PHYSICAL ACTIVITY, SEDENTARY BEHAVIOUR AND MELATONIN AMONG ROTATING SHIFT NURSES

by

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Abstract

**Background:** Shift work is associated with increased risk of cardiovascular disease and cancer, where decreased melatonin has been proposed as an intermediate in the causal pathway. The influence of physical activity on melatonin has rarely been studied in an observational setting, and it may be important in mediating the effects of shift work. We aimed to assess the influence of energy expended during physical activity of different intensities on melatonin among rotating shift nurses. We hypothesized that physical activity before the night shift would lessen the decrease in melatonin production that occurs with exposure to light at night.

**Methods:** 123 female rotating shift nurses working at Kingston General Hospital were recruited over a one-year period. Physical activity and sedentary behaviours for each participant were recorded during both a day and a night shift using activity diaries, and analysis was restricted to activities between 3 p.m. and 7 a.m. Concentrations of urinary 6-sulfatoxymelatonin, a melatonin metabolite, in morning void urine samples were analyzed for each shift.

**Results:** The average age of participants was 41 years, and 60% were overweight or obese (body mass index $\geq 25$ kg/m$^2$). An average of 6.9 and 5.2 hours of sleep were reported after the day shift and night shift, respectively. Sedentary behaviours such as standing and television watching accounted for over half of the total reported energy expenditure. During the day shift, energy expended in moderate and vigorous intensity physical activity between 3 p.m. and 7 a.m. was negatively associated with melatonin levels ($p=0.024, R^2 = 0.09$). During the night shift, energy expended in sedentary behaviours was negatively associated with melatonin levels ($p=0.008, R^2 = 0.03$).
Conclusions: Physical activity energy expenditure explains only a small amount of melatonin variation, suggesting that other factors are influencing melatonin production, or that melatonin production is minimally effected by these patterns of physical activity.
Co-Authorship

This thesis is the work of Mark McPherson in collaboration with his supervisors, Dr. Kristan Aronson and Dr. Ian Janssen. The study was designed by Dr. Kristan Aronson, Dr. Ian Janssen, Dr. Harriet Richardson, Dr. Joan Tranmer, Anne Grundy and Dr. Charles Graham. The statistical analysis was performed by Mark McPherson with input from Drs. Aronson and Janssen. Writing of the manuscript was performed by Mark McPherson with supervision by Drs. Kristan Aronson and Ian Janssen and editorial feedback from Anne Grundy, Dr. Joan Tranmer and Dr. Harriet Richardson. Mark McPherson wrote the other chapters of the thesis (introduction, literature review, methods, additional methods, and general discussion) with editorial feedback from Drs. Aronson and Janssen.
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<td>MVPA</td>
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Chapter 1

Introduction

1.1 General Introduction

Shift work is a major public health concern that has been associated with gastrointestinal pathologies, several cancers, and cardiovascular disease (Haus and Smolensky, 2006). Despite these potential health outcomes, nearly thirty percent of Canadians currently perform shift work (Shields, 2002; Williams, 2008). In the nursing profession, shift work is necessary for twenty-four hour medical care. Preventive measures have the potential to play a vital role in reducing the negative health impacts of shift work caused by altered sleeping and working patterns.

The hormone melatonin has been shown to be associated with many of the morbidities associated with shift work, including cardiovascular disease (Knutsson, 2003). Melatonin is a circadian hormone that is inhibited by light exposure and consequently varies by the daily sleep-wake cycle (Zawilsha et al., 2006). Working at night has been shown to decrease circulating levels of this hormone in shift workers (Touitou et al., 1990). There has been some evidence that physical activity at certain times during the day may function to increase melatonin, potentially protecting against the melatonin downregulation associated with working night shifts.

The relationship between physical activity and melatonin has been demonstrated in some animal models. Melatonin peaks were prolonged in exercised hamsters compared to sedentary controls (Lopez and Uruiqijo, 2007). Conversely, sled dogs were shown to have reduced peak melatonin levels after exercising (Dunlap et al., 2007). Experimental studies in humans have also shown similar inconsistencies, with studies depicting increased, decreased and no change in melatonin following short-term exercise (Atkinson et al., 2003). Two studies have determined the role of long term physical activity in athletes, and found inconsistent results, however sample sizes were very small (Lucia et al., 2001; Ronkainen et al., 1986).
Two published epidemiological studies have suggested that physical activity may be associated with levels of circulating melatonin. Knight et al. found that the total duration of exercise performed by a sample of healthy volunteers was significantly associated with urinary melatonin levels, and this relationship was moderated by the time of day of exercise (Knight et al., 2005). Most recently, Grundy et al. completed a pilot study using similar methodologies as this thesis, and found a non-significant positive relationship between self-reported physical activity and urinary melatonin levels in shift-working nurses during their day shift (Grundy et al., 2009). Though both of these studies did have some important limitations, they both suggest that physical activity could play a role in maintaining homeostatic melatonin levels in shift workers. No studies to date have observed the relationship between sedentary behaviour and melatonin in this at-risk population.

1.2 Overview of Objective and Study Design

This study aims to determine if there is an association between physical activity and sedentary behaviour with urinary melatonin levels among rotating shift workers. To evaluate this relationship, a cross-sectional study of rotating shift workers at Kingston General Hospital was conducted. Participants provided urine samples to determine their peak melatonin levels and completed a one-day diary to assess both recent and long-term physical activity. Each nurse participated during both a day and a night shift. Linear regression was used to determine the role of physical activity and sedentary behaviour in predicting melatonin levels during each shift.

1.3 Public Health Implications

Shift work has been associated with a plethora of diseases including cardiovascular disease, cancers and sleep-wake disorders (Haus and Smolensky, 2006). Melatonin has been proposed as a potential biological intermediate in these pathological pathways (Claustrat et al.,
An estimated 28-30% of Canadians perform shift work (Shields, 2002; Williams, 2008), so the population attributable risk of these diseases is substantial. Physical activity is a potential preventative mechanism by which the harmful effects of lowered melatonin levels can be evaded, and consequently could play an important role in reducing the impact of these morbidities.

1.4 Thesis Organization

This thesis conforms to the regulations outlined by the Queen’s University School of Graduate Studies and Research. The second chapter summarizes studies of relevance to melatonin and its relationship to physical activity, sedentary behaviour and cardiovascular disease. Chapter three provides a more detailed description of the methodological principles of this thesis including data collection and analysis strategies. The fourth chapter is comprised of the draft manuscript to be submitted for publication in an appropriate scientific peer-reviewed journal. The fifth chapter contains additional results describing the physical activity and sedentary behaviours of the study participants, and their associations with melatonin. Chapter six contains a general discussion of the findings, study conclusions and future research directions.
1.5 References


Chapter 2
Literature Review

2.1 General Introduction

Cardiovascular disease is currently the leading cause of mortality in developed countries, and is responsible for over 35% of all deaths globally (Rosamond et al., 2007). In Canada, cardiovascular disease is responsible for 32% of all deaths, averaging one death every seven minutes (Heart and Stroke Foundation of Canada, 2007). Several known risk factors for cardiovascular disease have been established, including smoking, obesity, physical inactivity, low income, diabetes and hypertension (Tanuseputro et al., 2003). Recent evidence has shown that working irregular shift patterns has also been shown to increase cardiovascular disease risk.

Shift workers have a 40% higher risk of cardiovascular disease compared to the general population (Knutsson, 2003). When coupled with the high prevalence of shift work [i.e., 28-30% of workers in health care and manufacturing industries (Shields, 2002; Williams, 2008)], the population etiologic fraction of shift work on cardiovascular disease has been estimated to be nearly 7% (Ha and Park, 2005). This increased cardiovascular risk in shift workers has been partially attributed to the behavioural and social changes associated with the altered sleep-wake cycle. These may include altered eating and socializing schedules, added stresses and reduced family time (Moore-Ede and Richardson, 1985). Disturbances in the body’s natural biological rhythms, or circadian disruption, have also been suspected as a mechanism of increasing cardiovascular disease risk in shift workers (Stevens, 2005). Much research has examined the behavioural and social impacts of shift work on cardiovascular disease, but the biological mechanisms explaining these associations are poorly characterized.

This chapter will highlight some of the research pertaining to the relationship between physical activity, sedentary behaviour and melatonin in shift workers. It will start by defining
some important terms, including physical activity, sedentary behaviour, melatonin, shift work and cardiovascular disease. The relevance of the hormone melatonin to diseases of the cardiovascular system and the impact of shift work on this hormone will then be described. The importance of physical activity and sedentary behaviour will be detailed in the context of reducing cardiovascular risk, and the role of shift work on these behaviours will also be discussed. Finally, evidence supporting the biological relationship between physical activity, sedentary behaviour and melatonin will be presented to provide a rationale for this thesis.

2.2 Key Definitions

*Shift work* describes work schedules that do not fall within the standard working period generally defined as 8:00 a.m. to 5:00 p.m. When the timing of these shifts varies over days or weeks, it is classified as rotating shift work (CCOHS, 2008). *Physical activity* is defined as any bodily movement produced by the skeletal muscles that results in an increase in energy expenditure (Dishman *et al.*, 2004). This incorporates exercise, leisure-time and occupational activity. *Sedentary behaviour* describes all behaviours in which bodily movement is minimal, and therefore little energy above the resting metabolic rate is expended. These behaviours include reading, watching television, driving a car and working on a computer (Dietz, 2007). *Circadian disruption* is the disturbance of the body’s natural biological rhythm (Stevens, 2005) resulting from changes in normal sleep-wake cycles, such as disruptions caused from working at night. Melatonin is the primary indicator of circadian rhythms in most animals including mammals, birds and reptiles (Goldman, 2003). *Cardiovascular disease* encompasses all pathological conditions relating to the cardiovascular system including myocardial infarctions, ischemic stroke and atherosclerosis.
2.3 Melatonin

As the primary circadian indicator in humans, melatonin (N-acetyl-5-methoxytryptamine) concentrations vary by the diurnal sleep-wake cycle (Zawilska et al., 2006). Melatonin is produced from the amino acid tryptophan in the pineal gland, and its release is regulated by the suprachiasmatic nucleus of the hypothalamus or the ‘master biological clock’ (Grant et al., 2009). Melatonin is responsible for sensations of fatigue, and its concentration increases by a factor of thirty at night (Atkinson et al., 2003). Studies have indicated that chronic insomniacs and those suffering from jet-lag have consistently low melatonin levels (Braam et al., 2008; Waterhouse et al., 2007).

Melatonin production is influenced by several factors including age (Savvidou, 2007), smoking (Ozguner et al., 2005), oral contraceptive use (Okatani et al., 2000) and anti-depressant medication (Härtter et al., 2001), but is primarily influenced by light exposure (Figure 2.1). Laboratory studies have shown that high levels of irradiant light at night reduce normal melatonin levels by a factor of thirty (Atkinson et al., 2003). Reduced melatonin levels in the summer caused by increased sunlight exposure have been attributed to mammalian seasonal breeding (Kovacs et al., 2000). Altered melatonin levels in humans have several proposed pathological implications such as sleep-wake disorders, depression, breast and prostate cancer and cardiovascular disease (Pandi-Perumal et al., 2008). This thesis will focus on the cardiovascular implications of reduced melatonin, though the products of this research may be generalized to the variety of health conditions that are associated with melatonin.
2.3.1 Melatonin and Shift Work

Melatonin levels in individuals with normal sleeping patterns begin to increase during the evening (~ 9:00 p.m.). Melatonin levels peak at around 2:00 a.m. and return to baseline around sunrise (~ 6:00 a.m.) (Zee and Manthena, 2007). Irregular sleeping patterns can lead to circadian disruption and shift the amplitude and timing of peak melatonin levels. Shift workers who consistently work at night may have shifted rhythms that coincide with their altered sleeping patterns (Haus and Smolensky, 2006). These shifted circadian rhythms may be less pronounced among rotating shift workers, such as the nurses who work two days followed by two nights, as reported by Grundy et al. (2009). Re-adaptation to normal circadian rhythmicity after the cessation of shift work has been shown to take from one week in the summer to three weeks during the winter (Midwinter and Arendt, 1991).

The majority of complaints from shift workers are associated with working the early morning shift (5 a.m. – 1 p.m.) when melatonin production in the pineal gland ceases (Touitou et
al., 1990). These include complaints of fatigue, gastrointestinal and nervous problems (Harma, 1996), all of which have been hypothesized to be associated with melatonin (Pandi-Perumal et al., 2008). Melatonin is responsible for sensations of fatigue, which could explain why 80-90% of shift working police officers, steel workers and meteorologists report complaints of sleepiness on the job. Approximately 70% of polled night shift train drivers and health care workers have admitted to falling asleep during the night shift (Akerstedt, 1988). Additionally, over one third of surveyed shift workers in the manufacturing industry report gastrointestinal and nervous problems (Harma, 1996) which have also been shown to be associated with melatonin (Pandi-Perumal et al., 2008).

Many observational studies have demonstrated a relationship between shift work and melatonin (Burch et al., 2005; Hansen et al., 2006; Schernhammer et al., 2004; Touitou et al., 1990); however, the magnitude of this relationship varies depending on the nature of the shift work under study. Touitou et al. (1990) observed four adult oil-refinery operators working a 3-4 day rotating shift cycle. Peak melatonin levels were higher in these workers compared to controls working only day shifts. The method of acquisition and the source of controls were not discussed, so their comparability cannot be confirmed. Burch et al. (2005) studied a shift working population at a medical device manufacturing company in the USA, and found that participants working permanent night shifts had lower melatonin levels than day workers, a difference that approached statistical significance (p=0.08). In their study, urine samples were collected for melatonin assessment after sleep and after work for each shift group studied, a collection strategy that assumed circadian rhythm shifted in the night shift group, which may not be the case according to recent work by Grundy et al. (2009). If indeed this assumption is not met, urine samples should have been collected at the same time of day for each shift group.
Shift workers have consistently been shown to have lower melatonin levels during the night shift (Hansen et al., 2006; Schernhammer et al., 2004), although several considerations need to be made when interpreting the results from these cross-sectional studies. Workers who are more susceptible to melatonin downregulation and are consequently less tolerant to working night shifts are most likely to select out of shift work. Therefore, the night workers under investigation are those who did not select out of shift work and may have been less likely to illustrate the true effect of shift work on melatonin levels (Stevens et al., 2007). Effect modification by genetics, particularly clock gene polymorphisms, may also be an important consideration that could influence the relationship between shift work and melatonin levels (Stevens et al., 2007). Despite the limitations to the existing epidemiological evidence, given the consistency of findings across studies, it appears that shift work poses an important public health concern when considering the pathological implications of insufficient and altered melatonin production.

2.3.2 Melatonin and Cardiovascular Disease

There is growing evidence that reduced melatonin is a risk factor for several cardiovascular morbidities, including hypertension and myocardial infarctions (Tengattini et al., 2008). Recently, laboratory experiments have shown that the heart itself may be responsible for the production of small amounts of melatonin (Reiter and Tan, 2009), which may be an evolutionary response to the cardio-protective effects of melatonin. For example, melatonin has been shown to sustain cardiac rhythm and prevent myocardial damage by autoimmune responses (Reiter and Tan, 2009; Scorza et al., 2008).

Indirect evidence of the relationship between melatonin and cardiovascular disease depicts a 10% - 400% increased risk of cardiovascular disease in shift-workers, who are more
susceptible to circadian disruption due to their inconsistent sleeping schedules (Knutsson, 2003). The effect of shift work on cardiovascular risk is likely multifactorial, and thus the pathway linking shift work to cardiovascular disease may not necessarily include melatonin down-regulation. Additionally the shift-working population may differ from the general population in other social, demographic, and behavioural risk factors, so this evidence is insufficient to depict a causal relationship between melatonin and cardiovascular disease.

Direct evidence linking melatonin with cardiovascular disease and its clinical risk factors (e.g., hypertension, high cholesterol) is less common. It has been shown that exogenous nighttime melatonin supplementation reduces systolic blood pressure by an average of 6 mm Hg compared to a placebo in male patients previously diagnosed with essential hypertension (p=0.04) (Scheer et al., 2004). However, this study used supplements that increased melatonin concentrations well above the natural physiological range, so the observed effects may not be consistent with melatonin at endogenous levels. Cross-sectional observational studies have shown that hypertensive cases have lower melatonin levels than normotensive patients (Sewerynek, 2002). However, due to the cross-sectional nature of data, it is not clear whether the high blood pressure preceded the low melatonin levels or vice versa.

The biological mechanism by which the association between melatonin and cardiovascular disease is plausible: melatonin can detect and destroy free radicals caused by oxidative stress (Tengattini et al., 2008). If uninhibited, these free radicals can lead to plaques and cellular damage in the arterial walls throughout the body. In addition to the radical mediated cardio-protective properties of melatonin, it has also been shown that melatonin acts as an antioxidant, anti-cholesterol and immuno-regulator (Tengattini et al., 2008). These properties have been demonstrated in animal models (Armanag et al., 2006; Wakatsuki et al., 2000), but have yet to be confirmed in human studies.
2.3.3 Biomarkers of Melatonin

Three biomarkers of circulating melatonin are commonly used in epidemiological and experimental studies. These include blood, saliva, and urine. Average concentrations of melatonin from each of these measurement techniques across a 24-hour period are illustrated in Figure 2.2. Additional details for each of these techniques are included in the following paragraphs.

**Figure 2.2** Average concentrations of melatonin in plasma (2), saliva (3) and 6-sulfatoxymelatonin (aMT6s) in urine (1) as measured by radioimmunoassay. (Arendt, 2006)

Direct measurement of melatonin in blood (serum) samples is considered to be the most accurate; validation studies commonly treat serum melatonin measurements as the gold standard when validating other biomarkers. However, blood samples require trained personnel and can be invasive for participants. Additionally, serum melatonin samples provide an instantaneous
measure of melatonin concentration; consequently, if researchers are interested in characterizing
daily circadian rhythms, several samples need to be taken to determine the circadian peak and
amplitude. Characterizing daily circadian rhythms is important because cumulative, peak and
instantaneous concentrations of melatonin may each have important independent health effects
(Hrushesky and Blask, 2004).

Salivary samples are a second method of assessing melatonin levels in humans. Unbound
circulating melatonin in blood passes through salivary glands into saliva. Since 61–78% of
melatonin is reversibly bound to serum proteins such as albumin (Di et al., 1998), salivary
melatonin represents approximately 30% of circulating melatonin. Although the inter-individual
differences in albumin-bound melatonin salivation rates (Kennaway and Voultsios, 1998) limits
the usefulness of salivary measurements for determining absolute melatonin levels, saliva
melatonin biomarkers are a useful and practical method to assess individual melatonin variability
(Voultsios et al., 1997).

Urine samples provide another method of assessing circulating melatonin levels by
measuring the concentration of its metabolite, 6-sulfatoxymelatonin. 6-sulfatoxymelatonin is the
main urinary melatonin metabolite, which is produced by a two step reaction in the liver. 6-
sulfatoxymelatonin concentrations have been shown to be representative of 70% of the circulating
melatonin (Bird et al., 1996). In contrast to blood and saliva melatonin measurements, morning
void urine samples measure cumulative concentrations of melatonin, providing an indication of
melatonin levels during its circadian peak. In adults with invariable sleeping schedules, nocturnal
peak urinary melatonin levels remain constant. However, in adults with disturbed sleeping
patterns, such as shift workers, these peaks may not be consistent so urinary 6-sulfatoxymelatonin
provides a practical and noninvasive means of detecting such changes in melatonin.
Urinary melatonin levels are commonly adjusted by levels of creatinine to compensate for varying volumes of urine excretion. Without this adjustment, lower volumes of urine would artificially inflate the concentration of 6-sulfatoxymelatonin in the urine. Creatinine is a product of the breakdown of creatine phosphate, a mobilizable energy reserve for skeletal muscles (Taylor et al., 2006). Creatinine is eliminated mainly through the kidneys, and is thus used as a marker of kidney function (Levey et al., 2006). Because it is eliminated at a constant rate, the concentrations of creatinine in urine provide a marker for total urine volume excreted. If a high volume of urine is excreted, the creatinine will appear more dilute (Thomas, 2010).

### 2.4 Physical Activity and Sedentary Behaviour

Physical activity is a complex construct composed of a variety of forms of energy expenditure. Physical activity encompasses exercise, leisure time, occupational and incidental (non-purposeful) movement (Tremblay et al., 2007). It is usually characterized by type, frequency, intensity and duration. Each of these facets of activity may have cumulative and independent roles on a variety of health outcomes (Haskell, 1994). Physical activity has both short-term and long-term effects on health. Short-term effects of recent activity may last from minutes to days, while long-term effects of regular physical activity may not be evident until decades after the fact (Dishman et al., 2004). Both the short-term and long-term effects have important roles on human health.

The study of sedentary behaviour, or inactivity, is of growing interest in physical activity epidemiology, though not traditionally considered when classifying intensity. Sedentary behaviour is at one extreme of the physical activity continuum and may include behaviours such as sitting and resting (Dietz, 2007). Sedentary behaviour has been shown to have health effects independent of low, moderate and vigorous intensity physical activity (Lamb and Brodie, 1990).
For example, the U.S. National Children and Youth Fitness Survey found that the amount of time a child watches television has an effect on the prevalence of obesity, independent of the duration of moderate-to-vigorous physical activity performed (Pate and Ross, 1987). Recently, researchers have begun exploring the mechanisms by which sedentary behaviours may impact health independent of physical activity. Several hypotheses have been generated, including hormonal and metabolic biological pathways, but no consensus has yet been developed to explain the independent health effects of physical activity and sedentary behaviours (Hamilton et al., 2008).

2.4.1 Measurements of Physical Activity and Sedentary Behaviour

In his editorial describing methods to assess physical activity, Richard Troiano explained that each time that he is asked the best measure of physical activity he must respond with the seemingly unsatisfactory statement, “It depends” (Troiano, 2009). There is no perfect quantification technique, demonstrated by the more than thirty commonly used physical activity measurement strategies in epidemiology (Laporte et al., 1985). Valid physical activity measurement proves challenging because of the multiple contributions of various components of physical activity, sedentary behaviour and energy expenditure (Kriska and Caspersen, 1997). The process is further complicated by the several dimensions of physical activity that influence health, including type, duration, intensity and frequency. Powell et al. (1987) determined that the number of detectable associations nearly doubled as the quality of physical activity measurements increased. Pedometers and questionnaires are two commonly used physical activity measurement strategies in epidemiologic studies, each with several important strengths and limitations.

Pedometers

Pedometers are small portable step counters that detect vertical displacement of the hips. Although they are usually considered to simply measure the number of steps taken, it is important
to recognize that other activities that result in vertical movements at the hip will also be detected by pedometers. Over the past decade pedometers have increased in popularity, likely due to their high practicality in large studies. A Medline search for studies using the keyword *pedometer* published in 2002 retrieved 12 English studies (Tutor-Locke *et al.*, 2002). When I performed the same search for 2009, I retrieved over ten times as many studies.

Pedometers have several strengths when measuring physical activity levels. Pedometers provide an objective measure of activity levels that is not susceptible to recall or self-report biases. The use of pedometers can also be cost-effective with meters averaging $20 U.S. per unit (Tudor-Locke and Myers, 2001). However, like all physical activity assessment tools, pedometers have several limitations. Pedometers cannot differentiate between intensities of activity. Running one kilometer requires fewer steps than walking the same distance, despite the fact that the higher intensity activity has a greater health benefit (Dishman *et al.*, 2004). The reliability of pedometers has also been brought into question, as they have been shown to be affected by several factors including the length of stride, abdominal adiposity and internal spring tension (Shepard *et al.*, 1999; Welk *et al.*, 2000). Validation studies comparing pedometers to accelerometers, a tool that can precisely measure the acceleration of movement, demonstrate substantial inter-sample variability. Kilanoski *et al.* (1999) found a Pearson correlation coefficient of 0.98 in a small cohort of children during recreational time, but a coefficient of 0.5 in the classroom. These validation studies must be interpreted with caution, however, because accelerometers also have several limitations (Corder and Brage, 2007) and thus are not considered by many researchers as the gold standard in physical activity assessment.

**Questionnaires**

Questionnaires are self-administered surveys requiring respondents to recall their activity for a specific timeframe of interest. They are the most commonly used method of assessing
physical activity and sedentary behaviour in epidemiological studies (Laporte et al. 1985), consequently substantial research has attempted to assess their validity, reliability and practicality.

Recall biases and errors are a common concern when reporting past physical activity. Self report often leads to over-reporting of both the intensity and duration of intensity (Rzewnicki et al., 2007). Understandably, errors have also been shown to be more common when respondents are asked about their long-term activity, such as activity in the previous year versus recent activity such as activity during the past twenty-four hours (Sallis and Saelens, 2000). Reliability and validity can also be affected by several factors including the length of questionnaire, age of respondent, and the type of activity under investigation (Corder and Brage, 2007; Sallis and Saelens, 2000). Exercise, defined as planned, structured and repetitive activity (Dishman et al., 2004), is most accurately recalled because participants are more likely to recall time they set aside for physical activity. Leisure time activities of lower intensity are commonly omitted from physical activity reports, and thus questionnaires should probe specifically for activities of a lower intensity (Shephard and Vuillemin, 2003).

Thorough questionnaires collect information on all four dimensions of physical activity: intensity, duration, frequency and type. Metabolic equivalents (METs) incorporate each of these important factors into a single measure of energy expenditure. A MET is a ratio of the metabolic rate of an activity compared to a standard resting metabolic rate (4.184 kJ \cdot kg^{-1} \cdot hr^{-1}), thus a MET of 3 would indicate that the activity requires 3 times as much energy expenditure as is required at rest (Ainsworth et al., 2000). Activities range from those of no intensity such as sitting (MET=1), to activities of high intensity such as running (MET=18). MET values can be calculated from questionnaire data obtained in epidemiological studies using the Compendium of Physical Activities (Ainsworth et al., 2000). This compendium includes a list of over 600
activities, and the MET values for this compendium were calculated using calorimetric data obtained in the laboratory setting. The intensities of activity can be determined from the MET values as depicted in Table 2.1. In summary, METs provide a comprehensive method of quantifying the energy expenditure of physical activities that is comparable between individuals of different weights and fitness levels.

Table 2.1 Intensity of activity based on Metabolic Equivalent score (Owen et al., 2000; Sarkin et al., 2000).

<table>
<thead>
<tr>
<th>MET value</th>
<th>Intensity of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 1.5</td>
<td>Sedentary behaviours</td>
</tr>
<tr>
<td>1.6 – 3.0</td>
<td>Low intensity activities</td>
</tr>
<tr>
<td>3.1 – 6.0</td>
<td>Moderate intensity activities</td>
</tr>
<tr>
<td>&gt;6.0</td>
<td>Vigorous intensity activities</td>
</tr>
</tbody>
</table>

The concept of a Metabolic Equivalent Minute or a MET·min can be used to compare and summate several activities at different intensities. For example, a two hour game of non-competitive volleyball (MET=3) accounts for 360 MET·min, which is equivalent to a 40 minute cross country run (MET=9) in terms of energy expenditure. Though the duration of the run is less than half of the volleyball game, the increased intensity results in the same amount of energy expended. Physical activity guidelines commonly recommend acquiring 600 MET·min of moderate or vigorous intensity per week to maintain or improve one’s health (Vašičková et al., 2008).

METs also prove useful for the measurement of sedentary behaviour. The compendium of physical activities include a wide variety of sedentary behaviours ranging from sitting and listening to music (MET=1) to driving a car (MET=1.3) (Ainsworth et al., 2000). In 2000, Talbot et al. quantified the physical activity and sedentary behaviours of 1296 Americans aged 18 to 94 using MET·min of activity per day. Female participants averaged 2270 MET·min per day, with sedentary activities contributing over half (1112.4 METmins) of the total energy
expenditure. High intensity activity only contributed 5% of the total activity-related energy expended by these women (Talbot et al., 2000). Though this study provides insight on the usefulness of METs in epidemiological studies, certain limitations could have influenced the results. Intensity of activity was assessed based on the activity performed, so for example, if tennis was reported it would be assigned an intensity based on the energy expenditure of an average tennis player. This exposure assessment strategy does not consider the fact that two individuals can perform the same activity at very different intensities. These MET assignments would likely underestimate the energy expenditure of younger fit individuals, while overestimate for older, less active adults (Bryne et al., 2005). In analytic studies, this error would bias the results towards the null, reducing the likelihood of detecting an association (Talbot et al., 2000).

METs have also been used to determine the role of physical activity on long-term health outcomes. METmins have been shown to be inversely associated with several health outcomes including body mass index, diabetes and cardiovascular disease (Bassett et al., 2004; Smith et al., 2005; Talbot et al., 2000). In a case-control study of 1050 Indians, respondents with less than 145 MET·min/day had a 2.3 times increased odds of ischemic heart disease than those with 145 or more MET·min/day, after controlling for smoking status, family history, household income and diet (Rastogi et al., 2004). This association is biologically plausible; Metsios et al. have shown that METmins are associated with several cardiovascular risk factors including hypertension, high cholesterol, and high levels of low-density lipoprotein (Metsios et al., 2009). Metabolic equivalents prove useful in epidemiological studies as an effective means of quantifying physical activity.

Despite the benefits of using metabolic equivalents to quantify the intensity, type, duration and frequency of activity, there are also some limitations that need to be considered. All reported physical activities and sedentary behaviours were tested in laboratory conditions and
consequently they may not reflect the activity conditions of the participant. For example, walking at a slow pace has a cited MET value of 2.5 (Ainsworth et al., 2000), but this was determined on a treadmill in a laboratory. When a respondent reports this activity, it is likely to be outside where other factors like waiting for crosswalks, or walking up hills would also affect the expenditure of energy. In addition, the baseline to which all METs are based (4.184 kJ kg\(^{-1}\) \(\text{hr}^{-1}\)) was determined from a 40 year old 70kg male. This baseline has been shown to overestimate basal metabolic rates in women and participants with low body mass indexes (Bryne et al., 2005). Consequently, it has been proposed that MET values should not be used to calculate the energy expenditure of an individual, but to characterize activity habits of a sample of individuals (Bryne et al., 2005).

### 2.4.2 Physical Activity and Shift Work

The emotional and physiological stress associated with working shifts has undeniable effects on the lifestyle habits of shift workers. Shift workers have been shown to have higher body mass indexes, blood pressures and cholesterol values than day workers (Nakamura et al., 1997). Several multifactorial hypotheses exist for these associations, most of which incorporate the role of physical inactivity.

In their observational study of 60 industrial blue-collar shift workers in Japan, Nakamura et al. determined that 69% of their shift working sample did not exercise at all, compared to 50% of day workers. Only 10% of shift workers exercised more than once a week (Nakamura et al., 1997). Since physical activity includes not only exercise, but also leisure-time and occupational activity, this study was unable to fully characterize the activity levels of each shift group. For example, night shift work may have been more labour-intensive than the day shift. Consequently, these workers may have participated in less exercise than the day workers, but could have substantially more occupational physical activity. Though this study only considered one aspect
of physical activity, it clearly demonstrates the role of altered working and sleeping patterns on levels of activity.

Several barriers exist to the implementation of effective physical activity programs in shift working populations. In his review of methods to promote and maintain health, Harma (1996) recommended that shift workers: 1) Perform moderate intensity activity; 2) Complete activity several hours prior to their sleeping period; 3) Participate in activity directly after each shift. These recommendations are evidence based; however, their practicality for a shift working population may be limited. Most shift workers work 12-hour shifts, much of which is spent standing and moving around. Shift workers report 2 - 4 hours less sleep than day workers (Åkerstedt, 1998), and nearly two thirds of shift workers report having fallen asleep on the job at least once a week (Moore-Ede and Richardson, 1985). Physical activity recommendations therefore need to take into account that moderate or vigorous intensity activities may not be feasible during workdays, and that there may not be ample time between shifts for adequate levels of activity. Sedentary behaviours, occupational and low-intensity activities thus must be considered when addressing the health of shift workers.

2.4.3 Physical Activity, Sedentary Behaviour and Cardiovascular Disease

The role of physical activity and sedentary behaviour on cardiovascular disease has been well established. In a review of 30 cohort and case-control studies investigating the role of physical activity on cardiovascular disease in women, active women had an 80% decreased risk of cardiovascular disease than inactive women \( p < 0.0001 \). Even one hour of walking per week was shown to decrease cardiovascular disease risk compared to those who participated in no physical activity (Oguma and Shinoda-Tagawa, 2004). Randomized controlled trials have demonstrated the role of activity in the secondary prevention of cardiovascular disease, with
physical activity halting the progression of coronary artery disease and reducing the plaque formation that leads to heart disease (O’Connor et al., 1989; Taylor et al., 2004).

Sedentary behaviour has been shown to be associated with several clinical risk factors of cardiovascular disease, independent of moderate and high intensity physical activity. Children who watch 3-5 hours of television/day have an odds ratio of 1.61 (95% CI: 1.31 – 1.70) of becoming overweight or obese compared to those who watch less than 2 hours/day (Tremblay and Willms, 2003). Overweight and obesity are strong predictors of cardiovascular disease (Dishman et al., 2004). Sedentary behaviour is also related to the metabolic syndrome, a combination of cardiovascular risk factors including abdominal obesity, hyperglycemia, and hypertension (Ford et al., 2005). American adults who spent more than four hours a day watching television or playing videogames outside of work had a 210% increased risk (95% CI 1.27 – 3.47) of metabolic syndrome, independent of total daily physical activity (Ford et al., 2005).

Although the etiological mechanism for the relationship between physical activity, sedentary behaviour and cardiovascular disease is unclear, it has been hypothesized that improved body composition, glucose management, and autonomic tone may play a mechanistic role in the biological pathway (Warburton, 2006). The role of the hormone melatonin may also influence this pathological pathway (Tengattini et al., 2008); however, this hypothesis remains largely untested in the literature.

2.5 Physical Activity, Sedentary Behaviour and Melatonin

The role of melatonin as a biological intermediate in the causal pathway between physical activity and cardiovascular disease has not been well established. This pathway is biologically plausible because physical activity up-regulates the catecholamine noradrenaline,
which promotes the synthesis of melatonin in the pineal gland (Atkinson et al., 2003). In addition, recent aerobic physical activity has been shown to increase sleep duration (Dishman et al., 2004), which could function to increase the length of time that melatonin can be synthesized by the pineal gland. Conversely, physical activity has been shown to increase levels of the stress hormone cortisol (Stupnicki et al., 1995), which may function to decrease pineal melatonin production (Buxton et al., 1997).

The relationship between physical activity and melatonin has been demonstrated in some animal models: Lopez and Uruiqijo found that melatonin peaks were prolonged in exercised Syrian hamsters compared to sedentary controls (Lopez and Uruiqijo, 2007). Conversely, Dunlap et al. (2007) found that exercised sled dogs had reduced peak melatonin levels at two different latitudes.

Similar inconsistencies have also been shown in experimental studies in humans; increased, decreased and no change in melatonin has been demonstrated in association with recent physical activity. Carr et al., who investigated the role of moderate intensity short-term endurance exercise on plasma melatonin in seven adult females, found that melatonin levels were up to 200% higher 30 minutes following the activity (Carr et al., 1981). Conversely, Montelone et al., who performed a similar study with seven adult males, found that melatonin levels were significantly lower for the three hours following a session of moderate intensity aerobic exercise, and this was proposed to occur through a cortisol-mediated pathway (Montelone et al., 1973). More recently, Miyazaki et al. found no change in melatonin levels in 46 healthy volunteers three hours after repeated moderate intensity aerobic exercise (Miyazaki et al., 2001). These conflicting findings may be due to small sample sizes and differences in melatonin analytical techniques, but are likely due to differences in light at night exposure and the time of day of the
physical activity. It is therefore important to examine the relative contribution of these factors in future analyses.

Two observational studies have investigated the role of long term physical activity participation on melatonin levels in athletes. Lucia et al. measured weekly urinary melatonin levels in nine professional cyclists at the Tour of Spain in 2001 (Lucia et al., 2001). Melatonin levels increased significantly the morning after each racing day, although concentrations were shown to decrease as the three week long race progressed. Ronkainen et al. performed a similar analysis of melatonin levels in eleven long distance runners, and no change in melatonin levels was found (Ronkainen et al., 1986). Long-term vigorous intensity exercise may influence several other physiological mechanisms that have a role in melatonin production including exercise-induced dehydration, fatigue and malnutrition, and these were not considered in either study. Further, sample sizes were very small.

Two published observational studies have attempted to determine the influence of physical activity on melatonin levels. In 2005, Knight et al. quantified physical activity using self-reported minutes of moderate or vigorous intensity exercise from 214 female volunteers. Exercise was significantly associated with creatinine-adjusted urinary melatonin levels (Beta=0.072, p=0.004), and this relationship was moderated by season, day length and the time of day exercise was performed (Knight et al., 2005). This study provides valuable information concerning the relationship between exercise and circadian disruption, but it does not examine the role of all physical activities. The study only examined structured exercise, omitting all other forms of physical activity that could contribute to melatonin up-regulation such as leisure and occupational activity. Intensity of activity was subjectively assigned as moderate or vigorous by the authors, which poses potential measurement error. The outcome of creatinine-corrected 6-sulfatoxymelatonin was a compilation of three separate measurements, urine volume, creatinine
concentration and levels of 6-sulfatoxymelatonin. Consequently, small errors in each measurement could contribute to a large overall error once combined. Twenty four percent of participants dropped out of the study after the first data collection, and no information was provided to demonstrate if these participants were different from the study population.

Using the same source population as this thesis, a pilot study was performed in which the methods and techniques required to determine the relationship between physical activity and urinary melatonin were developed and tested (Grundy et al., 2009). Sixty-one nurses employed at the Kingston General Hospital were recruited in 2006. Physical activity was measured using questionnaires and melatonin levels were assessed using urine samples. A non-significant positive association was found between self reported recent physical activity and urinary 6-sulfatoxymelatonin concentrations in the day shift group (linear regression coefficient: 0.46, p=0.13) (Grundy et al., 2009). Although this study provided evidence of the feasibility of several methodological principles, some important limitations were noted. One morning void urinary melatonin measurement was collected upon awakening for each participant; however, this was not at the same time for the day and night shift workers, so these concentrations could not be compared between the day and night shifts. Also, urinary melatonin levels were not adjusted for the total volume of urine excreted, which could function to dilute or concentrate the melatonin in each sample (Schernhammer and Hankison, 2009). Finally, the physical activity measurements were susceptible to self report biases due to the absence of objective quantification techniques.

2.5.1 Biologically Relevant Timeframes

An understanding of the timeframe in which physical activity impacts melatonin is vital for adequate exposure assessment. Animal models have indicated that only physical activity less than 16 hours prior to the commencement of sleep alters melatonin levels. Lopez and Urquijo
determined that only moderate or high intensity exercise at night resulted in changes in melatonin secretion in Syrian hamsters (Lopez and Urquijo, 2007).

In 1974, Webb and Agnew compared the circadian period of exercised and non-exercised subjects and found no statistically significant difference between groups (Webb and Agnew, 1974). Similarly, Wever compared the circadian rhythm of subjects during two week alternating periods of exercise and inactivity and no statistically significant differences were detected (Wever, 1979). Both research protocols required participants to exercise during the day. Two decades later, several studies have used similar methods and found that when the exercise is performed at night, the relationship between exercise and melatonin production is more apparent. Buxton et al. found that exercise at night elevated melatonin 30 minutes after the beginning of exercise, and continued for at least 80 minutes (Buxton et al., 1997). No such relationship was seen at any other point of the day.

It is possible that this relationship was confounded by light exposure patterns which would function to alter melatonin production independent of physical activity. However, Barger et al. observed the influence of exercise on melatonin rhythm in controlled lighting conditions, and found that the relationship between melatonin and exercise persisted, but again was highly dependant on the time of day of exercise (Barger et al., 2004).

The biologically effective timeframe of physical activity has been investigated directly in only one epidemiological study. Knight et al. compared the role of exercise in predicting melatonin levels during the morning, afternoon, evening and night (2005). Only activity performed during the evening and night (4 p.m. to 4 a.m.) was statistically associated with melatonin levels \((p=0.01)\). Grundy et al. considered all physical activity in the twenty-four hour study period prior to melatonin collection (Grundy et al., 2009), potentially contributing to the non-significant linear regression coefficients.
The moderating role of time of day is biologically plausible in both potential biological pathways linking physical activity and melatonin. In the first potential pathway, noradrenaline increases the rate of melatonin production by catalyzing the catabolism of tryptophan to melatonin. Although physical activity increases noradrenaline production at all times of the day (Gallivan et al., 1997), the pineal gland would not be producing melatonin during the day so there would be no reaction to catalyze. Noradrenaline has a fractional turnover of 6.8 ± 0.5 % per hour (Taubin et al., 1972), so it would not remain in circulation long enough to catalyze the reaction later in the evening. In the alternative biological pathway, physical activity increases sleep duration which would increase melatonin production (See section 2.5). Physical activity has its greatest effects on sleep duration during the late afternoon or evening (Tanaka et al., 2002), so any activity earlier in the day may not have much impact on sleep and consequently on melatonin.

An additional consideration when quantifying the relationship between physical activity and melatonin is the presence of a biologically relevant intensity of activity. In a study examining the role of exercise on circadian phase, Buxton et al. found that low intensity activity (40 – 60% maximum oxygen consumption) did not influence melatonin levels, but higher intensity activity (75% maximum oxygen consumption) at the same duration resulted in an increase in melatonin (1997). Previous epidemiological studies (Grundy et al., 2009; Knight et al., 2005) have only considered the role of moderate and vigorous physical activity on the relationship. No epidemiological research to date has aimed to determine if these two intensities of activity are the only biologically relevant predictors of melatonin in humans.

2.5.2 Potential Confounders

Confounders are variables associated with both the exposure and outcome, but are not intermediates on the causal pathway (Last et al., 2001). Several factors have been shown to
influence both physical activity and melatonin levels in humans. These factors may be potential confounding variables in the relationship between sedentary behaviour, physical activity and melatonin. Potential confounders include demographics, lifestyle factors, pharmacological agents and seasonal factors.

### Demographics

Levels of physical activity have been shown to decrease with age (Hawkins et al., 2009), as do melatonin levels (Figure 2.4). Women tend to participate in less physical activity than men, with 11.3% less women than men participating in regular sustained activity (Caspersen et al., 2000). Women have also been shown to have higher melatonin levels in some reports (Touitou et al., 1985); however, this relationship is not consistent in the literature (Beck-Friis et al., 1984). This thesis will use only female shift working nurses to eliminate the risk of sex confounding this relationship.

![Figure 2.3 Age variations of melatonin levels. (Savvidou, 2007)](image)

Figure 2.3 Age variations of melatonin levels. (Savvidou, 2007)
Lifestyle Factors

Caffeine, alcohol and smoking have all been shown to be associated with reduced melatonin levels (Härtter et al., 2006; Ozguner et al., 2005; Rupp et al., 2007) and may be related to overall health attitudes that influence physical activity. Melatonin is also influenced by several medications, including nonsteroidal anti-inflammatory drugs (Murphy et al., 1996), oral contraceptives (Okatani et al., 2000), anti-depressants (Härtter et al., 2001), hormone replacement therapy (Kos-Kudla et al., 2002) and sedatives (Djeridane and Touitou, 2003). These factors influence the synthesis and metabolism of melatonin in the pineal gland and liver. Medication use may also be related to physical activity, for example, physical activity is often prescribed by physicians to combat depression in conjunction with anti-depressants (Dishman et al., 2004). Additionally, sedentary behaviour is likely more prevalent after being prescribed sedatives.

Personal Characteristics

Body mass index (BMI) is defined as weight in kilograms divided by the square of height in metres (Calle et al., 1999). There is a strong association between BMI and long-term physical activity and sedentary behaviours (Mora et al., 2006). BMI has also shown to be inversely correlated with melatonin levels (Parry et al., 2008). An important consideration when considering BMI is that it may in fact be on the causal pathway by which physical activity influences melatonin levels.

Seasonal Factors

Melatonin is influenced by the number of hours of light exposure, which could be affected by the length of day and season. Physical activity and sedentary behaviours may also be related to both of these factors, as participants are more likely to be active during warm weather and longer days (Owen et al., 2000).
2.6 Additional Pathological Implications

In the 17th century, the French philosopher René Descartes described the pineal gland as the “seat of the soul” (Reppert and Weaver, 1995). Four centuries later, scientists are still uncovering the multisystem effects its main product has on human health. Melatonin has several pathological implications outside of its role in reducing cardiovascular disease risk. Reduced melatonin levels have been observed in several sleep-wake disorders including insomnia, jet-lag and narcolepsy (Brown et al., 2009; Reimann et al., 2002). Increased sleep duration has been shown to be important for the treatment of psychological conditions such as depression, mood disorders, and autism; and melatonin has been proposed as an intermediate in these pathways (Munesue et al., 2008). In addition, the role of melatonin in reducing breast cancer risk is of growing interest due to its established antioxidant mechanisms (Tengattini et al., 2008). Recently, the International Agency for Research on Cancer has classified shift work as a probable (type 2A) human carcinogen (Straif et al., 2007) and melatonin may have an important role in the pathogenesis of this common disease. If physical activity has the ability to increase melatonin levels, it may provide an alternate means for reducing the risk of these morbidities, in addition to its plethora of other pre-established benefits.

2.7 Summary of Literature

There is limited epidemiological evidence supporting an association between physical activity and the pineal hormone melatonin. Several experimental laboratory-based studies have examined the association between physical activity and melatonin levels, however many have conflicting results. Few epidemiological studies have attempted to detect this association, especially in a shift working population. These studies have used poor measurements of physical
activity and have not considered the role of sedentary behaviour. Several of the gaps that currently exist in the literature will be addressed in this thesis.

2.8 Rationale

Many critical aspects of modern life require twenty-four hour work and productivity, which explains why nearly 30% of Canadians are required to perform shift work (Shields, 2002; Williams, 2008). The global population etiologic fraction of shift work on cardiovascular disease has been estimated to be nearly 7% because of the high proportion of shift workers in the health and manufacturing industries (Ha and Park, 2001). Shift workers have a 40% increased risk of cardiovascular disease (Knutsson, 2003). Although this increased risk is likely multifactorial, most research has only focused on the behavioral and social risk factors associated with disturbed sleep cycles (Boggild and Knutsson, 1999). Physical activity has been well-established as a cardiovascular protective behaviour; however, few studies have determined its role in increasing melatonin levels in this at-risk population. If physical activity or sedentary behaviour does have a role in mediating melatonin levels in shift workers, occupation-specific preventative recommendations can be developed to combat the health issues associated with low melatonin levels.
2.9 References


Chapter 3
Methods

3.1 Overview of WSIB Study

Drs. Kristan Aronson, Ian Janssen, Harriet Richardson, Joan Tranmer, and Charles Graham received funding from the Workplace Safety and Insurance Board (WSIB) in 2008 to investigate the role of light exposure at night and several other factors as potential determinants of melatonin levels among rotating shift nurses at Kingston General Hospital (KGH). This thesis is a component of this larger study and looks specifically at the role of physical activity and sedentary behaviour on melatonin levels among shift working nurses in Kingston, Ontario. Implications of these associations on cardiovascular disease as the ultimate outcome are discussed since the MSc candidate is supported by a scholarship from the Heart and Stroke Foundation of Ontario.

The WSIB study was approved by Queen’s University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board (EPID-245-07) and this thesis obtained specific approval (EPID-297-09) (Appendix A).

The WSIB study was a longitudinal study of 123 healthy female nurses working at Kingston General Hospital. The participants were all shift working nurses, whose shift pattern consisted of two twelve hour days (7:00 a.m.–7:00 p.m.), two 12 hour nights (7:00 p.m.–7:00 a.m.), and five days off. Participants were asked to complete a questionnaire and diary outlining their physical activity habits, as well as provide biological samples for melatonin assessment.
3.1.1 WSIB Study Population

Female nurses from KGH were recruited from all medical units to participate in the study. Recruitment took place from April 2008 until February 2009. All full-time registered nurses and registered practical nurses were invited to participate, and a monetary honorarium was offered for their time. Recruitment strategies included posters, handouts and presentations. Interested nurses were advised to contact the study coordinator to arrange an initial interview. There were approximately 700 nurses who work rotating shifts at KGH, however, response rates could not be calculated as we had no information on what proportion of the population self-excluded because they did not meet the inclusion requirements.

Nurses were self-excluded from participation if they did not work the two twelve hour days (7:00 a.m.–7:00 p.m.) and two 12 hour nights (7:00 p.m.–7:00 a.m.) shift schedule, the typical rotating shift pattern at KGH. Participants were also excluded if they were pregnant or lactating in the preceding six months or if they were taking melatonin supplements at the time of enrollment, as these factors could greatly influence endogenous melatonin levels. Male nurses were not recruited for this study because the ultimate outcome under investigation was breast cancer. All exclusion criteria were explicitly outlined in the recruitment material (Appendix B).

Five participants who consented to participate did not complete the study for various reasons: injury (n=1), withdrawal (n=2), and loss to follow up (n=2). Demographic information is available for these participants, and they did not significantly differ by age, body mass index, or race (p>0.20). Five nurses withdrew from the study prior to completion of their second shift; four completed only the day shift study period and one completed the night shift study period only. Seven had incomplete outcome information for one of the two data study periods (day n=6, night n=1) (Figure 3.1).
Figure 3.1 Study flow diagram of the 123 participants recruited in Kingston, Ontario from 2008 - 2009

3.1.2 WSIB Study Methods

The study participants completed four study periods, consisting of a day and a night shift during both summer and winter. The two study periods in each season took place one month apart to reduce melatonin variability due to menstrual stage. Participants were divided into two cohorts due to limited study instruments. The first cohort completed all four study periods between April and December 2008, and the second participated between January and July 2009.

The day shift study period was on the first day shift of the two day, two night shift schedule and the night shift study period took place on the second night shift. Four urine samples were collected in each season, two during the day shift study period (7:00 a.m.–7:00 a.m.) and two during the night shift study period (7:00 p.m.–7:00 p.m.). For the day shift, the first urine sample was collected during the shift (3:00 p.m.–5:00 p.m.) and the second after awakening (5:00 a.m.–7:00 a.m.). During the night shift, urine was collected at the end of shift (7:00 a.m.–8:00
a.m.) and after awakening (3:00 p.m.–5:00 p.m.) (Appendices C & D). Because circadian rhythm does not appear to shift with the two-day, two-night shift schedule, the morning values should capture the nocturnal melatonin peaks for both shifts (Grundy et al., 2009), which was used as the primary outcome of interest for this thesis.

Four saliva samples were collected during each shift, as well as one blood sample among pre-menopausal nurses during their day shift.

Pedometers were worn for the full twenty-four hour study period, excluding time spent sleeping, bathing or swimming. The number of steps taken was recorded in the corresponding diary, as well as additional information regarding short term (defined here as previous 24 hours) and long term (defined here as previous month) physical activity and sedentary behaviours (Appendix E). Participants also completed a questionnaire detailing menopausal status, lifestyle habits and work history.

3.2 Physical Activity, Sedentary Behaviour and Melatonin among Rotating Shift Nurses

This thesis used data from the WSIB study to determine the association between physical activity and sedentary behaviour with melatonin levels in this shift working population. This study is a time-series cross-sectional study, and used data from both study periods (day and night) of the first season of participation, to maximize sample size. Exposure and outcome information from the summer of 2008 was used for the first cohort (n=73) and data collected in the winter of 2009 was used for the second cohort (n=50).

This thesis did not use the longitudinal data from WSIB study, as previous research has shown that physical activity has an acute effect on melatonin levels (Knight et al., 2005). The role of longer-term physical activity on melatonin was also assessed; however the longitudinal data was of limited use because we were interested in the role of a cumulative dose of physical activity
over time. The longitudinal data would only provide an indication of physical activity and sedentary behaviour several months prior to melatonin assessment, which would not reflect the activity during the entire biologically relevant time period. The longitudinal data also had varying lengths of follow-up and several additional drop-outs, so the cross-sectional data provides the most complete and biologically relevant information for the research question at hand.

Urinary, salivary and blood samples were collected in the WSIB study, however this thesis only utilized urinary measurements as they represent cumulative nocturnal peak melatonin levels (Mirick and Davis, 2008). Saliva samples can be used to detail individual melatonin variability (Voultsios et al., 1997); however, intra-individual variability does not relate to the objective of this study so this information was not utilized. Blood samples were only taken among pre-menopausal women, and only during the day shifts, so they were also impractical for this thesis.

3.3 Exposure Assessment

3.3.1 Physical Activity

Each participant was asked to complete a one-day diary for both the day and night study periods (Appendix E). Participants were asked to describe the timing, type, intensity, duration and frequency of physical activity and sedentary behaviours during each 24 hour study period. Nurses were provided with a list of fifteen common leisure-time activities and were provided space to indicate up to three additional activities. If applicable, for each activity, participants were asked to record the duration of participation and the intensity of activity. Intensity was recorded as low, moderate or vigorous based on perceived perspiration and respiratory rate. Occupational activity was also assessed with a question describing the amount of time spent performing higher intensity activities such as lifting or bathing patients. Physical activities were reported separately for the morning/afternoon (7 a.m.-3p.m.), the afternoon/evening (3 p.m.-11 p.m.), and the night shift (11 p.m.-7 a.m.).
p.m.) and the evening/morning (11 p.m.-7 a.m.). Participants were also asked to report their typical physical activities for the one month period prior to each 24-hour study period.

This diary was based on the physical activity assessment of the pilot study, with changes by Dr. Ian Janssen to encourage more detailed activity reports. The diary was designed for all objectives of the WSIB Study, and not all questions were relevant to this thesis.

3.3.2 Sedentary Behaviour

The duration of sedentary behaviour was recorded for the morning (7:00 a.m.–3:00 p.m.), afternoon (3:00 p.m.–11:00 p.m.), and evening (11:00 p.m.–7:00 a.m.) of the twenty-four hour period in the one-day diary. Respondents answered four questions regarding time spent watching television, using a computer, sitting, and performing light chores. Participants selected from five responses ranging from ‘None at all’ to ‘3 or more hours’. Two additional questions were added to describe the total amount of time spent on their feet and doing heavy lifting during their shift; responses could range from ‘None at all’ to ‘7 or more hours’.

Long term (previous 30 days) sedentary behaviour was also assessed using similar questions, but was divided into work days and non-work days, to capture the weekly variability in sedentary habits.

3.3.3 Metabolic Equivalents

Physical activity and sedentary behaviour variables were quantified by using Metabolic Equivalent Scores (METS) (Ainsworth et al., 2000). A MET score is a ratio of the metabolic rate of an activity compared to a standard resting metabolic rate (4.184 kJ · kg\(^{-1}\) · hr\(^{-1}\)), thus a MET of 3 would indicate that the activity requires 3 times as much energy expenditure as is required at rest (Ainsworth et al., 2000). Activities range from those of no intensity such as sitting (MET=1), to activities of high intensity such as running (MET=18). MET values were calculated using the
Compendium of Physical Activities (Ainsworth et al., 2000). This compendium includes a list of over six hundred activities, and the MET values for this compendium were calculated using calorimetric data obtained in the laboratory setting. If an activity was not listed, its MET value was determined from other peer-reviewed scientific publications.

Activities with MET values less than or equal to 1.5 are defined as sedentary behaviours (Owen et al., 2000), activities with a MET between 1.5 and 3 are classified as low intensity, and activities with a MET value > 3 are classified as being of a moderate or vigorous intensity (Sarkin et al., 2000). When a participant recorded performing a specific type of activity, a MET value was assigned that corresponded with the reported intensity. For example, if a participant reported taking a 30 min walk of low intensity, the MET value that corresponded to walking that fell between 1.5 and 3 was assigned to that activity. The MET scores were multiplied by the duration of activity in minutes and summed to attain units of MET-min for each intensity of activity (sedentary, low, moderate and vigorous). Moderate and vigorous intensity activity was combined into one variable due to the low levels of vigorous intensity activity, a common practice in exercise science and physical activity epidemiology (ex. Grundy et al., 2009; Scott et al., 2009).

### 3.3.4 Data Quality Check

Pedometer (Model SC-01, Stepscout Inc., Deep River, ON, Canada) counts were used to classify activity during both 24-hour data study periods. Pedometers were initially planned to be the primary indicator of recent activity, however several participants experienced at least one pedometer malfunction, especially during the winter study period as the batteries wore out. Pedometer data were excluded if i) The pedometer had no recorded reading; ii) The pedometer had an unreasonable reading [i.e. less than 500 steps per day (Hemmingsson et al., 2008)] iii) The participants claimed the pedometer was broken either in the ‘additional comments’ section of the study diary, or via conversations with study staff. Nearly 15% (n=32) of pedometer data were
excluded based on these criteria. A potential, although untested explanation for the high number of malfunctions is that the internal mechanisms of the pedometers, which are primarily composed of metallic materials, may have been affected by the electromagnetic radiation emitted from some of the hospital equipment.

The total low, moderate and vigorous intensity physical activity energy expenditure (in MET·min) based on the self-reported physical activity data was compared to the number of steps recorded on functioning pedometers (n = 192). Sedentary behaviour was not included in this analysis as these activities do not require much movement and consequently would not register on the pedometer. During the day shift, a statistically significant Spearman correlation coefficient of 0.32 (p = 0.0017) was calculated for the 92 functioning pedometers compared to self-reported low, moderate and vigorous intensity physical activity. A weaker non-significant association was found for the 100 functioning pedometers during the night shift, with a Spearman correlation of 0.17 (p = 0.09). Pedometer malfunctions were not related to the total physical activity performed. The standardized odds ratio of a malfunction by total MET·min of low, moderate and high intensity energy expenditure was 0.81 (95% CI: 0.47 – 1.41).

Although they are objective, pedometers are not the gold standard in physical activity measurement because of the potential for participants to increase their number of steps in reaction to the study. The validity of pedometers is highly influenced by the population under study (Tudor-Locke et al., 2002). More accurate measures of physical activity, such as those obtained by accelerometers, were economically unfeasible for this study.

3.3.5 Biologically Relevant Timeframe

In order to confirm the timeframe by which physical activity is most likely to influence melatonin levels, a series of linear regressions was carried out for physical activity at different times of the day. The role of physical activity and sedentary behaviours performed 9 hours (11
p.m.–7 p.m.), 16 hours (3 p.m.–7 a.m.), 24 hours (7 a.m.–7 a.m.), and 30 days prior to urine collection on levels of log-adjusted 6-sulfatoxymelatonin was assessed for both the day and night shifts. The model that explained the most variation in peak melatonin levels, as assessed by the R² values for each model, was compared to previous literature to confirm the biologically relevant timeframe that was used for the remainder of the analyses. It was predicted that this timeframe would be the same for both the night and day shifts.

If a participant declared that their reported activity in the 30 days prior to data collection was not typical of their usual activity, they were removed from the above analysis to determine their effect on the regression coefficients. If this model explained the highest variation in peak melatonin levels, only these individuals would have be utilized for the analyses and the biologically relevant timeframe would be determined to be greater than 30 days. This result would be inconsistent with previous literature (Knight et al., 2005).

3.4 Outcome Assessment

The outcome of interest for this thesis was the peak concentration of urinary creatinine-adjusted 6-sulfatoxymelatonin.

3.4.1 Data Collection

Each participant contributed two urine samples that reflect total peak melatonin levels, one after sleeping (5 a.m.–7 a.m.) for the day shift collection and the second at the end of their night shift (7 a.m.–8 a.m.). Participants were asked to record the time of urine collection in their one-day diary to ensure proper specimen protocol was followed. For their day shift study period, participants delivered their samples to the Core laboratory in KGH (preceding their next day shift). For the night shift, a courier was arranged by study staff to pick up the sample upon awakening. Samples were kept between 2-8°C except during transport. Urine was collected using
a standard 120 ml urine collection tube marked with a random identification number and placed in an airtight biohazard bag. Upon arrival at the Core laboratory, samples were picked up by the study staff within three days and centrifuged for 5 minutes at 2000 g. Particulate matter was removed and samples were aliquoted to fresh micro-tubes in the laboratory of Dr. Charles Graham and frozen at -80°C. All samples were handled using the biohazard handling procedures outlined by the Workplace Hazardous Materials Information System (Côté et al., 1998).

3.4.2 Melatonin Assessment

Levels of 6-sulfatoxymelatonin, the main urinary melatonin metabolite was measured using the Bühlmann 6-sulfatoxymelatonin enzyme-linked immunosorbant Assay (ELISA) kit (ALPCO Diagnostics, Salem, NH, USA). The Bühlmann 6-sulfatoxymelatonin ELISA test kit is a competitive immunoassay that uses capture antibody principles to determine levels of 6-sulfatoxymelatonin in human urine. Urine samples are added in duplicate to wells containing a goat immunoglobulin specific for 6-sulfatoxymelatonin. Polyclonal antibodies capture and bind to the immunoglobulin complexes and are identified by a colour change which can be analyzed using a microtiter plate reader at a wavelength of 450nm (Bird et al., 1996). When compared to the gold standard melatonin analytical technique, a serum 125–I–radioimmunoassay, the Bühlmann ELISA kit gives a Pearson correlation coefficient of 0.97 in a sample of 42 (Bird et al., 1996). It has been shown to have an intra-assay precision of 7.2%, an inter-assay precision of 11.9% and a dilution parallelism of 97.8% (Bird et al., 1996).

To ensure high quality laboratory data, samples were reanalyzed using appropriately adjusted dilutions if either duplicate was out of range of the standard curve, or if the standard curve fit the standards with an $R^2$ of less than 0.95. Coefficients of variation between duplicates were calculated for each sample, and retested if they exceeded 50. Two controls of known concentrations were also run in duplicate to confirm the accuracy of the results. Inter- and intra-
assay precision was not calculated due to financial restraints, as this would require purchasing additional test kits.

### 3.4.3 Creatinine Assessment

Creatinine measurement was required to determine the volume of urine excreted in each sample, which could function to dilute or concentrate melatonin in the urine. The Parameter Creatinine Assay (R&D Systems Inc, Minneapolis, MN, USA) was utilized to assess levels of creatinine in diluted urine samples. In this assay, an alkaline solution was added to urine samples that reacted with creatinine yielding a color change. The magnitude of this change was proportional to the concentration of creatinine and was quantified using a microtiter plate reader at a wavelength of 490nm. The optical density was compared to a standard curve to provide a concentration in mg/dL. The minimum detectable dose of the kit ranged from 0.01 – 0.07 mg/dL (Heinegard and Tederstrom, 1973), substantially lower than the concentration of creatinine produced by functioning kidneys (Surowiec et al., 2004). The intra-assay precision has been shown to range from 1.3 to 3.0% and the inter-assay precision is comparable, ranging from 2.3 to 3.9%. The assay has a dilution parallelism of 96% (Heinegard and Tederstrom, 1973). The same requirements for data quality were established for creatinine as presented for melatonin in Section 3.5.1, with the exception of the controls of known concentrations, which were unavailable for this assay. Urinary 6-sulfatoxymelatonin concentrations were divided by concentrations of creatinine and log-transformed to yield a distribution that approximated the normal distribution.

### 3.5 Confounder Selection

Variables identified in the literature review as potential confounders were assessed through interviews, the one-day diary, and a questionnaire. Potential confounders included time-dependant variables, which were measured during both shifts, and time-independent confounders
that remained constant for the entire study period. A confounder must be related to the exposure and outcome, but not on the causal pathway (Oleckno, 2002). The number of hours of sleep was not included as a potential confounder, as it is hypothesized as a potential intermediate on the causal pathway linking physical activity and melatonin (See Chapter 2.5).

3.5.1 Time-dependent Potential Confounders

Participants were asked to complete the diary during the twenty-four hour study period to limit any errors in recall. Diaries were returned using inter-hospital mail after sample collection. Participants were contacted by study staff if any responses were incomplete or ambiguous.

**Pharmaceuticals**

Participants were asked about their use of anti-depressants, beta-blockers, non-steroidal anti-inflammatory drugs, hormone replacement therapy, sedatives and oral contraceptives during the 24 hour study period. All of these variables were treated dichotomously due to insufficient data quality regarding the dose and frequency of medication.

**Seasonal Factors**

Melatonin has been shown to be affected by the duration of sunlight during the day. Information on the length of day of each shift was retrieved from the Institute for National Measurement standards (National Research Council of Canada, 2009), and was treated as a continuous variable in units of hours/day.

**Lifestyle habits**

Alcohol consumption was measured by the number of alcoholic drinks consumed during the study period. Alcohol consumption included wine, beer and spirits. Caffeine was similarly categorized and included tea, coffee, soda or other caffeinated beverages (i.e. energy drinks). Smoking habits during the twenty-four hour period were assessed from the question, “Please estimate the number of cigarettes you smoked today”, and treated as a continuous variable.
3.5.2 Time-independent Potential Confounders

Time-independent confounders were evaluated using both interviews and questionnaires. Information that required objective assessment such as height, weight and waist circumference (measured at iliac crest) was measured by the study coordinator to eliminate the risk of self report errors. Participants were provided with a questionnaire at the interview and asked to complete it at their convenience.

Demographics and Personal Characteristics

Age at the initial interview was included as a potential confounder and treated as a continuous variable. Body mass index was calculated as weight in kilograms divided by the square of their height in metres. Race was assessed from the questionnaire and treated dichotomously as “white” or “other” due to limited variability. The menopausal status of each participant was determined from the question, “Are you still menstruating?” in the day shift diary, and treated as a binary variable.

Seasonal Factors

The season of participation was included as either summer (May – Aug 2008) or winter (Jan – Mar 2009), based on the first shift of participation.

3.6 Statistical Analysis

3.6.1 Descriptive Analysis

Univariate analyses were used to describe the characteristics of the study sample. Means and standard deviations were computed to describe all continuous variables, and percentages were calculated for categorical variables. Potential predictor variables were stratified by shift and the Wilcoxon signed rank test was used to compare these continuous values for the day and night shift. Categorical variables were compared using McNemar’s test for paired data with Yate’s continuity correction.
All statistical analyses were completed using SAS (Version 9.1, SAS Institute, Cary, North Carolina, USA).

3.6.2 Regression analysis

Multivariate linear regression was used to determine the relationship between energy expenditure and melatonin. Separate linear regression models were used for each shift, to determine if the relationship between physical activity, sedentary behaviour, and peak melatonin levels was the same for both the day and night shift study periods. Creatinine-adjusted melatonin levels were log-adjusted to yield a distribution that approximated the normal distribution. The association between energy expended during MVPA, low intensity physical activity, and sedentary behaviours during the pre-determined biologically relevant timeframe on log-transformed creatinine-adjusted 6-sulfatoxymelatonin levels was assessed.

Regression assumptions were analyzed through residual plots, outlier assessments and collinearity diagnostics. Results were not adjusted to compensate for multiple comparisons.

3.6.3 Minimal Detectable Effect

Minimal detectable effect calculations were completed for all physical activity during the 24 hour study period. Variance information for the outcome of interest, urinary levels of 6-sulfatoxymelatonin, was obtained from the pilot data (Grundy et al., 2009). Since physical activity was measured differently for this study, variance information for the number of hours of light, moderate and vigorous physical activity was obtained from the completed diaries. With a sample size of 123 and a power of 0.8, regression coefficients greater than 0.164 or less than -0.164 could be detected with a type I error of 0.05. When the sample size is reduced to 116, after loss to follow up and withdrawals, the study could detect coefficients of +/- 0.169. The pilot data found a regression coefficient of 0.072 for recent activity. Since this study adjusted for urine
volume and used more thorough physical activity measurements, this thesis was more likely to
detect a relationship, if one exists. This study was therefore likely sufficient to determine the
influence of physical activity on urinary melatonin levels in this study population.

3.6.4 Random Effects Model

If the exposure-outcome relationship was similar for both shifts, consideration was given
to combining data across shifts to increase statistical power. Most participants contributed two
sets of data, one for each shift, so this data was not independent and therefore did not meet the
independency assumption for multiple linear regression. Consequently, a random effects (multi-
level) model was used to account for this data dependency (Laird and Ware, 1982). In this case,
each participant would represent an individual cluster of data, and if this is not considered in the
analysis, the standard error of the parameters would be incorrectly reduced, increasing the risk of
a type I error. Random effects statistical modeling can determine the role of clustering on the
standard error and adjust accordingly (Laird and Ware, 1982).

If the exposure-outcome association is modified by the shift worked (day or night), then a
random effects model would reduce the public health relevancy of the results, as interaction terms
are often difficult to interpret. In this case, only the stratified analysis will be presented in the
final manuscript.

3.6.5 Confounder Analysis

As a screening process for confounder selection, the bivariate association between the
creatinine-adjusted peak melatonin levels and each potential confounder was analyzed prior to
model building. Only variables associated with the outcome at p<0.25 in either shift were
considered as potential confounders. These variables were retained in the model as confounders if
they changed the exposure parameter estimates by more than 10% upon deletion.
3.7 Ethical Considerations

The WSIB study was approved by the Queen’s University Health Sciences Human Research Ethics Board, and this thesis also has also been granted specific approval. Consent was obtained from all study participants, and each was provided with a monetary honorarium of $200 as compensation for the time required to complete this study. All questionnaires were locked in a file cabinet at the research office of Kingston General Hospital. The information was entered into a database on a secure, password-protected computer at the Queen’s University Cancer Research Institute by the candidate and a research assistant. Three members of the study team, including the candidate and the study coordinator, had access to the study identification number key in order to contact participants for shift scheduling. All data and samples were analyzed without subject identifiers and only group data will be published.

3.8 Student Contribution

As a research assistant on the main WSIB study, the candidate has participated in participant recruitment and reimbursement, quality control and sample preparation. He aided in the creation, validation and entering of data into a survey database. Under the direction of study co-investigator, Dr. Charles Graham, the candidate completed a substantial portion of the laboratory analysis of salivary and urinary melatonin and creatinine. He created all exposure variables quantifying physical activity and sedentary behaviour with the assistance of Dr. Janssen, and performed all statistical analyses with the input of Drs. Aronson and Janssen. The candidate also led the writing of all chapters of this thesis, including the manuscript.
3.9 References


Chapter 4
Manuscript

Physical Activity, Sedentary Behaviour and Melatonin among Rotating Shift Nurses
ABSTRACT

Background: Shift work is associated with increased risk of cardiovascular disease and cancer, where decreased melatonin has been proposed as an intermediate in the causal pathway. The influence of physical activity on melatonin has rarely been studied in an observational setting, and it may be important in mediating the effects of shift work. We aimed to assess the influence of energy expended during physical activity of different intensities on melatonin among rotating shift nurses. We hypothesized that physical activity before the night shift would lessen the decrease in melatonin production that occurs with exposure to light at night.

Methods: 123 female rotating shift nurses working at Kingston General Hospital were recruited over a one-year period. Physical activity and sedentary behaviours for each participant were recorded during both a day and a night shift using activity diaries, and analysis was restricted to activities between 3 p.m. and 7 a.m. Concentrations of urinary 6-sulfatoxymelatonin, a melatonin metabolite, in morning void urine samples were analyzed for each shift.

Results: The average age of participants was 41 years, and 60% were overweight or obese (body mass index ≥ 25 kg/m²). An average of 6.9 and 5.2 hours of sleep were reported after the day shift and night shift, respectively. Sedentary behaviours such as standing and television watching accounted for over half of the total reported energy expenditure. During the day shift, energy expended in moderate and vigorous intensity physical activity between 3 p.m. and 7 a.m. was negatively associated with melatonin levels (p=0.024, R² = 0.09). During the night shift, energy expended in sedentary behaviours was negatively associated with melatonin levels (p=0.008, R² = 0.03).

Conclusions: Physical activity energy expenditure explains only a small amount of melatonin variation, suggesting that other factors are influencing melatonin production, or that melatonin production is minimally effected by these patterns of physical activity.

Keywords: physical activity, sedentary behaviour, melatonin, shift work

Word count: 3656 (excluding abstract, tables and references)
INTRODUCTION

Shift workers have a 40% higher risk of cardiovascular disease than the general population (Knutsson, 2003). With 28-30% of Canadians working shift work (Shields, 2002; Williams, 2008), the population etiologic fraction of shift work on cardiovascular disease has been estimated to be nearly 7% (Ha and Park, 2005). The increased risk of cardiovascular disease in shift workers has been attributed to behavioural and social changes that are associated with disrupted sleep-wake cycles. These may include altered eating and socializing schedules, added stresses, and reduced family time (Moore-Ede and Richardson, 1985). Although considerable research has examined the behavioural and social impacts of shift work on cardiovascular disease, the biological mechanisms explaining these associations are poorly characterized. Some research has suggested that disturbances in the body’s natural biological rhythms, or circadian disruption, could increase cardiovascular disease risk in the shift working population (Martino et al., 2008; Stevens, 2005).

Melatonin (N-acetyl-5-methoxytryptamine) is the primary circadian indicator in humans, and may play an important role on the biological pathway linking shift work and cardiovascular disease. Melatonin production is primarily influenced by light, and consequently shift workers may be susceptible to altered melatonin secretion patterns (Sharkey and Eastman, 2002) and lower circulating levels of melatonin (Schernhammer et al., 2004) due to their increased exposure to light at night. Melatonin can inhibit cardiovascular pathological processes by acting directly as a free radical scavenger or indirectly as an antioxidant (Tengattini et al., 2008). Exogenous melatonin administration reduces several cardiovascular risk factors including hypertension, dysrhythmias, and hypertrophic cardiomyopathy (Reiter and Tan, 2009; Scorza et al., 2008). Together, this suggests that the increased risk of cardiovascular disease in shift workers may be due, in part, to the reduction in melatonin levels associated with altered working patterns.
It is well-known that participation in moderate to vigorous intensity physical activity protects against the development of cardiovascular disease (Nocon et al., 2008). The proposed etiological mechanisms for this relationship include both the acute and chronic cardiovascular effects of physical activity including improved body composition, glucose management, and autonomic tone (Warburton et al., 2006). Melatonin may also influence this pathological pathway (Tengattini et al., 2008); however, this hypothesis remains largely untested.

Few studies have investigated the role of physical activity in affecting melatonin levels in humans, and those that have are mainly experimental and have a wide range of results. Researchers have reported an increase (Carr et al., 1981), decrease (Montelone et al., 1973), and no change (Miyazaki et al., 2001) in melatonin in response to a single bout of exercise. Both animal (ex. Lopez and Urquijo, 2007) and human (ex. Buxton et al., 1997) experimental studies have suggested that physical activity appears to have an acute effect on melatonin, with physical activity less than 12 hours prior to the commencement of sleep influencing circulating melatonin levels.

Two observational epidemiological studies have aimed to characterize the relationship between physical activity and melatonin. Knight et al. (2005) found that after stratifying by the timing of activity, there was a positive relationship between physical activity and melatonin in 213 female volunteers, and this relationship was strongest during the evening and night (4 p.m.–4 a.m.). In this study, physical activity explained 5.3% of melatonin variation. In a pilot project for the current study, Grundy et al. (2009) examined the role of low, moderate and high intensity acute physical activity on 61 rotating shift nurses. No consistent relationship was identified; however, a non-significant positive association was found between self-reported acute moderate and vigorous intensity physical activity and urinary 6-sulfatoxymelatonin concentrations among the nurses working their day shift (p=0.13).
The study of sedentary behaviour, or inactivity, is of growing interest in physical activity epidemiology, though it has not been traditionally considered when classifying intensity. Sedentary behaviour is at one extreme of the physical activity continuum and includes behaviours such as sitting and resting (Dietz, 2007). Sedentary behaviour has effects on health outcomes independent of low, moderate and vigorous intensity physical activity (Dietz, 2007). The influence of sedentary behaviour on melatonin has not been established in human or animal models, and consequently this study will examine the independent roles of energy expended in both physical activity and sedentary behaviours on circulating melatonin levels.

This study aims to determine the associations of both physical activity and sedentary behaviour with urinary melatonin levels among rotating shift nurses in an observational setting. If physical activity has a role in modulating the potential reduction in melatonin levels among shift workers, occupation-specific preventative recommendations can be developed to maximize health.

MATERIALS AND METHODS

Study Population

One hundred and twenty-three rotating shift nurses working full-time at Kingston General Hospital volunteered for this study. All participants worked the following rotating shift pattern: two 12 hour day shifts (7 a.m.-7 p.m.) directly followed by two 12 hour night shifts (7 p.m.-7 a.m.), followed by five days off. Participants self-excluded if they were taking melatonin supplements or if they had been pregnant or lactating in the six months prior to enrollment. Five participants were lost to follow-up or withdrew from the study prior to data collection. An additional twelve participants only completed one of two study periods, and consequently contributed one set of data for the final analyses.
**Procedures**

Nurses completed two 24 hour study periods, one during a day shift and one during a night shift. The day shift study period was on the first day shift of the two day, two night shift schedule and the night shift study period took place on the second night shift. Both study periods commenced at the beginning of each shift, 7 a.m. for day shifts and 7 p.m. for night shifts. The two study periods took place one month apart to reduce melatonin variability due to menstrual stage. The order of study periods (day and night) was based on the participant’s work schedule. For the day shift study period, a morning void urine sample was collected preceding their second day shift (5 a.m.–7 a.m.) of the two-day two-night rotational pattern. For the night shift study period, a morning void urine sample was collected after completion of their second night shift (7 a.m.–8 a.m.). Melatonin concentrations have been shown in our previous research to peak at night during both the day and night shifts when working this rotating shift schedule (Grundy et al., 2009), and consequently morning void samples were chosen to reflect the peak melatonin concentrations for both shifts. Urine samples were stored at -80°C preceding the laboratory analyses.

Participants completed a diary outlining the timing, duration, and intensity of physical activity and sedentary behaviours during each 24-hour study period (Appendix E). A list of fifteen common physical activities was provided on the diary; space was also provided to list up to three additional physical activities. For each activity, participants were asked to list the length of time they were engaged in that activity and the intensity. The intensity was recorded as low, moderate, or vigorous based on perceived perspiration and respiratory rate. Sedentary behaviours were assessed through five questions regarding time spent watching television, using a computer, sitting, light chores and standing. Physical activities and sedentary behaviours were reported separately for the morning/afternoon (7 a.m.-3 p.m.), the afternoon/evening (3 p.m.-11 p.m.) and
the evening/morning (11 p.m.-7 a.m.) time periods. Participants were also asked to report their typical physical activities and sedentary behaviours for the one month period prior to each 24-hour study period.

Information on potential confounders was collected at an initial interview, in a questionnaire completed at the commencement of the study, and in a diary. The questionnaire provided information on age, menopausal status, smoking history, and ethnicity. The diary included information on characteristics that could change for each study period such as alcohol and caffeine consumption and medication usage (non-steroidal anti-inflammatory drugs, sedatives, oral contraceptives and migraine medication). Height, weight and waist circumference were measured by a study nurse at the time of study enrollment.

**Exposure Assessment**

The type and intensity of each reported physical activity and sedentary behaviour was converted to a metabolic equivalent (MET) using the Compendium of Metabolic Equivalents (Ainsworth *et al.*, 2000). A MET is a ratio of the metabolic rate of an activity compared to a standard resting metabolic rate (4.184 kJ \cdot kg^{-1} \cdot hr^{-1}), thus a MET of 3 indicates that the activity requires 3 times as much energy expenditure as is required at rest (Ainsworth *et al.*, 2000). Activities range from those of no intensity such as sitting (MET=1) to activities of a very high intensity such as running (MET=18). Activities with MET values less than 1.5 are defined as sedentary behaviours (Owen *et al.*, 2000), activities with a MET between 1.5 and 3 are classified as low intensity, and activities with a MET value > 3 are classified as being of a moderate or vigorous intensity (Sarkin *et al.*, 2000).

The duration of each physical activity and sedentary behaviour reported in the diary was multiplied by its corresponding MET value to yield a metabolic equivalent minute variable.
(MET·min). MET·min values were summed for each intensity in each of the three time periods (7 a.m.-3 p.m., 3 p.m.-11 p.m., and 11 p.m.-7 a.m.) and are a representation of the total energy expended. The total MET·min of energy expended by physical activity during both study periods were compared to pedometer (Model SC-01, Stepscount Inc., Deep River, ON, Canada) counts (n=192), and Spearman correlation coefficients of 0.32 ($p=0.0017$) and 0.17 ($p=0.09$) were found for the day and night shift, respectively.

**Outcome Assessment**

Levels of 6-sulfatoxymelatonin, the main urinary melatonin metabolite were measured using the Bühlmann 6-sulfatoxymelatonin Enzyme-Linked Immunosorbant Assay (ELISA) kit (ALPCO Diagnostics, Salem, NH, USA). The Bühlmann 6-sulfatoxymelatonin ELISA test kit is a competitive immunoassay that uses capture antibody principles to determine levels of 6-sulfatoxymelatonin in human urine. 6-sulfatoxymelatonin concentrations have been shown to be representative of 70% of circulating melatonin (Bird et al., 1996). In contrast to blood and saliva melatonin measurements, morning void urine samples measure cumulative concentrations of melatonin, providing an indication of melatonin levels during the circadian peak. In adults with invariable sleeping schedules, nocturnal peak urinary melatonin levels remain fairly constant (Bojkowski et al., 1987). However, in adults with disturbed sleeping patterns, such as shift workers, the amplitude of these peaks may fluctuate so urinary 6-sulfatoxymelatonin provides a practical and noninvasive means of detecting such changes in circulating melatonin levels.

Creatinine measurement was required to determine the volume of urine excreted in each sample, which could function to dilute or concentrate melatonin in the urine. The Parameter Creatinine Assay (R&D Systems, Inc, Minneapolis, MN, USA) was utilized to assess levels of creatinine in diluted urine samples. Urinary 6-sulfatoxymelatonin concentrations were divided by
concentrations of creatinine and log-transformed to yield a distribution that approximated the normal distribution.

**Statistical Analysis**

Univariate analyses were used to describe the characteristics of the study sample. Means and standard deviations were computed for continuous variables and percentages were calculated for categorical variables. Potential predictor variables were stratified by shift and the Wilcoxon signed rank test was used to compare these continuous values for the day and night shift. Categorical variables were compared using McNemar’s test.

Multivariate linear regression was used to determine the relationship between energy expenditure between 3 a.m. and 7 p.m. and melatonin. This time period was representative of the biologically relevant timeframe determined from previous literature (Buxton *et al.*, 2003; Carr *et al.*, 1981; Knight *et al.*, 2005), and included both leisure-time and occupational activity. Additional analyses were conducted to confirm this timeframe (Chapter 6.2.1). Separate linear regression models were used for each shift, to determine if the relationship between physical activity, sedentary behaviour, and peak melatonin levels was the same for both the day and night shift study periods. Moderate and vigorous intensity physical activities (MVPA) were combined and treated as one variable, due to the low levels of vigorous intensity activity amongst the study sample.

Potential confounders were retained in the model if they changed the exposure parameter estimates by more than 10% upon deletion (Rothman *et al.*, 2008). All statistical analyses were completed using SAS (Version 9.1, SAS Institute, Cary, North Carolina, USA).
RESULTS

The study sample (n=118) was mainly Caucasian (95%) with an average age of 41 years (Table 1). Sleep problems were commonly reported (59%). Characteristics that may have varied over time for the 106 participants who completed both study periods are presented in Table 2. In comparison to their day shift, participants reported an average of 1.7 hours less sleep after their night shift. The geometric mean of creatinine-adjusted 6-sulfatoxymelatonin concentration was 27.1 mg/ng of creatinine after the day shift collection and 25.3 mg/ng of creatinine after the night shift collection, and these values did not differ (p=0.91).

Table 1 Characteristics of the 118<sup>a</sup> study participants recruited in Kingston, Canada from 2008 – 2009.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD&lt;sup&gt;b&lt;/sup&gt; or Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>41.1 ± 11.2</td>
</tr>
<tr>
<td>BMI&lt;sup&gt;c&lt;/sup&gt; (kg/m²)</td>
<td>27.7 ± 6.4</td>
</tr>
<tr>
<td>Underweight (≤18.5)</td>
<td>2 (1.7%)</td>
</tr>
<tr>
<td>Normal weight (18.5, 25]</td>
<td>45 (38.1%)</td>
</tr>
<tr>
<td>Overweight (25, 30)</td>
<td>39 (33.1%)</td>
</tr>
<tr>
<td>Obese (&gt;30)</td>
<td>31 (26.3%)</td>
</tr>
<tr>
<td>Missing</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>Smoking Status</td>
<td></td>
</tr>
<tr>
<td>Ever smoked</td>
<td>46 (40.0%)</td>
</tr>
<tr>
<td>Never smoked</td>
<td>72 (61.0%)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>112 (94.9%)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (3.4%)</td>
</tr>
<tr>
<td>Missing</td>
<td>2 (1.7%)</td>
</tr>
<tr>
<td>Sleep Problems</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>70 (59.3%)</td>
</tr>
<tr>
<td>No</td>
<td>43 (36.4%)</td>
</tr>
<tr>
<td>Missing</td>
<td>5 (4.2%)</td>
</tr>
<tr>
<td>Menopausal Status</td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>79 (66.9%)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>39 (33.1%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Includes participants who completed one or both 24-hour study periods  
<sup>b</sup> SD, standard deviation  
<sup>c</sup> BMI, body mass index
Table 2: Time variant characteristics\(^a\) of the 106\(^b\) study participants recruited in Kingston, Canada from 2008 – 2009.

<table>
<thead>
<tr>
<th>Study Period</th>
<th>Day Shift</th>
<th>Night Shift</th>
<th>(p) value (^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol Consumption (# drinks/day)</td>
<td>0.43 ± 1.9</td>
<td>0.09 ± 0.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Caffeine Consumption (# drinks/day)</td>
<td>2.8 ± 2.2</td>
<td>3.39 ± 2.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Sedatives (# participants)</td>
<td>5 (4.7%)</td>
<td>7 (6.6%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Pain Killers (# participants)</td>
<td>31 (29.2%)</td>
<td>28 (26.4%)</td>
<td>0.36</td>
</tr>
<tr>
<td>Oral Contraceptives (# participants)</td>
<td>22 (20.8%)</td>
<td>18 (17.0%)</td>
<td>0.1</td>
</tr>
<tr>
<td>Migraine Medication (# participants)</td>
<td>1 (0.9%)</td>
<td>2 (1.9%)</td>
<td>0.32</td>
</tr>
<tr>
<td>Sleep Duration (Hours)</td>
<td>6.9 ± 1.3</td>
<td>5.2 ± 2.0</td>
<td>0.03</td>
</tr>
<tr>
<td>Melatonin(^d) (mg/ng of Creatinine)</td>
<td>27.1 ± 2.9</td>
<td>25.3 ± 3.3</td>
<td>0.91</td>
</tr>
</tbody>
</table>

\(^a\)Data presented as mean ± standard deviation for continuous variables or prevalence (percent) for categorical variables

\(^b\)Includes only participants who completed both 24-hour study periods

\(^c\)\(p\)-values calculated by signed-rank test to account for the dependency of the data

\(^d\)Geometric means presented due to the skewness of the data

The MET·min values for low intensity physical activity and MVPA were not statistically different for the day and night 24-hour study periods (Table 3). During the biologically relevant timeframe (3 p.m.–7 a.m.), the MET·min for low intensity physical activity and sedentary behaviours were higher during the night shift study period than the day shift study period (\(p<0.0001\)). During the day shift study period, 45% of the daily energy expenditure occurred during the biologically relevant timeframe as compared to 90% for the night shift study period.

Table 3: Mean (± SD) MET·min/day of each intensity of activity for the 24-hour study period (7 a.m.–7 a.m.) and the biologically relevant time frame (3 p.m.–7 a.m.)\(^a\).

<table>
<thead>
<tr>
<th>Time Period of Physical Activity</th>
<th>Intensity of Activity</th>
<th>Day Shift</th>
<th>Night Shift</th>
<th>(p)-value(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 a.m.–7 a.m.</td>
<td>Sedentary</td>
<td>858 ± 297</td>
<td>852.3 ± 267</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>673 ± 658</td>
<td>580 ± 518</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>MVPA(^c)</td>
<td>284 ± 649</td>
<td>206 ± 530</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1815 ± 957</td>
<td>1638 ± 910</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3 p.m.–7 a.m.</td>
<td>Sedentary</td>
<td>423 ± 193</td>
<td>770 ± 190</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>252 ± 285</td>
<td>548 ± 459</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>MVPA(^c)</td>
<td>143 ± 298</td>
<td>162 ± 494</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>818 ± 450</td>
<td>1480 ± 813</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^a\)Includes only participants who completed both 24-hour study periods (n=106).

\(^b\)\(p\)-values calculated by signed-rank test to account for the dependency of the data

\(^c\)MVPA, moderate to vigorous intensity physical activity
Sedentary behaviours accounted for >45% of total MET-min during both study periods, although for the biologically relevant time period the MET-min for sedentary behaviour were greater for the night shift than the day shift (770 vs. 423, p<0.0001). The sedentary behaviours that contributed the most to the total MET-min during both 24-hour study periods were standing (76%), computer use (8%) and television watching (7%). Low intensity activities comprised 37% and 35% of the total MET-min during the day and night shift periods, respectively. These mostly consisted of lifting and bathing patients (56%) and walking (16%). MVPA during the day and night shift periods contributed to 9.4% and 11.8%, respectively, of total MET-min. During the month prior to each study period MVPA contributed to nearly 15% of total MET-min (data not shown).

Separate linear regressions were carried out for the day and night shift study periods for sedentary behaviour, low intensity, and MVPA between 3 p.m. and 7 a.m. Age was the only covariate included in the linear regressions analyses, as the other variables did not meet the modeling criteria for confounding. The $R^2$ values for the regression models for the day and night shift study periods were 0.086 and 0.034, respectively. Without age in the model, the adjusted $R^2$ changed to 0.01 and 0.039 for the day and night shift, respectively.

Table 4 depicts the standardized parameter estimates and levels of significance of sedentary behaviour, low intensity physical activity, and MVPA. The association between the different intensities of physical activity with melatonin differed for the day and night shift periods. For the day shift, MET-min accumulated during MVPA was negatively associated with melatonin levels (p=0.024), while MET-min accumulated during low intensity and sedentary behaviour were not (p>0.40). The partial regression coefficient was -0.21, indicating that for every one standard deviation (298 MET-min) increase in MVPA, melatonin decreased by 0.21 standard deviations (0.60 mg/ng of creatinine). This relationship is depicted in Figure 1. For the
night shift period, the MET-min accumulated during sedentary behaviour was negatively associated with melatonin ($p = 0.008$), such that every one standard deviation (190 MET-min) increase in sedentary behaviour was associated with a decrease in melatonin of 0.28 standard deviations (0.91 mg/ng of creatinine) (Figure 2). The associations persisted after removal of the six most influential points (data not shown).

**Table 4:** Standardized partial linear regression $\beta$ coefficients and significance for each intensity of physical activity energy expenditure during the biologically relevant time period (3 p.m.–7 a.m.) for the day and night study periods after controlling for age.

<table>
<thead>
<tr>
<th>Intensity</th>
<th><strong>Day Shift (n= 111)</strong></th>
<th></th>
<th><strong>Night Shift (n =113)</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>$p$-value</td>
<td>$\beta$</td>
<td>$p$-value</td>
</tr>
<tr>
<td>Sedentary behaviour</td>
<td>-0.08</td>
<td>0.38</td>
<td>-0.28</td>
<td>0.01</td>
</tr>
<tr>
<td>Low intensity physical activity</td>
<td>-0.02</td>
<td>0.83</td>
<td>0.02</td>
<td>0.82</td>
</tr>
<tr>
<td>MVPA$^a$</td>
<td>-0.21</td>
<td>0.02</td>
<td>0.07</td>
<td>0.47</td>
</tr>
</tbody>
</table>

$^a$ MVPA, moderate to vigorous intensity physical activity

**Figure 1:** Association between moderate to vigorous intensity physical activity (MVPA) energy expenditure (MET-min) and log-transformed 6-sulfatoxymelatonin concentration (mg/ng of creatinine) during the day shift study period (n=111).
DISCUSSION

This study aimed to determine if there was an association between physical activity, sedentary behaviour, and peak melatonin levels in a group of nurses working a rotating shift pattern. Physical activity and sedentary behaviours explained minimal variation (< 4%) in melatonin, although some of the associations were statistically significant. MVPA during the day study period was negatively associated with 6-sulfatoxymelatonin levels, but was unassociated with the outcome during the night study period. During the night shift period, sedentary behaviours were the only significant predictor of peak melatonin levels.
Although the association between MVPA and melatonin in this study is contrary to some previous epidemiological findings (Grundy et al., 2009; Knight et al., 2005), similar negative associations have been reported in both experimental and observational studies. Montelone et al. (1992) found that after 20 minutes of high intensity physical activity in human subjects, melatonin levels decreased for three hours. Similar results have also been shown in animal models (Atkinson et al., 2003). Reduced levels of melatonin may have been attributable to increased levels of cortisol which functions to reduce noradrenalin-stimulated melatonin release by the pineal gland. This relationship has been described in patients with hypercortisolemia, who show reduced levels of circulating melatonin (Soszynski et al., 1989).

An interesting observation was that energy expended in MVPA was associated with melatonin in the day shift while energy expended in sedentary behaviour was associated with melatonin in the night shift. The differences between shifts may indicate that the true biologically relevant timeframe by which physical activity and sedentary behaviour affects melatonin may be later than that used in this study (3:00 p.m.–7:00 a.m.). Since MVPA is largely unattainable during the 12 hour shift, most of the MVPA during the biologically relevant timeframe for the day shift study period would have occurred after the shift ended at 7:00 p.m. Conversely, most of the MVPA during the biologically relevant time frame for the night shift study period would have occurred before the 7:00 p.m. start time of the shift. For night shift, 90% of all daily sedentary energy expenditure occurred after 3:00 p.m., because nurses were more likely to engage in sedentary behaviours on the job. Therefore, during the night shift study period, the primary source of energy expenditure during the true biologically relevant timeframe was sedentary behaviour, hence its statistically significant association with melatonin. Thus, the timing of energy expenditure may play a more important role than the intensity.
This study is the third epidemiological study on this topic. Knight et al. found that exercise was positively associated with creatinine-adjusted melatonin levels in 214 healthy female volunteers (2005). Our methods improved on this study by including sedentary behaviours and low intensity physical activities. In addition, our study sample was rotating shift nurses who may be more susceptible to melatonin down-regulation due to their altered sleep-wake schedules compared to healthy volunteers. In a sample from the same working population as the current research, Grundy et al. found a non-significant positive association between physical activity and melatonin among those working the day shift, and a null association among those working the night shift (2009). This was a small pilot study (n=61), urine samples were taken at a different time for the day and the night shift, and melatonin measurements were not creatinine adjusted.

Our analysis indicated that physical activity and sedentary behaviour may decrease melatonin levels during the biologically relevant timeframe, though these exposures explained very little variation in melatonin levels. This decrease, though statistically significant, may not be clinically significant and consequently more consideration should be given to alternative means of preventing melatonin downregulation in shift workers. Before substantial policy recommendations are made, the role of melatonin on the pathway linking shift work and cardiovascular disease needs to be examined more thoroughly, as it is likely one of several multifactorial pathways leading to the increased rates of chronic diseases in shift workers.

A key strength of this study is that we assessed all intensities of physical activity and movement, not solely moderate and vigorous intensity exercise, the latter of which was uncommon in this working population. More than 60% of the activity-induced energy expenditure among these nurses was accrued through very low intensity sedentary behaviours that were performed over a prolonged period, such as standing and sitting. Activities of this intensity would not have been captured using most existing physical activity questionnaires, which focus
on MVPA. In addition, the use of urine biomarkers in this study provided a validated method of assessing melatonin levels.

Limitations include errors in recall of physical activity, which would function to bias the results towards the null. We do not expect that recall bias is an issue because participants were likely unaware of their melatonin status. Over-reporting and misclassification of physical activity and under-reporting of sedentary behaviours are a common concern with physical activity diaries and questionnaires (Sallis and Saelens, 2000), and may have influenced the reporting of the exposures of interest. General activity categories such as standing may introduce misclassification, as standing may include heavy lifting or other sources of energy expenditure not captured with this exposure assessment. Participants in this study were volunteers, which could introduce volunteer bias if the study sample differed from the general population. In terms of the external validity, the generalizability of the findings may be limited to female nurses working the 2-day, 2-night shift schedule.

Shift workers have a 40% increased risk of cardiovascular disease (Knutsson, 2003), and shift work has recently been named as a probable carcinogen (Straif et al., 2007). As melatonin may be one piece of this multifactorial puzzle, preventative mechanisms that stop melatonin downregulation need to be investigated in this at risk population. Physical activity energy expenditure explains only a small amount of melatonin variation, suggesting that other factors are influencing melatonin production. These factors should be investigated in the shift working population.

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Annie Langley, Lindsay Kobayashi, Shannyn MacDonald-Goodfellow and Dr. Charles Graham for their assistance with the laboratory analysis.

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REFERENCES


Chapter 5
Additional Results

This chapter provides additional information on the characterization of physical activity and sedentary behaviour, as well as their associations with 6-sulfatoxymelatonin. This information was judged as having too much detail for the manuscript, but provides important context for a more thorough understanding of the relationship between physical activity, sedentary behaviour and melatonin in this study.

5.1 Exposure Classification

5.1.1 Correlation Matrixes

Moderate and vigorous intensity physical activity, low intensity physical activity, and sedentary behaviour were weakly correlated during both shifts (spearman r <0.4), with the highest correlations between sedentary behaviours and low intensity activity during both shifts (Tables 5.1 and 5.2). Age was not statistically associated with MVPA energy expenditure during either shift.
Table 5.1: Spearman correlation coefficients of sedentary behaviour, low intensity activity, moderate and vigorous intensity activity energy expenditure (MET·min), and age for the 24-hour study period (7 a.m.–7 a.m.) and the biologically relevant time frame (3 p.m.–7 a.m.) during the day shift study period.

<table>
<thead>
<tr>
<th>Time Period of Physical Activity</th>
<th>MVPA\textsuperscript{a}</th>
<th>Low</th>
<th>Sedentary</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 a.m.–7 a.m.</td>
<td>MVPA\textsuperscript{a}</td>
<td>1</td>
<td>-0.06</td>
<td>0.185*</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>1</td>
<td>0.21*</td>
<td>-0.39*</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>1</td>
<td></td>
<td>-0.23</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 p.m.–7 a.m.</td>
<td>MVPA\textsuperscript{a}</td>
<td>1</td>
<td>-0.05</td>
<td>0.20*</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>1</td>
<td>0.21*</td>
<td>-0.35*</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>1</td>
<td></td>
<td>-0.21*</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} MVPA, moderate to vigorous intensity physical activity

* Statistically significant ($p < 0.05$)

Table 5.2: Spearman correlation coefficients of sedentary behaviour, low intensity activity, moderate and vigorous intensity activity energy expenditure (MET·min), and age for the 24-hour study period (7 a.m.–7 a.m.) and the biologically relevant time frame (3 p.m.–7 a.m.) during the night shift study period.

<table>
<thead>
<tr>
<th>Time Period of Physical Activity</th>
<th>MVPA\textsuperscript{a}</th>
<th>Low</th>
<th>Sedentary</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 a.m.–7 a.m.</td>
<td>MVPA\textsuperscript{a}</td>
<td>1</td>
<td>0.04</td>
<td>0.20*</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>1</td>
<td>0.39*</td>
<td>-0.14</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>1</td>
<td></td>
<td>-0.18*</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 p.m.–7 a.m.</td>
<td>MVPA\textsuperscript{a}</td>
<td>1</td>
<td>0.04</td>
<td>0.20*</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>1</td>
<td>0.39*</td>
<td>-0.13</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>1</td>
<td></td>
<td>-0.21*</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} MVPA, moderate to vigorous intensity physical activity

* Statistically significant ($p < 0.05$)
5.1.2 Metabolic Equivalents

Metabolic equivalent minutes (MET·min) were used to quantify physical activity and sedentary behaviours in this thesis. The total MET·min and minutes of the three most common activities at each intensity of physical activity are shown in Table 5.3. “Running” accounted for the largest energy expenditure (MET·min) in the vigorous intensity range; however, on average participants only participated in 0.9 and 1.6 minutes during the 24 hour day and night shift collection period, respectively. “Walking” in the moderate intensity range accounted for a much larger energy expenditure than did vigorous “running”. “Lifting and bathing patients” contributed to over 50% of all low intensity energy expenditure during both 24-hour collection periods. Similarly, “standing” contributed to over three quarters of all sedentary energy expenditure, and overall provided the greatest contribution to total energy expenditure.

Table 5.3: Average MET·min and minutes of the three most common activities at each intensity of physical activity or sedentary behaviour during both 24-hour collection periods.

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Type</th>
<th>MET·min (± SD)</th>
<th>Minutes (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day</td>
<td>Night</td>
</tr>
<tr>
<td>Vigorous</td>
<td>Running</td>
<td>10.0 ± 61.7</td>
<td>18.4 ± 88.9</td>
</tr>
<tr>
<td></td>
<td>Aerobics</td>
<td>3.9 ± 41.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Elliptical</td>
<td>3.2 ± 34.5</td>
<td>1.6 ± 16.9</td>
</tr>
<tr>
<td>Moderate</td>
<td>Walking</td>
<td>212.5 ± 630.8</td>
<td>141.2 ± 496.7</td>
</tr>
<tr>
<td></td>
<td>Bicycling</td>
<td>3.3 ± 22.4</td>
<td>0.7 ± 7.3</td>
</tr>
<tr>
<td></td>
<td>Strength Training</td>
<td>2.1 ± 11.6</td>
<td>1.4 ± 10.9</td>
</tr>
<tr>
<td>Low</td>
<td>Lifting &amp; Bathing Patients</td>
<td>377.9 ± 265.4</td>
<td>326.6 ± 232.0</td>
</tr>
<tr>
<td></td>
<td>Walking</td>
<td>127.4 ± 492.9</td>
<td>76.9 ± 350.7</td>
</tr>
<tr>
<td></td>
<td>Chores</td>
<td>78.9 ± 110.1</td>
<td>54.0 ± 68.4</td>
</tr>
<tr>
<td>Sedentary</td>
<td>Standing</td>
<td>644.4 ± 110.2</td>
<td>648.6 ± 219.8</td>
</tr>
<tr>
<td></td>
<td>Computer Use</td>
<td>77.4 ± 96.1</td>
<td>62.4 ± 71.1</td>
</tr>
<tr>
<td></td>
<td>Sitting</td>
<td>36.2 ± 36.3</td>
<td>75.6 ± 99.6</td>
</tr>
</tbody>
</table>

94
5.1.3 Pedometers

Pedometer counts were also used to assess physical activity during each 24-hour study period. Pedometers were initially planned to be the primary indicator of activity; however, several participants experienced at least one pedometer malfunction, especially towards the completion of the study when the batteries began to wear out. Pedometer data were excluded if: i) the pedometer had no recorded reading; ii) the pedometer had an unreasonable reading; or iii) the participant reported that the pedometer was broken. Nearly 15% (n=32) of pedometer data were excluded based on these criteria.

During the 24-hour day shift study period (7 a.m.–7 a.m.) participants were recorded as taking a mean of 8427 steps, with a standard deviation of 5741. During the 24-hour night shift study period (7 p.m.–7 p.m.) participants took nearly 50% more steps, averaging 12615 steps, with a standard deviation of 6334. There was a weak association between pedometer readings and self reported physical activity during the 24-hour study period (n =192). The total of low, moderate, and vigorous intensity physical activity was compared to the number of steps recorded on functioning pedometers. Sedentary behaviour was not included in this analysis as these activities do not require significant movement at the hip (where the pedometer is worn) and consequently would not register on the pedometer. During the day shift, a statistically significant Spearman correlation coefficient of 0.32 (p=0.0017) was calculated for the 92 functioning pedometers compared to self-reported low, moderate and vigorous intensity physical activity. A weaker association was found during the night shift, with a Spearman correlation of 0.17 (p=0.09) for 100 functioning pedometers.

A linear regression was performed comparing functioning pedometer readings to log-transformed creatinine-adjusted 6-sulfatoxymelatonin levels, and negative associations were found during both the day and night shifts. After adjusting for age, the standardized linear
regression coefficients for the day shift and night shift study periods were -0.088 (p=0.4) and -0.127 (p=0.2) for the day and night shifts, respectively. Both models explained less than 5% of the variance in melatonin, most of which was due to the inclusion of age in the model.

The relationship between several factors and pedometer malfunctions was examined (Table 5.4). Odds ratios for continuous variables were expressed per standard deviation. No factor was significantly associated with the probability of a pedometer malfunctioning. Pedometer malfunctions were not related to the total physical activity performed.

**Table 5.4:** Odds ratios and 95% confidence interval of potential predictors of pedometer malfunctions for the 192 functioning pedometers during both 24-hour study periods (day and night). Odds ratios were standardized for continuous exposures.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Day OR (95% CI)</th>
<th>Night OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Employment in the Emergency Dept</td>
<td>2.60 (0.61 – 11.05)</td>
<td>2.68 (0.63 – 11.41)</td>
</tr>
<tr>
<td>Employment in the Intensive Care Unit</td>
<td>1.62 (0.43 – 5.94)</td>
<td>1.35 (0.39 – 4.63)</td>
</tr>
<tr>
<td>Age</td>
<td>1.02 (0.60 – 1.75)</td>
<td>1.64 (0.93 – 2.91)</td>
</tr>
<tr>
<td>Total Energy Expenditure (MET·min)</td>
<td>0.81 (0.47 – 1.42)</td>
<td>0.84 (0.44 – 1.59)</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>1.33 (0.82 – 2.17)</td>
<td>1.05 (0.63 – 1.75)</td>
</tr>
</tbody>
</table>

*a Standardized odds ratio presented for continuous variables (Age, Total Energy Expenditure, Body Mass Index)*

**5.2 Outcome classification**
Creatinine-adjusted melatonin data were log-transformed to produce a distribution that yielded the normal distribution. Figure 5.1 and 5.2 depict the distribution of non-transformed and log-transformed creatinine-adjusted melatonin data, respectively, for both the day and night study periods.
5.3 Physical Activity, Sedentary Behaviour, and Melatonin

The relationship between the total energy expended by physical activity and sedentary behaviour with 6-sulfatoxymelatonin levels is presented in Table 5.5. Mean log-transformed creatinine-corrected 6-sulfatoxymelatonin levels were calculated for each quartile of total energy.
expenditure for both the day and night shift 24-hour collection periods. Melatonin levels appear to decrease with increasing energy expenditure, although a test for trend indicates that this decrease is not statistically significant \[ p=0.36 \text{ (day), } p=0.46 \text{ (night)} \].

**Table 5.5** Mean creatinine-corrected 6-sulfatoxymelatonin levels by quartile of total energy expenditure for both the day and night 24-hour collection periods.

<table>
<thead>
<tr>
<th>Total Energy Expenditure (MET·min)</th>
<th>Quartile</th>
<th>Day (n=111)</th>
<th>Night (n=113)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>50.7 ± 64.3</td>
<td>56.2 ± 99.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>42.2 ± 48.7</td>
<td>41.8 ± 31.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>73.0 ± 137.2</td>
<td>72.4 ± 225.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>29.8 ± 23.0</td>
<td>36.4 ± 36.6</td>
</tr>
</tbody>
</table>

### 5.3.1 Biologically Relevant Timeframes

To confirm the most biologically relevant timeframe by which physical activity may influence melatonin concentrations, separate multivariate linear regression models were run for the cumulative physical activity performed 9 hours (11 p.m. – 7 a.m.), 16 hours (3 p.m. – 7 a.m.), 24 hours (7 a.m. – 7 a.m.), and 30 days prior to melatonin assessment for the day and night shifts (Table 5.6). After controlling for age, physical activity energy expenditure occurring in the 16 hours (3 p.m. – 7 a.m.) prior to melatonin assessment explained the greatest variance in melatonin (adjusted $R^2 = 0.072$ for day shift collection and 0.043 for night shift collection). A smaller percentage of the variance in melatonin was explained by physical activity energy expenditure occurring in the 30 days prior to data collection (adjusted $R^2 = 0.062$ for day shift and 0.00 for night shift). A sensitivity analysis was conducted by removing all participants who claimed that their reported 30 day physical activity was not typical of their usual activity. Removing these individuals from the analysis had minimal influence on the predictability of the model, and usual
activity remained unassociated with melatonin levels. The direction of the association was the same for the 9 and 16 hour periods, but did change with the longer timeframes.

### 5.3.2 Random Effects Model

A random effects (multilevel) model was utilized to combine night and day shift data for each individual, while adjusting for the clustered nature of the data. The standardized parameter estimates are presented in Table 5.7. MVPA was shown to decrease peak melatonin levels, and this association approached statistical significance \((p=0.06)\). Shift (day or night) is significantly associated with the outcome \((p=0.03)\), in addition to an interaction between MVPA and shift \((p=0.04)\).

**Table 5.6**: Standardized partial linear regression \(\beta\) coefficients and adjusted \(R^2\) values for cumulative physical activity performed 9 hours (11 p.m. – 7 a.m.), 16 hours (3 p.m. – 7 a.m.), 24 hours (7 a.m. – 7 a.m.), and 30 days prior to melatonin assessment after controlling for age.

<table>
<thead>
<tr>
<th>Time Period of Physical Activity</th>
<th>Day Shift</th>
<th></th>
<th></th>
<th>Night Shift</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MVPA</td>
<td></td>
<td></td>
<td>MVPA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\beta)</td>
<td>p-value</td>
<td>Adj (R^2)</td>
<td>(\beta)</td>
<td>p-value</td>
<td>Adj (R^2)</td>
</tr>
<tr>
<td>9 hours</td>
<td>MVPA</td>
<td>-0.20</td>
<td>0.38</td>
<td>0.01</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>-0.01</td>
<td>0.93</td>
<td>0.039</td>
<td>0.03</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>-0.09</td>
<td>0.37</td>
<td>-0.04</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>16 hours</td>
<td>MVPA</td>
<td>-0.21</td>
<td>0.02</td>
<td>0.07</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>-0.02</td>
<td>0.83</td>
<td>0.086</td>
<td>0.02</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>-0.08</td>
<td>0.38</td>
<td>-0.28</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>24 hours</td>
<td>MVPA</td>
<td>-0.20</td>
<td>0.04</td>
<td>0.01</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.01</td>
<td>0.94</td>
<td>0.079</td>
<td>-0.03</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>-0.09</td>
<td>0.37</td>
<td>-0.19</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>30 days</td>
<td>MVPA</td>
<td>-0.08</td>
<td>0.45</td>
<td>0.15</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>-0.08</td>
<td>0.44</td>
<td>0.054</td>
<td>0.00</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>-0.07</td>
<td>0.51</td>
<td>-0.07</td>
<td>0.45</td>
<td></td>
</tr>
</tbody>
</table>

\(\text{MVPA, moderate to vigorous intensity physical activity} \)
Table 5.7: Standardized multilevel (random effects) linear regression β coefficients for both collection periods (n=226), after controlling for age.

<table>
<thead>
<tr>
<th>Intensity</th>
<th>β</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVPA</td>
<td>-0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>Low</td>
<td>0.00</td>
<td>0.77</td>
</tr>
<tr>
<td>Sedentary</td>
<td>-0.15</td>
<td>0.53</td>
</tr>
<tr>
<td>Shift (Night=1)</td>
<td>-1.30</td>
<td>0.03</td>
</tr>
<tr>
<td>MVPA*Shift⁶</td>
<td>8.60</td>
<td>0.04</td>
</tr>
</tbody>
</table>

⁶MVPA, moderate-to-vigorous intensity physical activity

5.4 Sleep Duration

Sleep duration was not included as a potential confounder of the relationship between physical activity, sedentary behaviour, and melatonin because of its potential to be on the biological pathway. Sleep duration, however, was not related to either the exposure or the outcome. During both shifts, the total energy expenditure of sedentary behaviour and each intensity of physical activity and sedentary behaviour after 3:00 p.m. were unrelated to the self-reported hours of sleep (r = 0.21, p>0.13 for both shifts). Similarly, hours of sleep had a negative, non-significant association with log-transformed, creatinine-adjusted 6-sulfatoxymelatonin levels [r = -0.11 (day), r = -0.01 (night), p>0.25].
Chapter 6
General Discussion

6.1 Summary of Main Findings

This thesis aimed to assess the influence of physical activity and sedentary behaviours on the circadian indicator melatonin among rotating shift nurses during two 24-hour collection periods. During the day shift collection period, moderate and vigorous intensity physical activity between 3:00 p.m. and 7:00 a.m. was inversely associated with morning void peak urinary melatonin levels. Sedentary behaviour was inversely correlated with melatonin levels during the night shift study period. Although significantly related, during both collection periods, physical activity and sedentary behaviour only explained a small proportion of the variability in urinary melatonin levels.

The low predictive ability of physical activity and sedentary behaviour on concentrations of melatonin is likely due to several factors. Melatonin is thought to be primarily influenced by circadian rhythm which is set by environmental triggers such as light. The purpose here was to investigate if there is an independent contribution of physical activity or sedentary behaviour on melatonin levels. These empirical results point to very little or no influence of these factors. Several other factors have also been shown to affect levels of this hormone and were not included in the analysis unless there was evidence of confounding by the variable. In their study of non-shift workers Knight et al. (2005) found a similarly low $R^2$ value of 0.053 when considering the relationship between duration of exercise between 4:00 p.m. and 4:00 a.m. and creatinine-adjusted 6-sulfatoxymelatonin levels.

The differences between the day and night shift collection periods are likely a result of the timing of the activities. The behaviours that the participants were more likely to undertake
closer to their melatonin peak were associated with decreased levels of the hormone. During the night shift, participants were unable to participate in moderate or vigorous intensity activities, and consequently sedentary behaviours, namely standing, sitting and computer use, were their main source of energy expenditure. Conversely, after their day shift, nurses were able to participate in physical activities of a higher intensity when melatonin levels were beginning to rise; consequently, these activities were associated with concentrations of this hormone. Thus, the timing of energy expenditure may play a more important role than the intensity at which it was accumulated. This hypothesis could be further investigated with objective measurement techniques that record the exact time of day of each activity performed (e.g., accelerometers), providing a narrower biological time window. A multilevel model was used to combine data from both shifts, but the moderating role of each shift limited its interpretability.

The negative association between energy expenditure and melatonin levels may be attributable to a cortisol-mediated pathway. Cortisol is often referred to as a stress hormone, and has antagonistic effects on melatonin, decreasing sensations of fatigue (Cleare et al., 2001). This relationship has been described in patients with hypercortisolemia, who show reduced levels of circulating melatonin (Soszynski et al., 1989). Physical activity and sedentary behaviour may influence cortisol secretion by promoting gluconeogenesis, the formation of glucose from proteins and fats (Brooks and Mercier, 1994). Cortisol may function to decrease melatonin levels, as demonstrated by laboratory studies that observe an increase in melatonin levels when cortisol is inhibited (Buxton et al., 1997). This association has been described in human (Montelone et al., 1992) and animal (Atkinson et al., 2003) experimental studies.

In this study, the biologically relevant timeframe by which physical activity and sedentary behaviours was considered to be between 3:00 p.m. and 7:00 a.m. for both day and night shifts. This is consistent with a previous epidemiological study by Knight et al. (2005) who
reported that exercise between 4:00 p.m. to 4:00 a.m. was associated with morning void urinary 6-sulfatoxymelatonin concentrations. Further analysis showed that energy expenditure during this timeframe was the most predictive of melatonin levels, though the differences were quite modest. This moderating role of the time of day of activity on melatonin production has also been demonstrated in experimental studies, even after controlling for light exposure patterns (Barger et al., 2004). Longer term (30 day) physical activity was unrelated to melatonin levels in this study, indicating that physical activity likely has an acute role, if any, on melatonin production. A previous study of long distance runners supports this finding (Ronkainen et al., 1986).

Sleep duration was proposed as a potential mediator of the relationship under study. Physical activity has been shown to increase the quality and duration of sleep, which could function to increase total melatonin production. In this sample, sleep duration was not related to the exposure or the outcome, indicating that it likely did not have a mediating role in this relationship in these rotating shift nurses.

6.2 Strengths and Limitations

This study was able to characterize all forms of physical activity, not solely exercise of a moderate-to-vigorous intensity, which was uncommon during both study periods. Much of the energy expenditure of these nurses occurred during their shift, which would not have been be captured by measures of exercise. Sedentary behaviour contributed to the highest proportion of energy expenditure during both study periods, and thus the inclusion of these behaviours is a major strength of the study. In addition, errors in recall were minimized because the physical activity diary was completed during each study period.

The duration, intensity, frequency and type of physical activity and sedentary behaviours were quantified through the use of Metabolic Equivalents (METs). Metabolic equivalent minutes
provide a well established method of comparing and summatng various types of activities. Two activities may both be at a high intensity (MET>6), but they may not provide equivalent rates of energy expenditure. Previous epidemiological studies have used the duration of moderate or vigorous intensity activity (e.g., Knight et al., 2005), which cannot compensate for the fact that each type of activity provides different levels of energy expenditure.

Melatonin levels were assessed using a validated biomarker: 6-sulfatoxymelatonin can measure total peak melatonin levels, and does not require highly invasive specimen collection. Creatinine corrections permitted adjustment for the total urine excreted. The pilot study was able to ensure the practicality of urine collection in this population, and demonstrated that a morning void urine sample would capture the melatonin peak for both day and night shifts.

As with any study, this study was not without its limitations. The physical activity diary required subjective responses, which may have been susceptible to errors associated with self report. Though the timeframe covered by the physical activity diary was short (16 hours) the study participants had to recall their activities during their shift when they were likely preoccupied with occupational duties. In addition, the time categories (i.e. 3:00 p.m. – 11:00 p.m.) were not reflective of functional time points of their day, increasing the probability of recall error. For example, it may have been easier for nurses to recall their activities between their lunch break (12:00 p.m.) and the end of their shift (7:00 p.m.). Since it is unlikely that any errors in recall would be related to melatonin levels, any resulting misclassification would be non-differential. Pedometers did not provide a reliable objective comparison to measure errors in exposure assessment due to the high number of malfunctions and the additional limitations associated with step counters (Basset et al., 1996).

The use of metabolic equivalents may have also been a potential source of misclassification, as all the recorded MET values were tested in laboratory conditions and
consequently they may not reflect the activity conditions of the participant. In addition, the baseline to which all METs are based (4.184 kJ kg\(^{-1}\) hr\(^{-1}\)) was determined from a 40 year old 70 kg male. This baseline has been shown to overestimate basal metabolic rates in women and participants with a low body mass index (Bryne et al., 2005). Since melatonin was not shown to be influenced by body mass index, and the study was restricted to women, this potential misclassification would likely be random and non-differential.

Metabolic equivalent scores were assigned based on self-perception of relative intensity, which may have been influenced by levels of physical fitness. Two individuals may perceive the same activity to be at different intensities based on their level of fitness, despite the activity requiring similar levels of energy expenditure. Unfit individuals may be more likely to perceive an activity to be at a higher intensity, whereas fit individuals may have adapted to higher intensity activities and consequently underreport the intensity. Measures of physical fitness were unavailable for this study, so its role on the relationship could not be determined. Fitness levels are unlikely to be related to melatonin, so this potential error would be unlikely to bias the results.

The timing of urine collection was pertinent to ensuring that peak melatonin levels were assessed. There was no means of ensuring compliance to study protocol to confirm that these samples were reflective of the appropriate stage of the circadian cycle. Inter-individual differences in melatonin metabolism may also increase the measurement error of the outcome. The assumption that morning void urinary samples provide peak melatonin measurements for both day and night shifts was based on information from the pilot study (Grundy et al., 2009), however if this is not the case for certain individuals any relationship between energy expenditure and melatonin would be more difficult to detect.

Selection biases occur when the study sample differs systematically from the base population (Rothman et al., 2008). Selection biases are of minimal concern in this study, as it is
unlikely that the biological mechanisms explaining the association in the study sample differed from the general population. Participants in this study were volunteers, which could introduce volunteer bias if the study sample differed from the target population. Although a response rate could not be calculated due to the unavailability of a suitable denominator, we do not predict that response biases would alter our estimates of effect as participants were not aware of their melatonin levels.

In terms of external validity, the generalizability of our findings may be limited to female nurses working the 2-day, 2-night shift schedule. The healthy worker effect may limit the generalizability of the findings to the general population. Individuals who are more susceptible to the adverse effects of melatonin downregulation, such as gastrointestinal pathology, stress and fatigue (Moore-Ede and Richardson, 1985), are more likely to select out of shift work, and consequently they would not be eligible for this study. This may explain why melatonin levels were similar after the day and night shifts, as those individuals who experienced circadian disruption, and consequently had lower melatonin levels during the night shift, selected out of shift work.

6.3 Future Directions

Shift workers have a 40% increased risk of cardiovascular disease (Knutsson, 2003), and shift work has been named as a “probable” carcinogen (Straif et al., 2007). Although melatonin may be one piece of this multifactorial puzzle, it will also be important to consider in future studies the quantity and timing of physical activity and sedentary behaviours since it makes sense from a biological point of view that these exposures could have a moderating effect.

Future studies should use more precise exposure assessment techniques such as accelerometers. Accelerometers store minute-by-minute physical activity data, so the precise
timing and duration of each activity could be measured. These tools would eliminate any threat of self-report error, and could provide a tool by which the biologically relevant timeframe linking physical activity and sedentary behaviours with melatonin could be further investigated. The validity of these results are dependent on the appropriate classification of the biologically relevant timeframe by which physical activity and sedentary behaviours affect melatonin production, which could have been misclassified by the broad timeframes used to classify the exposure in this study.

Cortisol measurements would provide a means by which the biological mechanisms could be further examined, and our research group is planning a new study that will examine the relationship between melatonin and cortisol in this study population. Consideration should be given to the timing of the sample collection, to ensure that the cortisol measurements reflect the biologically relevant timeframe of physical activity.

Additional information on potential effect modifiers including physical fitness levels and lighting conditions would provide a more thorough look at the variety of predictors of melatonin. This information would help to characterize one of the many multifactorial pathways that may link shift work to cardiovascular disease. In addition, future research should focus on the non-circadian based cardiovascular risk factors associated with shift work. These may include social, dietary and physiological mechanisms.

The role of physical activity and sedentary behaviour on melatonin should also be examined in other shift working populations with different shift schedules. An estimated 41% of women working shift work in Canada participate in rotating shift work; the additional 59% report working evening, night and irregular shifts (Williams, 2008). The prevalence of these alternative shift schedules suggests that they may have an important impact on the population etiological
fraction of melatonin on cardiovascular disease; however, this study was unable to capture these other types of shift work.

Finally, this relationship should also be examined in men. Men comprise 57% of the total Canadian shift working population (Williams, 2008), and the relationship between physical activity and circadian rhythm may be moderated by gender.

6.4 Contribution of Research

This study found that peak melatonin levels did not significantly differ between day and night study periods. From the point of view of nurses working this rotating shift pattern, this is a promising result, indicating that the two day, two night shift schedule may not influence melatonin levels. If this is the case, research should investigate other shift schedules, to direct healthy public policy recommendations for the healthiest shift work patterns.

The analyses of this thesis indicate that although physical activity and sedentary behaviour may decrease melatonin levels during the biologically relevant timeframe; these exposures explained very little variation in melatonin levels. This decrease, although statistically significant, may not be clinically significant and consequently more consideration should be given to alternative means of preventing melatonin down-regulation in night shift workers. Before substantial policy recommendations are made, the role of melatonin on the pathway linking shift work and cardiovascular disease needs to be examined more thoroughly, as it is likely one of several multifactorial pathways leading to the increased rates of chronic diseases in shift workers.

The hypothesis that the protective association between physical activity and cardiovascular disease may be partially due to increased melatonin levels is not supported by this research. The inverse association between physical activity and melatonin in this study should not
be interpreted as rationale to support a sedentary lifestyle in shift working nurses. The benefits of physical activity in reducing cardiovascular disease risk are overwhelming, and thus physical activity should be encouraged amongst this at-risk population. The results of this research indicate that the majority of this shift working population does not meet the recommended guidelines for physical activity (Public Health Agency of Canada, 1998), and the health implications of a sedentary lifestyle far exceed the potential morbidity associated with the very modest decrease in melatonin.

6.5 Conclusion

In the 17th century, the French philosopher René Descartes described the pineal gland as the “seat of the soul” (Reppert and Weaver, 1995). Four centuries later, scientists are still uncovering the multisystem effects that its main product has on human health. This thesis examined the role of physical activity and sedentary behaviour on melatonin in relation to increased cardiovascular risk; however, melatonin has several pathological implications outside of its role in reducing cardiovascular disease risk. Reduced melatonin levels have been observed in several sleep-wake disorders including insomnia, jet-lag and narcolepsy (Brown et al., 2009; Reimann et al., 2002). Increased sleep duration has been shown to be important for the treatment of psychological conditions such as depression, mood disorders, and autism, and melatonin has been proposed as an intermediate in these pathways (Munesue et al., 2008). In addition, the role of melatonin in reducing breast cancer risk is of growing interest due to its established antioxidant mechanisms (Tengattini et al., 2008). If physical activity and sedentary behaviours do have a clinically meaningful role on melatonin levels, the implications of these sources of energy expenditure would expand beyond those of cardiovascular morbidity.
Many critical aspects of modern life require 24-hour work and productivity, which explains why nearly 30% of Canadians are required to perform shift work (Shields, 2002; Williams, 2008). The global population etiologic fraction of shift work on cardiovascular disease has been estimated to be nearly 7% because of the high proportion of shift workers in the health and manufacturing industries (Ha and Park, 2005). Shift workers have a 40% increased risk of cardiovascular disease (Knutsson, 2003). Although this increased risk is likely multifactorial, most research has only focused on the behavioral and social risk factors associated with disturbed sleep cycles (Boggild and Knutsson, 1999). Physical inactivity has been shown to be a strong predictor of cardiovascular related mortality, and consequently public health recommendations should continue to promote activities of moderate and high intensity in the shift working population. The results of this thesis indicate that these recommendations may not be sufficient to reduce the conditions that lead to decreased melatonin levels in this population of female rotating shift nurses.
6.6 References


Appendix A

Ethics Approval

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

September 28, 2009

Mr. Mark McPherson
Department of Community Health and Epidemiology
Cancer Research Institute
Division of Cancer Care and Epidemiology
16 Stuart Street
Queen's University

Dear Mr. McPherson,

Study Title: The association between physical activity and sedentary behavior with melatonin levels in rotating shift nurses
Co-Investigators: Dr. K. Aronson and Dr. I. Janssen

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/rob.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

[Signature]
Date

Study Code: EPID-297-09

➢ 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
Recruitment Handout

How does ambient light and physical activity affect cancer risk factors in nurses?

The International Agency for Research on Cancer has recently classified long-term night shift work as a probable carcinogen. Disruption of circadian rhythms due to light exposure at night has been suggested to explain this effect through its impact on the hormone melatonin.

Researchers at the Queen’s Cancer Research Institute are looking for **female nurses**:

- who have worked DDNN rotational shifts for the last year
- not currently pregnant
- not pregnant or lactating within the past 6 month
- not taking melatonin supplements

Participants will be offered a $200 honourarium

If you are interested in learning more about this project, please contact:
Deborah Emerton Tel.: 613-548-2389 or ext. 2389
E-mail: emertonod@kgk.kari.net
Appendix C
Day Shift Study Procedures

Day Shift #1:
0500-0700hrs
- Put on light meter and pedometer when you get dressed
- Collect saliva sample #1 (Blue Trim label) and place in individual Biohazard bag and refrigerate or send directly to lab
- Begin one day diary
1500-1700
- Collect saliva sample #2 (Yellow coloured label) and place in individual Biohazard bag and refrigerate or send directly to lab
- Collect urine sample #1 (Yellow coloured label) and place in individual Biohazard bag and refrigerate or send directly to lab
2300 – 0100 (or at bedtime)
- Collect saliva sample #3 (Green coloured label) and place in individual Biohazard bag and refrigerate or send directly to lab

Day Shift #2:
0500 – 0700
- Collect saliva sample #4 (Pink coloured label) and place in individual Biohazard bag and refrigerate or send directly to lab
- Collect urine sample #2 (Pink coloured label) and place in individual Biohazard bag and refrigerate or send directly to lab
- For pre-menopausal women – collect blood, after 8 hours fast 3 tubes must be protected from light – wrap in foil and place in individual Biohazard bag and refrigerate or send directly to lab.
1900
- Remove light meter and pedometer. Complete study diary.
Appendix D
Night Shift Study Procedures

Night Shift #1:

1900
  - Put on light meter and pedometer before starting first night shift

After First Sleep
1500-1700 (upon awakening)
  - Collect saliva sample #1 (Blue trim label) and place in individual Biohazard bag and refrigerate or bring to work to send directly to lab
  - Start study diary

During second night shift
2300-0100
  - Collect saliva sample #2 (Yellow coloured label) and place in individual Biohazard bag and refrigerate or send directly to lab
0700 (before leaving work)
  - Collect saliva sample #3 (Green coloured label) and place in individual Biohazard bag and refrigerate or send directly to lab
  - Collect urine sample #1 (Yellow coloured label) and place in individual Biohazard bag and refrigerate or send directly to lab

After Second Sleep Day
1500 – 1700 (or upon awakening)
  - Collect saliva sample #4 (Pink coloured label) and place in individual Biohazard bag and refrigerate.
  - Collect urine sample #2 (Pink coloured label) and place in individual Biohazard bag and refrigerate.
  - Remove light meter and pedometer.
  - Complete study diary
Appendix E
Physical Activity Diary

STUDY OF LIGHT AT NIGHT, PHYSICAL ACTIVITY AND MELATONIN 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.

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 your cooperation.

Thank you!
SAMPLE TIMES AND Pedometer Values

Please record the exact time and date of collection for all urine and saliva samples. Please also record the value on your pedometer at the time of each saliva sample collection.

Saliva Samples:
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: 

Urine Samples:
Time and date of urine sample #1 collection: 
Time and date of urine sample #2 collection: 

Pedometer Values:
Value at time of saliva sample #1 collection: 
Value at time of saliva sample #2 collection: 
Value at time of saliva sample #3 collection: 
Value at time of saliva sample #4 collection: 
Study ID

Today’s Date __________/__________/_________  Shift worked during specified 24-hr period (day/night): __________
Day  Month  Year  Shift worked yesterday (day/night/off): __________

HEALTH BACKGROUND

1) Are you still menstruating?
   ❑ Yes → what was the date of the first day of your last menstrual period? __________
          month/day/year
   ❑ No

2) 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 #3)  ❑ 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>

3) 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 #4)  ❑ 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>
HEALTH BACKGROUND (cont’d)

4) During the 24-hour period specified on the front cover of this one-day diary, did you suffer from a migraine?
   - No (go to question #5)
   - Yes → Please provide details of the medication taken (including brand name, dose and number of tablets)

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Dose (milligrams)</th>
<th>Number of Tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5) Have you taken prescribed birth control medication in the 24-hour period specified on the front cover of this diary? (e.g. Norplant, Norinyl, Demulen, Depo-Provera, Tri-Cyclen, Alesse, etc.)
   - No (go to question #6)
   - Yes → Please provide details of the medication taken (including brand name, dose and number of tablets)

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Dose (milligrams)</th>
<th>Number of Tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LIFESTYLE HABITS - Smoking

6) Did you smoke during the 24-hour period specified on the cover of this one-day diary?
   - No (go to question #7)
   - Yes → Please estimate the number of cigarettes you smoked today. ____________

7) 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

8) 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 #9)

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

LIGHTING CONDITIONS

9) Please answer the following questions:

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?

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

10) When you sleep at night do you usually wear a sleep mask? ☐ Yes ☐ No

11) When you sleep during the day do you usually wear a sleep mask? ☐ Yes ☐ No
PHYSICAL ACTIVITY (Past 24 hours)

In the table below, please record which physical activities that you have participated in during the 24-hour period specified on the front cover of this one-day diary. It is important to indicate the duration you performed each of these activities in the morning-afternoon (7:00 AM - 3:00 PM), afternoon-evening (3:00 PM – 11:00 AM), or evening-morning (11:00 PM – 7:00 AM). It is also important to indicate whether the intensity of the physical activity was light, moderate, and heavy. These activities include both sports/exercise and active forms of transportation (e.g., walking to work).

The 3 intensity categories can be defined as follows:
- **Light**: Require minimal physical effort such as slow walking
- **Moderate**: Activities that are not exhausting, but that increase heart rate and breathing rate slightly and may cause some light sweating
- **Heavy**: Activities that substantially increase heart rate and breathing and cause heavy sweating

<table>
<thead>
<tr>
<th>Type of Activity</th>
<th>Duration Performed (in minutes) at Different Times of the Day</th>
<th>Typical Intensity of Activity (Please check only one for each activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morning-Afternoon (07:00-15:00)</td>
<td>Afternoon-Evening (15:00-23:00)</td>
</tr>
<tr>
<td>Walking (at least 10 minutes)</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Bicycling (stationary or outdoor)</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Jogging or running (outdoors or treadmill)</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Aerobics class</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Yoga</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Strength training (including lifting weights)</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Rowing</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Tennis</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Calisthenics (sit-ups, push-ups, etc.)</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
</tbody>
</table>
### PHYSICAL ACTIVITY (Cont'd)

12) (cont’d)

<table>
<thead>
<tr>
<th>Type of Activity</th>
<th>Duration Performed (in minutes) at Different Times of the Day</th>
<th>Typical Intensity of Activity (Please check only one for each activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morning-Afternoon (07:00-15:00)</td>
<td>Afternoon-Evening (15:00-23:00)</td>
</tr>
<tr>
<td>Hiking</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Swimming</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Golfing</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Dancing</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>OTHER (please list below)</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

*In the above box be sure to indicate up to 3 additional activities that were not listed but which you performed in the specified 24 hour period. For these activities also list the duration and intensity of the physical activity session.*

13) The following are questions about your household activity levels.

a) In the 24 hour period specified on the cover of this one-day diary, about how many hours did you watch television (including videos and DVDs)? Please mark one box for each of the 3 times of day listed

<table>
<thead>
<tr>
<th>Morning – Afternoon (07:00 – 15:00 hours)</th>
<th>Afternoon – Evening (15:00 – 23:00 hours)</th>
<th>Evening – Morning (23:00 – 07:00 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ None at all</td>
<td>□ None at all</td>
<td>□ None at all</td>
</tr>
<tr>
<td>□ About half an hour</td>
<td>□ About half an hour</td>
<td>□ About half an hour</td>
</tr>
<tr>
<td>□ About 1 hour</td>
<td>□ About 1 hour</td>
<td>□ About 1 hour</td>
</tr>
<tr>
<td>□ About 2 hours</td>
<td>□ About 2 hours</td>
<td>□ About 2 hours</td>
</tr>
<tr>
<td>□ 3 or more hours</td>
<td>□ 3 or more hours</td>
<td>□ 3 or more hours</td>
</tr>
</tbody>
</table>
PHYSICAL ACTIVITY (Cont’d)

13) (cont’d)

b) In the 24 hour period specified on the cover of this one-day diary, about how many hours did you use the computer (including Internet, email, chatting, etc.)? Please mark one box for each of the 3 times of day listed

<table>
<thead>
<tr>
<th>Morning – Afternoon (07:00 – 15:00 hours)</th>
<th>Afternoon – Evening (15:00 – 23:00 hours)</th>
<th>Evening – Morning (23:00 – 07:00 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None at all</td>
<td>None at all</td>
<td>None at all</td>
</tr>
<tr>
<td>About half an hour</td>
<td>About half an hour</td>
<td>About half an hour</td>
</tr>
<tr>
<td>About 1 hour</td>
<td>About 1 hour</td>
<td>About 1 hour</td>
</tr>
<tr>
<td>About 2 hours</td>
<td>About 2 hours</td>
<td>About 2 hours</td>
</tr>
<tr>
<td>3 or more hours</td>
<td>3 or more hours</td>
<td>3 or more hours</td>
</tr>
</tbody>
</table>

c) In the 24 hour period specified on the cover of this one-day diary, about how many hours did you sit quietly around the home doing things such as reading, knitting, playing board games, etc.? This does not include time spent watching television or on the computer. Please mark one box for each of the 3 times of day listed

<table>
<thead>
<tr>
<th>Morning – Afternoon (07:00 – 15:00 hours)</th>
<th>Afternoon – Evening (15:00 – 23:00 hours)</th>
<th>Evening – Morning (23:00 – 07:00 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None at all</td>
<td>None at all</td>
<td>None at all</td>
</tr>
<tr>
<td>About half an hour</td>
<td>About half an hour</td>
<td>About half an hour</td>
</tr>
<tr>
<td>About 1 hour</td>
<td>About 1 hour</td>
<td>About 1 hour</td>
</tr>
<tr>
<td>About 2 hours</td>
<td>About 2 hours</td>
<td>About 2 hours</td>
</tr>
<tr>
<td>3 or more hours</td>
<td>3 or more hours</td>
<td>3 or more hours</td>
</tr>
</tbody>
</table>

d) In the 24 hour period specified on the cover of this one-day diary, about how many hours did you perform light chores around the home such as cooking and cleaning? Please mark one box for each of the 3 times of day listed

<table>
<thead>
<tr>
<th>Morning – Afternoon (07:00 – 15:00 hours)</th>
<th>Afternoon – Evening (15:00 – 23:00 hours)</th>
<th>Evening – Morning (23:00 – 07:00 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None at all</td>
<td>None at all</td>
<td>None at all</td>
</tr>
<tr>
<td>About half an hour</td>
<td>About half an hour</td>
<td>About half an hour</td>
</tr>
<tr>
<td>About 1 hour</td>
<td>About 1 hour</td>
<td>About 1 hour</td>
</tr>
<tr>
<td>About 2 hours</td>
<td>About 2 hours</td>
<td>About 2 hours</td>
</tr>
<tr>
<td>3 or more hours</td>
<td>3 or more hours</td>
<td>3 or more hours</td>
</tr>
</tbody>
</table>
PHYSICAL ACTIVITY (Cont’d)

14) The following questions are about activities you performed while at work in the 24 hour period specified on the front of this diary.

a) About how much time did you spend on your feet while at work?
   - None at all
   - About half an hour
   - About 1 hour
   - About 2 hours
   - About 3 hours
   - About 4 hours
   - About 5 hours
   - About 6 hours
   - 7 or more hours

b) About how much time do you spend doing heavier activities such as lifting or bathing patients?
   - None at all
   - About half an hour
   - About 1 hour
   - About 2 hours
   - About 3 hours
   - About 4 hours
   - About 5 hours
   - About 6 hours
   - 7 or more hours
**PHYSICAL ACTIVITY (Past Month)**

15) Please indicate which of the following physical activities you have performed in the last 30 days (1 month). For each activity you performed, indicate the number of times you have participated in the past 30 days, the average length/duration of participation for a given session, and whether the typical intensity was light, moderate, and heavy. These activities include both *sports/exercise* and active forms of *transportation* (e.g., walking to work).

The 3 intensity categories can be defined as follows:

- **Light:** Require minimal physical effort such as slow walking
- **Moderate:** Activities that are not exhausting, but that increase heart rate and breathing rate slightly and may cause some light sweating
- **Heavy:** Activities that substantially increase heart rate and breathing and cause heavy sweating

<table>
<thead>
<tr>
<th>Type of Activity</th>
<th>Number of times performed in past 30 days</th>
<th>Average duration of physical activity session</th>
<th>Typical Intensity of Activity (Please check only one for each activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking (at least 10 minutes)</td>
<td></td>
<td></td>
<td>□</td>
</tr>
<tr>
<td>Bicycling (stationary or outdoor)</td>
<td></td>
<td></td>
<td>□</td>
</tr>
<tr>
<td>Elliptical Trainer</td>
<td></td>
<td></td>
<td>□</td>
</tr>
<tr>
<td>Jogging or running (outdoors or treadmill)</td>
<td></td>
<td></td>
<td>□ □ □</td>
</tr>
<tr>
<td>Aerobics class</td>
<td></td>
<td></td>
<td>□</td>
</tr>
<tr>
<td>Yoga</td>
<td></td>
<td></td>
<td>□</td>
</tr>
<tr>
<td>Strength training (including lifting weights)</td>
<td></td>
<td></td>
<td>□</td>
</tr>
<tr>
<td>Rowing</td>
<td></td>
<td></td>
<td>□</td>
</tr>
<tr>
<td>Tennis</td>
<td></td>
<td></td>
<td>□</td>
</tr>
<tr>
<td>Squash/racquetball</td>
<td></td>
<td></td>
<td>□</td>
</tr>
<tr>
<td>Calisthenics (sit-ups, push-ups, etc.)</td>
<td></td>
<td></td>
<td>□</td>
</tr>
<tr>
<td>Hiking</td>
<td></td>
<td></td>
<td>□</td>
</tr>
<tr>
<td>Swimming</td>
<td></td>
<td></td>
<td>□</td>
</tr>
<tr>
<td>Golfing</td>
<td></td>
<td></td>
<td>□</td>
</tr>
</tbody>
</table>
### PHYSICAL ACTIVITY (Cont’d)

15) (cont’d)

<table>
<thead>
<tr>
<th>Type of Activity</th>
<th>Number of times performed in past 30 days</th>
<th>Average duration of physical activity session</th>
<th>Typical Intensity of Activity (Please check one for each activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dancing</td>
<td></td>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Hiking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTHER (please list below)</td>
<td></td>
<td></td>
<td>□</td>
</tr>
</tbody>
</table>

*In the above box be sure to indicate up to 3 additional activities that were not listed but which you performed in the past month. For these activities also list the number of times you have participated, the average duration, and the typical intensity.*

16) Was the amount of physical activity you performed in the past month typical for you? Please check the correct response option.
- □ Yes
- □ No, I usually perform more physical activity
- □ No, I usually perform less physical activity

17) The following questions are about your household activity levels.

a) About how many hours a day in the past 30 days did you usually watch television (including videos and DVDs) in your free time? *(Please mark one box for days that you work and one box for days that you have off)*

<table>
<thead>
<tr>
<th>Days that you work</th>
<th>Days that you have off</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ None at all</td>
<td>□ None at all</td>
</tr>
<tr>
<td>□ About half an hour a day</td>
<td>□ About half an hour a day</td>
</tr>
<tr>
<td>□ About 1 hour a day</td>
<td>□ About 1 hour a day</td>
</tr>
<tr>
<td>□ About 2 hours a day</td>
<td>□ About 2 hours a day</td>
</tr>
<tr>
<td>□ About 3 hours a day</td>
<td>□ About 3 hours a day</td>
</tr>
<tr>
<td>□ About 4 hours a day</td>
<td>□ About 4 hours a day</td>
</tr>
<tr>
<td>□ About 5 hours a day</td>
<td>□ About 5 hours a day</td>
</tr>
<tr>
<td>□ About 6 hours a day</td>
<td>□ About 6 hours a day</td>
</tr>
<tr>
<td>□ About 7 hours or more a day</td>
<td>□ About 7 hours or more a day</td>
</tr>
</tbody>
</table>
## PHYSICAL ACTIVITY (Cont’d)

17) (cont’d)

b) About how many hours a day in the past 30 days did you use the computer (including Internet, email, chatting, etc.)? *(Please mark one box for days that you work and one box for days that you do not work)*

<table>
<thead>
<tr>
<th>Days that you work</th>
<th>Days that you have off</th>
</tr>
</thead>
<tbody>
<tr>
<td>None at all</td>
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<tr>
<td>About half an hour a day</td>
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</tbody>
</table>

c) About how many hours a day in the past 30 days do you sit quietly around the home doing things such as reading, knitting, playing board games, etc. This does not include time spent watching television or on the computer? *(Please mark one box for days that you work and one box for days that you do not work)*

<table>
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<tr>
<td>About 7 hours or more a day</td>
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</tbody>
</table>

d) About how many hours a day in the past 30 days do you perform light chores around the home such as cooking and cleaning? *(Please mark one box for days that you work and one box for days that you do not work)*

<table>
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</table>
PHYSICAL ACTIVITY (Cont’d)

17) (cont’d)

c) About how many hours a day in the past 30 days do you perform moderate chores around the home such as cooking and cleaning? (Please mark one box for days that you work and one box for days that you do not work.)

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<tr>
<td>About 7 hours or more a day</td>
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</tbody>
</table>

f) About how many hours a day in the past 30 days do you perform heavy chores around the home such as gardening, shoveling snow, etc.? (Please mark one box for days that you work and one box for days that you do not work.)

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</table>
PHYSICAL ACTIVITY (Cont’d)

18) The following questions are about activities you performed while at work in the past 30 days.
   a) About how much time do you spend on your feet while at work?
      ❑ None at all
      ❑ About half an hour a day
      ❑ About 1 hour a day
      ❑ About 2 hours a day
      ❑ About 3 hours a day
      ❑ About 4 hours a day
      ❑ About 5 hours a day
      ❑ About 6 hours a day
      ❑ About 7 hours or more a day

   b) About how much time do you spend doing heavier activities such as lifting or bathing patients?
      ❑ None at all
      ❑ About half an hour a day
      ❑ About 1 hour a day
      ❑ About 2 hours a day
      ❑ About 3 hours a day
      ❑ About 4 hours a day
      ❑ About 5 hours a day
      ❑ About 6 hours a day
      ❑ About 7 hours or more a day
CHANGES MADE TO LIGHTING CONDITIONS

19) 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.

20) 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.