorporating objective measures of light (10,23). The precise measurement of both light exposure and melatonin in this thesis provided sufficient power to detect associations with a relatively small sample size. Additionally, the use of multiple saliva samples over a 24-hour period meant that this study was able to characterize melatonin secretion patterns among sub-groups of those working on their day and night shifts. Comparisons of chronological time points (similar times of day) revealed that the circadian rhythms of melatonin production among subjects working at night were not altered, such that peak melatonin production occurred at night when working and not during the day while sleeping. This observation highlighted the importance of accounting for 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 (10,23). From the perspective of workplace policy, the absence of a change in the timing of melatonin secretion may suggest that if it is the change in timing of melatonin production that impacts on cancer risk, then rotating shift patterns that do not allow these changes to occur may have a smaller impact on cancer risk among shift workers than shift work patterns that do alter the timing of melatonin production.

This study also contributes observational evidence regarding the relationship between physical activity and melatonin, since the majority of previous studies have been
laboratory based (12-16, 24). By measuring recreational, household and occupational physical activity, this study also provides a more complete physical activity exposure assessment than previous work which has focused primarily on the role of exercise (11). Finally, the assessment of both recent and usual physical activity meant that this study was able to contribute knowledge regarding both the acute and chronic effects of physical activity on melatonin levels in the context of an observational study.

Despite these strengths, this study also has several limitations. The main limitation was the presence of confounding by circadian stage at the time of melatonin assessment in the urine samples. In order to account for this source of confounding, the analysis was stratified by shift group. However, stratification produced a reduced sample size, which limited the power of the study to detect associations of both light intensity and physical activity with melatonin levels. The poor representation in saliva samples of the absolute melatonin levels measured in urine limited the use of salivary melatonin levels as an outcome measure and prevented their use as a method to control for confounding by circadian rhythms.

Another limitation of this study was the use of self-report to collect information regarding recent and usual physical activity. Physical activity questions in both the study diary and questionnaire were based on the previously validated Lifetime Physical Activity Questionnaire (25). However, the use of a self-report measure means that the accuracy of physical activity data depends on the ability of study subjects to correctly report their activity levels and inaccuracy in recall would lead to exposure misclassification. Existing studies of the accuracy of self-reported physical activity measures have demonstrated that misclassification of physical activity is common in
Also, while questionnaires usually describe sedentary and heavy intensity activities well, their ability to capture activity of light and moderate intensity is more limited. Since activities of both moderate and heavy intensity were included in the analysis, inaccuracies in the reporting of moderate intensity activities could have led to either an under or over estimation of total activity, depending on whether participants over or under reported moderate intensity activities. Furthermore, in this study there were inconsistencies in the detail with which participants reported activity, particularly with respect to recent occupational activity where some subjects reported specific activities at work, while others simply reported 12 hours of nursing. As only moderate and heavy intensity activities were included in the analysis in order to ensure that activity and not inactivity was being measured, higher intensity activities performed by subjects who provided general activity descriptions could have been missed, leading to exposure misclassification. However, the amount of detail present in activity reporting is not expected to be influenced by melatonin levels, thus any misclassification present would be non-differential, biasing effect estimates towards the null.

Participants in this study were volunteers, meaning that volunteer bias, which occurs when those who volunteer to participate are systematically different from those who do not, may be present. For example, nurses participating in the study might have been more conscious of the potential effects of shift work on their health, and thus may have made more of an effort to make their bedrooms dark when sleeping during the day. This would reduce the variability of light exposure levels during sleep and would make those working at night more similar to those working during the day, which may have reduced the ability of this study to detect associations between light exposure and
melatonin. Another potential source of selection bias was the decision to target specific hospital nursing units to recruit participants for the study. If working conditions, such as light levels during night shifts, were different among units included in the study compared to those that were not, there could have been effects on melatonin levels that were not captured in this study. For example, if light levels in the emergency department, which was not included in this study, were higher at night than in other areas of the hospital, effects of this high light intensity on melatonin levels would have been missed.

Finally, only one rotating shift pattern (two 12-hour days, two 12-hour nights, five days off) was included in this study. Although the study demonstrated that this pattern of rotating shifts had no impact on the timing of melatonin secretion among those working nights, the impact of different rotation schedules, such as a greater number of consecutive days or nights or shorter shifts, on melatonin secretion could not be evaluated. A currently funded study will also assess if long duration of rotating shift work affects the timing or magnitude of melatonin production, compared to short durations of working a rotating shift schedule. Therefore, it is impossible to determine if it is rotating shifts in general, or simply this specific rotation pattern, that are responsible for the absence of a change in the timing of daily melatonin variations when working nights.

6.3 Future Directions:

The results of this thesis suggest an inverse association between light intensity exposure and melatonin levels. However, the presence of confounding by circadian stage at the time of melatonin assessment in urine samples means that this result should be interpreted with caution. As salivary melatonin levels did not provide an accurate
representation of absolute melatonin levels and were thus a poor melatonin outcome
measure, future studies among rotating shift workers should incorporate the role of
circadian variations in melatonin levels at the design stage. This could be accomplished
through a urine assessment strategy that allows for the comparison of similar
chronological as well as functional time points between those working day and night
shifts, as seen in Figure 6.1. This method of melatonin assessment would allow
investigations to see if melatonin secretion patterns are altered among rotating shift
workers when working at night, especially since both existing research and the results of
this thesis suggest that, given the rotating shift pattern in this population, the assumption
that the timing of melatonin production changes to the day among those working at night
is not valid (28-30). If the confounding influence of circadian stage is removed in this
way, stratification of the analysis by shift group would not be necessary. This would
increase the power of the study to detect associations of both light exposure and physical
activity with melatonin.

**Figure 6.1: Revised urine and saliva collection timeline**

<table>
<thead>
<tr>
<th>Time of Day:</th>
<th>5 - 7 AM</th>
<th>3 - 5 PM</th>
<th>11PM - 1AM</th>
<th>5 - 7AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Point:</td>
<td>Upon Awakening</td>
<td>Mid-Shift</td>
<td>Before Sleep</td>
<td>Upon Awakening</td>
</tr>
<tr>
<td>Functional:</td>
<td>Saliva Sample 1</td>
<td>Saliva Sample 2</td>
<td>Saliva Sample 3</td>
<td>Saliva Sample 4</td>
</tr>
<tr>
<td>Day Workers:</td>
<td>5 - 7 AM</td>
<td>3 - 5 PM</td>
<td>11PM - 1AM</td>
<td>5 - 7AM</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time of Day:</th>
<th>3 - 5 PM</th>
<th>11PM - 1AM</th>
<th>5 - 7 AM</th>
<th>3 - 5PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Point:</td>
<td>Upon Awakening</td>
<td>Mid-Shift</td>
<td>Before Sleep</td>
<td>Upon Awakening</td>
</tr>
<tr>
<td>Functional:</td>
<td>Saliva Sample 1</td>
<td>Saliva Sample 2</td>
<td>Saliva Sample 3</td>
<td>Saliva Sample 4</td>
</tr>
<tr>
<td>Night Workers:</td>
<td>3 - 5 PM</td>
<td>11PM - 1AM</td>
<td>5 - 7 AM</td>
<td>3 - 5PM</td>
</tr>
</tbody>
</table>
This thesis also failed to detect a relationship between both recent and usual physical activity and melatonin levels; however, a trend towards increasing melatonin with increasing recent physical activity was seen among day workers. Previous observational studies have suggested that the association between physical activity and melatonin may be small (11) and misclassification of activity levels through the use of self-report in the study diary and questionnaire may have limited our ability to detect small associations. Given that physical activity is a modifiable behaviour and that it has the potential to positively impact on melatonin levels (11,12,24), future studies should use more precise and objective measures of physical activity, such as pedometers or accelerometers, to obtain higher quality physical activity data and reduce exposure misclassification. If shown to have a positive impact on melatonin levels, physical activity could be used to reduce the potential negative impact of light at night exposure on melatonin among rotating shift workers who must work at night.

In light of these limitations, future studies that employ a longitudinal design allowing rotating shift workers to be followed on both their day and night shifts are required. This method of assessment as it would allow for comparisons to be made both between and within individuals working day and night shifts. Existing research indicates that while the phasing and amplitude of melatonin rhythms are fairly consistent within individuals, the ability to synthesize melatonin differs, leading to wide inter-individual variations in peak melatonin levels (31,32). Thus, the ability to make comparisons within individuals on different shifts would reduce the impact of these inter-individual
melatonin variations and could increase the ability of such studies to detect associations of both light at night and physical activity with melatonin levels.

6.4 Contribution of Research and Conclusions:

The overall aim of this thesis was to describe the associations of light intensity exposure and physical activity with melatonin levels. The use of a light intensity data logger to objectively assess light exposure provided an improvement upon many previous studies where proxies of light exposure, such as the number of years of night shift work, have been used (1,2,21,22). This precise method of exposure assessment allowed the study to specifically investigate the impact of light on melatonin levels, an improvement upon studies that used proxy measures.

The use of two melatonin biomarkers (saliva and urine) allowed us to characterize the melatonin secretion patterns of both day and night rotating shift workers and demonstrated that the melatonin secretion patterns among rotating shift workers who work at night do not differ from a day-oriented pattern. This observation suggested that urinary melatonin measures in this study would be confounded by differences in circadian stage at the time of melatonin assessment when comparisons were made between day and night workers. While in the full study population an inverse relationship between light exposure and melatonin was seen, stratification of this analysis by shift group revealed differences in the observed associations between shift groups and demonstrated the importance of accounting for the role of circadian melatonin variations in studies of rotating shift workers. This methodological consideration had not been
highlighted in previous research (10,23) and represents an important contribution to knowledge.

This study also found that salivary melatonin measures were unable to capture the pattern of absolute melatonin levels measured in urine, as predicted by the literature (3-7). This meant that salivary melatonin could not be used as an absolute melatonin outcome measure in this study. Furthermore, no relationship was found between light intensity and the magnitude of salivary melatonin variation. However, the poor correlation between urinary and salivary melatonin measures, combined with the presence of missing data from the salivary melatonin analysis, suggested that the magnitude of salivary melatonin variation was not a reliable outcome measure in this study.

This thesis did not demonstrate any significant differences in melatonin production across levels of physical activity for either recent or usual activity. The majority of previous research surrounding the relationship between physical activity and melatonin has been laboratory-based (11-16), such that this thesis contributed observational evidence regarding this relationship. Since most existing studies of this relationship have focused primarily on the role of exercise (11-16), this study also provided a more complete exposure assessment of physical activity by incorporating recreational, household and occupational physical activities.

Finally, this thesis found that there was not a significant correlation between sleep duration and melatonin levels. Therefore it concluded that, in this population, sleep duration was not a good proxy for melatonin production. However, stratification of the analysis by shift group reduced the variability of both sleep duration and melatonin levels.
and may have meant that this study lacked the statistical power to detect a relationship between sleep duration and melatonin, meaning that future studies should evaluate the accuracy of sleep duration as a proxy for melatonin production in other populations before incorporating the use of this proxy into study designs.

Future studies of light at night exposure, physical activity and melatonin, proposed intermediates in the causal pathway to cancer, will clarify whether melatonin is indeed a mechanism through which shift work impacts on cancer risk. These studies will also allow us to determine whether rotating shift patterns, such as the pattern seen in this study, are actually able to affect the timing or magnitude of melatonin variations. This will be important from a policy perspective as it will facilitate the assessment of whether rotating shifts are better for health than permanent night shifts, and whether one particular rotation pattern is preferable to another.
References


Appendix A: Ethics Approval

QUEEN’S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD

September 26, 2007

Ms. Anne Grundy
Department of Community Health and Epidemiology
c/o Queen’s Cancer Research Institute
10 Stuart Street, Second Floor
Queen’s University

Dear Ms. Grundy,

Study Title: Determinants and methods of assessment of melatonin levels among rotating shift nurses
Co-Investigators: Dr. K. Aronson and Dr. C. Graham

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 list 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. (see http://www.queensu.ca/vpr/reb.htm).

- **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.

- **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, Research Ethics Board

Date: Sept 27, 2007

Study Code: EPID-246-07

- 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.
Appendix B: Study Diary
STUDY OF PHYSICAL ACTIVITY, LIGHT AND MELATONIN LEVELS IN NURSES

ONE-DAY DIARY

This one-day diary is part of our research study to understand the relationship between a woman’s environment, behavioural patterns and melatonin production. This one-day diary should be completed over a twenty-four hour period, beginning ______________ and ending ______________. All questions included in this one-day diary pertain to activities completed and conditions experienced within this twenty-four hour period only. We expect that it will take approximately fifteen to thirty minutes to complete.

The answers that you share with us will be kept strictly confidential and identified by a study ID number, known only by selected members of our research team. Please note that although there are questions in this one-day diary that bear some similarity to those found in the questionnaire completed on the first day of study participation, it is imperative that you answer all questions. Your honesty is important for the success of this research, and a partial answer is better than no answer at all.

We appreciate all of your cooperation tremendously.

Thank you!

**TIME AND DATE OF SAMPLE COLLECTION**

Time and date of saliva sample #1 collection: ______________
Time and date of saliva sample #2 collection: ______________
Time and date of saliva sample #3 collection: ______________
Time and date of saliva sample #4 collection: ______________
Time and date of urine collection: ______________
Today’s Date: _____ / ________ / _____  Shift worked during specified 24-hr period (day/evening):
        Day    Month    Year  Shift worked yesterday (day/evening/off):

PHYSICAL ACTIVITIES

1) In the table below, please record **sedentary**, **light**, **moderate** and **heavy** activities that you have done during the 24-hour period specified on the front cover of this one-day diary. These activities could be **sports/exercise** or **around the home/leisure** (not including activities done at work). A sample list of activities is provided for reference at the end of this diary.

The four categories of physical intensity levels are:

- **Sedentary**: Activities that involve sitting only, with minimal walking
- **Light**: Activities that require minimal physical effort such as those activities that are done standing or with slow walking
- **Moderate**: Activities that are not exhausting, that increases the heart rate slightly and may cause some light perspiration
- **Heavy**: Activities that increase the heart rate and cause heavy sweating.

<table>
<thead>
<tr>
<th>Description of Activity (Please indicate distance if appropriate, i.e. jogging example below)</th>
<th>Morning – Afternoon (7:00AM – 3:00PM)</th>
<th>Afternoon – Evening (3:00PM – 11:00PM)</th>
<th>Evening – Morning (11:00PM – 7:00AM)</th>
<th>Intensity of Activity (Please check only one for each activity)</th>
<th>Is this a typical amount of activity for you? Yes or No → Do you usually do more or less?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hrs and/or mins</td>
<td>Hrs and/or mins</td>
<td>Hrs and/or mins</td>
<td>Sedentary</td>
<td>Light</td>
<td>Moderate</td>
</tr>
<tr>
<td>Sports/Exercise:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e.g. jogging (distance ~5 km)</td>
<td>1hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e.g. aerobics class</td>
<td>1hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Around the home/Leisure:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e.g. laundry</td>
<td>2 hrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PHYSICAL ACTIVITIES (cont’d)

2) In the table below, please record sedentary, light, moderate and heavy occupational duties, as well as the estimated time to complete them, that you have done during the shift which includes the 24-hour time period specified on the front cover of this one-day diary.

The four categories of physical intensity levels are:

- **Sedentary**: Activities that involve sitting only, with minimal walking
- **Light**: Activities that require minimal physical effort such as those activities that are done standing or with slow walking
- **Moderate**: Activities that are not exhausting, that increases the heart rate slightly and may cause some light perspiration
- **Heavy**: Activities that increase the heart rate and cause heavy sweating.

<table>
<thead>
<tr>
<th>Description of Occupational Duty</th>
<th>Estimated total number of hours and/or minutes to complete task(s)</th>
<th>Intensity of Activity (Please check only one for each activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sedentary</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3) Is this a typical amount of activity for you?
   - Yes ☐
   - No, I usually do more ☐
   - No, I usually do less ☐

LIFESTYLE HABITS - Smoking

4) Did you smoke during the 24-hour period specified on the cover of this one-day diary?
   - ☐ No (go to question #5)
   - ☐ Yes → Please estimate the number of cigarettes you smoked today.

5) On average, how many hours, during the 24-hour period specified on the cover of this one-day diary, were you exposed to someone else’s tobacco smoke?

<table>
<thead>
<tr>
<th>The number of hours exposed to “second-hand” tobacco smoke</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>☐</td>
</tr>
</tbody>
</table>
LIFESTYLE HABITS – Alcohol and caffeinated products consumption

6) Did you drink any of the following in the 24-hour period specified on the front cover of this one-day diary?

<table>
<thead>
<tr>
<th></th>
<th>Beer</th>
<th>Wine</th>
<th>Spirits</th>
<th>Coffee</th>
<th>Tea</th>
<th>Other caffeinated beverages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>No</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

On average, how many drinks did you consume? _One drink=1 glass of wine or 1 bottle of beer or 1 oz of spirits._ For caffeinated beverages, specify size (e.g. small/single shot) and type (e.g. espresso). (If “no” to all, i.e. beer, wine, spirits, coffee, tea, other caffeinated beverages, go to question #7)

<table>
<thead>
<tr>
<th>Number of Drinks</th>
<th>Specify average size and type</th>
<th>Specify average size and type</th>
<th>Specify average size and type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LIGHTING CONDITIONS

7) Please describe the lighting conditions you experience, if you were indoors or outdoors, throughout the 24-hour period specified on the front cover of this one-day diary.

<table>
<thead>
<tr>
<th>Time of Day</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning – Afternoon (7:00 AM – 3:00 PM)</td>
<td><em>e.g. Stayed indoors this morning. Incandescent lights.</em></td>
</tr>
<tr>
<td>Afternoon – Evening (3:00 PM – 11:00 PM)</td>
<td><em>e.g. Worked in garden for 2 hours. It was sunny.</em></td>
</tr>
<tr>
<td>Evening – Morning (11:00 PM – 7:00 AM)</td>
<td><em>e.g. Had night shift, fluorescent bulbs on floor.</em></td>
</tr>
</tbody>
</table>
8) Please answer the following questions:

<table>
<thead>
<tr>
<th>Study ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIGHTING CONDITIONS (cont’d)</td>
</tr>
</tbody>
</table>

| What time did you wake-up at the start of the 24-hour period specified on the front cover of this one-day diary? |
| What time did you go to sleep? |
| What time did you wake-up at the end of the 24-hour period specified on the front cover of this one-day diary? |

| Did you read while in bed, just prior to going to sleep? |
| NO | YES For how long? ____ minutes |

| If sleep was interrupted, were lights turned on? (Please choose N/A if sleep was not interrupted.) |
| NO | YES | N/A |

| If the lights were on, were they on for more than 1 hour? (Please choose N/A if sleep was not interrupted.) |
| NO | YES | N/A |

9) Please answer the following questions about indoor lighting conditions you experienced during the 24-hour period specified on the front cover of this one-day diary.

<p>| How would you describe the indoor lighting conditions at home that you were exposed to during the day? |</p>
<table>
<thead>
<tr>
<th>All artificial light</th>
<th>Almost all artificial light</th>
<th>Even mixture</th>
<th>Almost all natural light</th>
<th>All natural light</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

<p>| How would you describe the indoor lighting conditions at work that you were exposed to during the day? |</p>
<table>
<thead>
<tr>
<th>Very dim light</th>
<th>Somewhat dim light</th>
<th>Neither dim nor bright</th>
<th>Somewhat bright light</th>
<th>Very bright light</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

<p>| How would you describe the indoor lighting conditions at home that you were exposed to when it was dark outside (i.e. at night)? |</p>
<table>
<thead>
<tr>
<th>Very dim light</th>
<th>Somewhat dim light</th>
<th>Neither dim nor bright</th>
<th>Somewhat bright light</th>
<th>Very bright light</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

<p>| How would you describe the indoor lighting conditions at work that you were exposed to when it was dark outside (i.e. at night)? |</p>
<table>
<thead>
<tr>
<th>Very dim light</th>
<th>Somewhat dim light</th>
<th>Neither dim nor bright</th>
<th>Somewhat bright light</th>
<th>Very bright light</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
10) What were the main sources of lighting used in the room(s) that you spent at least 50% of your time in when it was dark outside (i.e. at night) during the 24-hour period specified on the front cover of this one-day diary? Please check all that apply.

<table>
<thead>
<tr>
<th>Location</th>
<th>Overhead (non-recessed/track)</th>
<th>Recessed (pot lights)</th>
<th>Track</th>
<th>Stand alone lamps</th>
<th>Other (Specify)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Work</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

11) The following questions pertain to the physical environment of your bedroom while you slept. Please answer the questions as it pertains to conditions experienced while you slept during the 24-hour period specified on the front cover of this one-day diary. If the question does not apply to you (for instance, if there are no streetlights visible from your bedroom, as asked in the first question) please choose N/A.

<table>
<thead>
<tr>
<th>Question</th>
<th>Option</th>
</tr>
</thead>
<tbody>
<tr>
<td>If there are streetlights visible from your bedroom window(s), were they on while you slept?</td>
<td>NO, YES, N/A</td>
</tr>
<tr>
<td>If there are shades/curtains/blinds or other window treatments on your bedroom window(s), were they closed while you slept?</td>
<td>NO, YES, please specify the type of window treatment</td>
</tr>
<tr>
<td>If you sleep with a light on in the bedroom (such as a nightlight), was it on while you slept?</td>
<td>NO, YES, please specify the type of light</td>
</tr>
<tr>
<td>If there is a light visible from your bedroom (such as a hall light), was it left on while you slept?</td>
<td>NO, YES, please specify the type of light</td>
</tr>
<tr>
<td>If the moon/stars were shining brightly, could they be seen in the room while you slept?</td>
<td>NO, YES, N/A</td>
</tr>
<tr>
<td>If the sun was shining brightly, could it be seen in the room while you slept?</td>
<td>NO, YES, N/A</td>
</tr>
</tbody>
</table>

12) What best describes the ambient light in your bedroom?

- Dark (you wear a mask in bed) ☐
- Medium (can see to the end of the bed) ☐
- Light (can almost read without a light) ☐
LIGHTING CONDITIONS (cont’d)

13) What were the main type(s) of light bulbs in use in the room(s) where you spent at least 50% of your time, when it was dark outside (i.e. at night), during the 24 hour period specified on the front cover of this one-day diary? Please check all that apply.

<table>
<thead>
<tr>
<th>Location</th>
<th>Incandescent (specify wattage or volts)</th>
<th>Fluorescent</th>
<th>Other Specify</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td></td>
<td>Wattage _______</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Volts _______</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>Work</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td></td>
<td>Wattage _______</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Volts _______</td>
<td>☐</td>
<td></td>
</tr>
</tbody>
</table>

USE OF NSAID’s, SEDATIVES AND MUSCLE RELAXANTS

14) Have you taken aspirin, ibuprofen, other nonsteroidal anti-inflammatory (NSAIDs) pain medication or Tylenol/other acetaminophen pain medication in the 24-hour period specified on the front cover of this one-day diary??

☐ No (go to question #15) ☐ Yes → Please provide details. If you do not remember the brand name, fill in the type, dose and number of tablets taken.

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Dose (milligrams)</th>
<th>Number of Tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example: Tylenol</td>
<td>200</td>
<td>1</td>
</tr>
</tbody>
</table>

15) Have you used sedatives or muscle relaxants in the 24-hour period specified on the front cover of this one-day diary??

☐ No (go to question #16) ☐ Yes → Please provide details. If you do not remember the brand name, fill in the type, dose and number of tablets taken.

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Dose (milligrams)</th>
<th>Number of Tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example: Methocarbamol (Robaxin)</td>
<td>200</td>
<td>1</td>
</tr>
</tbody>
</table>
16) By participating in this study, have you changed the lighting conditions in your home and/or bedroom?

☐ No

☐ Yes → Please provide details in the space provided specifying what type of changes you have made.

17) By participating in this study, will you change the lighting conditions in your home and/or bedroom?

☐ No

☐ Yes → Please provide details in the space provided specifying what type of changes you will make.

Because we want to be able to use all the information you have provided, please take a moment to review each page, making sure that you did not skip any pages.

If you have any additional comments, please provide them in the space provided below.

Thank you again for the information you have provided!
Your input is very valuable to us
Sample List of Activities for Physical Activity Question #1

When describing physical activity please include as much detail as possible and attempt to make your list as complete as possible. Some examples of activities to include are as follows; however, do not feel limited to the activities that are listed.

**Sports/Exercise Activities**
- Bicycle - Leisure (stationary or not)
- Bicycle - Fast (stationary or not)
- Rowing (Stationary machine)
- Skating (leisure vs vigorous)
- Elliptical Trainer
- Lifting Weights
- Running (please try to include distance)
- Walking - Leisure
- Walking - Power/quickly
- Tennis
- Yoga
- Squash
- Push-ups
- Sit ups
- Aerobics Class (include class type)

**Around the Home/Leisure Activities**
- Shopping (grocery with cart, clothes, mall or downtown or department store)
- Housework Indoors (Vacuuming, mopping, doing dishes, ironing)
- Housework Outdoors (Shovelling snow, gardening, raking leaves etc)
- Childcare - (sitting or standing or running)
- Lying down (watching TV)
- Standing (cooking, working, talking on the phone, miscellaneous)
- Sitting (reading, writing, miscellaneous)
- Swimming (specify type of stroke used)
- Dancing
- Typing
- Knitting
- Eating
- Showering
- Driving
- Playing Musical Instrument (specify type of instrument)
- Loading/unloading the car
- Carrying Baby (pushing stroller, holding baby standing still or walking)
- Dressing/Undressing
Appendix C: Study Questionnaire
This questionnaire is part of our research study to understand the relationship between a woman’s environment, behavioural patterns and melatonin production. The specific objectives are to investigate the association between melatonin levels produced by the body and exposure to certain environmental factors, including light exposure and physical activity.

The following questions should be completed on ________________, the first day of study participation. We expect that it will take approximately fifteen to thirty minutes to complete.

The answers that you share with us will be kept strictly confidential and identified by a study ID number, known only by selected members of our research team. Your honesty is important for the success of this research, and a partial answer is better than no answer at all.

We appreciate all your cooperation tremendously.

Thank you!
Today’s Date: ____________________________

Month / day / year

1) What is your date of birth? ____________________________

Month / day / year

2) What is your height? ________ (cm) or ________ (feet and inches)

3) What is your weight? ________ (kg) or ________ (lbs)

4) How would you best describe you and your grandparents’ race, ethnicity or colour? Please specify as many as applicable:

<table>
<thead>
<tr>
<th>Race, ethnicity or colour</th>
<th>Yourself</th>
<th>Maternal Grandmother</th>
<th>Maternal Grandfather</th>
<th>Paternal Grandmother</th>
<th>Paternal Grandfather</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native/Aboriginal peoples of North America</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filipino</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japanese</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Korean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Asian (e.g. East Indian, Pakistani, Punjabi, Sri Lankan)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South East Asian (e.g. Cambodian, Indonesian, Laotian, Vietnamese)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arab/West Asian (e.g. Armenian, Egyptian, Iranian, Lebanese, Moroccan)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latin America</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (Specify)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**HEALTH BACKGROUND**

5) How old were you when you had your first menstrual period?

_______ years of age  □ Have never menstruated (go to question #8)
HEALTH BACKGROUND (cont’d)

6) Are you still menstruating?
   - No → How many years has it been since your last menstrual period?
     _____ years
   - Yes (go to question #8)

7) How did your menstrual periods stop?
   - Naturally (through onset of menopause)
   - As a result of radiation or chemotherapy
   - As a result of a hysterectomy
   - Other – please specify: ______

8) Are you currently pregnant?
   - No
   - Yes

9) Are you currently breast-feeding?
   - No (go to question #10)
   - Yes → Please indicate the number of months you have been breast-feeding. ______

10) Have you ever been pregnant?
    - No (go to question #12)
    - Yes → Please indicate the total number of times you have been pregnant (please include any live births, miscarriages or abortions). ______

11) Do you have any biological children?
    - No (go to question #12)
    - Yes → Please indicate how old you were when you gave birth to your first child. ______

12) How many of your first-degree blood relatives (mother, sisters and/or daughters) have been diagnosed with breast cancer?
    - None
    - 1
    - > 1
    - Unknown
HEALTH BACKGROUND (cont’d)

13) These questions are about breast lumps or cysts that you may have had.

<table>
<thead>
<tr>
<th>Question</th>
<th>Response Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you ever had a lump or cyst in your breast?</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>(go to question #14)</td>
<td></td>
</tr>
<tr>
<td>How old were you when the first lump/cyst appeared?</td>
<td>Age (years)</td>
</tr>
<tr>
<td>Did you have any of the lumps/cysts examined by a doctor?</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Did you have a biopsy or fine needle aspiration for any of the lumps/cysts?</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>If yes, how many?</td>
<td>______</td>
</tr>
<tr>
<td>Did a doctor diagnose any of the lumps/cysts as atypical hyperplasia?</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Did a doctor diagnose any of the lumps/cysts as carcinoma in situ?</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Did a doctor diagnose any of the lumps/cysts as breast cancer?</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

14) Have you taken aspirin, ibuprofen, other nonsteroidal anti-inflammatory (NSAIDs) pain medication or Tylenol/other acetaminophen pain medication in the last 2 weeks?

☑ No (go to question #15)

☐ Yes → Please provide details. If you do not remember the brand name, fill in the type of drug, dose and the number of tablets (frequency) taken per week. Please indicate how many you have taken within the last 24-hours.

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Dose (milligrams)</th>
<th>Frequency (per week)</th>
<th>Within the last 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example: Tylenol</td>
<td>200</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

132
HEALTH BACKGROUND (cont’d)

15) Have you taken any muscle relaxants in the last 2 weeks?
   - No (go to question #16)
   - Yes → Please provide details. If you do not remember the brand name, fill in the type of drug, dose and the number of tablets (frequency) taken per week. Please indicate how many you have taken within the last 24-hours.

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Dose (milligrams)</th>
<th>Frequency (per week)</th>
<th>Within the last 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example: Methocarbamol (Robaxin)</td>
<td>500</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>

16) Are you currently taking any contraceptive medication (e.g. Alesse, Triphasil, Ortho-Cyclen, etc.)?
   - No (go to question #17)
   - Yes → Please provide details. If you do not remember the brand name, fill in the medication type (e.g. oral, patch, injection etc.), date started and number of year(s) you have taken the drug.

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Medication Type</th>
<th>Date Started</th>
<th>Total Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example: Alesse</td>
<td>Oral</td>
<td>Sept 2000</td>
<td>5 yrs</td>
</tr>
</tbody>
</table>

LIFESTYLE HABITS

17) Are you currently smoking?
   - No (go to question #19)
   - Yes

18) How many cigarettes a day or per week, over the last two weeks, have you smoked? ___________________________ cigarettes/day
    OR ___________________________ cigarettes/week

19) Have you quit smoking within the last 12 months?
   - No (go to question #20)
   - Yes → When did you quit? _________
    How many cigarettes would you smoke per day?
**LIFESTYLE HABITS (cont’d)**

20) Does anyone in your household currently smoke?

- ❑ No (go to question #21)
- ❑ Yes → Please provide details in the table below.

<table>
<thead>
<tr>
<th>MEMBER 1</th>
<th>MEMBER 2</th>
<th>MEMBER 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>On average, how many cigarettes a day does s/he usually smoke? (cigarettes/day)</td>
<td>_____ cigarettes/day</td>
<td>_____ cigarettes/day</td>
</tr>
<tr>
<td>On average, how many hours per week are you exposed to each household member’s tobacco smoke?</td>
<td>_____ hours</td>
<td>_____ hours</td>
</tr>
</tbody>
</table>

21) On average, in the last 2 weeks, how many hours per week, including in the workplace, were you exposed to someone else’s tobacco smoke?

<table>
<thead>
<tr>
<th>Hours per week exposed to “second-hand” tobacco smoke</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>❑</td>
</tr>
</tbody>
</table>

22) This question asks about your alcohol and caffeine consumption habits. Have you drank the following more than twice in the last 2 weeks?

<table>
<thead>
<tr>
<th>Beer</th>
<th>Wine</th>
<th>Spirits</th>
<th>Coffee</th>
<th>Tea</th>
<th>Other caffeinated beverages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>❑</td>
<td>❑</td>
<td>❑</td>
<td>❑</td>
<td>❑</td>
</tr>
<tr>
<td>No</td>
<td>❑</td>
<td>❑</td>
<td>❑</td>
<td>❑</td>
<td>❑</td>
</tr>
</tbody>
</table>

In the last two weeks, how many drinks per day or week did you consume? *One drink=1 glass of wine or 1 bottle of beer or 1 oz of spirits.* For caffeinated beverages, specify size (e.g. small/single shot) and type (e.g. espresso). (If “no” to all, i.e. beer, wine, spirits, coffee, tea, other caffeinated beverages, go to question #23)

<table>
<thead>
<tr>
<th>Drinks/day OR Drinks/week</th>
<th>Beer</th>
<th>Wine</th>
<th>Spirits</th>
<th>Coffee</th>
<th>Tea</th>
<th>Other caffeinated beverages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinks/week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>❑</td>
<td>❑</td>
<td>❑</td>
<td>❑</td>
<td>❑</td>
<td>❑</td>
<td></td>
</tr>
</tbody>
</table>

Specify average size and type

Specify average size and type

Specify average size and type
LIFESTYLE HABITS (cont’d)

23) Please answer the following questions about your sleeping habits over the last 2 weeks.

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>What time, on average, were the lights turned off for bed?</td>
<td></td>
</tr>
<tr>
<td>What time, on average, did you wake up?</td>
<td></td>
</tr>
<tr>
<td>Did you read while in bed, just prior to going to sleep?</td>
<td>☐ NO ☐ YES → For how long? _____ minutes</td>
</tr>
<tr>
<td>If sleep was interrupted, were lights usually turned on? (Please choose N/A if sleep was not interrupted.)</td>
<td>☐ NO ☐ YES ☐ N/A</td>
</tr>
<tr>
<td>Were lights usually turned on for more than 1 hour? (Please choose N/A if sleep was not interrupted.)</td>
<td>☐ NO ☐ YES ☐ N/A</td>
</tr>
</tbody>
</table>

LIGHTING CONDITIONS

23) What are the main sources of lighting used in the room(s) that you spend at least 50% of your time in when it is dark outside (i.e. at night)? Please check all that apply.

<table>
<thead>
<tr>
<th>Location</th>
<th>Overhead (non-recessed/track)</th>
<th>Recessed (pot-lights)</th>
<th>Track</th>
<th>Stand alone lamps</th>
<th>Other Specify:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐______________</td>
</tr>
<tr>
<td>Work</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐______________</td>
</tr>
</tbody>
</table>

24) What are the main types of light bulbs in use in the room(s) that you spend at least 50% of your time in when it is dark outside (i.e. at night)? Please check all that apply.

<table>
<thead>
<tr>
<th>Location</th>
<th>Incandescent</th>
<th>Fluorescent</th>
<th>Other Specify:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home</td>
<td>☐</td>
<td>☐</td>
<td>☐______________</td>
</tr>
<tr>
<td></td>
<td>Wattage _____</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Volts _______</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Work</td>
<td>☐</td>
<td>☐</td>
<td>☐______________</td>
</tr>
<tr>
<td></td>
<td>Wattage _____</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Volts _______</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
25) Please answer the following questions about the average indoor lighting conditions you have experienced over the last two weeks.

<table>
<thead>
<tr>
<th>All artificial light</th>
<th>Almost all artificial light</th>
<th>Even mixture</th>
<th>Almost all natural light</th>
<th>All natural light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

How would you describe the indoor lighting conditions **at home** that you are usually exposed to during the day?

<table>
<thead>
<tr>
<th>Very dim light</th>
<th>Somewhat dim light</th>
<th>Neither dim nor bright</th>
<th>Somewhat bright light</th>
<th>Very bright light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

How would you describe the indoor lighting conditions **at work** that you are usually exposed to during the day?

<table>
<thead>
<tr>
<th>Never</th>
<th>Almost Never</th>
<th>Sometimes</th>
<th>Almost Always</th>
<th>Always</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

How would you describe the indoor lighting conditions **at home** that you are usually exposed to when it is **dark outside** (i.e. at night)?

<table>
<thead>
<tr>
<th>Never</th>
<th>Almost Never</th>
<th>Sometimes</th>
<th>Almost Always</th>
<th>Always</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

How would you describe the indoor lighting conditions **at work** that you are usually exposed to when it is **dark outside** (i.e. at night)?

<table>
<thead>
<tr>
<th>Never</th>
<th>Almost Never</th>
<th>Sometimes</th>
<th>Almost Always</th>
<th>Always</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The following questions pertain to the physical environment of your bedroom just prior to going to sleep. Please answer the questions as it pertains to conditions experienced in the last 2 weeks. If the question does not apply to you (for instance, if there are no streetlights visible from your bedroom, as asked in the first question) please choose N/A.

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
</table>
| If there are streetlights visible from your bedroom window(s), were they on while you slept? | □ NO  
□ YES  
□ N/A                                                                 |
| If there are shades/curtains/blinds or other window treatments on your bedroom window(s), were they closed while you slept? | □ NO  
□ YES → please specify the type of window treatment  
□ N/A                                                                 |
| If you sleep with a light on in the bedroom (such as a nightlight), was it on while you slept? | □ NO  
□ YES → please specify the type of light  
□ N/A                                                                 |
| If there is a light visible from your bedroom (such as a hall light), was it left on while you slept? | □ NO  
□ YES → please specify the type of light  
□ N/A                                                                 |
| If the moon/stars were shining brightly, could they be seen in the room while you slept? | □ NO  
□ YES  
□ N/A                                                                 |
| If the sun was shining brightly, could it be seen in the room while you slept? | □ NO  
□ YES  
□ N/A                                                                 |

27) What best describes the ambient light in your bedroom?
- Dark (you wear a mask in bed)  
□
- Medium (can see to the end of the bed)  
□
- Light (can almost read without a light)  
□
29) Please list **sedentary, light, moderate** and **heavy** activities that you have done **in the last 2 weeks**. These activities could be **sports/exercise** or **around the home/leisure**. A sample list of activities is provided for reference at the end of this questionnaire.

The four categories of physical intensity levels are:

**Sedentary:** Activities that involve sitting only, with minimal walking

**Light:** Activities that require minimal physical effort such as those activities that are done standing or with slow walking

**Moderate:** Activities that are not exhausting, that increases the heart rate slightly and may cause some light perspiration

**Heavy:** Activities that increase the heart rate and cause heavy sweating.

<table>
<thead>
<tr>
<th>Description of Activity (Please indicate distance if appropriate, i.e. jogging example below)</th>
<th>Morning – Afternoon (7:00AM – 3:00PM)</th>
<th>Afternoon – Evening (3:00PM – 11:00PM)</th>
<th>Evening – Morning (11:00PM – 7:00AM)</th>
<th>Intensity of Activity (Please check only one for each activity)</th>
<th>Is this a typical amount of activity for you? Yes or No→ Do you usually do more or less?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hrs and/or mins</td>
<td>Hrs and/or mins</td>
<td>Hrs and/or mins</td>
<td>Sedentary</td>
<td>Light</td>
<td>Moderate</td>
</tr>
<tr>
<td><strong>Sports/Exercise:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e.g. jogging (distance ~5 km)</td>
<td>1hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e.g. aerobics class</td>
<td>1hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Around the home/Leisure:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e.g. laundry</td>
<td>2 hrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Thank you very much for completing this questionnaire.**

Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page, making sure that you did not skip any pages.
30) Please tell us about your work schedule for the **last 2 weeks**.

<table>
<thead>
<tr>
<th>Week</th>
<th>Average # of hours worked/week</th>
<th>Total number of shifts worked for each shift type</th>
<th>Usual hours worked at each type of shift</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
<td>Evening</td>
<td>Late-night</td>
</tr>
<tr>
<td></td>
<td>Start</td>
<td>End</td>
<td>Start</td>
</tr>
<tr>
<td>e.g.</td>
<td>36</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Sample List of Activities for Physical Activity Question #29

When describing physical activity please include as much detail as possible and attempt to make your list as complete as possible. Some examples of activities to include are as follows; however, do not feel limited to the activities that are listed.

### Sports/Exercise Activities

- Bicycle - Leisure (stationary or not)
- Bicycle - Fast (stationary or not)
- Rowing (Stationary machine)
- Skating (leisure vs vigorous)
- Elliptical Trainer
- Lifting Weights
- Running (please try to include mph or km/h and terrain)
- Walking - Leisure (include terrain)
- Walking - Power/quickly (include terrain)
- Tennis
- Yoga
- Squash
- Push-ups
- Sit ups
- Aerobics Class (include class type)

### Around the Home/Leisure Activities

- Shopping (grocery with cart, clothes, mall or downtown or department store)
- Housework Indoors (Vacuuming, mopping, doing dishes, ironing)
- Housework Outdoors (Shovelling snow, gardening, raking leaves etc)
- Childcare - (sitting or standing or running)
- Lying down (watching TV)
- Standing (cooking, working, talking on the phone, miscellaneous)
- Sitting (reading, writing, miscellaneous)
- Swimming (specify type of stroke used)
- Dancing
- Typing
- Knitting
- Eating
- Showering
- Driving
- Playing Musical Instrument (specify type of instrument)
- Loading/unloading the car
- Carrying Baby (pushing stroller, holding baby standing still or walking)
- Dressing/Undressing