

Circadian Rhythmicity in the Intensive Care Unit (ICU): Understanding
Melatonin Patterns and Their Relationship to Delirium in ICU Patients

By

Alanna Livia Ash

A Thesis

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfillment of the requirements of the degree of

MASTER OF NURSING

College of Nursing

University of Manitoba

Winnipeg, Manitoba

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Abstract

The circadian rhythm is an internal body cadence, responsible for regulation of sleep in all mammals. In humans, this clock is altered by several factors, including light and secretion of the hormone melatonin. Within the intensive care unit (ICU) population, it is well evidenced that patients suffer from circadian dysregulation, often for long periods of time. Additionally, many parallels have been noted between severely fragmented sleep and delirium, an acute neurological condition frequently observed in ICU patients. A prospective cohort pilot study of five subjects was undertaken to enable a greater understanding of both sleep in the ICU and the relationship between circadian rhythm and delirium. From a total of thirty-six urine samples per subject, excretion of 6-sulphatoxymelatonin (aMT6s), the urinary metabolite of melatonin was analyzed. T-test comparison ($p=0.05$) of mean aMT6s (ng/mL) revealed significant differences in the nighttime excretion between subjects in this study and healthy individuals. No significant differences were observed with t-test comparison of mean aMT6s of the first 24 hours from the current study to ICU subjects in previous literature. No subjects were identified as delirious in the study and therefore no relationship could be found between circadian rhythmicity, as evidenced by melatonin excretion and delirium in this study population.

Acknowledgements

There are a number of people who have contributed so very much, in a variety of ways, to enable me to reach my goal of receiving a Masters in Nursing. I will always be thankful to the following individuals/groups for their help:

- God. I am thankful to Him who has given me the gift to care for others, and reach this goal.
- My family. My children and best friend Jason Chau, who have continued to be my motivation to complete this project.
- My best friend in the world, Shanna Mae Olson. Shanna has provided with me babysitting to attend classes, editing and most importantly, heaps of emotional support and encouragement.
- Dr. Diana McMillan, my supervisor, friend and mentor. Dr. McMillan's enthusiasm inspired me from the first moment I met her. A wonderful and knowledgeable advisor, she has been there to teach me, help me understand the process of growth during a Masters, and has provided me insightful advice during the rough times in my program.
- Dr. William-Diehl Jones, for providing me with his laboratory facilities and for his positive attitude at all times.
- Dr. Rakesh Arora, for his continuous attempts to support me in whatever means were available to him. Additionally, for his expert knowledge in the area of delirium.

- Shelley Dollbeck, Patient Care Manager, for showing interest when no one else would, and providing unwavering support for me to complete my project on her nursing unit.
- The bedside nurses on the Medical Intensive Care Unit for their assistance with sample collection and making this study a priority while they were already very busy at work.
- The Manitoba Centre for Nursing and Allied Health Research for their assistance with statistical analysis.
- Buhler Research Lab in the Health Sciences Centre, Winnipeg, Manitoba.
- Kathleen and Winifred Ruane Foundation for the research grant which enabled this project.
- Andrew Walker for his help with statistical analysis.

Dedication

To my beloved children: Andrew, Emilyn, Calum and Isla Grace, I love you all so very much! You inspire me to dream big and motivate me to reach my goals and fulfill my educational pursuits.

And to my mother, Jillian Dacyk, a piece of this book is yours. You have been my biggest supporter and helped me tirelessly, no matter the time of day. My love, always.

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Chapter 1: Statement of the Problem

It is widely known that the quantity and quality of patients' sleep within the Intensive Care Unit (ICU) setting is of poor quality and quantity. Reports indicate that 61-71% of patients describe significantly restricted sleep during their ICU stay (Simini, 1999; Richardson, Allsop, Coghill, & Turnock, 2007). Multiple factors result in circadian dysrhythmicity, an alteration in the normal mammalian body clock. Unfortunately, these variations can ultimately cause significant disruptions in sleep, which can have detrimental health consequences (Spiegel, Leproult, & Cauter, 1999; Weinhouse & Schwab, 2006), and although a great deal of research has been directed towards this problem within the ICU patient population, the concern of altered sleep persists.

One noteworthy condition that is reported to have a relationship to altered sleep is delirium (Figueroa-Ramos et al., 2009). Delirium is an acute neurological condition characterized by a reduced level of consciousness, and the condition is reported to be present in 16 -87% of ICU patients (Balas et al., 2007; Bergeron, Dubois, Dumont, Dial, & Skrobik, 2011; Ely et al., 2001; Lingehall, Smulter, Engstrom, Gustafson, & Olofsson, 2013; Papanmekaporn et al., 2014). Delirium carries with it long-term consequences, including greater hospital costs (Milbrandt et al., 2004), greater length of admission time (Ely et al., 2001), and increased mortality rates (Ely et al., 2004; Pisani et al., 2009).

Previous research has established the habitual sleep patterns in healthy humans differ significantly to the sleep patterns of ICU patients (Aurell & Elmqvist, 1985; Elliott, McKinley, & Cistulli, 2011; Friese, Diaz-Arrastia,

McBride, Frankel, & Gentilello, 2007). Additionally, secretion of the sleep-promoting hormone melatonin in the ICU patient population has been measured in secreted urine, saliva and via blood collection (Benloucif et al., 2008a; Bojkowski, Arendt, Shih, & Markey, 1987; de Almeida et al., 2011a). Urinary melatonin sampling is reported to be affordable, easily accessible, practical and, unlike salivary samples, free from contamination by food or drink (Benloucif et al., 2008). What remains unknown is whether a relationship exists between melatonin secretion and delirium rates. More specifically, it is not known whether the alterations noted in urine melatonin of ICU patients display a relationship with the fluctuating level of consciousness observed in this population.

In this chapter, our purpose is to detail the importance of studying circadian rhythmicity as reflected by melatonin secretion and delirium in ICU patients. This discussion will include a description and the significance of both the human circadian rhythm and the condition of delirium. Included in this discussion will be the purpose of this study, and the primary and secondary hypotheses, which are guiding this research project.

Circadian Rhythmicity in the ICU

Circadian rhythmicity refers to the body's ability to regulate biological outcomes, including glucose availability, hormone release, and temperature and sleep regulation; a process observed in all mammals (Chan, 2012; Rosenwasser & Turek, 2011). The intricate physiological processes underlying the circadian rhythm are complex and encompass many body systems; however, only the regulation of sleep shall be discussed, as it has significant importance to this

research study. Both internal and external synchronizers exist which modify the body's ability to regulate sleep. Within the ICU, it is most often external factors which alter the normal sleep rhythm. The majority of studies of sleep in the ICU point to light, noise and frequent disruptions as the largest external dysregulation culprits (Drouot, Cabello, d'Ortho & Brochard, 2008; Krachman et al., 1995; Weinhouse & Schwab, 2006).

Celik et al. (2005) noted that nursing activities caused frequent interruptions in patient sleep. Over a period of three days, the authors found an average of 51 interactions per patient, per night. The majority of these interruptions consisted of procedures such as endotracheal suctioning, bathing, dressing changes and mouth care. The time in which most of these activities occurred was between the hours of 0200 and 0500; interestingly, around the same time as the natural peak of melatonin, a hormone needed for the promotion of sleep. Interruptions were also studied by Meyer et al. (1994), who stated that in the ICU, it is this type of disturbance that ultimately prevents consolidated sleep periods.

A great deal of research has been devoted to understanding the nature and impact of environmental disruptions on sleep and the circadian rhythm. Of these, light exposure is particularly problematic, as light possesses the strongest influence of all stimuli on the circadian rhythm. Exposure to light is not only disruptive for the patient, but also responsible for a physiologic change. Light suppresses the normal circadian secretion of melatonin, a hormone associated with sleep. As this hormone is a valuable component of sleep continuance,

suppression contributes to an alteration in the patient's sleep pattern (Arendt, 2005). Accurate measurement of circadian rhythmicity in ICU patients is difficult to achieve for several reasons. Only a handful of accurate modalities exist to measure circadian rhythmicity, and with each of these are barriers to achieving optimal recordings. Polysomnography is described as the gold standard by which to do such measurements. This process of recording and analyzing electrophysiologic arousal waves allows researchers to capture a picture of sleep architecture and duration (Knauert et al., 2014). Polysomnography is, however, both time-consuming and costly, requiring specific equipment and technical support to record and interpret the results. Furthermore, this method of analysis can be uncomfortable for critically-ill patients (Drouot, et al., 2008).

Actigraphy is yet another method used for measuring sleep. This option uses an accelerometer, usually in the form of a watch or bracelet to record the subject's movement, with the goal of estimating sleep through patterns of rest and activity. However, in the ICU population, this method of measurement may not be accurate, as often these patients are restrained, either physically or chemically, causing limited movement of their limbs, thereby over-estimating sleep time (Bourne, Minelli, Mills & Kandler, 2007). Additionally, according to Tryon (2004), over estimation may also be a result of actigraphy sensing sleep at an earlier phase within the sleep onset than does polysomnography.

Lastly, understanding a picture of individual circadian rhythmicity can be captured through measurement and graphing of melatonin secretion. Multiple studies in the ICU have used this method to best-understand circadian rhythm, as

it is accurate and reasonably affordable (Drouot, et al., 2008; Pisani et al., 2015). Measurement can occur via blood, saliva or urine. Between the three aforementioned methods, research has demonstrated good correlations in both amplitude and timing (Bojkowski et al., 1987; Elliott et al., 2002). Arendt (2005) highlights, however, that disadvantages exist for all three methods, and that preservation of blood volume, invasiveness, and desired intervals should all be considerations when selecting a mode of collection. The standard for the frequency of collection of these biological measures has been set by previous research, and is often in one, two, or four-hour increments (Mundigler et al., 2002; Olofsson et al., 2004; Seifman, et al., 2014). Of these options, the most accurate depiction of circadian rhythm is achieved with the greatest frequency of collection, enabling the researcher to map a participant's rhythm over a 24-hour period, providing a better overall picture of the subject's sleep health.

Understanding Circadian Rhythmicity

Although extensive research data exists on sleep in the ICU patient population, the majority of this research is not directed at understanding the circadian rhythm specifically, but to understanding the types and frequency of disruptions in sleep and the Total Sleep Time (TST) of patients (Bosma, et al., 2007; Cooper et al., 2000; Fanfulla et al., 2011). One could argue that it is possible that a chicken-and-egg phenomenon exists; although it is known that frequent disruptions are problematic, it also is plausible that altered circadian rhythmicity of patients causes such poor sleep. What is known is that detrimental side effects of abnormal sleep are observed within this environment (Pisani et al.,

2015; Weinhouse & Schwab, 2006). Whether this is due specifically to the abnormal physiologic change to the circadian rhythm causing circadian dysrhythmia or a drastic reduction in sleep is not always clear.

ICU patients exhibit abnormal circadian rhythms and severely fragmented and decreased sleep (Billings & Watson, 2015; Boesen et al., 2015). It is difficult to ascertain the magnitude of impact that altered sleep has on ICU patients. The vast majority of sleep deprivation studies utilize healthy volunteers rather than the critically ill. After observing the numerous neurophysiologic complications in healthy subjects, one could speculate that the symptoms would be magnified in unstable, critically ill patients.

When taking specific body systems into consideration, it seems plausible that the effects of altered sleep architecture, either total sleep loss or fragmented sleep, impact all systems. These side effects can include delirium (Figueroa-Ramos et al., 2009), weakened respiratory muscles (Chen & Tang, 1989), and alterations in glucose tolerance (Schmid et al., 2011). The consequences of sleep loss have potential for long-term complications, reinforcing the notion that sleep loss places our patients at great risk.

In addition to the physiological side effects, the stressful nature of sleep in the ICU has previously been recounted by patients. Reports have indicated that 61-71% of patients describe their sleep as being considerably restricted during their admission on the unit (Simini, 1999; Richardson, Allsop, Coghill, & Turnock, 2007). Nelson et al. (2001) described that cancer patients receiving ICU care found sleep disruption the second most stressful factor in their ICU stay. It is

no surprise, as patients' sleep is altered drastically in this environment. Various reports indicate that patients' TST varies anywhere from 1 hour 50 minutes to 10.5 hours a night (Appendix A), a departure from the recommended average of 7.5-8.5 hours per night (Hirshkowitz et al., 2015). It has been reported, however, that even when the duration of sleep in ICU patients has been long, the sleep architecture can be grossly abnormal. In their 2015 review, Drouot and Quentin describe these types of sleep abnormalities as frequent waking, unusual distribution of time spent in each sleep stage, altered or abolished REM sleep and a large quantity of daytime sleep (Drouot & Quentin, 2015).

Although patient sleep has been identified as a very important issue in many nursing studies (Gellerstedt et al., 2015; Ulras et al., 2015), it remains difficult to estimate with precision the amount of sleep each patient is receiving (Nesbitt & Goode, 2014). Inaccuracies have been found, with estimations of TST by both patients and nurses (Aurell & Elmquist, 1985; Beecroft et al., 2008; Elliott, McKinley, & Cistulli, 2011; Toublanc et al., 2007), demonstrating that although healthcare providers may be attempting to promote sleep, it may be difficult to assess, and difficult for patients to understand whether proper rest is being achieved.

As previously noted, circadian dysrhythmia is considerable and warrants further investigation. Furthermore, a greater understanding of the relationship between circadian rhythm and delirium would be beneficial, as clearly little research exists.

Understanding Delirium

The relationship between sleep and delirium is complex; as Figuero-Ramos (2009) states, it is a reciprocal relationship in which sleep loss has been identified as a causative factor in the development of delirium, and vice versa. Furthermore, contributing factors, including neurotransmitter imbalance and irregular melatonin secretion, are a shared link between both sleep deprivation and delirium. According to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), the criteria for diagnosis of delirium includes:

(a) a disturbance attention; (b) the disturbance develops over a short period of time (usually a few hours or days), represents a change from baseline attention and awareness, and tends to fluctuate in severity during the course of the day; (c) an additional disturbance in cognition; (d) the disturbances in Criteria A and C are not better explained by another preexisting, established, or evolving neurocognitive disorder and do not occur in the context of a severely reduced level of arousal, such as a coma; and e) there is evidence from the history, physical examination, or laboratory findings that the disturbance is a direct physiological consequence of another medical condition, substance intoxication or withdrawal.

In addition to the term delirium, the condition within ICU is also commonly referred to as encephalopathy, ICU psychosis or ICU syndrome. Authors such as Caplan (2008) advise against this terminology, however, stating

that diagnosis of a condition based upon geographical location alone is “nonsensical” (p. 2287). Moreover, the author argues that focusing on the location as a causative agent negates further investigation into additional causes of the delirium.

How significant is the issue of sleep loss and fragmentation? Of all side effects associated with sleep irregularities, delirium may be the most clinically important. It has been reported that between 16-87% of patients develop this condition during their ICU stay (Balas et al., 2007; Bergeron, Dubois, Dumont, Dial, & Skrobik, 2011; Ely et al., 2001). The percentage cited demonstrates a very wide range of results. This number may speak to the wide variety and accuracy of the tools used to determine delirium and also to the need to continue researching this condition.

Patients who develop delirium during their ICU stay can expect a greater number of complications while admitted and post-discharge. Delirium has been found as the strongest determinant of length of stay in the hospital (Ely et al., 2001). The presence of delirium also accounts for a 39% increase in ICU cost (median cost of \$22,346 for patients with at least one episode of delirium and \$13,332 without), and 31% greater hospital cost (Milbrandt et al., 2004). Furthermore, delirium is associated with a higher 6-month mortality compared to those who did not develop the condition during their ICU stay (34% vs. 15%) (Ely et al., 2004). Additionally, according to Pandharipande et al. (2013), evaluation at three and twelve months post ICU discharge revealed diminished executive functioning and cognition in those previously identified as delirious.

Significance of This Research Study

As previously detailed, both circadian dysrhythmicity and delirium result in considerable consequences to ICU patients. Due to the time-consuming nature of studies of circadian rhythmicity, few report the circadian rhythmicity of ICU patients as reflected by urinary melatonin. Additionally, in studies where researchers have collected and interpreted this data, collection periods are often limited to 1-2 days (Gehlbach et al., 2012a; Mundigler et al., 2002a). Being that a great variance in daily sleep rhythms exists among ICU patients (Drouot & Quentin, 2015), it would be advantageous to gain a better understanding of the sleep process in this patient group. A longer analysis enables a more detailed depiction of the circadian rhythmicity in ICU patients, thereby adding to the existing knowledge in this area (Elliott & Nathaney, 2014).

Delirium in ICU patients is a well-studied subject. The relationship shared with melatonin, however, has received very little attention. Although a great deal of research describes the connection that is present between sleep and delirium in the ICU, the literature often describes similarities between neurotransmitter release, and behavior patterns (Watson et al., 2012). Two measures of circadian rhythm patterns and delirium, namely, urine melatonin and Confusion Assessment Method for Intensive Care Units (CAM-ICU) score, have not previously been used in conjunction to understand this correlation. This research study is significant as it may provide insight into the relationship between these two measures and thereby lay groundwork for future research in this area. Both

measures are affordable, non-invasive means to capture circadian rhythm patterns and delirium, causing less disruption to patient care.

Hypotheses

The primary hypothesis guiding this research study is as follows:

- Patients admitted to the ICU will demonstrate an altered circadian rhythm as evidenced by q2h urinary melatonin analysis.

The secondary hypothesis guiding this research study is as follows:

- A correlation exists between circadian dysregulation and delirium as evidenced by a presence of atypical melatonin secretion and positive CAM-ICU score in ICU patients.

Substantial evidence supports the need for a greater understanding of sleep in ICU patients. Both physical and psychological consequences in patient health are observed when a lack of sleep is noted. Delirium, although well studied, remains persistent in the ICU population, causing significant health sequelae and prolonged hospital stays for patients. As previously described, this study provides an opportunity to understand these two problematic components of an ICU stay and understand their relationship. Furthermore, although it is known that a connection exists between sleep and delirium, understanding the relationship specifically between urinary melatonin and CAM-ICU scores will add to the knowledge in this field. Any addition of information in this area may inform future interventions to support both improved sleep and delirium rates in the future; two health outcomes predicted to decrease psychological and physical side effects, while shortening hospital stays for ICU patients.

Chapter Two: Literature Review

This chapter will detail the process of sleep and circadian rhythmicity. Special attention will be provided to description of melatonin, its role in the circadian rhythm and variations observed in this hormone within the ICU environment. Additionally, delirium will be discussed, including its diagnosis, prevalence within the ICU and relationship with sleep and circadian rhythmicity.

Sleep

Understanding of sleep has varied greatly over centuries. Antiquated views of sleep observed the process as a transitional state between alertness and death with no differentiation understood between sleep and coma-like or hypnotic states (Roehrs, 2011). Waking was considered a reversal of the sleep process, virtually an opposing course. Contemporary theories about sleep conceive it as a dynamic process involving specific hormone release and electrical activity in the brain (Roehrs, 2011). This comprehension was achieved, however, with a great deal of research and experimentation to understand sleep, wakefulness and the detrimental consequences of sleep deprivation in both animals and humans (Gulevich, Dement & Johnson, 1966; Rechtschaffen, Gilliland, Bergmann, Winter, 1983).

The Sleep Process.

The focus of this review is to clarify the importance of sleep, specifically in ICU patients. A brief explanation of the other elements of the sleep matrix will be provided to enhance an understanding of the process as a whole. Sleep is an intricate process which involves a myriad of cerebral activity, including

neuroendocrine influences, activation and inhibition of the arousal system, and input for the chronobiologic clock (Landis, 2011). Sleep cannot be explained in successive steps, as often the processes occur simultaneously; and for this reason, following a brief explanation of sleep, each domain shall be explained separately.

Sleep is staged based upon recordings of brain wave activity (electroencephalography), measurement of muscle tone (electromyography) and measurement of eye movement (electro-oculography) (Landis, 2011). According to Carskadon and Dement (2011), sleep can be defined as “a reversible behavioral state of perceptual disengagement from and unresponsiveness to the environment” (p.16). Within sleep, two broad stages can be recognized: Rapid Eye Movement (REM) sleep and Non Rapid Eye Movement (NREM) sleep (Iber, et al. (2007). From this point, NREM sleep can further be subdivided as stages N1, N2 and N3 (often referred to as slow wave sleep), a categorization determined by distinct brainwaves during each phase. A cyclical pattern can be observed in sleep. The normal architecture, or pattern of sleep, includes one period of NREM sleep followed by a period of REM sleep, which completes one cycle. Although individual variations can be observed, this pattern typically totals 90-110 minutes per cycle. The first third of the night is predominately SWS, followed by a mix of waking episodes, NREM and REM sleep, while the latter third of the night is typically dominated by REM sleep (Carskadon & Dement, 2011).

The human body requires sleep. Although this statement is self-evident, it is the specific architecture of “normal” sleep the body requires. Many studies have demonstrated the multi-system effect of total sleep deprivation, including

hallucinations (Mullaney, Kripke, Fleck et al., 1983; Patrick & Gilbert, 1896), increase in seizure activity in epileptics (Scalise, Desiato, Gigue et al., 2006), subclinical anxiety, depression and paranoia (Kahn-Green, Killgore, Kamimori et al., 2007) and difficulty with thermoregulation (Romeijn et al., 2012). However, it is partial deprivation, or fragmented sleep, that is most often observed in today's society. The consequences of chronic sleep loss in ICU patients can be just as severe, resulting in alterations of neurobehavioral function, motor function, mood and cognition (Durmer & Dinges, 2005; Van Dongen, Maislin, Mullington & Dinges, 2003). This type of deprivation is a result of an increase of N1 and N2 sleep, at the expense of Slow Wave Sleep (SWS) and REM sleep, which can occur for a variety of reasons.

Several neuroendocrine mediators exist within the sleep process including a variety of hormones which are key in activation, maintenance and termination of sleep. A large portion of this chapter however, will be devoted to explanation of the hormone melatonin and its counterpart cortisol. The description of these hormones has been integrated throughout the chapter and for this reason no specific section will be devoted to neuroendocrine influence.

The Arousal System.

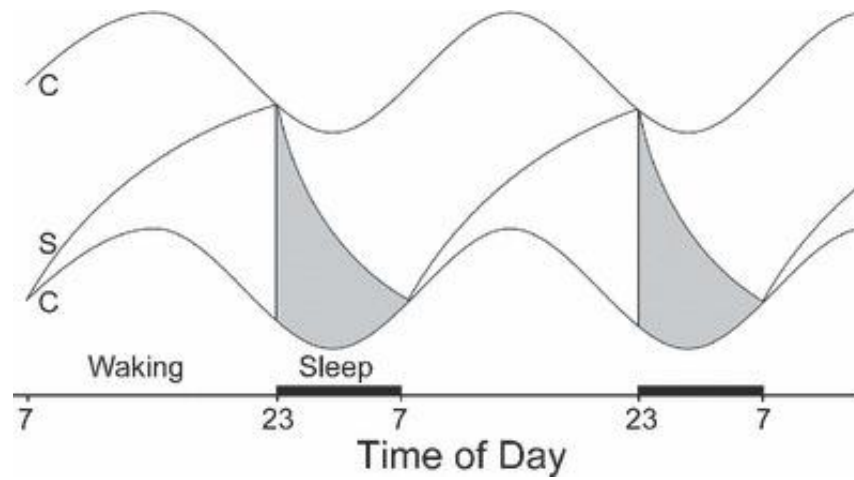
Sleep and wakefulness is maintained via two parallel neural pathways each with distinct cell varieties and neurotransmitters (Saper, Scammell & Lu, 2005). These pathways are constructed of long neuronal axons, which affect widespread brain regions, causing near global involvement for the sleep/waking process (Shwartz & Ross, 2008). Originating in basal forebrain, the first branch of

the arousal system is composed of cholinergic neurons which innervate the thalamus, and specifically Principal Thalamic Nuclei (PTN) (Hallanger, Levey, Lee, Rye, & Wainer, 1987). The PTN neurons serve as gating mechanisms of arousal, which are most active during wakefulness and REM sleep, and slowed during NREM sleep. The second branch extends into the lateral hypothalamus, basal forebrain and cerebral cortex (Jones, 2003; Saper, Chou, & Scammell, 2001). Composed of numerous monoaminergic cell types, including dopaminergic, serotonergic, and histaminergic neurons, this tract discharges most frequently during wakefulness, is slowed during NREM sleep and demonstrates little activity during REM sleep (Fuller, Gooley, & Saper, 2006). While timed differently, additional orexin/hypocretin neuronal cells exist within the same tract and these serve an important role in REM specific homeostasis. In rats, lesions in this area of the brain cause insomnia and stimulation induces NREM sleep onset (Gerashchenko, Blanco-Centurion, Greco, & Shiromani, 2003).

Both tracts are activated in a synchronized process by signals from the Reticular Activating System (RAS), which adjusts timing of these signals by acting as an internal clock. Signal modification from the RAS results from simultaneous processes, including hormone release and the mammalian circadian rhythm (Reppert & Weaver, 2002). Diurnal animals, including humans, often receive these sleep-promoting signals from the RAS in the evening or during times of low lighting, and experience the opposite effect during daylight hours (Reppert & Weaver, 2002).

Activation of either wake-promoting or sleep-promoting neurons can achieve either effect (Landis, 2011). In short, for sleep to occur, the arousal system must be inactivated, and vice versa. This process operates as a mutually inhibitory switch and has been depicted by a two-process model (Borbely, 1982). The model describes a balance between Process S (sleep-dependent process) and Process C (a sleep-independent circadian process) (Figure 1). Though the model specifies a very complex course, it illustrates that sleep duration and propensity are determined by the collective action of the two processes. Process S, a measure of prior waking time, is balanced against Process C, the variation of sleep propensity, controlled by the circadian clock (Borbely). The author detailed that the cyclic occurrence of NREM and REM sleep is a result of interaction between these processes.

Figure 1. Two Process Model of Sleep



Reprinted from Daan, S., Beersma, & D. G., Borbely, A. A. (1984). Timing of human sleep: Recovery process gated by a circadian pacemaker. *The American of Physiology*, 246, 161-183. Retrieved from <http://www.physiology.org>.

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Circadian Rhythmicity

Overview

Compared to all other animals, one feature that distinguishes mammals from fish, birds and insects is the presence of a biological clock. As an attempt for the body to adapt to the solar day and night, while promoting concentrated periods of activity during the daylight hours, adaptation of the circadian rhythm was established (Mistlberger & Rusak, 2011). The circadian clock (circadian is approximately a day in Latin) is typically close to 24 hours in duration, although individual variations can be observed, and length can be altered by a variety of factors (Chan et al., 2012). The biological clock provides endogenous direction for processes, including glucose availability, hormone release, and temperature and sleep regulation (Chan, 2012; Rosenwasser & Turek, 2011). According to Turek (2011), of all the functions of the circadian clock, one could argue that the sleep-wake cycle embodies the master clock itself, as the control of the sleep rhythm by the suprachiasmatic nucleus (SCN) in turn controls many other downstream bodily rhythms.

Physiology of the Circadian Sleep and Wake Cycle

As previously described, location of the master clock is within two bilaterally-paired regions of the brain: the SCN and the hypothalamus (Turek, 2011). Through a multitude of receptors (primarily photic receptors) the clock is entrained to a day/night rhythm by surrounding stimuli. Of all stimuli, light is the most instrumental in regulation of the bodily clock (Turek). In addition to this form of input, however, are other environmental *zeitgebers* (German for time-

givers), which aid in reinforcing the daily rhythm. Such *zeitgebers* include eating/drinking patterns, social interactions and activity level (Gooley & Saper, 2011).

In addition to communication by way of effect pathways, signals are sent via tens of thousands of single-cell circadian oscillators, which coordinate to produce circadian output signals (Reppert & Weaver, 2001). Various signals are sent through numerous neurotransmitters and both central and peripherally located clock cells. Moreover, these signaling processes assist in control of hormone release of corticosteroids and production of the sleep-promoting hormone melatonin (Gooley & Saper, 2011). Activation of these clock cells takes place at a molecular level, initiating sleep and wakefulness through multiple, specifically targeted feedback loops (Rosenwasser & Turek, 2011).

Observations in Intensive Care Patients.

To date, there is no way to directly observe the circadian rhythm. Several measurements can be used which are indicative of circadian activity, but these means cannot expressly monitor the action of the circadian pacemaker. Common modalities that are reflective of circadian activity include frequent measurement of temperature, and the hormones cortisol and melatonin (Pisani, 2015). Although sleep and circadian rhythmicity are not one and the same, examination of sleep structure, paired with an understanding of biological measurements, remain the most constructive measures by which to understand the circadian clock. The inability to collect measurements is stated to clarify that although studies have

been undertaken within the ICU, results only depict a connection to circadian cadence rather than to definitively describe it.

Temperature has been a commonly measured biomarker when evaluating circadian rhythm in ICU patients. In 2013, Gazendam et al. evaluated warmth via temperature-sensitive Foley urine catheter or a rectal tube every 5 minutes for a total of 48 hours (N=21). The authors found both advances and delays in the subjects' rhythmicity, but noted that although a circadian rhythm was present, it was altered from the known rhythm in healthy controls. Furthermore, the degree of abnormality was correlated with the severity of illness observed in subjects. Additional researchers have found similar results within the ICU population (Paul & Lemmer, 2007; Tweedie et al., 1989). In a sample of 24 sedated patients, Paul and Lemmer evaluated circadian rhythmicity via tympanic temperature in patients both with and without cerebral injury. Although abnormalities were identified in both groups, those with cerebral injury had more severe irregularities.

Although cortisol can be reflective of circadian activity, it is not without controversy. In healthy individuals, cortisol, a stress hormone, is released by the adrenals, under direct control of the hypothalamic-pituitary-adrenal axis, which is then linked to the circadian pacemaker (Mazzoccoli et al., 2011). In healthy subjects, a diurnal pattern of cortisol release has been well depicted (Weitzman et al., 1971; Czeisler et al., 1999). However, the ICU department houses patients with a variety of acute medical conditions. Multiple studies have described acute variations and insufficiency of stress hormones related to acute illness (Boonen & Van Den Berghe, 2014; Marik, 2009; Mazzoccoli et al., 2011; Yang et al.,

2014). Furthermore, in his review, Gomez-Sanchez (2013) highlighted a variety of conditions common to the ICU and explained a relationship between each ailment and atypical synthesis, release and/or metabolism of cortisol. Due to these results, one could question the robustness of this biomarker to illustrate a circadian rhythm accurately in this population.

In Paul and Lemmers' study (2007), only 37% of subjects demonstrated a 24-hour cortisol rhythm. In both brain-injured and non-brain-injured subjects abnormalities were observed, though deviations from normal were more pronounced in the cerebral injury group, which the authors state is not uncommon. In yet another neurological ICU setting, Bartanusz et al. (2015) described a loss of diurnal cortisol variation during the first week after acute injury. In contrast to these studies, only one recent study could be found examining cortisol as a means by which to understand circadian rhythmicity in an ICU population. In their study, Frisk et al., (2004) demonstrated that patients still exhibited a cortisol profile, although abnormally patterned and with higher free cortisol than compared to healthy controls. The authors also studied the hormone melatonin, a far more common trend when researching circadian rhythmicity in the ICU, also a topic to be covered further in this chapter.

Melatonin

Physiology.

Melatonin is often understood as a sleep hormone. It should be noted, nevertheless, that melatonin secretion occurs in all species, whether their activity is diurnal or nocturnal (Arendt, 2005). For this reason, it cannot be called a sleep

hormone specifically, but a hormone that assists in synchronization of the circadian rhythm. As previously covered, photic information is transferred from the eye, to the SCN, then further to the PVN; and subsequently to the pineal gland through multi-synaptic pathways (Gooley & Saper, 2011). The hormone melatonin originates from the kynurenine/tryptophan pathway in which synthesis takes place in the pineal gland of the brain the retina and the intestinal tract (Buysse, 2011). The kynurenine/tryptophan path can lead two directions: ultimately resulting in the production of either melatonin or N-acetyltransferase (NAT) (Majewski, Kozłowska, Thoene, Lepiarczyk, & Grzegorzewski, 2016). Through the first pathway, tryptophan is converted via enzymes and oxidization to serotonin, followed by melatonin.

More complex, the second pathway has a multitude of steps before reaching its final product. Researchers have identified metabolites created in the third step of the pathway, when with the interaction of Kynurenine Aminotransferase (KAT) causes the conversion of L-Kynurenine to kynurenic acid, may play a role in brain dysfunction (Adams Wilson et al., 2012; Devlin & Skrobik, 2012). Interestingly, these metabolites have been studied within the ICU, demonstrating an association between elevated plasma kynurenine kynurenine/tryptophan ratio and fewer living days without brain dysfunction (Adams Wilson et al., 2012). The authors stated the need for further research in the area. One can question whether this could be another link between melatonin and delirium, understanding that melatonin synthesis and brain dysfunction related to metabolites can occur from activation of the same pathway.

Through a feedback loop, melatonin synthesis and secretion adjusts activity of the internal pacemaker via day/night signaling. The release of melatonin can be altered by numerous factors, including light exposure, altered sleep and wake activity, seasonal variations and pathologic illness (Guardiola-Lemaitre & Quera-Salva, 2011). Via various signaling pathways, circadian patterns are regulated in organs, tissues and glands, as melatonin receptors are plentiful through the body. When speaking broadly of all mammals, over 100 structures have been found within the central nervous system, which contain melatonin receptors (Guardiola-Lemaitre & Quera-Salva, 2011).

Endogenous melatonin levels are lowest during daytime and increase after dark onset, to a peak, between 0400-0500 (Benloucif, et al., 2008; Lewy, Sack & Singer, 1985), although in one study, subjects showed a bimodal peak (Wehr, et al., 1995). Similarly, the peak sensitivity of the multiple receptors occurs into the evening, paralleling the natural hormone's secretion pattern. In other mammals exposed to exogenous melatonin, it has been observed that the medication does not achieve the desired response when administered during the day, as the necessary receptors have not been optimized at a cellular level (Barrett et al., 2003). Although its secretion plays a role in sleep onset, it cannot be used in isolation to understand sleep. It can, however, serve as a biomarker to observe the circadian rhythmicity of mammals. Compared to other commonly used measurements of rhythmicity such as core body temperature, melatonin has proved to be a more precise biomarker; and as Arendt (2005) states [its secretion is] "the most direct peripheral link to the circadian clock" (p. 292). Measurement

of melatonin can be achieved by collection and analysis of saliva, plasma, or by its key metabolite, 6-sulfatoxymelatonin, found in urine (Guardiola-Lemaitre & Quera-Salva, 2011). Little difference has been found between these three means of measurement; therefore, collection can be achieved through multiple methods, providing added options to researchers (Benloucif, et al., 2008; de Almeida et al., 2011).

Although its role may still appear ambiguous, it may be due to the fact that melatonin has been called a “mysterious” hormone (Turek, 2011, p. 361). Its mystery has been maintained, even with the vast amount of research which has been executed examining its function. One noteworthy observation, however, is the outcome of exogenous melatonin administration in aiding to alter/rest the circadian rhythm, which can attest to its importance (Brzezinski et al., 2005). Numerous studies have examined groups exhibiting altered secretion patterns due to multiple causes, and encouraged re-entrainment of their circadian rhythm via use of supplemental melatonin. These groups include: those who are employed in positions necessitating shift work (Jorgenson & Witting, 1998; Roth, 2012; Sadegniat-Haghighi, Aminian, Pouryaghoub & Yazdi, 2008); patients experiencing delayed sleep phase disorder (Saxvig, et al., 2014); blind subjects with free-running circadian rhythm (Lockley & Arendt, 2007); and as a treatment for jet lag (Petrie, Conaglen, Thompson & Chamberlain, 1989; Srinivasan et al., 2008).

Melatonin Secretion in Intensive Care Patients.

Melatonin levels have been studied in various ICU patient populations. These populations include non-septic and septic patients (Mundigler et al., 2002), patients receiving continuous intravenous sedation (Gehlbach et al., 2012), mechanically ventilated patients (Elliott & Nathaney, 2014) and those with neurological injuries (Seifman, et al., 2014). Some notable studies include those completed by Mundigler et al. (2002) and Olofsson et al. (2004). Mundigler et al. studied a sample of 47 participants, which included septic, non-septic and healthy control subjects. Subjects were not further stratified based upon ventilation status. The results revealed an abolished circadian rhythm in the septic group, but a clear presence of a rhythmic secretion of melatonin in the non-septic group. In Olofsson et al.'s design, eight mechanically ventilated subjects were studied for three consecutive nights. Only one of their patients possessed a rhythmic melatonin secretion. Within their study, some patients were septic and others were not, although the authors state that sepsis was the dominant diagnosis. Shilo et al. (1999) also revealed similar results when studying circadian rhythm in ventilated and non-ventilated groups. In their group of 14 ventilated ICU patients, all had altered melatonin secretion when compared to the control group, and 12 of 14 had an abolished rhythm altogether (1am and 4am mean 6-SMT results of 1786 +/- 1842 ng compared to 5814 +/-3137 ng, respectively).

In a more recent study, Seifman et al. (2014) compared serum melatonin samples in three ICU patient populations: those with traumatic brain injury (TBI) (n=18), those with trauma but no TBI (n=14), and general medical conditions

(n=13). The groups were compared to healthy volunteers and demonstrated a presence of a diurnal rhythm, though markedly altered when compared to the healthy controls. Similarly, Elliott and Nathaney (2014) described a presence of a diurnal melatonin rhythm in a majority (81%) of ventilated patients but noted several abnormalities in their sleep architecture (N=22). Within the several studies that have been highlighted from various ICU patient populations, a common thread of altered melatonin secretion in ICU patients is apparent. Some patient groups exhibit an abolished rhythm entirely, whereas others maintain a diurnal rhythm with considerable differences when compared to healthy controls.

Sleep in the Intensive Care Unit

Sleep Duration and Quality.

Reports have indicated that 61-71% of patients describe considerably restricted sleep (Simini, 1999; Richardson, Allsop, Coghill, & Turnock, 2007) and Nelson (2001) described that cancer patients receiving ICU care found sleep disruption the second most stressful factor in their ICU stay. Only pain superseded the distress experienced by this patient group. Based on the busy and often chaotic environment of critical care, it is no surprise that patients' sleep is altered drastically in this environment. Various reports indicate that patients' Total Sleep Time (TST) varies anywhere from 1 hour 50 minutes to 10.5 hours a night (Appendix A), a far reach from the recommended amount of 7-9 hours per night (Hirshkowitz et al., 2015). It has been reported, however, that even when the duration of sleep has been long, the sleep architecture is abnormal, with sleep mostly composed of stage N1 and N2 sleep, but lacking in sufficient REM sleep

(Elliott, McKinley, & Cistulli, 2011). Furthermore, inaccuracies have been found in the estimations of TST by both patients and nurses (Aurell & Elmquist, 1985; Beecroft et al., 2008; Elliott, McKinley, & Cistulli, 2011; Toublanc et al., 2007), demonstrating that it may be difficult for healthcare providers to assess, and patients to voice whether proper rest is being achieved.

The current study was undertaken to gain a greater understanding of circadian rhythmicity, of which sleep is one component. A variety of studies within the ICU have also examined sleep and sleep deprivation specifically. It should be noted that although interrelated, these are two separate concepts. When examining the feasibility of biomarkers measurement to understand sleep, one could look broadly at biomarkers reflective of circadian rhythmicity or those that are more specific to sleep itself. As detailed above, previous studies have measure TST and architecture within the ICU. It should be noted however, that new modalities are presently being explored which are indicative of physiologic sleep need.

Biomarkers indicative of cellular oxidative stress have demonstrated in several studies to be increased due to total sleep loss. Oxidative stress results from an imbalance which occurs during creation and elimination of reactive oxygen and nitrogen (Turrens, 2003). Known as free radicals, these molecules naturally occur in the body but have been associated with a variety of health conditions and diseases (Dröge, 2002). In 2015, Periasamy, Hsu, Fu, & Liu described elevations in oxidative stress biomarkers in mice including Glutamic oxaloacetic transaminase (GOT), Creatine Phosphokinase-Myocardial Band (CKMB), Tumor

Necrosis Factor (TNF), Total Bilirubin (TIL), Blood Urea Nitrogen (BUN), Lactic Dehydrogenase (LDH) and glutamic Pyruvic Transminase (GPT). The elevations occurred after sleep deprivation up to 72 hours and post deprivation histology of liver, heart, kidney and pancreas cells revealed organ damage resulting from oxidative stress. In addition, Chang et al. (2008) found that not only were the biomarkers of oxidative stress elevated after sleep deprivation, but phosphatidylcholine, an important component of plasma lipoproteins was significantly reduced. The authors describe that the high oxidative stress and ensuing lipid peroxidation could be a cause of metabolic disease. Welje et al. (2015) similarly described a potential link between oxidative stress and metabolic dysfunction in parallel rat and human studies. The authors found notable decreases in oxalic acid and diacylglycerol 36:3 post deprivation which normalized after recovery sleep, both of which are metabolites associated with oxidizing environments. Though not commonly used in clinical settings yet, these tests may provide a greater opportunity to measure sleep and sleep loss in our patients on a molecular level in the future.

Causes of Sleep Disruption.

Multiple factors are responsible for sleep loss in the ICU. This sleep loss is often called sleep fragmentation, disruption or deprivation (either full or partial). The physiologic status of the patient, prior medications and environmental disruptions (including noise, light, and frequent nurse interruptions), all significantly impact a patient's sleep (Drouot, Cabello, d'Ortho & Brochard, 2008; Krachman et al., 1995; Weinhouse & Schwab, 2006). It is noteworthy to

understand that of all the contributing sources of sleep disruption, however, the greatest quantity of research has been devoted to studying environmental disruptions, citing these to cause the majority of sleep interruptions.

Severe illness appears to be independently associated with sleep loss in ICU patients. This conclusion, however, comes from limited data and studies, often with small sample sizes. Gabor et al. (2003) noted that when comparing sleep disruption between seven critically ill, mechanically ventilated patients and six healthy subjects, the healthy group was observed to sleep quite well. This conclusion highlighted that illness severity and mechanical ventilation were specifically identified as causes of sleep disturbance. In a larger study examining sleep in ICU patients (N=22), Fanfulla et al. (2011) observed a direct correlation between increasing pH and incidence of poor sleep. The authors stated that abnormalities in sleep architecture, as measured by PSG recording, were associated to both alkalosis and severity of illness.

Interestingly, Fanfulla and colleagues (2011) found that sleep disturbance was not related to mechanical ventilation. This finding is in contrast to a multitude of studies identifying the use of a ventilator as a cause of sleep deprivation and disruption in ICU (Bosma et al., 2007; Parthasarathy & Tobin, 2002).

In 1994, Meyer et al. measured adverse environmental conditions, including patient interruptions, noise and light levels in several different ICU settings. Their measurement of sound revealed mean daytime peak sound levels between 82.6 +/- 0.1 and 83.1 +/- 0.1 dB. Though peak sound levels at night (2400-0600) were not as high, they remained consistently louder than the

Environmental Protection Agency's (EPA) recommendations at the time of hospital noise no louder than 45 dB in the daytime, and 35 dB at night (Meyer et al.). Outside of North America, there have been many other studies reporting similar findings, with Li (2011) finding a mean dB level at 59.2 in Taiwan; Elliott, McKinley and Eager (2010) describing mean sound levels of 56.22 \pm 1.65 dB in Australia; and Akansel and Kaymakci (2008) measuring sound between 49-89 dB with a mean of 65dB in Turkey. A multitude of studies have consistently reported noise levels above the recommended level (Elliott, McKinley & Eager, 2010), exposing this disturbing trend in ICUs on a global level.

Studies have identified that ambient light in the ICU setting may be detrimental to patient health, though the severity of its influence on health has been debated (Hu, Hegadoren, Wang, & Jiang, 2016; Walder, Francioli, Meyer, Lançon, & Romand, 2000). Although studies are being undertaken to investigate the efficacy of increased natural light and/or cycled lighting (Engwall, Fridh, Johansson, Bergbom, & Lindahl, 2015; Kamdar et al., 2016; Ritchie, Stothard, & Wright, 2015; Verceles et al., 2013), more work may need to be done for healthcare providers to understand the necessity of these interventions (Kamdar et al., 2016).

Contrary results have described the frequency of disruption in an ICU patient's sleep. In 2003, Gabor et al. found that although disruptions due to environmental light, noise and nursing activities were frequent, they only accounted for approximately 30% of sleep disturbances observed in the ICU. In contrast, Celik et al. (2005) noted a significant number of interruptions to patient

sleep as a result of nursing activities. Over a period of three days, the authors found an average of 51 interactions per patient, per night. The majority of these interruptions consisted of endotracheal suctioning, bathing, dressing changes and mouth care. The time in which most of these activities occurred was between the hours of 0200 and 0500; interestingly, around the same time as the natural peak of melatonin, a hormone needed for the promotion of sleep.

Meyer et al., (1994) stated that in the ICU, it is the frequent interruptions that ultimately prevent consolidated sleep periods. According to Le et al. (2012), when examining sleep in multiple ICU subtypes (N=200), nursing staff indicated that 13.9% of their nocturnal nursing interruptions could be omitted. For those activities that could not be omitted, however, nurses felt that the majority of these activities could be safely clustered to encourage patient sleep.

Consequences of Sleep Disruption.

Disturbed sleep in the ICU has been noted to have a detrimental effect on the patient's mind and body health (Weinhouse & Schwab, 2006). While the majority of studies describing the physiologic side effects of altered sleep architecture have not been executed in the ICU environment, the results of these studies will be included when describing the effects of both acute and chronic sleep deprivation by body system. Wang and Greenberg (2013) affirm that the abnormal sleep architecture observed in ICU patients parallels the sleep observed in deprivation studies. Moreover, one could argue that sleep disturbance in the ICU population specifically would yield more severe consequences in comparison to the healthy volunteers in many of the studies; the patients are acutely ill at the

outset, compounding potentially catastrophic health crises, including multisystem failure.

Physiologic Side Effects Related to Sleep Disturbance

Sleep disturbance has a global effect on the body and these consequences have been well described. One well-known outcome of sleep fragmentation is alteration in immune function (Born et al., 1997; Bryant & Curtis, 2013). Besedovsky, Lange and Born (2012) described that immune function is tightly coupled with the circadian rhythm. Fragmented sleep results in immunological alterations in the following ways: decreased resistance to illness (Cohen et al., 2009; Orzech et al., 2013; Wilder-Smith et al., 2013), impairment of immunological memory (Spiegel, Sheridan & Van Cauter, 2002), decreased response to both Hepatitis B vaccination (Prather et al., 2012) and Influenza vaccination (Benedict et al., 2012). Interestingly, the reverse is also true. In 2011, Lange et al. established that immunological memory could be boosted by ample sleep post-vaccine.

Cardiac side effects have been observed in both acute and chronic sleep loss. The most significant cardiac risk factors are related to inflammatory biomarkers. A substantial amount of evidence has pointed to a link between sleep loss and an increase in pro-inflammatory cytokines (Chennaoui, et al., 2011; Meier-Ewert, et al., 2004; van Leeuwen et al., 2009), increased C-Reactive Protein (CRP) with total sleep loss (Meier-Ewert, et al., 2004), and increases in both CRP and Interleukin 17 (IL-17) observed after reduced sleep loss for two nights (van Leeuwen, et al., 2009).

Conflicting information exists regarding whether sleep loss causes a decrease of both the hypoxic and hypercapnic response in ventilated patients (Spengler & Shea, 2000; White, Douglas & Pickett, 1983). In patients with pulmonary disease, sleep loss results in decreases in both Forced Expiratory Volume (FEV) and Forced Vital Capacity (FVC), small changes were also noted in the Maximal Voluntary Ventilation (MVV) and the Maximal Inspiratory Pressure (MIP), although the authors found these changes consistent with reduced muscular strength (Phillips, Cooper & Burke, 1987) which was previously described by Chen and Tang (1989).

Various studies have reported impairments in glucose tolerance and potential weight gain in response to sleep loss (Gottlieb, 2005; Schmid et al., 2011; Spiegel, Leproult, & Cauter, 1999; Van Cauter, 2011). More recently, Schmid et al. (2011) found similar results, citing that glucose tolerance was impaired and insulin sensitivity blunted after food intake in sleep-restricted individuals. Within an ICU population, these alterations in blood glucose could have significant consequences as stringent control of blood glucose in this population was associated with decreased organ dysfunction, ICU stay and mortality (Krinsley, 2004).

Neurologic side effects may be the most identifiable results of sleep loss, although the majority of these are easily reversible when provided with adequate sleep (Bonnet, 2011). With acute sleep loss, changes such as blurred speech, hand tremor and mild nystagmus have been noted (Koller, et al., 1968, as cited in Bonnet). Other neurologic alterations include changes in pain perception, core

body temperature and decreased task performance, decision-making ability and memory (Bonnet). Additionally, Dawson and Reid (1997) compared acute sleep deprivation to intoxication, noting similarities between the impedance on judgment and cognition. It is not only acute sleep deprivation that is known to hinder cognition; chronic sleep loss is known to affect cognitive performance tests and mood to a greater degree than acute sleep loss (Pilcher & Huffcutt, 1996).

The Relationship Between Sleep and Delirium

Within the ICU environment, it is chronic sleep loss that may pose the greatest challenge to patients, as its relationship to delirium is well known (Watson, Ceriana, & Fanfulla 2012). The relationship is complex; as Figuero-Ramos states (2009), it is a reciprocal relationship, in which sleep loss has been identified as a causative factor in the development of delirium, and vice versa. Furthermore, contributing factors, including neurotransmitter imbalance and irregular melatonin secretion, are a shared link between both sleep deprivation and delirium. Watson, Ceriana and Fanfulla state that although a link is clear, it is difficult to discern whether a causal relationship exists or that simply a physiologic pathway is shared, common to both sleep and delirium.

In 2001, Shigeta et al. (2001) evaluated delirium in postoperative patients and observed that those with delirium had lower plasma melatonin than their baseline level preoperatively, and those without delirium had normal plasma levels. In another study, Miyazaki et al. (2003) found a significant correlation in post esophagectomy patients. In their sample of 41 participants, 26.8% developed delirium, and of those subjects, a majority of them demonstrated significantly

lower serum melatonin levels than the non-delirious subjects. It should be noted that although the authors chose not to use a validated tool to diagnose delirium, they modeled their diagnosis criteria after the CAM-ICU.

Delirium

Definitions.

Multiple descriptions of delirium exist. Of these, however, those descriptions found in the Diagnostic and Statistical Manual of Mental Disorders (DSM) and the International Classification of Disease (10th edition) remain the most recognized globally (Theuerkauf, Guenther & Putensen, 2012). According to the fifth and most recent edition of the DSM (V), delirium is a disturbance of consciousness and cognition that cannot be explained by a pre-existing condition of dementia. This state develops over shorter periods of time (for example, hours to days rather than over weeks and months). Furthermore, based upon assessment, diagnostic testing and physical exam, the condition is secondary to illness, substance use, medication use, exposure to a toxic substance or a combination of these elements.

Although the DSM-V is the most current edition, it should be noted that the vast majority of research has been completed using its predecessor, the DSM-IV. As the criteria for diagnosis remain largely unchanged between the editions, the diagnostic criteria for the 4th edition has not been included. The tenth revision of International Classification of Disease (World Health Organization, 1993) has described delirium “not induced by alcohol or other psychoactive substances” (pg. F05) as characterized by six features. These features include: A) a clouded

consciousness; B) a disturbance of cognition; C) motor disturbances; D) interruption of sleep; E) rapid onset and fluctuation of symptoms; and lastly, F) objective evidence pointing towards underlying cerebral or systemic disease. In their 2012 review, Theuerkauf, Guenther and Putensen described that use of the DSM criteria is a more inclusive tool, capturing a higher incidence of delirium in acute patients, whereas the ICD-10 was better suited in delirium identification postoperatively, rather than for broad usage.

Delirium Subtypes.

Based upon assessment with standardized screening tools, delirium has been described by subtype: hypoactive, hyperactive, or a mixed-type (Peterson, et al., 2007). Hypoactive delirium is characterized by a decrease in movement, responsiveness and attention, whereas the hyperactive form includes restlessness, agitation, hyperactivity, and at times, hallucinations. The mixed subtype combines aspects from both the hypo and hyper subtypes (Lipowski, 1989; Pandharipande et al., 2007). Peterson et al. described that of these three types mixed-type is the most prevalent (54.9%), followed by hypoactive and hyperactive (43.5% and 1.6%, respectively) (N=614). The outcome from Pandharipande et al. (2007) differed substantially. The authors found when evaluating 100 ICU patients in either a surgical group or trauma group that delirium was present in 70% of their entire population. Furthermore, in the surgical group subtypes (total delirium 73%) results were as follows: hypoactive 64%, mixed 9% and hyperactive 0%. Within the trauma group subtypes (total delirium rate 67%) results were as follows: hypoactive: 60%, mixed 6% and hyperactive 1%.

Prevalence.

The issue of delirium in the ICU is a significant one. It has been reported that between 16-87 % of patients develop this condition during their ICU stay (Balas et al., 2007; Bergeron, Dubois, Dumont, Dial, & Skrobik, 2011; Ely et al., 2001; LingeHall, Smulter, Engstrom, Gustafson, & Olofsson, 2013; Pipanmekaporn et al., 2014). Typically, the condition develops within 24-48 hours post-admission, with a duration ranging from 1-4 days, although in post-operative ICU delirium has been reported lasting weeks, including up to and post-discharge (Inouye et al., 2007; Pisani et al., 2007; Pisani et al., 2009; Theuerkauf et al., 2012).

A well-known correlation exists between delirium and ventilated inpatients. In their seminal paper in 2001, Ely et al. (2001) described the incidence of delirium in 85% of ventilated ICU patients, a nearly twofold increase than observed in non-ventilated patients (Thomason, et al., 2005). Additionally, studies have affirmed a significant correlation with sedative medication, analgesics and delirium (Pisani et al., 2009; Riker et al., 2009). A 2006 study by (Pandharipande et al. demonstrated that sedatives and benzodiazepine medications increase the risk of delirium significantly. The authors found that use of the medication Lorazepam, commonly used for anxiety in ICU patients, was independently associated with a 20% increased risk of developing delirium. Although the authors also noted an increase in delirium rates in those using analgesic medications such as Fentanyl and Morphine and the sedative Propofol, the results were not statistically significant. Due to this

relationship, the difficulty of maintaining pain control and comfort for ICU patients while trying to minimize their delirium risk presents a challenge for many healthcare providers.

Diagnosis.

Until recent years, assessment and diagnosis of delirium was completed using the criteria set forth by either the DSM or ICD, completed by a physician. Development of a variety of tools within the past decade has allowed for bedside health care professionals, including nurses, residents and ICU medical staff, to now complete the assessment and identify those suffering with delirium.

To date, approximately 11 tools are well known for the purpose of bedside identification and diagnosis of delirium (Wong, Holroyd-Leduc, Simel, & Straus, 2010). Most frequently used worldwide include the Intensive Care Screening Delirium Checklist (ISDSC), the Nursing Delirium screening Checklist (NUDESC), a tool developed specifically for use by the bedside ICU nurse, and Confusion Assessment Method for ICU (CAM-ICU). Although all three of these tools are validated means for assessment, studies have demonstrated a wide variation in their sensitivity and specificity.

In 2001, Bergeron et al. established a sensitivity and specificity of 99% and 64% respectively when assessing use of the ISDSC in 93 ICU patients. Although this tool revealed an excellent sensitivity due to its 64% specificity, the authors concluded at that time that it was recommended as a screening, but not diagnostic, tool. Gusamo-Flores, Salluh, Chalhub and Quarantini conducted a meta-analysis of numerous delirium screening tools, revealing a pooled sensitivity

and specificity of the ISDSC of 80% (95% CI: 77-82.6) and 95.9% (95% CI: 94.8-96.8), respectively.

The Nu-DESC has shown strength in a multitude of settings, including cardiac surgery (Lingehall, Smulter, Engstrom, Gustafson, & Olofsson, 2013), the Post Anesthesia Care Unit (PACU) (Radtke et al., 2008), the postoperative Surgical ward (Radtke et al., 2010) and in geriatric inpatients (Leung et al., 2008). Although the sensitivity and specificity of the tool has remained relatively consistent, with reports of sensitivities between 83%-95% and specificities of 87%-92%, it is not until very recently that the tool was validated for use in an ICU setting (Luetz et al., 2010; Radke et al., 2008; Radke et al., 2010). The wide range of sensitivities and specificities noted may be attributed to the extensive array of environments in which it was tested. Furthermore, its ability to capture specific subtypes of delirium may have placed the tool at a disadvantage when looking at statistical data alone. Lingehall et al. (2010) reported that although the NU-DeSC is a robust tool for screening and diagnosis, its rigor lies in identification of the hyperactive subtype specifically.

Several studies have compared bedside screening and diagnostic tools for delirium, showing conflicting results. Two studies were completed by Radtke et al. (2008, 2010) comparing the diagnostic ability of the CAM-ICU, Nu-DESC and the DDS. Both projects took place in non-ICU environments utilizing the DSM-IV as the gold standard by which to make comparisons. Authors in both studies concluded that the NU-Desc was the most sensitive tool for both screening and diagnostics. It should be noted, however, that although both the CAM-ICU and

DDS had good specificity, in one study the sensitivity of the DDS was a dismal 14% (Radke et al., 2008). Leutz et al. (2010) found similar results in their prospective cohort study (N=156). The authors concluded that ultimately, the use of the DDS as a screening tool should be discontinued based upon the poor validity established in the study.

Some studies have asserted that the CAM-ICU was the superior instrument when compared to the ISDSC for non-psychiatric-based screening and diagnosis of delirium. In 2012, three studies illustrated that both tools are suitable for their intended purpose, although two of three authors reported superiority with the CAM (Gusamo- Flores, Salluh, Chalhub & Quarantini; Tomasi et al.). In the third study, conducted by Fagundes et al., both tools were found to be equal, establishing strength within different data sub-sets. Though the trial boasted a large sample size (N=595), the design was lacking in a gold standard against which to measure the tools, thereby not genuinely validating either instrument.

Confusion Assessment Method for ICU

As stated, there have been conflicting reports of superiority in delirium screening tools. There is little doubt from the literature, however, that the CAM-ICU is a robust tool. Wong et al. (2010) completed a large-scale systematic review comparing the usage of bedside tools for diagnosis of delirium in adults. Twenty-five prospective studies were included (N=3027) and compared for accuracy of diagnosis (only studies which used the DSM as a reference standard were included), ease of use and time to administer. The authors concluded that the CAM-ICU was the most suitable tool for the charge when compared to 10 others.

Pooled sensitivity was reported at 86% (95% CI: 74-93) and specificity 93% (95% CI: 87-96).

Subsequent recent systematic reviews have found similar results. Gusamo-Flores, Salluh, Chalhub & Quarantini; Tomasi et al. (2012) described the CAM-ICU as “excellent for the detection of delirium in critically ill ICU patients, regardless of the sub group evaluated.” In their review, both the CAM-ICU and the ICDSC demonstrated high sensitivities, and specificities, with the CAM-ICU being superior with 80% specificity (95% CI: 77.1-82) and 95.9% specificity (95% CI: 94.8-96.8). In a systematic review and meta-analysis, Shi et al (2013) described a pooled sensitivity and specificity of 81% (95% CI: 57-93) and 98% (95% CI: 86-100) respectively. The authors noted in their discussion that the CAM-ICU shows a higher specificity than sensitivity (a theme common among systematic reviews) and therefore should not be used as a replacement for clinical judgment but rather as an adjunct to clinical evaluation.

Within the literature, the strengths of the CAM have been consistently described. Multiple studies have documented the ease of use of the tool, requiring staff only minutes to administer and the ability of multiple professionals to administer the screening (Radke et al., 2008; Shi et al., 2013; Wong et al., 2010). Furthermore, it has been highlighted that CAM-ICU has been translated into multiple languages, which has made the tool available at a transcontinental level (Pipanmekaporn et al., 2014). Assortments of limitations of the tool have been expressed in many studies. Neufeld et al. (2013) described high specificity and a 28% sensitivity when utilizing the tool to evaluate post-operative delirium in the

elderly. The authors concluded that in this specific population the tool could not adequately identify delirium.

In 2012, Woien, Balsliemke and Stubhaug stated that deep sedation of their patients made diagnosis difficult, finding that staff under-diagnosed delirium in their subject group. Using a commonly used and validated ten-point scale for sedation (-5 to +5), the Richmond Agitation Scale (RASS) (Sessler et al., 2002), the authors found that diagnosis of delirium was possible at a RASS score of 0 (alert and calm), but difficult in the sedated range of patients (-1 to -5), although the CAM-ICU manual indicates the test is valid to a RASS score up to and including -3. These complications were also found by Leutz et al., 2010, who stated that due to sedation, eye contact and verbal response was difficult. Moreover, the authors suggested a possible revision of the sedation inclusion requirements, stating that trained professionals found it difficult to assess the CAM-ICU score at a sedation level of RASS -3.

Other concerns regarding the use of the tool include inability for nurses to correctly identify delirium while using the bedside tool. Mistarz et al. (2011) found a significant discrepancy between CAM-ICU results from a bedside nursing assessment compared to a formal delirium assessment. El Hussain, Hirst and Salyers (2015) describe this inability for bedside nurses to identify delirium as a common phenomenon, which ultimately reduces the quality of nursing care. The authors suggest that a more thorough process is used in validation of tools and that proper educational programs be offered before implantation of the tool begins.

Swan (2014) confirmed the need for educational training for use of bedside tools. The author found that when studying educational programs for CAM-ICU use, that the percent of Unable to Assess (UTA) results decreased by 41% after implementation of a short educational session once the tool was already in use. As a result of the decreased UTAs, a greater number of patients were more accurately identified as either positive or negative for delirium using the CAM-ICU.

The importance of the circadian rhythm during sleep is well documented. In an inpatient setting it has been established that sleep plays an important physiologic role and shares a unique, yet little understood relationship with delirium. As significant morbidities are associated with delirium in ICU, a greater understanding of the causes and process of the condition could provide better outcomes for ICU patients.

Chapter 3: Method

In this methods chapter we will detail the methodology utilized to collect and analyze both urinary 6-sulphatoxymelatonin samples and Confusion Assessment Method for ICU (CAM-ICU) scores in ICU patients. This description includes the details of the processes used to recruit participants and collect and analyze the data. The design chosen for the study was a prospective cohort study. No previous literature existed examining the relationship between urinary melatonin and CAM-ICU scores within an acutely ill population. This specific design supports analysis of both circadian rhythmicity, as reflected by melatonin, and delirium score over the period of enrollment. Ideally, this shall accurately paint a picture of whether delirium is present concurrently when greatest deviations in circadian rhythmicity occur. A pilot trial facilitated an understanding of this relationship, which may direct a larger scale study in this area at a later date.

Setting

For the sample collection, an adult medical ICU in a tertiary care centre in Winnipeg, Manitoba was chosen. The unit consisted of eleven functional beds, nine allotted to patients with medical and surgical needs, and two for cardiac care. The patients admitted to this unit were both ventilated and non-ventilated, requiring an advanced level of care for acute health crises, including pneumonia, sepsis, and multi-system failure. The unit was selected for the research study for two reasons: 1) as a medical ICU, the patient population includes diverse health conditions and needs. As we were not aiming to block enrollment based upon

health condition but rather to examine the melatonin and CAM-ICU scores on the “typical” patient in ICU, this setting was ideal; and 2) the ICU is the largest in Manitoba, allowing for enrollment to occur at a steady rate.

Sampling

A small sample size was utilized in this pilot study. A goal of 10 patients was planned in order to identify circadian rhythm via melatonin secretion, positive CAM-ICU scores and the feasibility of regular urine measurements and CAM assessments in this population. With the enrollment of ten subjects, assuming all four days of data could be collected would have yielded a total of forty-eight samples each, a total of four hundred and eighty urine samples. Eight CAM-ICU scores could be collected during this time, with a total of eighty for all subjects.

Inclusion criteria for the research study were as follows:

- a. 18 years or older
- b. Ability to provide informed consent or was in the presence of a family member and/or guardian who had the ability to provide consent
- c. Admission to the Medical ICU of Health Sciences Centre
- d. English speaking patient and/or family members

Exclusion criteria were as follows:

- a. Previously diagnosed sleep disorder
- b. Class II or greater liver disease
- c. Patients with expected admission ≥ 4 days.

Measurement Tools

The measurement tools for the research study included both biological and psychosocial end points to evaluate changes to our primary and secondary outcome respectively. Additionally, data pertaining to participant demographics was collected for the purpose of sub-group analysis.

Urinary Melatonin.

For the research study, the researchers sought to use a form of measurement that accurately reflected human circadian rhythmicity, while not causing any greater harm to these acutely ill individuals. For this reason, we decided to collect urine samples for measurement of 6-sulphatoxymelatonin (aMT6s), the urinary metabolite of melatonin. These samples were processed using the Enzyme Linked Immunosorbent Assay (ELISA) method (GenWay Biotech #40-371-25006) (Appendix B).

Urine samples were collected from urine collection bags attached to previously inserted Foley urinary catheters, an intervention considered as standard of care within the ICU. Collection was completed by the Principal Investigator (PI) every two hours during the day and by the bedside nurse at night. The collection of urine specifically over other forms of body fluids for melatonin analysis caused no discomfort and the least disruption to the patients. The urine samples were then stored for up to twelve hours in the designated laboratory refrigerator in the ICU, and then moved to a -80 °C degree freezer housed in the Research Centre of the affiliated hospital.

Analysis of the melatonin samples was completed with an Enzyme Linked Immunosorbent assay (ELISA) technique using melatonin ELISA kits (GenWay Biotech #40-371-25006, San Diego, CA). The mean values of the duplicates were used for analysis. The analytical sensitivity of the ELISA procedure (limit of detection) is expected at 1.0 ng/mL, with an intra-assay sensitivity range between 5.2-204 ng/mL and a coefficient of variation between 5.2-12.2% (melatonin sulphate ELISA, GenWay Biotech, 371-25006).

CAM-ICU Screening Tool.

The Confusion Assessment Method for ICU (CAM-ICU) (Ely et al., 2001) scale was used to identify the presence or absence of delirium in the participants. The scale is scored as a dichotomy, either positive or negative. Four features are measured on the worksheet: a) acute or fluctuating mental status; b) inattention; c) altered level of consciousness, and; d) disorganized thinking. Each feature was scored if present, and the overall CAM-ICU was scored as positive if features one and two plus three or four were present.

Each of the four features, if present, is given a checkmark. Once all four features have been assessed, the overall CAM-ICU score is calculated. To be considered as having a positive CAM-ICU score (the patient is delirious), the patient must meet the following criteria: Feature one (acute or fluctuating mental status) plus feature two (inattention) and either feature three (altered level of consciousness) or feature four (disorganized thinking) present (Appendix C). The standard of care in the ICU is for the bedside nurse to complete and document the CAM-ICU assessment every four hours. For this study, two CAM-ICU

assessments were performed daily (0800, 1600) were transcribed onto our study worksheets. Ely et al. described the total time to complete the assessment as two minutes with a standard deviation of one minute. As completion of the CAM-ICU is the current standard of care in the ICU, for the purpose of our study, these assessments caused the participants no additional duress nor did they impose any additional work for the bedside nurse. Furthermore, as the staff was previously trained in use of this tool, no further evaluation or training of its use was provided before commencement of the study.

Several studies have validated the CAM-ICU as a viable tool in the measurement of delirium in the ICU (Ely et al., 2001; Flores, Salluh, Chalhoub, & Quarantini, 2012). The authors of the tool describe sensitivities of 100% and 93% and specificities of 98% and 100% (Ely et al., 2001). In a systematic review, comparing the efficacy of the tool to another popular delirium rating scale, the CAM-ICU was found to have pooled sensitivity and specificity of 80% and 95.9%, respectively (Flores et al., 2012). Furthermore, the use of this tool has become so widespread, published reports detail its use internationally (Tomasi et al., 2012; Woien, Balsliemke, & Stubhaug, 2012). Within the Winnipeg Regional Health Authority, the CAM-ICU has become the standard of care in delirium assessment in the ICU, with its use adopted in all ICUs in the region.

Participant Demographic Information.

The participant demographic worksheets (Appendix D) is a tool that was created by the PI to collect information about the participants, which allowed for a better understanding of the diversity of the sample and to ensure the sample was

representative of previously described ICU populations. Additionally, this information allowed for later sub-group analysis. The tool consisted of four questions, including age, sex, admitting diagnosis and daily sedative use. Age, sex and admitting diagnosis were collected by the PI at the time of enrollment. Sedative use was collected daily by the PI including the name of each sedative medication and the total 24-hour dose.

Preparation for the Research Study

Prior to commencement of the study, approval was obtained from the University of Manitoba's Education/Nursing Research Ethics Board (ENREB) (Appendix E) and Intensive Care Patient Care Manager (Appendix F). Permission was granted for use of the laboratory at the John Buhler Research Centre (Appendix G). A general permission for use of the CAM-ICU model was granted by the authors and is available on their website (Appendix H).

Staff Readiness.

Several documents were created by the principal investigator in order to facilitate proper execution of the research study. First, before the study commenced, a poster was placed on the education board on the unit, detailing the purpose of the study, the protocol and the contact information for the PI. Secondly, data collection sheets were created for the research study to capture daily therapy time, urinary sample collection time and daily CAM-ICU scores (Appendix C).

Once site approval had been given, three staff information sessions were provided to staff during their monthly staff meetings to prepare the staff for the

upcoming research study. Management, nursing staff and administrative staff were invited to hear details regarding the purpose of the study, enrollment procedures and approximate length of both the enrollment and treatment phases. Staff were encouraged to ask the PI questions in order to provide clarification of study procedures, facilitating enrollment and the initiation of treatment. By holding repeated sessions during meetings, a greater number of staff were able to attend and gain an understanding of the upcoming study.

Upon commencement of the study, the PI explained the research study to each bedside nurse and other health care team members who were interested. Additionally, the PI encouraged the staff to phone with any concerns or questions related to the study or nighttime data collection.

Procedure

Enrollment.

Within 24 hours of admission, patients were approached by the Clinical Resource Nurse (CRN) to determine interest in participating in the research study. When patients were cognitively unable, or in the presence of a language barrier, the patient's family was utilized as translators or to provide consent if necessary, as per the ENREB approval. Once interest in the study was determined, a research assistant described the study to the patient and/or family members, provided them with a written consent form and obtained consent. Additionally, patients were approached to provide assent in all circumstances (Appendix I, J).

The assent process ensured that researchers were making participants aware of the planned urine collection, and providing an opportunity to decline if

they so desired. In all instances distress to the participants was minimized.

Participants, and families when applicable, were provided with signed copies of their consent forms, which included contact information for the PI should they have any further questions or desire to discontinue participation in the study.

Sample Collection.

Within an hour of enrollment, the first urine sample was collected for aMT6s analysis. Collection of the urine samples was obtained passively from urine in the pre-existing urine collection bag attached to the participant's urinary catheter. The sample was collected into 5mL aliquot tubes. Samples were collected every two hours on each participant until the end of four days of enrollment (48 samples), removal of their urinary catheter or discharge. The time required to collect the urine sample was estimated to be approximately one to two minutes and caused no discomfort to the patient. Collection was done with consideration to eliminate or minimize disruption or inconvenience to patient, family and staff. After completion, the numbered urine samples were then stored temporarily in the designated laboratory refrigerator in the ICU, then moved by the PI to a -80 C freezer housed in the lab at the affiliated hospital, and analyzed at a later time.

Sample Analysis.

Due to financial constraints, samples were analyzed from time points every four hours, rather than every two as originally intended, this provided a total of seventy-nine samples. All samples for analysis were thawed, mixed and 1mL samples were taken from the 5mL aliquot tubes. The 1mL samples were then

frozen at -80C until analysis. For each ELISA, samples were thawed and 10uL was collected and diluted into 500uL of assay buffer. ELISA was then carried out according to manufacturer's recommendation. At the end of the procedure, plates were read at an absorbance of 450nm. For each plate a new standard curve was developed by plotting the optical density of the standards (y-axis), against the concentration (x-axis). Four replications of each sample were completed to ensure accuracy of the measurement, from which a cumulative standard curve was developed. The curve was developed based upon the reciprocal of the absorbance values and the equation of the line was calculated. A typical calibration curve and expected results has been detailed by the manufacturer (Appendix B). From the calculations completed, based upon dilution, the concentration in each sample could be determined.

CAM-ICU Assessment.

During the first nursing assessment following enrollment, the CAM-ICU score was recorded by the bedside nurse in keeping with standard practices. Twice daily, (~0800 and 1600) the CAM-ICU score was determined and recorded by the bedside nurse. Each score was additionally transcribed onto the study data collection sheets by the P.I. The CAM-ICU assessments followed the standard of care and did not deviate from the usual schedule. Each assessment took approximately 1-2 minutes to complete. All CAM assessments were completed by the nursing and/or medical staff rather than members of the research team.

Data Analysis

The data analysis plan was determined with the assistance of a statistician. Data was entered into PROC MIXED of the SAS program (version 9.3) by the PI, who also completed the majority of the statistical calculations. A significance level of 0.05 was assigned, and 10 participants were desired for this pilot project. It was not possible to estimate an effect size in advance due to the complex nature of the repeated measures experiment.

For the primary outcome, the goal of statistical analysis was to measure the standardized difference between the standard or “normal” melatonin concentration levels and the melatonin concentration levels found in the urine samples of the patient. Intra and inter subject comparison of melatonin concentration levels were also completed. The mean, minimum and maximum aMT6s results (in ng/mL) were calculated for each subject for a daytime (DT) (1000-2200) and nighttime (NT) (2200-1000) period. Furthermore, the overall mean of the aMT6s quantity, the minimum mean, maximum mean and standard deviation for the DT period was then compared between days 1-4 for each subject. Furthermore, the same descriptive statistics were calculated using data from all four DT periods for each subject and used for inter subject comparison.

The same calculations were completed using data from the NT period; once data was available from both DT and NT, a night/day ratio (NDR) was calculated. The NDR was calculated by examining smaller increments, the Night Period (NP) which were the hours between 2300-0700 and the Day Period (DP), the hours between 1100-1800. The NDR was calculated by averaging the first two

nighttime collection periods and dividing that number by the mean of the daytime period for data from the first 24-hours. The calculation was completed to parallel those done by Mahlberg et al. (2006), completed in healthy subjects to use as a comparison. These time intervals were reflective of the normal peak and trough of urinary melatonin (Figure 2), and enabled comparison to studies examining both healthy subjects and those in the ICU who also used the same time window. Additionally, an overall 24-hour mean melatonin calculation was completed by averaging both the DT and NT means for each subject for further comparison to previous studies again both in healthy subjects and within the ICU population.

To determine if the data was significantly different than results of previous studies, a comparison of confidence intervals was undertaken. Although limited by a small data set, using a significance level of 0.05, a 95% Confidence Interval (CI) was developed using the mean, standard deviation and subsequently the standard error of four specific time points. These time points were selected based upon previous research examining aMT6s in both healthy adults and ICU patients. The previous literature reported mean aMT6s excretion during the period of 0700-1100, 1100-1800, 1800-2300 and 2300-0700 in healthy (Mahlberg et al., 2006) and ICU subjects (Gehlbach et al., 2012). Comparing the CIs of this study and the previous two studies allowed the PI to determine whether the intervals overlapped, or demonstrated significant difference from aMT6s in healthy subjects, and or aMT6s excretion patterns in the previous ICU study.

When analyzing the CAM-ICU scores, the total number of positive scores for each participant during the study was calculated. Although the score is

calculated in a numeric fashion, it is reported categorically as either positive or negative. For this reason, relative frequency of the positive scores was calculated for each subject and compared to previous literature to determine if the study sample was representative of the “typical” ICU population.

Using a prospective cohort study design, enrollment of a small sample of patient participants from one medical ICU was undertaken to determine the feasibility of future research in this area on a larger scale, while further understanding patient circadian rhythmicity and delirium. Frequent urine-based melatonin sampling provided a more detailed picture of the circadian rhythm in this patient population and delirium was measured by CAM-ICU assessment; both assessments were completed in conjunction with standard of care practices, causing no additional distress to the patients.

Chapter 4: Results

This chapter shall offer a summary of the results of the research study. This will include a description of participant demographics and an overview of findings relating to the primary and secondary hypotheses of the study.

Subject Demographics

In total, 11 participants were enrolled into the study; 7 females (64%) and 4 males (36%, with a mean age of 53.5 years (SD 13.4). No pattern was observed in admitting diagnosis and almost all subjects came in due to different causes. All subjects were discharged from the unit either during data collection or shortly following it, with the exception of one subject upon whom care was withdrawn and who passed away shortly after. Once the decision was made to withdraw care, no further urine samples were collected. A total of 325 aliquots were collected. The number of days of specimen collection ranged from two to six, with 8 to 46 samples per participant collected in total. Due to financial constraints and missing data, a subset of the participant data and a q4h aliquot sample frame was selected for this pilot analysis.

The following analysis is based on the five participants analyzed. The five participants were selected with the greatest number of consecutive samples, without any missing data. Therefore, the final study sample consisted of five participants, all of whom were admitted to the medical ICU of a tertiary care centre in Manitoba. Subjects were approach and enrolled on their first day of admission into the ICU. The five participants were comprised of three males, and two females. All participants in the final analysis were between the ages of 50 to

65 years of age with a mean age of 55.8 years (SD 4.1). Of the participants enrolled, greater than half were not on sedative infusions and greater than half of the subjects were ventilated (See Table 1).

Table 1

Characteristics of Study Sample

Sample Characteristics (N = 5)

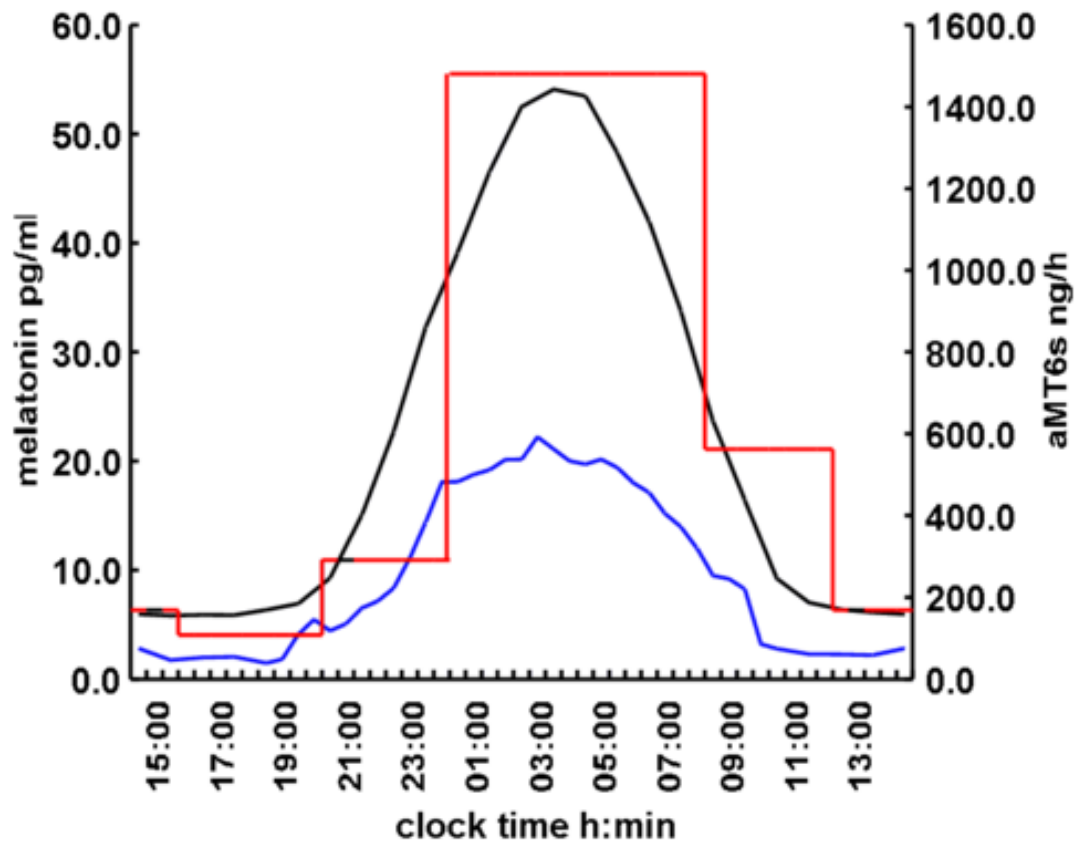
Variable	Frequency	Percent
Sex		
Males	3	60
Females	2	40
Age (years)		
50 - 55	2	40
>55 - 60	2	40
> 60 - 65	1	20
Sedated	2	40
Non-sedated	3	60
Ventilated	3	60
Non-ventilated	2	40

Primary Study Hypothesis

Hypothesis one: Patients admitted to the ICU will demonstrate an altered circadian rhythm as evidenced by q2h urinary melatonin analysis. Urine collection for the subjects was q2h; however, due to financial constraints, measurement of the urine samples was only possible at four-hour intervals. Figure 2 depicts the normal curve of melatonin excretion, including 6-

sulphatoxymelatonin (aMT6s), the urinary metabolite of melatonin in healthy subjects over a 24-hour period. Note that average concentrations of melatonin in plasma (black, n=133), saliva (blue, n=28), and urine red (n=88).

Figure 2. Melatonin Secretion in Humans in Plasma, Urine and Saliva



Reprinted from L. J. De Groot, G. Chrousos, K. Dungan, K. R. Feingold, A. Grossman, J. M. Hershman, . . . A. Vinik (Eds.), *Endotext*. South Dartmouth (MA): MDText.com, Inc. Retrieved from <http://www.ncbi.nlm.nih.gov/books/NBK279108/>. Reprinted with permission.

Note the distribution of the curve in the healthy subjects; a large surge of melatonin excretion is observed during the nighttime period, but remains low during the day. In comparison, none of the subjects in the study demonstrated normal aMT6s distributions over the three-day period. Each subject showed great variability in their urinary melatonin patterns, demonstrating a dissimilar picture from a healthy curve.

Table 2

Daytime (1000-2200) Mean, Minimum and Maximum aMT6s Values by Subject and Day (ng/mL)

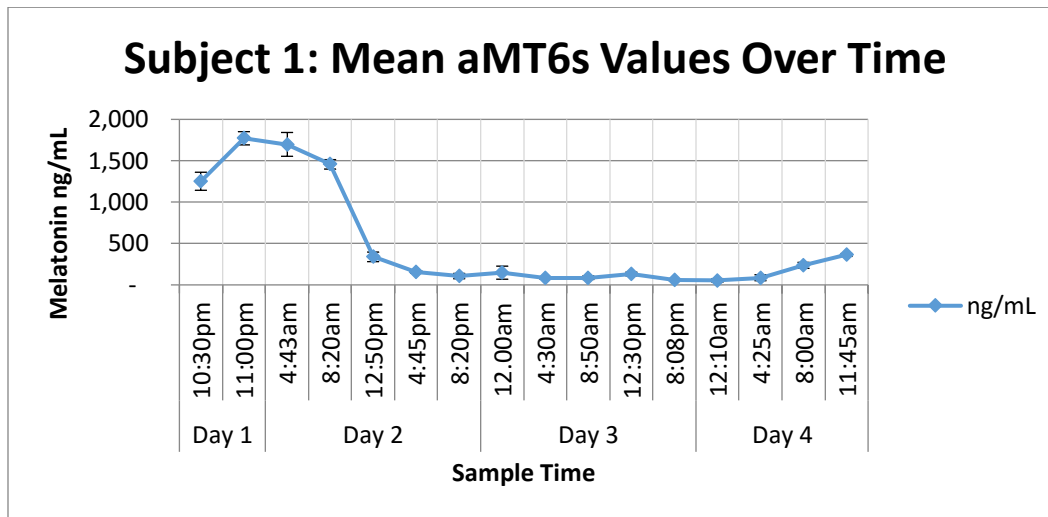
Day Number	Sample Mean (ng/mL)	Minimum Mean (ng/mL)	Maximum Mean (ng/mL)
Subject One			
1	1252	1252	1252
2	201	107	339
3	94	59	132
4	362	362	362
Cumulative	456	445	521
Subject Two			
1	41	41	41
2	254	194	363
3	73	19	149
4	169	169	169
Cumulative	134	105	180
Subject Three			
1	230	152	284
2	319	309	325
3	188	98	238
4	528	528	528
Cumulative	316	271	344
Subject Four			
1	584	554	606
2	558	298	821
3	1495	1242	1819
Cumulative	894.1	698	1082
Subject Five			
1	939	595	1120
2	2395	2084	2927
3	2149	1013	3399
Cumulative	1828	1231	2482

Table 3

Nighttime (2200-1000) Mean, Minimum, and Maximum aMT6s Values by Subject and Night

Night Number	Mean (ng/mL)	Minimum (ng/mL)	Maximum (ng/mL)
Subject One			
1	1642	1457	1772
2	105	82	83
3	125	87	86
Cumulative	625	542	647
Subject Two			
1	105	61	148
2	558	360	726
3	160	92	210
Cumulative	274	171	361
Subject Three			
1	219	165	273
2	220	164	275
3	206	170	242
Cumulative	215	166	263
Subject Four			
1	434	372	505
2	1538	1184	1747
3	1683	1683	1683
Cumulative	1218	1079	1311
Subject Five			
1	2079	2079	2079
2	1509	1459	1559
3	2461	2074	2930
4	1961	1961	1961
Cumulative	2003	1893	2132

Figure 3. Subject One Mean aMT6s Values Over Time

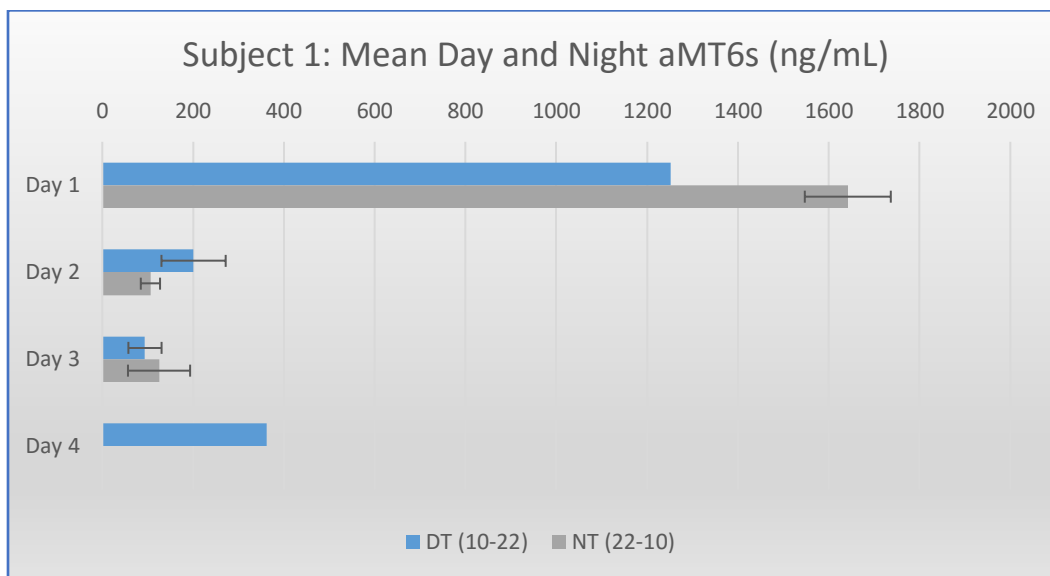


Subject One's aMT6s data was analyzed in a broad fashion [Daytime (DT=1000-2200) and Nighttime (NT=2200-1000) means, 24-hour means and Night Day Ratios (NDRs)] and also in smaller, more precise time periods in four hour intervals which we utilized later for t-test analysis. Daytime and Nighttime means were calculated for each participant based upon an average of samples collected during 1000-2200 and 2200-1000, respectively. The DT and NT mean demonstrated significant intra-subject variation.

Subject One had the most aberrant rhythm of all that were analyzed. The visual representation demonstrates one peak on the first day, followed by a plateau for the remaining days of collection. The patient was enrolled later in the day, which attributes to a lone sample for the first daytime period. A cumulative mean was calculated for all DT (1000-2200) samples. The descriptive statistics for days 1-4 were as follows: Mean 477 ng/mL (SD 528 ng/mL), minimum 59 ng/mL, maximum 1252 ng/mL, and range 1193 ng/mL.

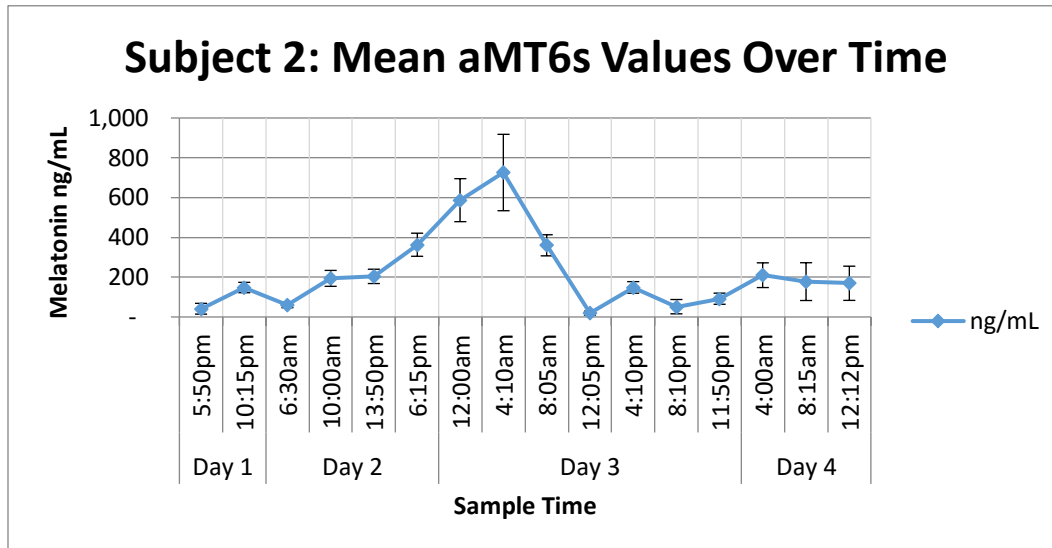
NT calculations (2200-1100) for subject one revealed greater intra-subject differences than daytime measurements. A cumulative mean was calculated for all nighttime (2200-1000) samples. The descriptive statistics for nights 1-3 were as follows: mean 625 ng/mL (SD 881 ng/mL), minimum 82 ng/mL, maximum 1771 ng/mL, and range 1689 ng/mL. Figure 4 illustrates both the daily and nightly mean aMT6s measurements for Subject One.

Figure 4. Daytime and Nighttime aMT6s Mean Values for Subject One



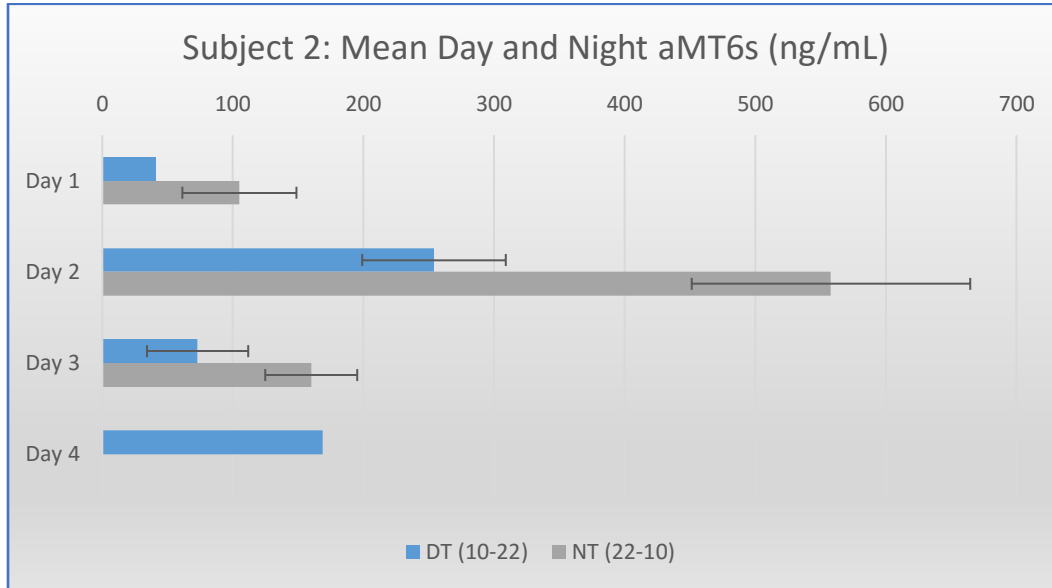
The calculated 24-hour means per study day were as follows: Day 1:1447 ng/mL, Day 2: 153 ng/mL, Day 3: 110 ng/mL and; Day 4: 362 ng/mL, cumulative 24-hour mean: 518 ng/mL. Using the NP and DP mean the NDR was be 3.7.

Figure 5. Subject Two Mean aMT6s Values Over Time



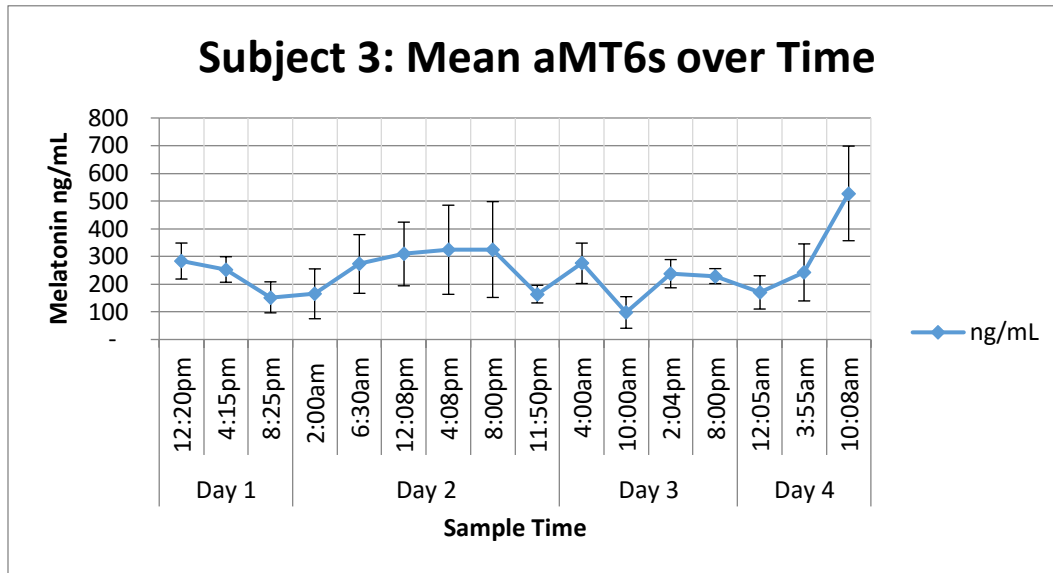
As noted in Figure 5, the rhythm demonstrated by Subject Two was quite aberrant. A cumulative mean was calculated for all DT (1000-2200) samples. The descriptive statistics for days 1-4 were as follows: mean 134 ng/mL (SD 97 ng/mL), minimum 41 ng/mL, maximum 363 ng/mL, and range 322 ng/mL. A cumulative mean was calculated for all nighttime (2200-1000) samples. The descriptive statistics for nights 1-3 were as follows: mean 274 ng/mL, (SD 247 ng/mL), minimum 61 ng/mL, maximum 726 ng/mL, and range 665 ng/mL. Figure 6 illustrates both the daily and nightly mean aMT6s measurements for Subject Two. Note that, based upon the time that enrollment began, Subject Two did not have a Day 4 NT period.

Figure 6. Daytime and Nighttime Means for Subject Two



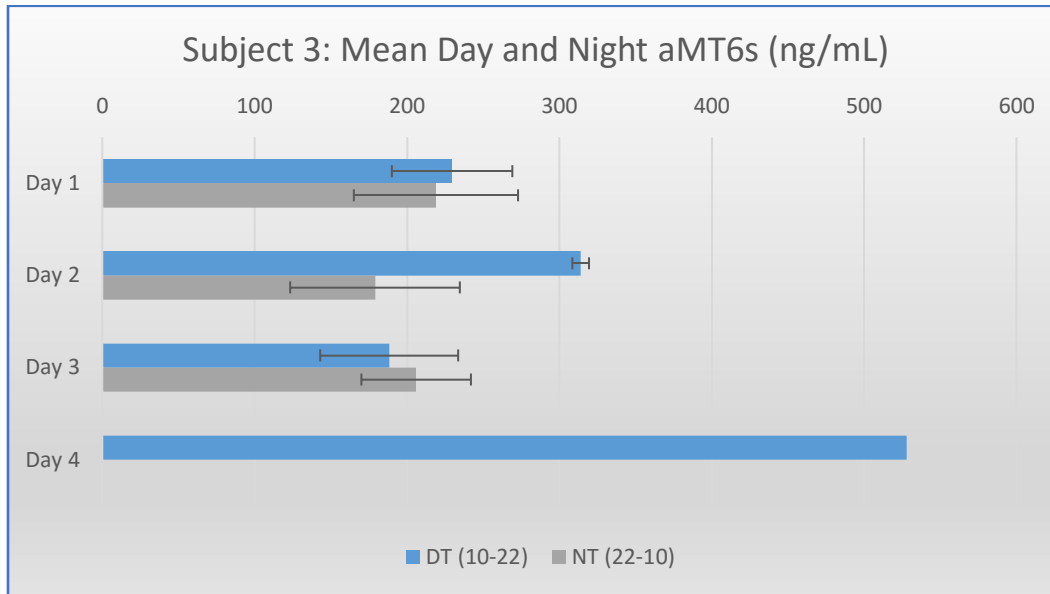
Both a 24-hour aMT6s and NDR were calculated. The calculated 24-hour means per study day were as follows: Day 1: 73ng/mL, Day 2: 406 ng/mL, Day 3: 117ng/mL, Day 4: 169 ng/mL; and cumulative 24-hour mean: 191 ng/mL. Based upon the NP and DP means, the NDR was 1.8.

Figure 7. Subject Three Mean aMT6s Values Over Time



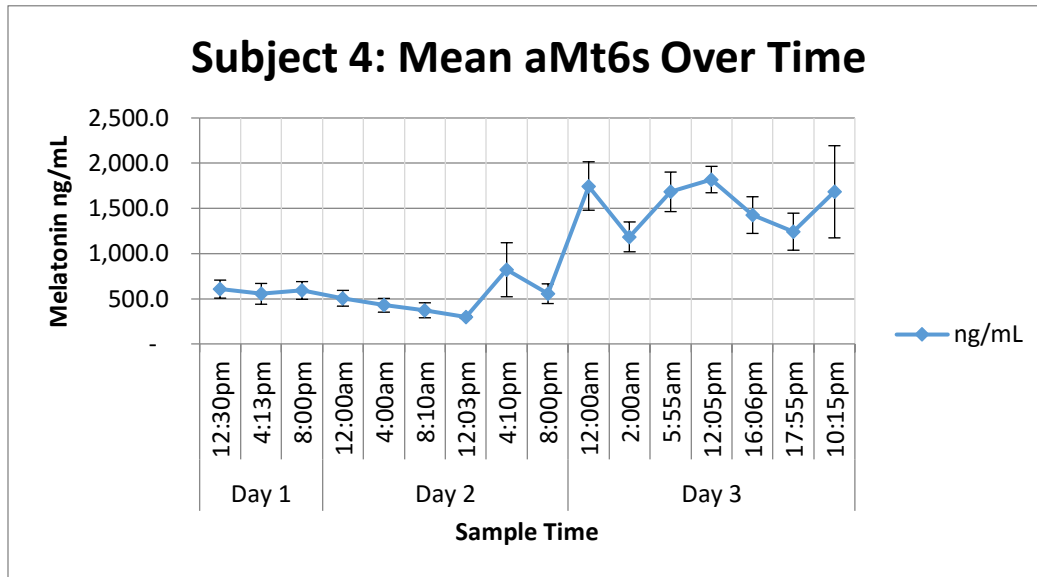
The descriptive statistics for Subject Three for days 1-4 were as follows: mean: 316 ng/mL (SD151 ng/mL), minimum 98 ng/mL, maximum 528ng/mL and range 430 ng/mL. A cumulative mean was calculated for all nighttime (2200-1000) samples. The descriptive statistics for nights 1-3 were as follows: mean 215 ng/mL (SD 8 ng/mL), minimum 164 ng/mL, maximum 273 ng/mL and range 109 ng/mL. Figure 8 illustrates both the daily and nightly mean aMT6s measurements for Subject 3. Note that, based upon the time that enrollment began, Subject Three did not have a Day 4 NT period.

Figure 8. Daytime and Nighttime Means for Subject Three



Both a 24-hour aMT6s and NDR were calculated. The calculated 24-hour means per study day were as follows: Day 1: 225 ng/mL, Day 2: 260 ng/mL, Day 3: 197 ng/mL and; Day 4: 528 ng/mL and 24-hour cumulative mean: 305 ng/mL. Note that, due to Day 4 only having one measurement which was quite high both the DT mean and the 24-hour mean are significant outliers from previous days. Based upon the NP and DP, the NDR was calculated to be 0.69.

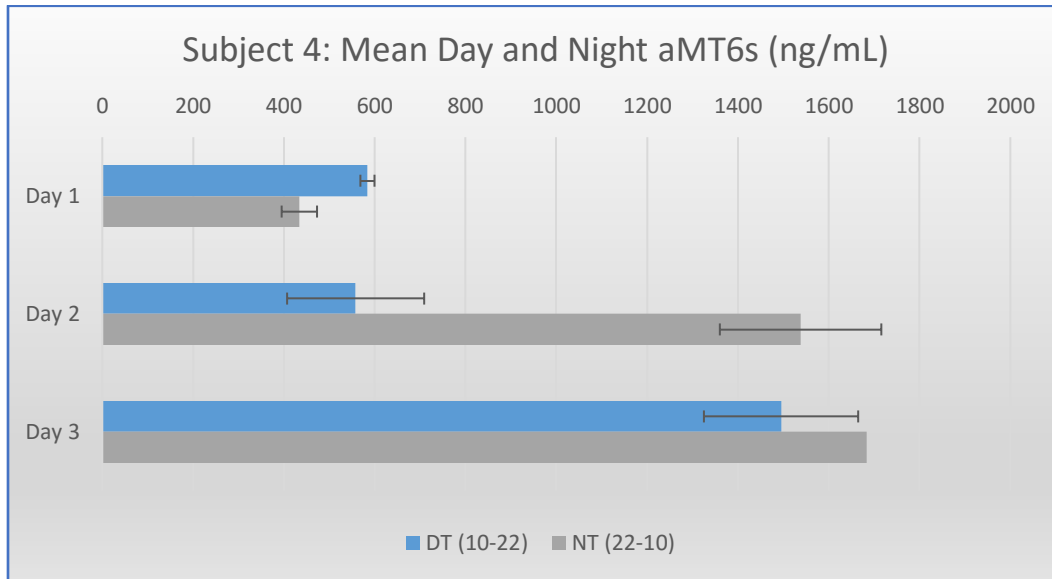
Figure 9. Subject Four Mean aMT6s Values Over Time



A cumulative mean was calculated for Subject Four for all DT (1000-2200) samples. The descriptive statistics for days 1-4 were as follows: Mean 879 ng/mL (SD 534 ng/mL), minimum 298 ng/mL, maximum 1819 ng/mL, and range 1521 ng/mL.

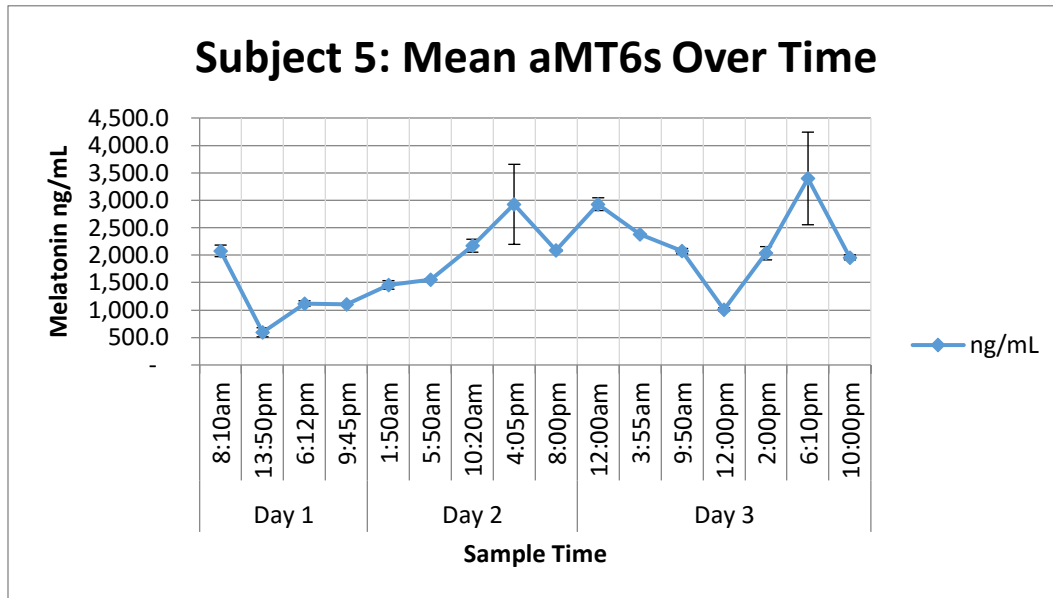
NT calculations (2200-1100) for Subject four revealed greater intra-subject differences than daytime measurements. A cumulative mean was calculated for all nighttime (2200-1000) samples. The descriptive statistics for nights 1-3 were as follows: mean 1218 ng/mL (SD 683 ng/mL), minimum 372 ng/mL, maximum 1747 ng/mL and range 1375 ng/mL. Figure 10 illustrates both the daily and nightly mean aMT6s measurements for Subject Four.

Figure 10. Daytime and Nighttime Means for Subject Four



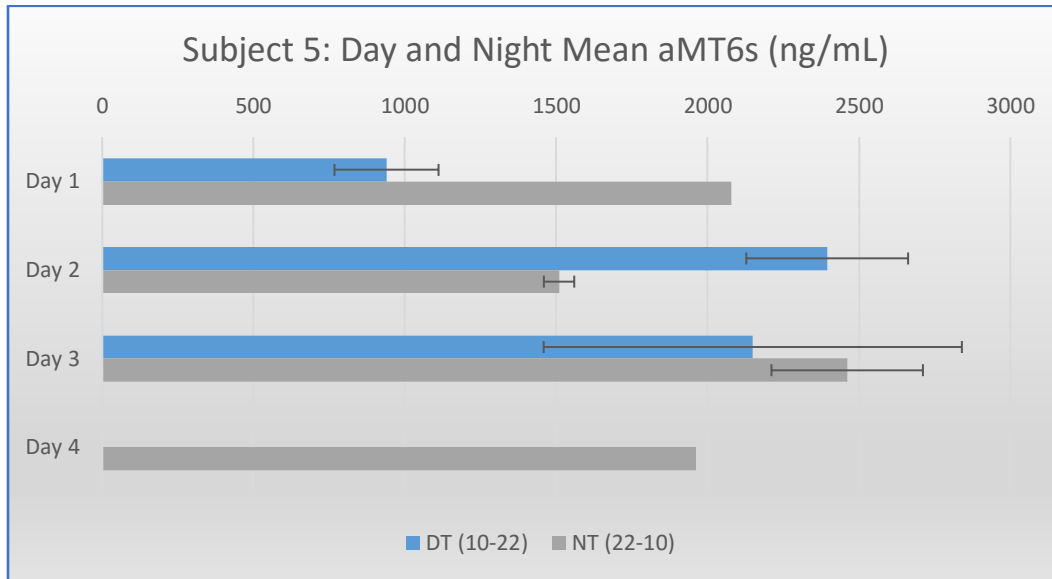
Both a 24-hour aMT6s mean and a night/day ratio (NDR) were calculated. The calculated 24-hour means per study day were as follows: Day 1: 509 ng/mL, Day 2: 1048 ng/mL and Day 3: 1059 ng/mL. The cumulative 24-hour mean: 872 ng/mL. Based upon NP and the DP, the NDR was calculated to be 1.8.

Figure 11. Subject Five Mean aMT6s Over Time



A cumulative mean was calculated for Subject Five for all DT (1000-2200) samples. The descriptive statistics for days 1-3 were as follows: Mean 1828 ng/mL (SD 779 ng/mL), minimum 595 ng/mL, maximum 3399 ng/mL, and range 2864 ng/mL.

A cumulative mean was calculated for all NT (2200-1000) samples. The descriptive statistics for nights 1-4 were as follows: mean 2003 ng/mL (SD 392 ng/mL), minimum 1459 ng/mL, maximum 2430 ng/mL, and range 1471 ng/mL. Figure 11 illustrates both the daily and nightly mean aMT6s measurements for Subject Five. Note that, based upon the time that enrollment began, Subject five did not have a Day 4 DT period.

Figure 12. Daytime and Nighttime Means for Subject Five

Both a 24-hour aMT6s mean and a night/day ratio (NDR) were calculated. The calculated 24-hour means per study day were as follows: Day 1: 1509 ng/mL, Day 2: 1952 ng/mL, Day 3: 2305 ng/mL and Day 4: 1961 ng/mL. The cumulative 24-hour mean: 1932 ng/mL. Using the first two NP and DP, the NDR was calculated to be 0.7.

Inter-Subject Comparison

In order to understand the differences between the Subjects, the mean DT, NT, 24-hour aMT6s mean in ng/mL and the NDR were plotted and are represented in Figure 13 and Figure 14, respectively.

Figure 13. Daytime aMT6s Mean for all Subjects

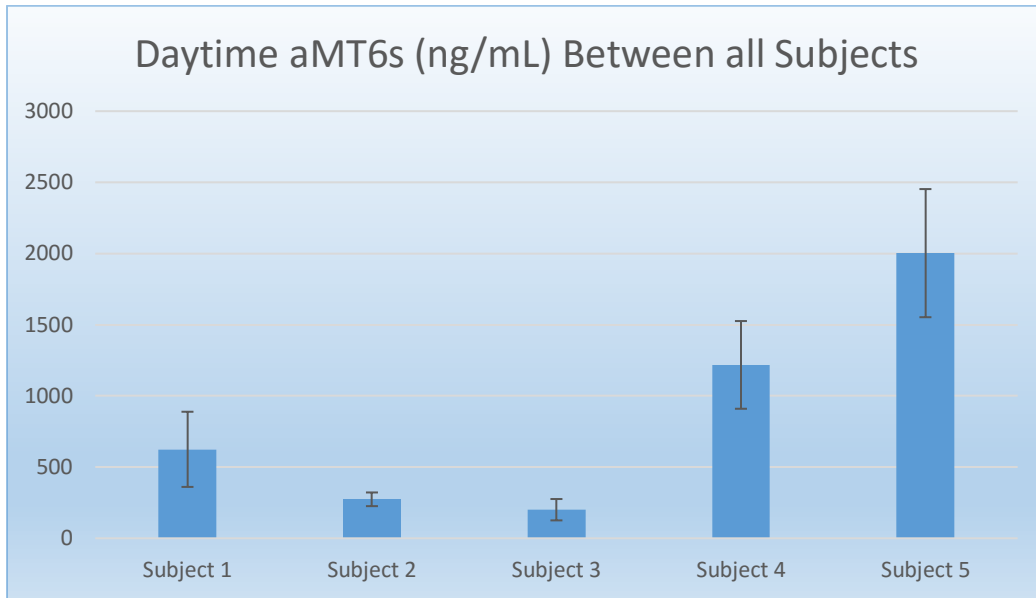


Figure 14. Nighttime aMT6s Mean for All Subjects

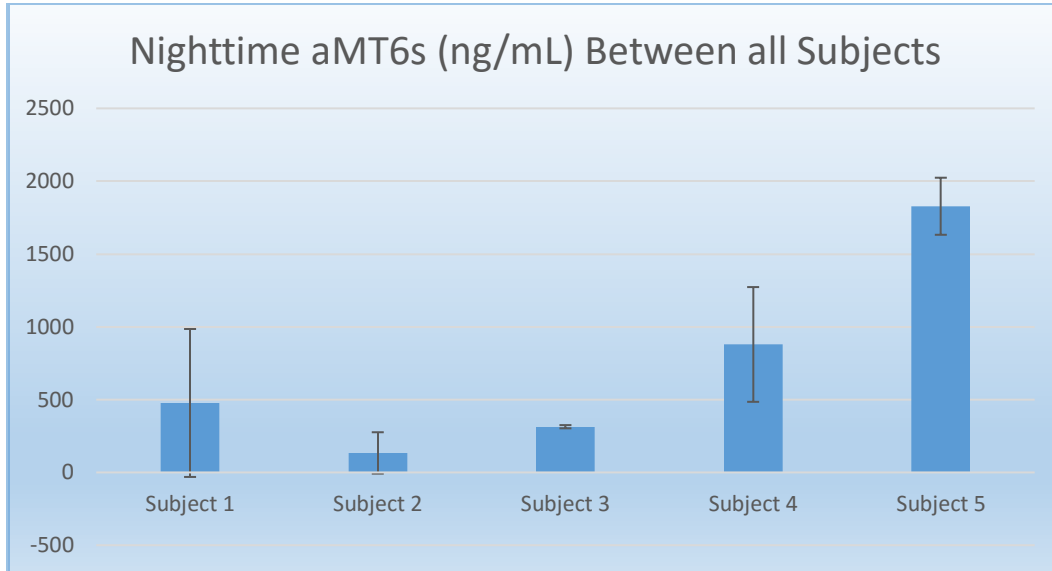


Figure 15. 24-Hour Total aMT6s Mean for all Subjects

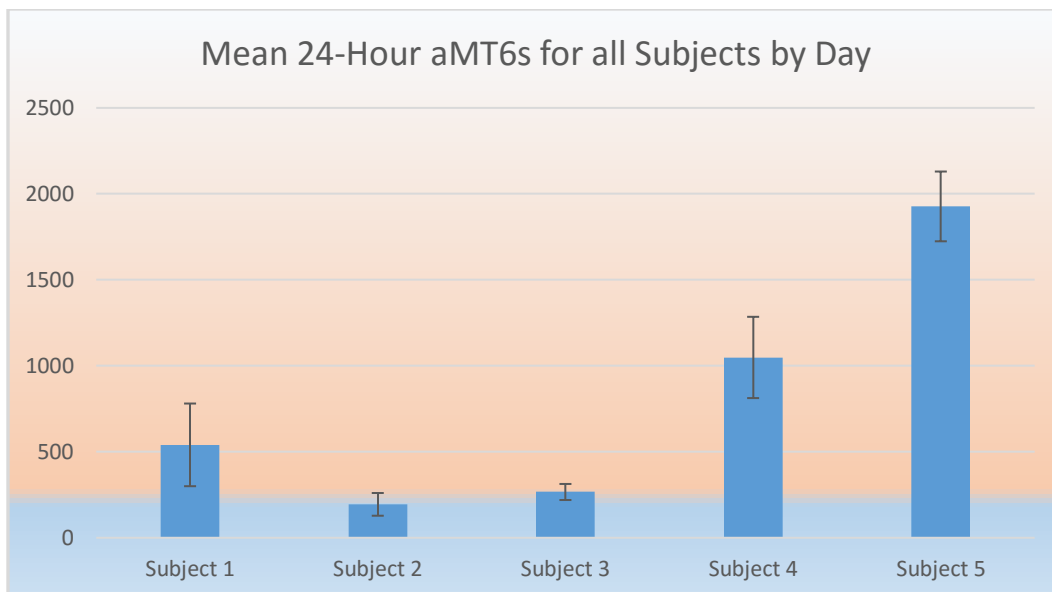
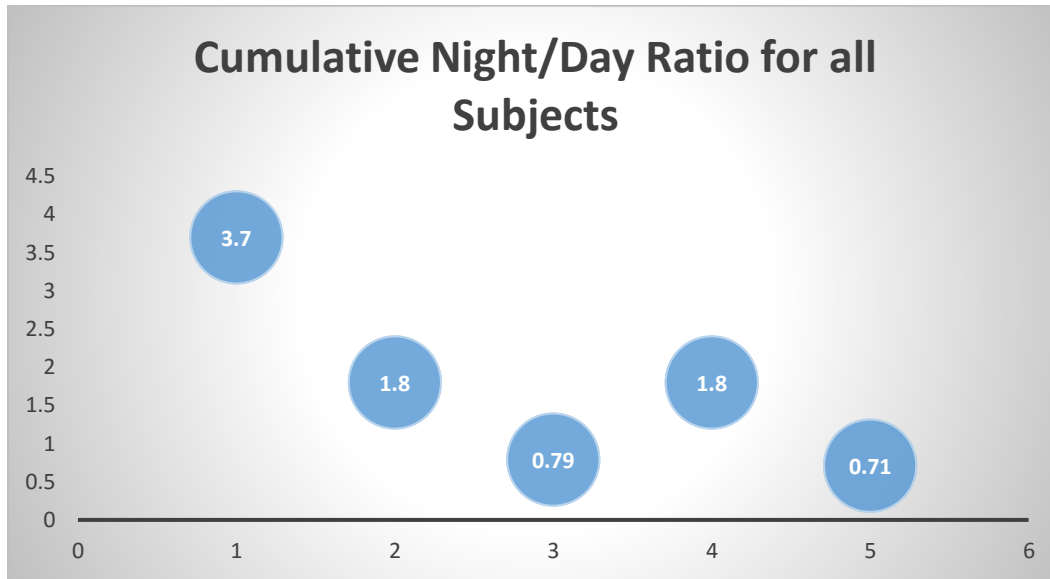


Figure 16. Night/Day Ratio for all Subjects for the First 24-hours



Trying to establish whether a significant difference in aMT6s excretion was observed when comparing with healthy and previous ICU Subjects, a CI and subsequently a t-score for each time period was calculated. The first study day was divided into four periods for analysis: Evening Period (1800-2300), Night Period (2300-0700), Morning Period (0700-1100) and Daytime Period (1100-1800) calculations were based upon data collected during these four intervals of the first twenty-four hours only, as this was equal to the comparative data. Table 4 presents the mean, SD, standard error and upper and lower confidence interval (CI) limit of this study's first 24 hours of aMT6s data of all subjects.

Table 4

Cumulative aMT6s Values for all Subjects in the First Twenty-four Hours

	Mean Evening	Mean Nighttime	Mean Morning	Mean Daytime
<i>Mean</i>	650.8	794.2	1025.6	346.5
<i>SD</i>	519.2	764.54	897.4	237.3
<i>Standard Error</i>	232.2	341.9	448.7	106.1
<i>95%CI lower limit</i>	195.7	124.0	146.1	138.5
<i>95%CI upper limit</i>	1105.9	1464.3	1905.1	554.5

Table 5 and 6 depict data from the current study compared to healthy subjects and previously described ICU subjects, respectively. Table 5 illustrates the total sample mean aMT6s values found in this study for four time periods versus comparable reported data of healthy subjects (Mahlberg et al., 2006). A t-value was calculated based upon the CI of each time period for the 24 hours, and from the t-value it could be determined when comparing to the previous literature if the data from this study was significantly different. A large variation can be observed in the standard error due to two factors: a) with a small sample size, the mean is more sensitive to each measurement than in a larger sample; and b)

variation between the time periods would even be expected in healthy individuals based upon the release of melatonin, even more so, however, in ICU subjects as deviation from normal melatonin excretion was expected.

Table 6 depicts the data compared to the previous literature from ICU subjects. A t-value was calculated based upon the CI of each time period for the first 24 hours, and from the t-value it could be determined when comparing to the previous literature if the data from this study was significantly different.

Table 5

Comparison of Mean aMT6s to aMT6 of Healthy Volunteers During Day One Evening, Nighttime, Morning and Daytime Period

Time Period	ICU Subjects (N = 5)			Healthy Adults (N =75) Mahlberg et al. (2006)			Standard Error	t-value	P value	Significant Difference (alpha 0.05)
	Mean (Ng/mL)	Lower 95% CI	Upper 95% CI	Mean (Ng/mL)	Lower 95% CI	Upper 95% CI				
Evening Period	650.87	195.77	1105.97	525	433.57	616.43	46.65	0.53	0.05	No
Nighttime Period	794.18	124.03	1464.33	2004	1751.2	2256.8	128.98	-3.31	0.05	Yes
Morning Period	1025.63	146.11	1905.15	1175	987.15	1362.85	95.84	-0.33	0.05	No
Daytime Period	346.54	138.51	554.57	473	418.91	527.09	27.60	-1.15	0.05	No

Note: Evening Period = 1800-2300; Nighttime Period = 2300-0700; Morning Period = 0700-1100; Daytime Period=

1100-1800

Table 6

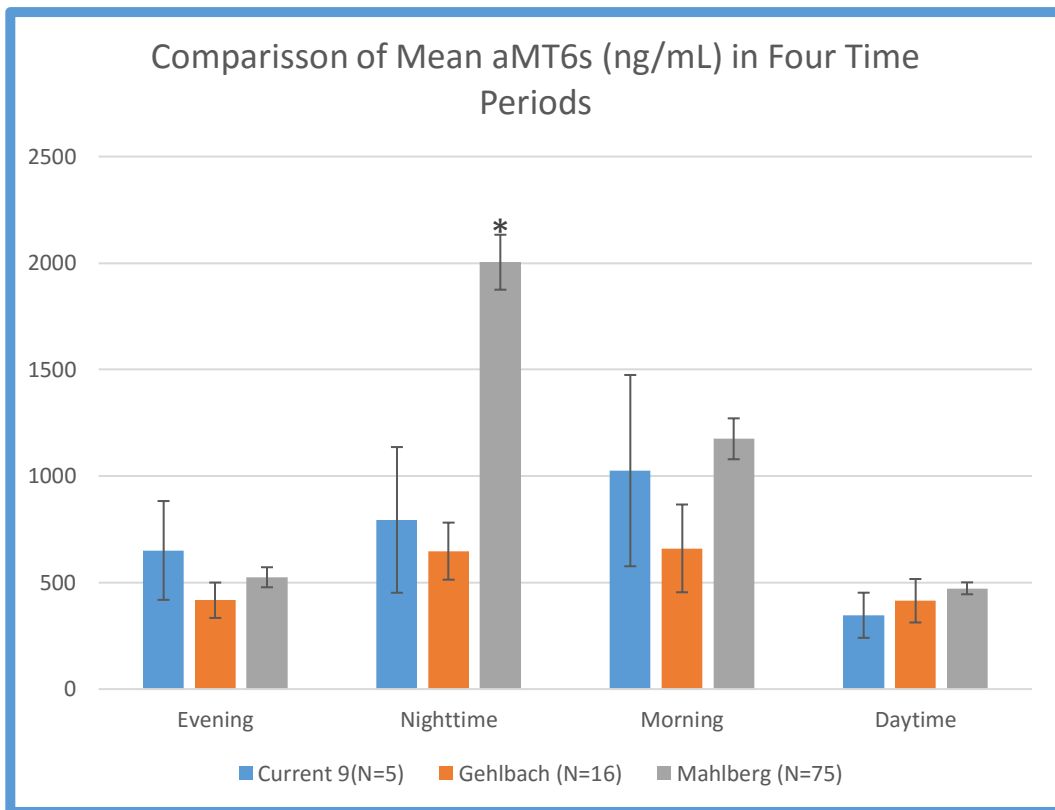
Comparison of Mean aMT6s to aMT6 of ICU Subjects During Day One Evening, Nighttime, Morning and Daytime Period

Time Period	ICU Subjects (N = 5)			ICU Subjects (N =16) Gehlbech et al. (2006)			Standard Error	t-value	P value	Significant Difference (alpha 0.05)
	Mean (Ng/mL)	Lower 95% CI	Upper 95% CI	Mean (Ng/mL)	Lower 95% CI	Upper 95% CI				
Evening Period	650.87	195.77	1105.97	417.1	253.64	580.56	83.4	0.95	0.05	No
Nighttime Period	794.18	124.03	1464.33	647.5	385.40	909.60	133.73	0.40	0.05	No
Morning Period	1025.63	146.11	1905.15	660.5	256.35	1064.65	206.2	0.74	0.05	No
Daytime Period	346.54	138.51	554.57	414.7	212.97	616.43	102.93	-0.46	0.05	No

Note: Evening Period = 1800-2300; Nighttime Period = 2300-0700; Morning Period = 0700-1100; Daytime Period = 1100-1800

Figure 17 provides a visual depiction of the mean aMT6s (ng/mL) for the current study population, compared to the mean aMT6s for the same time period in both previous ICU subjects and healthy volunteers. Note, however, that these data come from the first 24 hours in each study only, and therefore cannot provide an overall picture of aMT6s throughout the study period.

Figure 17. Comparison of Mean aMT6s (ng/mL) in Four Time Periods



Note: * denotes significantly different findings from current study

Secondary Study Hypothesis

Hypothesis two: A correlation will be observed between circadian dysregulation and delirium as evidenced by a presence of atypical melatonin secretion and positive CAM-ICU score in ICU subjects. During the research study, the CAM-ICU score was to be reported on the subject's in-hospital patient flow sheet, as per usual care. Although the practice of scoring and recording the CAM score was already the standard of care within the unit, the P.I. also spoke with each bedside nurse providing care to encourage this score being collected with each assessment (every four hours).

Five subjects were included in the analysis. Each subject was enrolled in the study for approximately three days, thereby providing a total of 8 CAM scores per subject (including their baseline score before their initial blood draw). Surprisingly, none of the subjects were scored with a positive score, indicating delirium, during the study. Results of CAM-ICU scores for the study sample have been presented in Table 7.

Table 7

CAM-ICU Completions and Scores for the Study Sample

<i>Subject Number</i>	<i>Number of Assessments Completed (%)</i>	<i>Missed Scores- No Reason Given (%)</i>	<i>Not Done Scores Attributed to Being Either “Too Sedated/ Not Applicable” Scores (%)</i>	<i>Positive Scores (%)</i>	<i>Negative Scores (%)</i>
<i>1</i>	2/8(25)	3/8	3/8(37.5)	0	2/8(25)
<i>2</i>	0/8(0)	1/8	7/8(87.5)	0	0/8
<i>3</i>	8/8(100)	0/8	0/8(0)	0	8/8(100)
<i>4</i>	7/8(87.5)	1/8	0/8(0)	0	7/8(87.5)
<i>5</i>	0/8(0)	3/8	5/8(62.5)	0	0/8
<i>Cumulative</i>	<i>17/40(42.5)</i>	<i>8/40(20)</i>	<i>15/40(37.5)</i>		

It was noted by the P.I. that in the five subjects that were recruited but were not included in analysis, these participants also commonly had missing, “too sedated” or “NA” scores.

Based upon urine sampling q4h the five subjects enrolled demonstrated aberrant circadian rhythmicity when compared to visual depictions of healthy subjects as reflected by aMT6s analysis. With the exception of the nighttime period, these differences were not found to be statistically significant from previous healthy controls. No significant differences were found compared to

previous ICU subjects related to aMT6s excretion, demonstrating similarity between these two groups. Due to a lack of completed CAM scores, it is unknown if any subjects in the study were in fact delirious. Furthermore, due to this missing CAM data, no relationship between anomalous melatonin levels and delirium could be inferred.

Chapter 5: Discussion

The purpose of this study was to gain a better understanding of the circadian rhythmicity of ICU patients and furthermore, to understand the relationship between circadian rhythmicity as reflected by melatonin secretion and delirium. A significant amount of research has been devoted to examining both sleep and delirium in the ICU population. However, this is one of the few studies to explore urinary melatonin secretion over multiple time points and multiple days, and the first known study to attempt to evaluate a relationship between melatonin and delirium. The data supports that based on urinary melatonin secretion evaluation, subjects in the ICU experience significantly aberrant circadian rhythms compared to healthy individuals and that there is variation in urinary melatonin levels between and within individuals as measured by aMT6s. The limited available CAM-ICU data did not support statistical evaluation between melatonin and delirium measures. This chapter will provide discussion relating to subject demographics, the primary and secondary study hypotheses and an evaluation of the present study, including its strengths and limitations.

Subject Demographics

As reported in the results chapter, 11 participants were enrolled in the study but due to missing data and financial constraints, a subset of 5 participants, with the most complete data were selected to form the final study sample for this analysis. This final sample included both men and women, with a mean age of 55.8 years (4.1), and included sedated and non-sedated subjects, and ventilated

and non-ventilated subjects. Although the sample is small, and therefore inherently not as diverse, it is helpful to consider how the sample characteristics of this study compare to other reported ICU populations.

In 2013, Garland et al., completed an observational study (N = 6061) in Manitoba and described the population demographics within their ICU in a tertiary care centre. In their study, the majority of subjects were male (72% versus 42% female). The authors describe that rather than this difference demonstrating a lack of access to ICU services for females, it has been skewed by including coronary ICU admissions in this number, of which the majority are known to be men. The unit in which this research study was completed did not include coronary care.

Interestingly, the proportion of males to females did shift when the participants with the most complete data were selected from the original data set of 11 participants. The final sample of 5 subjects included 3 males and 2 females, compared to the 4 males and 7 females originally enrolled subjects. The basis for the disproportionate amount of missing data in female subjects is not known, final sample was based only upon those which had the most complete data sets. No data was available regarding patient demographics for the unit in which the study was completed on.

In the study reported by Garland et al. (2013), the majority of subjects were within the ages of 58.5-60.8 years with a mean and SD of 64.4 (16.5). This mean age was nearly ten years older than the mean age in this study. Furthermore, a population-based epidemiologic study in ICU (N=1293) in the

province of Alberta also found the mean age of admission as 64.4 years, with the majority age between 50.6-74 years (Laupland, 2004).

Within the study sample, 60% of subjects utilized mechanical ventilation, compared to 40% non-ventilated. The percentage of patients in ICU utilizing mechanical ventilation is quite high. With respect to mechanical ventilation use within the first 24 hours of ICU admission, Wunsch et al. (2013) report 68% of UK patients were mechanically ventilated, compared to 27.4% of patients in the US (Wunsch, Angus, Harrison, Linde-Zwirble, & Rowan, 2011). Although this is a small sample size and may not be representative of the unit population, it is noteworthy to consider ventilation status in relation to sleep and delirium. Use of mechanical ventilation has been observed to decrease total sleep time and negatively impact sleep architecture (Drouot, Cabello, d'Ortho, & Brochard, 2008). Moreover, use of mechanical ventilation has also been found to result in higher delirium rates in ICU (Morandi, Brummel, & Ely, 2011).

Primary Study Hypothesis

A wide array of aMT6s secretion patterns was observed in the five ICU subjects included in the study analysis. In addition, each subject demonstrated an altered urinary melatonin secretion pattern compared to the normal urinary melatonin patterns reported in healthy subjects (Mahlberg et al., 2006).

Substantial research has previously shown that circadian rhythmicity is altered in ICU patients, whether measured by actigraphy (Freedman, Gazendam, Levan, Pack, & Schwab, 2001; Shilo et al., 1999), melatonin secretion (Mundigler et al., 2002b; Olofsson, Alling, Lundberg, & Malmros, 2004; Shilo et al., 1999) or

polysomnography (Buckle, Pouliot, Millar, Kerr, & Kryger, 1992; Freedman, Kotzer, & Schwab, 1999; Olofsson et al., 2004) The current research study is significant in its ability to capture markers of this circadian rhythm for a longer period of time, rather than just providing a glimpse over a one-day period (Gehlbach et al., 2012b; Mundigler et al., 2002). As there are many differences in collection methods, timing and analysis methods, it is difficult to provide a direct comparison to previous research. Due to the many variables, only three studies were available with similar method and timing to compare to; two studies examining ICU patients (Gehlbach et al., 2012; Riutta, Ylitalo & Kaukinen, 2008) and one looking at aMT6s in healthy adults (Mahlberg, et al., 2006).

Gehlbach et al. (2012) completed their collection of aMT6s excretion in ICU patients, specifically those who were mechanically ventilated and receiving continuous sedation. Sixteen participants had their aMT6s levels measured for twenty-four hours, ten of those individuals continued for another twenty-four hours. The means provided by the author were for the first 24 hours of data collection (n=16). The t-test results depicted no significant difference in any of the above noted time periods between Gehlbach et al. and the present study, which also used data from the first 24 hours only. Although few conclusions can be drawn from a small sample size, results found commonality with those reported by Gehlbach et al., demonstrating impaired circadian rhythmicity as evidenced by aMT6s excretion in ICU patients.

In a study of ICU patients, Riutta, Ylitalo and Kaukinen (2008) measured urine aMT6s secretion over a three-day period every 12 hours (N=40, Day 1; n

=36, Day 2; n= 20, Day 3). Although the times intervals were different than those reported by Gehlbach et al. (2012) and Mahlberg et al. (2006) which were used for analysis, similarities were observed between their reported DT and NT means and standard deviations. The authors reported DT (1000-2200) means (SD) of all subjects over the three days as 680 ng/mL (750) and NT (2200-1000) means (SDs) of 1180 ng/mL (890). By comparison, the overall mean (SD) for the DT and NT period in this study were 657 ng/mL (714) and 932 ng/mL (852).

When examining the DT means (SD) for all subjects combined for each study day, interesting dissimilarities were noted across study days and nights. The DT means (SD) of study Days 1, 2, 3 and 4 were 609 ng/mL (497), 745 ng/mL (932), 800 ng/mL (962) and 353 ng/mL (180) respectively; the first three DT means were quite similar, with a notable decrease in the fourth DT mean. Furthermore, substantial variation was observed for the DT means, which validates the wide range of aMT6s results for the daytime sample. NT means (SD) for each study Nights 1, 2, and 3 were 895 ng/mL (902), 786 ng/mL (693), and 927 ng/mL (1081). Night 4 had only one sample and therefore a mean and SD could not be calculated. Less variation in NT samples was observed. A common characteristic observed however was the large standard deviations, sometimes larger than the mean. Similar sized standard deviations can be observed in results from both Gehlbach et al. (2012) and Ruitta et al. (2008). In all studies significant individual variation can be noted in both DT and NT periods. Ruitta et al. observed a twentyfold inter-subject variation, which has been described previously (Benloucif et al., 2008; Mahlberg et al., 2006).

In 2006, Mahlberg et al. analyzed sleep via aMT6s excretion in healthy adults between the ages of 20-84. The collection periods were divided into four time periods, then reported by age group. Due to the manner in which the data was reported by age group, direct comparison to the study was challenging. The authors explained that four features were observed in their data: a) Melatonin secretion decreases with age, b) excretion can have a twenty-fold variance between individuals, c) daytime melatonin excretion does not change with age, and d) nighttime melatonin secretion is 94% responsible for the individual variations in melatonin. It should be mentioned that the authors studied 24-hours of aMT6s secretion only and thus could not serve as an ideal comparison for this study and its longevity of collection.

Mahlberg et al. (2006) used the NP and DP to calculate and NDR for each participant based upon the first two NP and first DP. The NDRs were reported by age group for the following ages: 20-35, 36-50, 51-65 and >65. The NDR for the age group of 51-65 which nearly all participants from this study fall into, was 2.9 with a range of 1.5-11.6, compared to a mean of 1.7 in this group with a range of 0.69-3.7. Although the ratio cannot provide the pattern of melatonin secretion for each subject, it can help one understand the difference between night and day rhythms both individually and collectively.

The t-test completed between the current study's data and the results from Mahlberg et al. (2006) showed little differentiation in three of the four time intervals compared. The EP (1800-2300), NP (2300-0700), MP (0700-1100) and DP (1100-1800) periods were compared, and a statistical difference was observed

the NP between Mahlberg et al. and the current study. As the authors previously described that 94% of variance in melatonin secretion patterns were observed in the night-time period, rather than the day, it is not surprising that this period was significantly different than in healthy subjects.

Due to the abnormalities observed in the participants' rhythms in this study, it was expected that greater differences would have been observed between the current participants and the healthy subjects. This thought was founded upon the unique architecture detected in the aMT6s profiles of each subject. While visually the rhythm appeared aberrant and the levels of melatonin may have been somewhat elevated for some subjects, on some days during periods with normally low secretion levels, no statistically significant differences were found outside of the NP. As seen in Figures 5 and 20, the greatest normal surge in urinary melatonin secretion occurs during the night-time period, with normally significantly less melatonin secreted during normal periods of wake (Benloucif et al., 2008; de Almeida et al., 2011). Given that melatonin levels are suppressed by light and sleep disruption (Benloucif et al. (2005), low daytime levels would be predicted.

The largest expected discrepancy between melatonin secretion levels in healthy individuals and subjects, given normal secretion patterns and the sleep environment in ICU, would occur during the night period, which was found here, with a t-value of 3.31 reported (alpha 0.05) when comparing mean aMT6s levels between the healthy individuals and this sample.

A larger sample size would be beneficial in determining whether this night-time suppression of melatonin is a generalized phenomenon for ICU patients, and for exploring whether smaller but potentially consistent aberrations from normal occur during non-night-time periods. Furthermore, in the current study, samples were not synchronized across participants, leaving it difficult to have the same number of samples in each time interval during later analysis. Future studies using synchronized data collection time points for long time periods would help to establish whether patterns exist regarding time after admission in which aberrancies are noted and the type of deviations observed. Furthermore, this manner of data collection will facilitate greater intra and inter-subject analysis.

Secondary Study Hypothesis

Due to factors detailed, the secondary hypothesis could not be proven. Although ultimately no participants tested positive for delirium, a large number of delirium assessments were not completed. According to recent studies in medical ICUs, delirium rates range from 23%-71% (Boesen et al., 2016; Ritchie et al., 2016; Woien, Balsliemke & Stubhaug, 2013); a very large range, but still very different findings than observed in this study, even given the small sample size. As noted on Table 7, 57% of possible assessments for this study were not completed by bedside staff. Of those not completed 65% were due to “too sedated” or “NA”, 35% were left blank with no reason provided. These negative findings can be attributed to two possible causes: a) The subjects genuinely were negative for the presence of delirium; or b) The delirium was not adequately

captured by the bedside staff. As the unit was previously using the CAM-ICU, no further education was provided in using the screening tool. Potential contributing factors to these results will be discussed below.

Evaluation of the Present Study

As this was a pilot study, much was to be learned about the process of executing this type of collection. Several limitations for the study were identified; along with recommendations for future research.

Limitations.

The most significant limitation of the research study was as small sample size. Although a sample of ten subjects was originally enrolled, due to financial and quality assurance constraints, the analysis was limited to only half of the collected samples. Although the samples analyzed were still able to provide an accurate picture of the circadian rhythm of the ICU subjects, a greater number of samples would have allowed for a more detailed picture, with measurements every two hours, rather than four.

Another significant limitation was the lack of CAM-ICU measurements completed by the bedside staff. The study data reflected the assessments as completed, but may have failed to adequately reflect the actual delirium in the selected sample. When looking at the bedside worksheets, it was noted that often the subject was scored as “NA”, or not applicable or “too sedated.” Although this can be likely, given the environment and need for heavy sedation, it was also noted that often the subject’s sedations score, completed using the Richmond Agitation and Sedation Score (Sessler et al., 2002), was within the range in which

the CAM-ICU tool is validated for use. This discrepancy between level of sedation and delirium assessment implies that more education was needed with the staff regarding assessment of sedated patients; and furthermore, begs to question what percentage of delirious patients are going unnoticed in this ICU.

In 2014, Swan noted a large percentage of patients were deemed “unable to assess” during bedside CAM assessments. The author detailed that a 10-minute, one-on-one session to review sedation levels and use of the CAM was provided to each bedside staff member. By completion of the education program, patients were 41% less likely to receive a determination of “unable to assess” by bedside staff. In the case of over sedation, Woien, Balsliemke and Stubhaug (2013) found a similar issue in using the tool in 2013. The authors questioned whether sedation levels in ICU were too high when in their study they often found assessment with the CAM tool impossible to complete due to significant sedation. Whether the participants were genuinely too sedated for use of the tool or the staff were not using the tool correctly is difficult to ascertain. For the benefit of the patients, however, this could be an area of further research in the future.

Missing data was another limitation in analysis of samples. This occurred for many reasons, including subject health status, staff workload and communication issues. All of these factors, however, can be present in all ICUs and therefore could present as a possible limitation for any future study in this area. One subject enrolled in the study had very limited urinary output, and for this reason did not consistently provide a sample every two hours for analysis. Although the P.I. was present to collect urine samples during the day, and

provided daily education to the staff on the unit, 7 urine samples were discarded by another discipline who found them in the refrigerator and was unsure what they were for. Moreover, overnight when staff were busy, several times “too busy” was written on the urine collection worksheet in place of the time the urine sample was collected for that desired time. Unfortunately, given the busy nature of the ICU environment, future studies would need the ability to dedicate collection personnel, available 24 hours a day, and so complete mitigation of this issue may not be possible.

It is difficult to determine whether issues with data collection were based upon time management, heavy assignments or overall interest in the research project. Older, well-cited research indicates that 76.4% of nurses have an unfavourable, or lukewarm attitude towards research participation and utilization (Olande, 2003). The author found that the limited interest was based upon a multitude of factors, and varied among the bedside nurses’ education, with those having a baccalaureate degree in nursing showing the most interest.

In more recent data, published in 2011 Riley et al., described similarities to Olande’s results, and also pointed to a strong correlation between educational preparedness of staff and interest in research. Additionally, the authors described that 48% of nurses surveyed felt that research was very important in nursing practice, and that the majority of these nurses felt more comfortable with research due to prior exposure through formal education, or within their workplace. Both the study in 2003 and 2011 emphasized the importance on the administration in facilitating interest in research, either through informal arrangements such as

journal clubs or by way of more formal measures including establishment of a research mentorship program.

Anecdotally, the author of this study found limited interest expressed by some bedside nurses at the beginning of the project. Attitudes changed, however, and interest increased towards the study after staff were aware of the P.I.'s role in bedside nursing care and subsequently expressed interest to actively participate in data collection. For this study, the ability for these connections to happen earlier was limited, as the P.I. completing the study was an outsider, visiting the hospital for data collection purposes only. Any future, nursing-based study such as this should take into account the nursing culture in which the study is taking place, and question the overall comfort level of staff to be involved in such a process.

When reflecting on the method of collection, it can be noted that the manner in which urine was collected was additionally a limitation of this study. Data collection took place over multiple one-week periods, as the P.I. moved out of the province during the study. Due to this, a greater number of subjects could not be enrolled, and enrolment over consecutive weeks could have provided greater consistency. While samples were analyzed according to the manufacturer's recommendations (Appendix B), a possibility remains that the samples could have partially degraded while waiting to be moved to the freezer. Furthermore, the possibility of user error is always present during the pipetting and analysis process.

Although collection took place every two hours, this collection was not at the same interval (for example 0800, 1000, 1200) for all subjects, but rather at the

time of enrolment and every two hours after. Having samples collected at these variable times prevented the possibility of analysis at multiple time points and intervals. Moreover, lacking the standardized collection times limited the comparative literature that could be used for further analysis.

Strengths of this Study.

In this study, a picture of the urinary derivative of melatonin in ICU subjects was captured over a longer period of time than in some previous research (Gehlbach et al., 2012b; Mundigler et al., 2002). Data from this study was also compared to findings in both the ICU and healthy adult populations, and despite a small sample size, meaningful sample and comparative findings were generated. This information can be added to the body of literature devoted to understanding circadian rhythmicity in ICU patients, as another piece of an exceptionally complex puzzle. As indicated, many authors have sought to understand the altered circadian rhythms through a variety of avenues, and limitations exist based upon participant condition and cost for these precise measurements.

Testing for the predicted correlation between circadian rhythmicity and delirium has been a difficult feat. Limited data exists to describe the association; thus far, some evidence points to a relationship, but authors call for further research (Balan et al., 2003; Miyazaki et al., 2003; Ouimet, Kavanagh, Gottfried, & Skrobik, 2007). In contrast, one study found no relationship between the two variables (Nuttall, Kumar, & Murray, 1998) The secondary aim of this study was to contribute to the existing body of knowledge in this regard. Due to several unforeseen factors, this study was not able to test whether there is a link between

these two factors. The pilot study, however, was able to direct and inform future research in the area, which is the objective of all pilot research. Therefore, when evaluating the aim of this study in a broader capacity for examining the study's potential to inform future research, the goals of the study were soundly met.

Sleep within the ICU is a well-known concern. Understanding of the circadian rhythm specifically, in this environment is difficult due to the needed precision of tests, health status of the patient and cost. Although many studies have examined the circadian rhythm and sleep in ICU patients, including the significant health consequences related to sleep loss, little has changed. The purpose of this study was to gain a better understanding of the circadian rhythm of ICU patients over a longer period of time. Furthermore, this study aimed to understand if any relationship can be observed between circadian rhythmicity and delirium. In addition to understanding these two aspects, as a pilot trial, the aim was to understand the feasibility of a study such as this on a larger scale.

The results demonstrated altered circadian rhythmicity as reflected by aMT6s secretion in all subjects. Subjects had aberrant circadian rhythm from normal with significant individual variability, comparable to similar ICU populations. Significant and atypical melatonin secretion was observed, specifically in the night-time period, compared to healthy individuals. Measurement of delirium using the CAM-ICU revealed negative scores in several participants and "too sedated" or "NA" in many participants. These results can lead one to question whether in the unit where the research was completed, if delirium rates are being accurately identified. Previous research has echoed

similar results finding that staff education programs can help to decrease “unable to assess” scores. Additionally, to compensate for the potential of unable to assess score, a power calculation should be completed to determine what the ideal sample size should be for future studies in this area. The CAM tool has been validated as having both high pooled sensitivity (86%) and specificity (93%) (Wong et al., 2010) and therefore if being used correctly, a large sample size may not be needed to identify delirium.

The literature clearly shows that noteworthy short and long-term health consequences exist from both sleep loss and delirium. Therefore, there is no doubt in the value in future studies to gain a better understanding of the sleep process and its relationship with delirium. With a better understanding of these processes, one can hope that successful interventions can be implemented to provide improved long-term health outcomes for our patients.

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Appendix A

Summary of Sleep Research in The ICU

Author and Year	Study Title	Sample Size (N)	Method	Total Sleep Time (TST)
Aurell & Elmqvist, 1985	Sleep in the surgical intensive care unit: continuous polygraphic recording of sleep in 9 patients receiving postoperative care	9	Postoperative patients studied for 2-4 days with continuous polygraphic recordings.	TST ~1hour, 50 minutes per night with REM sleep almost completely suppressed. Nurses' estimation of patient sleep grossly misjudged.
Beecroft et al., 2008	Sleep monitoring in the intensive care unit: comparison of nurse assessment, actigraphy and polysomnography	n=12	Stable, ventilated ICU patients. Comparison of accuracy between bedside overnight nurse assessment, actigraphy and polysomnography to assess total sleep time and sleep architecture.	TST of 3.10 (median) with ~65% agreement between actigraphy and polysomnography. Nurse assessment of total sleep and number of awakenings was underestimated.
Bosma, et al., 2007	Patient-ventilator interaction and sleep in mechanically ventilated patients: Pressure support versus proportional assist ventilation	n=13	Patients weaning from mechanical ventilation were randomized to either propositional assist ventilation or pressure support ventilation. Between 2200 and 0800, polysomnography, light noise, esophageal pressure and airway flow were measured.	TST (in minutes) was 334+/-124 in the proportional assist group and 314+/-140 in the pressure support ventilation group per night
Cooper et al., 2000	Sleep in critically ill patients requiring mechanical ventilation	n=20	Polysomnography was used in patients for 24 continuous hours and patients were divided into 3 groups based upon results: Disrupted sleep group, atypical sleep group and coma group.	The disrupted sleep group and atypical sleep group averaged 7.0+/- 4.8, 10 +/-5 hours of TST respectively. No total sleep time was given for the coma group.
Fanfulla et al., 2011	Sleep disturbances in patients admitted to a step down unit after ICU discharge: The role of mechanical ventilation	n=22	Patients in the step down unit underwent 24 hour polysomnography in two groups: Mechanically ventilated and those breathing spontaneously.	Data was available for 21 patients showing TST of 613+/-249 minutes and 645+/-326 in the mechanically ventilated and spontaneous breathing

				groups respectively. A predominance of stage 1 sleep was observed.
Friese, et al., 2007	Quantity and quality of sleep in the intensive care unit: Are our patients sleeping?	n=16	Polysomnography was used to monitor patients for up to 24 hours	Total polysomnographic time was 315 hours (mean 19.7 hours per patient). TST was 132 hours (mean 8.28 hours per patient). Slow wave and REM sleep were decreased from normal.
Freedman et al., 2001	Abnormal sleep/wake cycles and the effect of environmental noise on sleep distribution in the intensive care unit	n=22	Polysomnography and noise measurements was used for 24-48 hours in a medical ICU to determine the effect of noise on sleep distribution	21 separate 24 hour day/night periods showed the mean number of sleep periods/24 hours as 41+/-28, with the mean length of each period 15+/-9 minutes. 56%+/-18% of sleep occurred during the daytime
Gabor et al., 2003	Contribution of the intensive care environment to sleep disruption in mechanically ventilated patients and healthy subjects	n=7 ventilated patients, n=6 healthy subjects	Polysomnography was used to measure the effect of disruptions on sleep on mechanically ventilated patients in an open unit and healthy subjects in both an open unit and in a private room	TST per day of 6.2 +/- 2.5 in the ventilated group, with 54% of sleep during the daytime, compared to 8.2 +/- 1.4 and 9.5+/-1.7 hours per day in the healthy subjects in either an open unit or private room subjectively. Daytime sleep ~30% in this group.
Hilton, 1976	Quantity and quality of patients' sleep and sleep-disturbing factors in a respiratory intensive care unit	n=10	Continuous polysomnography was used for 48 hours to determine the quality and quality of sleep in the intensive care unit	TST ranged from 15 minutes to 13.3 hours in a 24-hour period. Decreased REM sleep was noted and incomplete sleep cycles in all subjects

Toublanc, et al., 2007	Assist-control ventilation vs. low levels of pressure support ventilation on sleep quality on intubated ICU patients	N=10 in the ACV/PS V group, n=10 in the PSV/AC V group	Patients were randomized to one of two groups: Either assist control followed by pressure support ventilation or the opposite between the hours of 2200-0600, while monitored with polysomnography.	Sleep was similar in both groups. In the assist control/pressure support group TST was 217 +/-31, the pressure support/assist control group showed a TST of 228 +/-23 minutes.
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Appendix B



Instructions for Use

Melatonin Sulfate ELISA

Enzyme immunoassay for the quantitative determination of melatonin sulfate (synonyms: 6-hydroxymelatonin sulfate, 6-sulfatoxymelatonin) in human urine.

REF 40-371-25006

 96

   2-8°C

U.S.: *For research use only.*
Not for use in diagnostic procedures.

GenWay Biotech Inc.

6777 Nancy Ridge Drive ■ San Diego ■ CA 92121
Phone: 858-458-0866
Fax: 858-458-0833

techline@genwaybio.com
www.genwaybio.com

1. INTENDED USE

Enzyme immunoassay for the quantitative determination of melatonin sulfate (synonyms: 6-Hydroxymelatonin Sulfate, 6-Sulfatoxymelatonin) in human urine.

2. SUMMARY AND EXPLANATION

The pineal gland ("corpus pineale") has been called a neuroendocrine transducer because of its important role in photoperiodism. The major hormone of the pineal gland is N-acetyl-5-methoxy-tryptamine or melatonin which is synthesized from the amino acid tryptophane. Melatonin has its highest levels in plasma during nighttime. Its characteristic nocturnal surge appears to encode temporal information such as length of night. Regulation of the melatonin secretion is under neural control. Sympathetic innervation seems to play a major role via its release of noradrenaline. Altered patterns and/or levels of melatonin secretion have been reported to coincide with sleep disorders, "jet lag", depression, stress, schizophrenia, hypothalamic amenorrhea, pregnancy, anorexia nervosa, some forms of cancer, immunological disorders as well as control of sexual maturation during puberty.

Most of the circulating melatonin is metabolized in the liver to 6-hydroxymelatonin and subsequently to 6-sulfatoxymelatonin which is excreted into the urine.

The concentration of 6-Hydroxymelatonin Sulfate in urine correlates well with the total level of melatonin in the blood during the collection period.

3. TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. After the substrate reaction the intensity of the developed color is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

4. WARNINGS AND PRECAUTIONS

1. For research use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact GenWay in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2-8°C.

Melatonin Sulfate ELISA (40-371-25006)

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6. SPECIMEN COLLECTION AND STORAGE

Urine

It is possible to use spontaneous as well as 24 h urine. The total volume of urine excreted during a 24 h period should be collected and mixed in a single bottle. Preservation is not necessary. Determine total volume for calculation of results. Mix and centrifuge samples before use in the assay.			
Storage:	2-8°C	-20°C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles. For more details see: Griefahn et al. (2001).
Stability:	4 d	15 y	

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12x8	MTP	Microtiter Plate Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal).
1 x 5 mL	ANTISERUM	Melatonin Sulfate Antiserum Ready to use. Contains Antiserum (rabbit), Tris buffer, 0.01 % Thimerosal.
1 x 0.2 mL	ENZCONJ CONC	Enzyme Conjugate, Concentrate (40x) Contains Melatonin Sulfate, conjugated to peroxidase, phosphate buffer, 0.01 % Thimerosal.
1 x 7 x 0.1 mL	CAL A-G	Standard A-G 0; 1.7; 5.2; 15.6; 46.7; 140; 420 ng/mL 0; 5.2; 15.9; 47.6; 142; 427; 1281 nmol/L Ready to use. Contains Melatonin Sulfate, Tris buffer, 0.01 % Thimerosal.
1 x 2 x 0.1 mL	CONTROL 1+2	Control 1+2 Ready to use. Contains 0.02 % Thimerosal. Concentrations / acceptable ranges see QC Certificate.
1 x 60 mL	ASSAYBUF	Assay Buffer Red colored. Ready to use. Contains Tris buffer, BSA, 0.01 % Thimerosal.
1 x 50 mL	WASHBUF CONC	Wash Buffer, Concentrate (20x) Contains phosphate buffer, Tween, 0.1 % Thimerosal.
1 x 0.9 mL	TMB SUBS CONC	TMB Substrate Solution, Concentrate (31x) Contains TMB, Buffer, stabilizers.
1 x 27 mL	TMB BUF	TMB Substrate Buffer Store protected from light. Ready to use. Contains H ₂ O ₂ , citrate buffer, stabilizers.
1 x 12 mL	TMB STOP	TMB Stop Solution Ready to use. 1 M H ₂ SO ₄ .
3 x	FOIL	Adhesive Foil

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volumes: 10; 50; 100; 1000 µL
2. Round-bottom polystyrene test tubes (12 x 75 mm)
3. Rack for test tubes
4. Orbital shaker (500 rpm)
5. Vortex mixer
6. 8-Channel Micropipettor with reagent reservoirs
7. Wash bottle, automated or semi-automated microtiter plate washing system
8. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
9. Bidistilled or deionised water
10. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. The contents of the kit for 96 determinations can be divided into 3 separate runs. The volumes stated below are for one run with 4 strips (32 determinations).
2. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.

Melatonin Sulfate ELISA (40-371-25006)

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3. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
4. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
5. Some components contain a 250 µL solution. Take care that the solution is completely on the bottom of the vial before opening.
6. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
7. Use a pipetting scheme to verify an appropriate plate layout.
8. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
9. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled evenly with Wash Buffer, and that there are no residues in the wells.
10. Humidity affects the coated wells/tubes. Do not open pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch with desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of Lyophilized or concentrated Components

Dilute/dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
15 mL	Wash Buffer	ad 300 mL	bidist. Water	1:20	Resolve crystals at 18-25°C.	2-8°C	4 w
50 µL	Enzyme Conjugate	with 2 mL	Assay Buffer	1:41	Prepare freshly and use only once.	18-25°C	30 min
300 µL	TMB Substrate Solution	with 9 mL	TMB Substrate Buffer	1:31	Prepare freshly and use only once.	18-25°C	10 min

10.2. Dilution of Standards, Controls and Patient Urine Samples

1.	Pipette 10 µL of each Standard, Control and patient urine sample into polystyrene, polypropylene or glass tubes. Avoid direct sun light.
2.	Pipette 500 µL of Assay Buffer into each tube. Vortex.

Samples containing concentrations higher than the highest standard have to be further diluted with Assay Buffer.

11. TEST PROCEDURE

1.	Pipette 50 µL of each <u>diluted</u> Standard, <u>diluted</u> Control and <u>diluted</u> patient sample into the respective wells of the Microtiter Plate.
2.	Pipette 50 µL of freshly prepared Enzyme Conjugate into each well.
3.	Pipette 50 µL of Melatonin Sulfate Antiserum into each well.
4.	Cover plate with adhesive foil. Incubate 2 h at RT (18-25°C) on an orbital shaker (500 rpm).
5.	<i>Approx. 10 min before end of incubation prepare TMB Substrate Solution.</i>
6.	Remove adhesive foil. Discard incubation solution. Wash plate 4 x with 250 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
7.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
8.	Pipette 200 µL of freshly prepared TMB Substrate Solution into each well.
9.	Incubate 30 min at RT (18-25°C) on an orbital shaker (500 rpm).
10.	Stop the substrate reaction by adding 100 µL of TMB Stop Solution into each well. Briefly mix

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	contents by gently shaking the plate.
11.	Measure optical density with a photometer at 450 nm (Reference-wavelength: 600-650 nm) within 60 min after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards and kit controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

It is recommended to participate at appropriate quality assessment trials.

13. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Calculate the 24 h excretion for each urine sample: $\mu\text{g}/24\text{h} = \mu\text{g}/\text{L} \times \text{L}/24\text{h}$

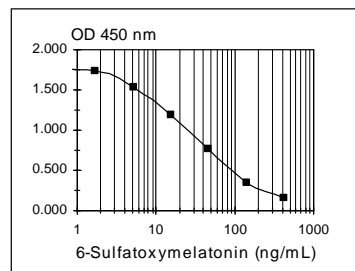
Conversion:

Melatonin Sulfate (ng/mL) x 3.05 = nmol/L

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Melatonin Sulfate (ng/mL)	Mean OD	OD/OD _{max} (%)
A	0.0	1.805	100.0
B	1.7	1.741	96.5
C	5.2	1.536	85.1
D	15.6	1.185	65.7
E	46.7	0.773	42.8
F	140.0	0.341	18.9
G	420.0	0.164	9.1



14. EXPECTED VALUES

Apparently healthy subjects show the following values:

Age	n	Melatonin Sulfate			
		24h (µg)		night fraction (µg/h)	
		Mean	90% percentile	Mean	90% percentile
20-35	26	36.8	15.6 - 58.1	2.8	0.9 - 5.6
36-50	17	29.6	9.9 - 52.9	2.1	0.6 - 3.6
51-65	16	20.4	12.3 - 32.8	1.5	0.9 - 2.5
> 65	16	15.8	7.5 - 32.7	1.0	0.3 - 2.3

It is recommended that each laboratory establishes its own range of normal values.

Melatonin Sulfate ELISA (40-371-25006)

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15. LIMITATIONS OF THE PROCEDURE

Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

For cross-reactivities, see PERFORMANCE.

16. PERFORMANCE

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)		Cross-reactivity of other substances tested < 0.0001 %
	Melatonin Sulfate	100		
	Melatonin	0.002		
	6-OH-Melatonin	0.001		
	N-Acetyl-L-OH-Tryptamine	0.0005		
	N-Acetyl-L-Tryptophane	< 0.0001		
	5-Methoxy-Tryptamine	< 0.0001		
	Tryptamine	< 0.0001		
	5-HIAA	< 0.0001		
Analytical Sensitivity (Limit of Detection)	1.0 ng/mL	Mean signal (Zero-Standard) - 2SD		
Precision	Range (ng/mL)	CV (%)		
	Intra-Assay	5.8 - 204	5.2 - 12.2	
	Inter-Assay	12.4 - 220	5.1 - 14.9	
Linearity	Range (ng/mL)	Serial dilution up to	Range (%)	
	96.5 - 248.8	1:32	80 - 116	
Recovery	Mean (%)	Range (%)	% Recovery after spiking	
	105.8	91 - 122		
Method Comparison versus RIA	GenWay -Assay = 1.15 x RIA + 4.2		r = 0.96; n = 40	

Appendix C

Confusion Assessment Method for ICU (CAM-ICU) Worksheet

CAM-ICU Worksheet

Feature 1: Acute Onset or Fluctuating Course	Score	Check here if Present
Is the pt different than his/her baseline mental status? OR Has the patient had any fluctuation in mental status in the past 24 hours as evidenced by fluctuation on a sedation scale (i.e., RASS), GCS, or previous delirium assessment?	Either question Yes →	<input type="checkbox"/>
Feature 2: Inattention		
Letters Attention Test (See training manual for alternate Pictures) Directions: Say to the patient, "I am going to read you a series of 10 letters. Whenever you hear the letter 'A,' indicate by squeezing my hand." Read letters from the following letter list in a normal tone 3 seconds apart. <p style="text-align: center;">S A V E A H A A R T</p> Errors are counted when patient fails to squeeze on the letter "A" and when the patient squeezes on any letter other than "A."	Number of Errors >2 →	<input type="checkbox"/>
Feature 3: Altered Level of Consciousness		
Present if the Actual RASS score is anything other than alert and calm (zero)	RASS anything other than zero →	<input type="checkbox"/>
Feature 4: Disorganized Thinking		
Yes/No Questions (See training manual for alternate set of questions) 1. Will a stone float on water? 2. Are there fish in the sea? 3. Does one pound weigh more than two pounds? 4. Can you use a hammer to pound a nail? Errors are counted when the patient incorrectly answers a question. Command Say to patient: "Hold up this many fingers" (Hold 2 fingers in front of patient) "Now do the same thing with the other hand" (Do not repeat number of fingers) *If pt is unable to move both arms, for 2 nd part of command ask patient to "Add one more finger" An error is counted if patient is unable to complete the entire command.	Combined number of errors >1 →	<input type="checkbox"/>
Overall CAM-ICU	Criteria Met →	<input type="checkbox"/> CAM-ICU Positive (Delirium Present)
	Criteria Not Met →	<input type="checkbox"/> CAM-ICU Negative (No Delirium)
Feature 1 <u>plus</u> 2 <u>and</u> either 3 <u>or</u> 4 present = CAM-ICU positive		

Appendix D

Subject Worksheets

Subject Demographic Information

PART A: TO BE COMPLETED AT ADMISSION ONLY

Study Number _____

Treatment Group _____

Age _____

Sex M F

Admitting
Diagnosis _____

Sedative Medication

PART B: TO BE COMPLETED DAILY

Check all that apply

- ◇ Propofol (Diprivan) total daily dose: _____
- ◇ Ativan (Lorazepam) total daily dose: _____
- ◇ Versed (Midazolam) total daily dose: _____
- ◇ Fentanyl total daily dose: _____
- ◇ Morphine total daily dose: _____
- ◇ Other. Total daily dose: _____

Data Collection Worksheet

Any Questions? Call Alanna at [REDACTED]

Subject # _____

DAY 1

- | | |
|-----------------------|------------------------------|
| 1. /Aug/2014 @ _____ | Actual time collected: _____ |
| 2. /Aug/2014 @ _____ | Actual time collected: _____ |
| 3. /Aug/2014 @ _____ | Actual time collected: _____ |
| 4. /Aug/2014 @ _____ | Actual time collected: _____ |
| 5. /Aug/2014 @ _____ | Actual time collected: _____ |
| 6. /Aug/2014 @ _____ | Actual time collected: _____ |
| 7. /Aug/2014 @ _____ | Actual time collected: _____ |
| 8. /Aug/2014 @ _____ | Actual time collected: _____ |
| 9. /Aug/2014 @ _____ | Actual time collected: _____ |
| 10. /Aug/2014 @ _____ | Actual time collected: _____ |
| 11. /Aug/2014 @ _____ | Actual time collected: _____ |
| 12. /Aug/2014 @ _____ | Actual time collected: _____ |

CAM-ICU Score


1. /Aug/2014 @0800 Actual time _____
2. /Aug/2014 @01600 Actual time _____

Sedative medications: to be completed by the researcher

- Fentanyl: _____
 Versed: _____
 Propofol: _____
 Other: _____

Appendix E

University of Manitoba's Education/Nursing Research Ethics Board (ENREB) Approval

 UNIVERSITY OF MANITOBA	Research Ethics and Compliance <small>Office of the Vice-President (Research and International)</small>	Human Ethics 208-194 Dufour Road Winnipeg, MB Canada R3T 2N2 Phone +204-474-7122 Fax +204-269-7173
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APPROVAL CERTIFICATE

October 3, 2013

Kathleen and Winfred Ruane Research Grant

TO: Alanna Chau (Advisor D. McMillan)
Principal Investigator

FROM: Lorna Guse, Chair
Education/Nursing Research Ethics Board (ENREB)

Re: Protocol #E2013:085
"Circadian Rhythmicity in the ICU: Understanding Melatonin Patterns and their Relationship to Delirium in ICU Patients"

Please be advised that your above-referenced protocol has received human ethics approval by the Education/Nursing Research Ethics Board, which is organized and operates according to the Tri-Council Policy Statement (2). This approval is valid for one year only.

Any significant changes of the protocol and/or informed consent form should be reported to the Human Ethics Secretariat in advance of implementation of such changes.

Please note:

- If you have funds pending human ethics approval, please mail/e-mail/fax (261-0325) a copy of this Approval (identifying the related UM Project Number) to the Research Grants Officer in ORS in order to initiate fund setup. (How to find your UM Project Number: <http://umanitoba.ca/research/ors/mrt-faq.html#pr0>)
- if you have received multi-year funding for this research, responsibility lies with you to apply for and obtain Renewal Approval at the expiry of the initial one-year approval; otherwise the account will be locked.

The Research Quality Management Office may request to review research documentation from this project to demonstrate compliance with this approved protocol and the University of Manitoba *Ethics of Research Involving Humans*.

The Research Ethics Board requests a final report for your study (available at: http://umanitoba.ca/research/orec/ethics/human_ethics_REB_forms_guidelines.html) in order to be in compliance with Tri-Council Guidelines.

umanitoba.ca/research

Appendix F
Letter of Support

July 08/2013

Alanna Chau

[REDACTED]

[REDACTED]

[REDACTED]

Attention: Alanna Chau

I am writing to you in response to your request for support in conducting a thesis research study/project that you would like to execute at Health Sciences Centre MICU.

With the support of your thesis committee, my understanding is that you will be analyzing urinary melatonin levels in order to understand participant's circadian rhythm among patients in MICU, as well as chart reviews to gain knowledge of patient CAM scores.

I also understand that approval to carry out this research study will be pre-approved through both Nursing and Education Research Ethics Board, as well as the Centre itself.

I support this request and wish you all the best in your Masters of Nursing Program with the University of Manitoba. If I can be of further assistance to you, let me know.

Sincerely,

Shelley Dolbeck
Manager of Patient Care
MICU/CCU
Health Sciences Centre
700 William Avenue
Winnipeg, Manitoba
R3E 0Z3

Appendix G

Letter of Support for use of Laboratory

July 17, 2013

To Whom it Concerns –

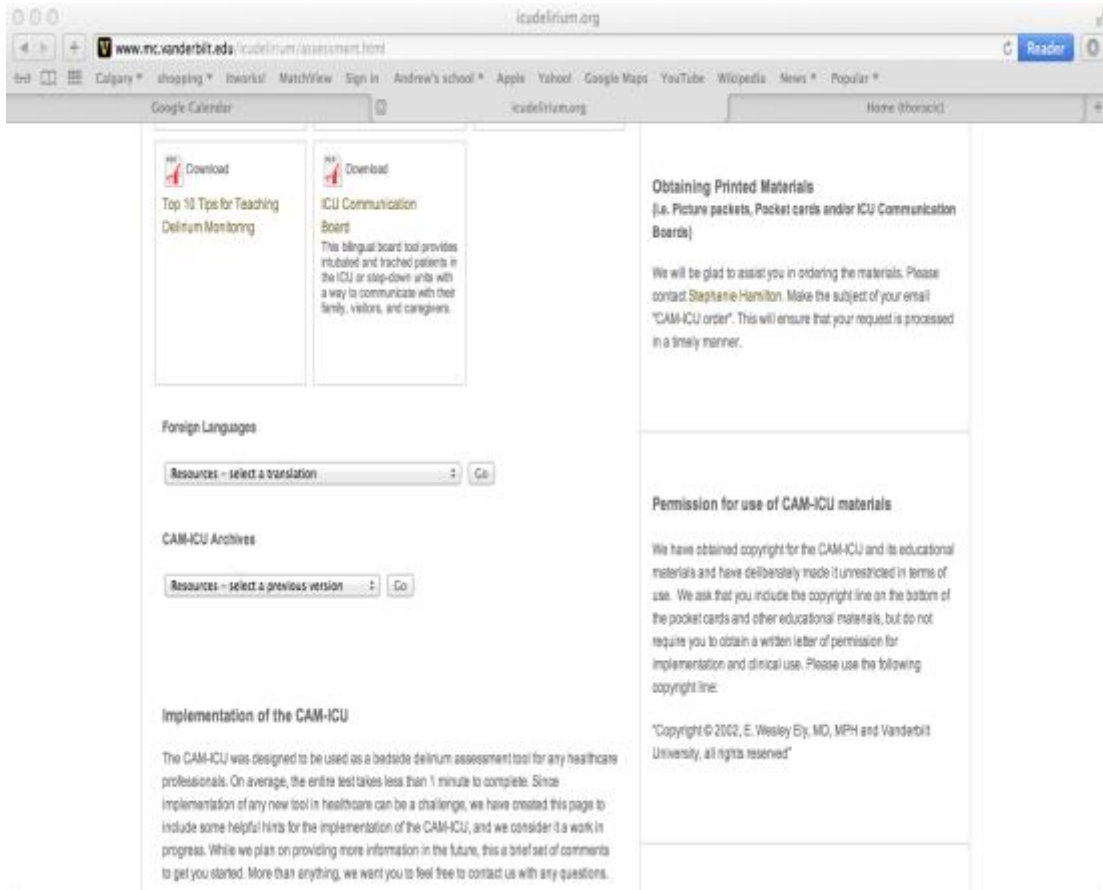
I am pleased to write a letter of support for the project as outlined by Alanna Chau. I am a Scientist at the Manitoba Institute for Child Health, and hold a Nil appointment in the Department of Biological Sciences at the University of Manitoba. I have a research lab at the John Buhler Research Center, 715 McDermot Avenue, with equipment that will be essential for Ms. Chau's research project. The equipment includes plate readers and plate washers for ELISA analysis of saliva samples, as well as ancillary equipment such as analytical balances, spectrophotometers, and pipettors. I am more than happy to avail these to Ms. Chau, and to assist her in her project.

Sincerely,

Dr. William Diehl-Jones
Scientist,
Manitoba Institute for Child Health
Bill_Diehl-Jones@umanitoba.ca

Appendix H

CAM-ICU Permission



Appendix I

Letter of Invitation into the Research Study

**Research Project Title:
Circadian Rhythmicity in the ICU: Understanding Melatonin Patterns and their
Relationship to Delirium in ICU Patients.**

Dear ICU patient,

This letter is an invitation to participate in a research study. As a full-time Masters student in the Faculty of Nursing at the University of Manitoba, I am working with Dr. Diana McMillan to understand the bodily clock of ICU patients and its relationship to delirium, a state of confusion often experienced by ICU patients.

Study Purpose

Due to the complex nature of illness in the ICU, frequent disruptions to patients and a variety of medications used within the ICU environment, ICU patients are often found to have abnormal sleep and waking patterns. Furthermore, ICU patients are often lacking in exposure to normal day/night light exposure as well as other cues that tell them when to wake and rest. These abnormalities often result in disruption of the patient's bodily clock, or circadian rhythm.

Previous research has looked at circadian rhythm disruption of ICU patients and it is well documented that abnormal sleep and rest patterns can be related to delirium, a temporary state of confusion often observed in ICU patients.

The purpose of this research study is to further understand the circadian rhythm of ICU patients, and determine if a relationship exists between circadian rhythm abnormalities and confusion level. This will be achieved by measuring urine melatonin levels (a hormone involved in the timing of sleep), and comparing it to the results of a confusion assessment completed by the bedside nurse. In addition, the participant's age, gender, admitting diagnosis and daily doses of any sedative-type medication will be collected from the hospital chart.

Your Involvement

We are looking for patients who may be interested in participating in this research study. Once enrolled, participants will have urine collected once per hour from the pre-existing urine collection bag attached to their catheter for a total of four days.

All other treatment of the participants will follow the usual standard of care.

Risks/Benefits

It is unlikely that you will experience any risks or benefits from participation in this research study. It is possible that others may benefit from results of this research study in the future, however.

Contact Information

If you have any questions regarding this study or are interested in participating, please call me at [REDACTED] or email me at umchau4@cc.umanitoba.ca. You may also contact my supervising professor, Dr. Diana McMillan, at 204-474-7295 or email her at Diana.mcmillan@ad.umanitoba.ca. If you have any concerns or complaints, you are able to contact the above named persons or the Human Ethics Secretariat at (204) 474-7122, or by email to Margaret_bowman@umanitoba.ca.

I assure you that the study has been reviewed and approved by the Education/Nursing Research Ethics Board through the University of Manitoba, as well as approval from Health Sciences Centre. However, the final decision to participate is yours and is totally voluntary, and it will not affect the care you receive.

Thank you in advance for your interest and assistance with this research,

Alanna Chau, RN, BScN
Masters student, Faculty of Nursing
University of Manitoba

Letter of Invitation into the Research Study for Family Members

Research Project Title:

Circadian Rhythmicity in the ICU: Understanding Melatonin Patterns and their Relationship to Delirium in ICU Patients.

To the family members of an ICU patient,

This letter serves as an invitation for your family member to participate in a research study. It has been determined by your family member's health care providers that at this time, your family member (the ICU patient) is lacking in the cognitive awareness required for him/her to make decisions regarding participation in this study. For this reason, you as a family member are being approached with an invitation for your family member to participate in this research study.

As a full time Masters student, in the Faculty of Nursing at the University of Manitoba, I am working with Dr. Diana McMillan to understand the bodily clock of ICU patients and its relationship to delirium, a state of confusion often experienced by ICU patients.

Study Purpose

Due to the complex nature of illness in the ICU, frequent disruptions to patients and a variety of medications used within the ICU environment, ICU patients are often found to have abnormal sleep and waking patterns. Furthermore, ICU patients are often lacking in exposure to normal day/night light exposure as well as other cues that tell them when to wake and rest. These abnormalities often result in disruption of the patient's bodily clock, or circadian rhythm.

Previous research has looked at circadian rhythm disruption of ICU patients and it is well documented that abnormal sleep and rest patterns can be related to delirium, a temporary state of confusion often observed in ICU patients.

The purpose of this research study is to further understand the circadian rhythm of ICU patients, and determine if a relationship exists between circadian rhythm abnormalities and confusion level. This will be achieved by measuring urine melatonin levels (a hormone involved in the timing of sleep), and comparing it to the results of a confusion assessment completed by the bedside nurse. In addition, the participant's age, gender, admitting diagnosis and daily doses of any sedative-type medication will be collected from the hospital chart.

Your Family Member's Involvement

We are looking for patients to participate in this research study. Once enrolled, participants will have urine collected once per hour from the pre-existing urine collection bag attached to their catheter for a total of four days.

All other treatment of the participants will follow the usual standard of care.

Risks/Benefits

It is unlikely that your family member will experience any risks or benefits from participation in this research study. It is possible that others may benefit from results of this research study in the future, however.

Completion of the Study

Should your family member at a later time decide that he/she would not like to complete the study, their participation should be discontinued immediately, and all confidential information destroyed.

Contact Information

If you have any questions regarding this study or are interested in participating, please call me at [REDACTED] or email at umchau4@cc.umanitoba.ca. You can also contact my supervising professor, Dr. Diana McMillan at 204-474-7295 or email at Diana.mcmillan@ad.umanitoba.ca. If you have any concerns or complaints, you are able to contact the above named persons or the Human Ethics Secretariat at (204) 474-7122, or email Margaret_bowman@umanitoba.ca.

I assure you that the study has been reviewed and approved by the Education/Nursing Research Ethics Board through the University of Manitoba as well as approval from Health Sciences Centre. However, the final decision to participate is yours and is totally voluntary and it will not affect the care you receive.

Thank you in advance for your interest and assistance with this research,

Alanna Chau, RN, BScN
Masters student, Faculty of Nursing
University of Manitoba

Appendix J

Informed Consent Form

Informed Consent

Research Project Title:

Circadian Rhythmicity in the ICU: Understanding Melatonin Patterns and their Relationship to Delirium in ICU Patients.

Principal Investigator and contact information:

Alanna Chau, RN, BScN, [REDACTED]

Research Supervisor and contact information:

Diana McMillan, RN, PhD, (204) 474-7295

This consent form, a copy of which will be left with you for your records and reference, is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included here, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

It has been determined by your family member's health care providers that at this time, your family member (the ICU patient) is lacking in the cognitive awareness required for him/her to make decisions regarding participation in this study. For this reason, you as a family member are being approached to provide consent for your family member to participate in this research study.

Purpose of the Study: We are asking permission for your family member to participate in a research study at Health Sciences Centre. The purpose of this study is to understand the bodily clock, or circadian rhythm of ICU patients, and explore the relationship between sleep patterns and delirium, a state of confusion often observed in the ICU.

Procedure: If you agree to allow your family member to participate, a sample of urine will be taken hourly from their pre-existing urinary catheter drainage bag for a total of four days. The patient will not be directly touched or disturbed for this collection. The urine sample will be used to analyze 6-sulphatoxymelatonin, a natural hormone associated with sleep. Other information including CAM-ICU (a delirium rating score) age, sex, admitting diagnosis and daily sedative and analgesic medication intake will also be obtained from their chart. Collection of the urine samples and medical information will be completed by the principal investigator, or a research assistant.

Risks and Benefits: There are no expected risks or benefits of participating in this study. The urine collection is expected to cause no discomfort as it is obtained from a urine collection bag attached to the urinary catheter, not from you directly. It is unlikely that there will be any direct

benefits from participation in this study. It is hoped, however, that data collected in this research study may help others in the future by helping researchers to understand to the sleep/wake cycles of ICU patients, and their connection decrease their delirium.

Confidentiality: All information collected for study purposes will be kept confidential. Each participant will be given a study number, not related to his or her personal health number. Although the data collected in this study will later be used in scholarly presentations and publications, there will be no way to link the information to any specific study participants. Additionally, a presentation will be made to ICU staff detailing the results of the research study. If you would like a summary of the finding, they will be available by May 2014. Please provide you information at the end of this form to receive this summary.

The data collected will be kept secure. Paper files will be stored in a locked filing cabinet in the principal investigator's home, electronic files will be stored on a password protected computer with an encryption feature. Only the principal investigator, Alanna Chau, and the research supervisor, Dr. Diana McMillan, will have access to these files. Raw data (numerical data which is non-identifiable) will be viewed by the University of Manitoba statistician during the data analysis portion of the research study. All data related to the study will be kept until January 2021, at which time it will be destroyed using a confidential shredder.

Your decision for your family member to participate in the study is completely voluntary. Whether you should decide for them to participate, not participate or withdraw from the study at a later date will not affect the care they receive at the Health Sciences Centre. Should you enroll them, their ICU physician and nurses will be aware of their enrolment. There will be no financial compensation for participation in the study.

Your signature on this form indicates that you have understood the purpose and risks and potential benefits of this study. In no way does this waive your legal rights, the rights of your family member, or release the researchers or involved institutions from their legal and professional responsibilities. You, and your family member (the ICU patient), are free to withdraw from the study at any time and are free to contact the researcher with any questions or concerns you may have.

This study is being conducted by Alanna Chau and RN and Masters of Nursing student at the University of Manitoba (telephone number [REDACTED], email umchau4@cc.umanitoba.ca). Her research supervisor is Dr. Diana McMillan (telephone number (204) 474-7294, email Diana_mcmillan@ad.umanitoba.ca). This study has been approved by the University of Manitoba Education/Nursing Research Ethics Board and the Health Sciences Centre. If you have any concerns or complaints, you are able to contact the above named persons or the Human Ethics Secretariat at (204) 474-7122, or email Margaret_bowman@umanitoba.ca. A copy of this consent has been given to you to keep for your records. The University of Manitoba may look at your research records to see that the research is being done in a safe and proper way.

Participants Signature _____ Date _____

Researchers Signature _____ Date _____

Note to Participants: Please print your name, address OR email address here if you wish to receive a copy of the results of the study:

Informed Consent Form

Informed Consent for Family Members

Research Project Title:

Circadian Rhythmicity in the ICU: Understanding Melatonin Patterns and their Relationship to Delirium in ICU Patients.

Principal Investigator and contact information:

Alanna Chau, RN, BScN, [REDACTED]

Research Supervisor and contact information:

Diana McMillan, RN, PhD, (204) 474-7295

This consent form, a copy of which will be left with you for your records and reference, is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included here, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

Purpose of the Study: You (or your family member) are being asked to participate in a research study at Health Sciences Centre. The purpose of this study is to understand the bodily clock, or circadian rhythm of ICU patients, and explore the relationship between sleep patterns and delirium, a state of confusion often observed in the ICU.

Procedure: If you agree to participate, a sample of urine will be taken hourly from your urinary catheter drainage bag for a total of four days. You will not be directly touched or disturbed for this collection. The urine sample will be used to analyze 6-sulphatoxymelatonin, a natural hormone associated with sleep. Other information including CAM-ICU (a delirium rating score) age, sex, admitting diagnosis and daily sedative and analgesic medication intake will also be obtained from your chart. Collection of the urine samples and medical information will be completed by the principal investigator, or a research assistant.

Risks and Benefits: There are no expected risks or benefits of participating in this study. The urine collection is expected to cause no discomfort as it is obtained from a urine collection bag attached to the urinary catheter, not from your family member directly. It is unlikely that there will be any direct benefits from participation in this study. It is hoped, however, that data collected in this research study may help others in the future by helping researchers to understand to the sleep/wake cycles of ICU patients, and their connection decrease their delirium

Confidentiality: All information collected for study purposes will be kept confidential. Each participant will be given a study number, not related to his or her personal health number.

Although the data collected in this study will later be used in scholarly presentations and publications, there will be no way to link the information to any specific study participants. Additionally, a presentation will be made to ICU staff detailing the results of the research study. If you would like a summary of the finding, they will be available by May 2014. Please provide you information at the end of this form to receive this summary.

The data collected will be kept secure. Paper files will be stored in a locked filing cabinet in the principal investigator's home, electronic files will be stored on a password protected computer with an encryption feature. Only the principal investigator, Alanna Chau, and the research supervisor, Dr. Diana McMillan, will have access to these files. Raw data (numerical data which is non-identifiable) will be viewed by the University of Manitoba statistician during the data analysis portion of the research study. All data related to the study will be kept until January 2021, at which time it will be destroyed using a confidential shredder.

Your decision to participate in the study is completely voluntary. Whether you should decide to participate, not participate or withdraw from the study at a later date will not affect the care you receive at the Health Sciences Centre. Should you enroll, your ICU physician and nurses will be aware of your enrolment. There will be no financial compensation for participation in the study.

Your signature on this form indicates that you have understood the purpose and risks and potential benefits of this study. In no way does this waive your legal rights or release the researchers or involved institutions from their legal and professional responsibilities. You are free to withdraw from the study at any time. If you decide to withdraw, this can be done by notifying the principal investigator, at that time, all your confidential data would be destroyed. You are free to contact the researcher with any questions or concerns you may have.

This study is being conducted by Alanna Chau and RN and Masters of Nursing student at the University of Manitoba (telephone number) [REDACTED], email umchau4@cc.umanitoba.ca). Her research supervisor is Dr. Diana McMillan (telephone number (204) 474-7294, email Diana_mcmillan@ad.umanitoba.ca). This study has been approved by the University of Manitoba Education/Nursing Research Ethics Board and the Health Sciences Centre. If you have any concerns or complaints, you are able to contact the above named persons or the Human Ethics Secretariat at (204) 474-7122, or email Margaret_bowman@umanitoba.ca. A copy of this consent has been given to you to keep for your records. The University of Manitoba may look at your research records to see that the research is being done in a safe and proper way.

Participant's Signature _____ Date _____

Researcher's Signature _____ Date _____

Note to Participants: Please print your name, address OR email address here if you wish to receive a copy of the results of the study:

Appendix K

Permission for Reprints

Figure 1:

Daan, S., Beersma, & D. G., Borbely, A. A. (1984). Timing of human sleep: Recovery process gated by a circadian pacemaker. *The American of Physiology*, 246, 161-183. Retrieved from <http://www.physiology.org>

Dear Ms. Ash:

Please feel free to use the figure you sent me and include the reference to its origin in your thesis.

I wish you good luck with your interesting project, wherever on earth it is carried out.

With kind regards,

Serge Daan

Prof. Dr. Serge Daan

Emeritus, Niko Tinbergen chair in Behavioral Biology

Linnaeusborg, University of Groningen

private address:

Hoofdweg 274, 9765CN Paterswolde, The Netherlands

Tel [+31-50-3093999](tel:+31-50-3093999); Cell [+31-6-21107299](tel:+31-6-21107299)

Email: s.daan@rug.nl

Figure 2:

Melatonin Secretion in Humans in Plasma, Urine and Saliva. Arendt, J. (2000). The pineal gland and pineal tumours. In L. J. De Groot, G. Chrousos, K. Dungan, K. R. Feingold, A. Grossman, J. M. Hershman, . . . A. Vinik (Eds.), *Endotext* (). South Dartmouth (MA): MDText.com, Inc.

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