

**THE IMPACT OF MELATONIN, MELATONIN ANALOGUES, CAFFEINE, AND
BRIGHT LIGHT ON SLEEP AND THERMOREGULATORY PHYSIOLOGY**

by

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ABSTRACT

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The Impact of Melatonin, Melatonin Analogues, Caffeine, and Bright Light on Sleep and Thermoregulatory Physiology

Thesis directed by Associate Professor Kenneth P. Wright, Jr.

The thermoregulatory system is tied to the regulation of sleep and wakefulness. Research protocols have examined the unmasked effect of endogenous melatonin on these systems by giving exogenous doses under controlled conditions during the daytime, when endogenous levels are low. Study findings have demonstrated that exogenous melatonin improves sleep, increases peripheral heat loss, and decreases core body temperature (CBT). These thermoregulatory adjustments mimic those that occur around habitual bedtime, when endogenous melatonin levels are high. The emergences of artificial light and stimulants i.e., caffeine have impacted the behavior and physiology that normally precede sleep. Caffeine may independently impact sleep/wakefulness, or in conjunction with the thermoregulatory system. Bright light during the biological night suppresses melatonin and changes the thermoregulatory pattern that precedes nocturnal sleep; these changes may ultimately impact the sleep/wakefulness system. To improve our understanding of physiological mechanisms promoting and disrupting sleep/wakefulness, it is important to examine the connection between melatonin and the sleep/wakefulness and thermoregulatory systems, and the impact of environmental and behavioral factors on these systems. Therefore, the aims of this dissertation were to: 1) determine the effect of a melatonin receptor analogue ramelteon, on daytime sleep and body temperature, and the relationship between the two variables; 2) determine the effect of daytime exogenous melatonin on resting

energy expenditure, (REE); and 3) determine the individual and compound effects of caffeine and bright light on thermoregulatory and sleep physiology at night.

Consistent with our hypotheses, 1) ramelteon significantly improved daytime sleep, lowered CBT, and increased peripheral heat loss 2) exogenous melatonin decreased REE during the daytime, and 3) caffeine delayed the nocturnal rise in peripheral heat loss, attenuated the fall in CBT, while the combination of caffeine and bright light decreased slow wave sleep and increased sleep onset latency.

These findings suggest that melatonin may play an important role in the regulation of sleep/wakefulness as evidenced by the effect of daytime ramelteon administration on sleep and thermoregulatory physiology and the effect of daytime exogenous melatonin on REE. Finally, caffeine and bright light had a negative impact on nocturnal sleep and these effects may be mediated in part by their impact on the thermoregulatory system.

DEDICATION AND ACKNOWLEDGMENTS

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CHAPTER 1

SLEEP AND THE THERMOREGULATORY SYSTEM: THE IMPACT OF MELATONIN, CAFFEINE, AND BRIGHT LIGHT

Rachel R. Markwald

Introduction

Due to many social, economic, and work-related pressures modern society has become an environment where most individuals no longer retire at dusk and rise at dawn. The invention of artificial light allowed for the extension of daily activities to occur at all hours of the biological night, while the use of central nervous system stimulants such as caffeine has become a common strategy to counteract sleepiness. Melatonin is a hormone responsible for mediating many physiological, endocrinological, and behavioral processes involved with sleep, and the internal circadian clock system (Wright KP Jr. 2007). In particular, it seems to be intimately involved in the processes of sleep and thermoregulation. In humans, melatonin levels are highest at night when sleep typically occurs and core body temperature level (CBT) is low. Additionally, exogenous melatonin given during the daytime decreases CBT and increases sleep efficiency in a nap opportunity (Cajochen, Krauchi et al. 1996; Cajochen, Krauchi et al. 1997; Hughes and Badia 1997). Further, there is evidence that this is more than just a temporal association between sleep and thermoregulation. In fact, they may be causally related to one another, with melatonin acting as a link between the two systems (Krauchi, Cajochen et al. 1999; Aoki, Zhao et al. 2008). Bright light and caffeine have impacted the behavior and physiology that normally precedes sleep during the biological night, thus impacting the relationship between these two processes. This chapter addresses what is known about melatonin, melatonergic analogues, caffeine, and bright light as they relate to sleep, thermoregulation, and the relationship between these two processes. This review begins with an introduction to the sleep and circadian systems followed by a review of research concerning melatonin and melatonergic analogues. Next, the association between thermoregulation and sleep will be examined. Finally, this review will conclude with a

detailed discussion regarding the impact of two behavioral/environmental factors (caffeine and bright light) on sleep and thermoregulation.

Sleep and Circadian Regulation of Sleep/Wakefulness

Sleep Regulation

Sleep is under the control of two distinct central nervous system processes. The first process is referred to as the homeostatic drive for sleep, or accumulation of sleep need that occurs with the accumulation of time awake and which translates to a physiological pressure to sleep. This homeostatic sleep drive is thought to promote sleep through inhibition of wakefulness promoting brain centers and activation of sleep promoting brain centers. Sleep/wakefulness regulation in diurnal animals involves an extensive circuitry of brain regions and cell types situated throughout the cortex and brain stem. Several nuclei are involved in the ascending arousal system including: the histaminergic tuberomammillary nuclei, serotonergic dorsal and median nuclei and raphe nuclei, noradrenergic locus coeruleus, acetylcholinergic basal forebrain and brain stem, and dopaminergic ventral periaqueductal grey matter (Saper, Scammell et al. 2005). The major monoaminergic systems each send afferent projections to a small cell group identified as an important somnogenic region in the brain termed the ventral lateral preoptic area (VLPO) of the hypothalamus (Chou, Bjorkum et al. 2002). During wakefulness, these arousal cell groups actively inhibit the VLPO. With time awake, however, the homeostatic drive to sleep becomes greater and the VLPO is disinhibited. It then inhibits arousal through direct *gamma*-Aminobutyric acid (GABA)ergic and galaninergic projections to each of these nuclei, with innervation of the tuberomammillary nuclei, linked to transitions between arousal and sleep (Lu, Bjorkum et al. 2002; Ko, Estabrooke et al. 2003). It has been shown in cats that the nucleotide

adenosine (the byproduct of energy metabolism; breakdown of ATP) builds up throughout the day in the basal forebrain, and dissipates with sleep (Porkka-Heiskanen, Strecker et al. 1997). Further, it has been proposed that adenosine acts on adenosine A1 and A2 receptors located in the VLPO and cortex to promote sleep (Saper, Scammell et al. 2005), thus the homeostatic drive to sleep might be mediated in part via the gradual accumulation of adenosine acting on A1 and A2 receptors.

Circadian Regulation

The second process that influences sleep/wakefulness is the master circadian clock located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus. Additionally, there are numerous peripheral clocks located in tissue, e.g. liver, vasculature (Ekmekcioglu, Haslmayer et al. 2001) that appear to be synchronized to the master circadian clock. The SCN consists of gene regulatory feedback loops which oscillate to a near 24 hr rhythm (Shearman, Sriram et al. 2000). This feedback loop is preserved when brain temperature fluctuations occur such as those observed with the circadian rhythm of body temperature. Circadian regulation of sleep/wake patterns of diurnal animals involves the extensive circuitry mentioned above with the addition of the master circadian clock in the SCN. The SCN has afferent and efferent projections to many of the wakefulness promoting nuclei including the neighboring dorsal medial nucleus and paraventricular nucleus (Dai, Swaab et al. 1997). When the circadian clock is promoting wakefulness, these arousal cell groups actively inhibit the VLPO. As mentioned earlier, the VLPO inhibits wake-promoting nuclei, and this is accomplished when the circadian wakefulness promoting signal is low and homeostatic drive is high.

Circadian regulation of sleep/wakefulness, and its localization to the SCN became apparent through several animal studies. Complete lesions of the SCN appear to abolish sleep and temperature circadian rhythms in rodents (Eastman, Mistlberger et al. 1984). Therefore, it is thought that the oscillating output of the clock gives time context to most physiological processes and behaviors including sleep, and ensures proper entrainment of internal rhythms to the daily light–dark cycle. Thus, the distribution of sleep over the 24-h day is strongly determined by the circadian system (Franken and Dijk 2009).

The average circadian period of the circadian clock in humans is slightly longer on average than 24 hrs (Czeisler and Klerman 1999; Wyatt, Ritz-De Cecco et al. 1999; Wright, Hughes et al. 2001) and is reset daily by light exposure. Light modifies the circadian rhythm output generated by the SCN, changing neuronal firing rates and synchronizing the internal clock to the external environment. Ganglionic photoreceptors in the retina directly transmit light/dark signals through the retinohypothalamic tract via glutamatergic input to the SCN and SNS input to the pineal gland. Additionally, the SCN receives indirect input from retinal recipient neurons of the intergeniculate leaflet via the geniculohypothalamic tract (Morin 1994). A dense serotonergic innervation from the median raphe nucleus modulates this photic input via serotonin 1B (5-HT_{1B}) presynaptic receptors on retinal glutamatergic terminals (Meyer-Bernstein and Morin 1996), and this function is thought to set the gain of light input to the SCN (Pickard and Rea 1997). Finally, melatonin production, under control of the circadian clock, provides feedback to the SCN through receptor binding to further modify activity of the internal master circadian clock.

Pineal Gland Regulation of Melatonin

Melatonin was found to be a hormone responsible for mediating SCN circadian rhythm output since removal of its major site of synthesis, the pineal gland, abolished free running rhythms in birds housed in darkness. Pineal transplants in these same birds restored rhythmicity (Redman, Armstrong et al. 1983). The SCN regulates the near-24-hour circadian rhythm in melatonin levels such that under normal conditions, melatonin levels are low during the solar day and high during solar darkness. High circulating levels of melatonin represent the biological night in nocturnal and diurnal species (Arendt 1988). It has been estimated in rats that blood flow to the pineal gland (4 ml/min/g) is higher than any other endocrine organ excluding the kidney (Goldman and Wurtman 1964). Additionally, in most mammals, including rats and humans, the pineal gland lacks an endothelial blood-brain barrier (Macchi and Bruce 2004). Therefore, melatonin can directly enter the circulation, as well as enter the cerebral spinal fluid via the pineal recess. The most developed pineal afferent is the sympathetic noradrenergic pathway from cell bodies in the superior cervical ganglion. These post-ganglionic neurons receive regulatory input from the SCN, which, in turn, receives direct retinal input from retinal ganglion cells as described above (Morin 1994). Using radioimmunoassay methods, mean melatonin production has been estimated in healthy adults at 28.8 µg/day (Lacoste 1993), with pronounced differences between individuals (Bergiannaki, Soldatos et al. 1995). It is important to note that melatonin has been found to display a circadian rhythm when assayed from plasma, urine, and salivary samples, thus allowing an accurate method for determining the timing of the internal circadian clock. The circadian melatonin rhythm represents the phase, amplitude, and period of the master internal clock (Wright KP Jr. 2007).

Melatonin Receptor Physiology

There are two identified membrane bound melatonin receptors in mammals, MT1 and MT2 (previously termed Mel1a and Mel1b when first discovered) (Reppert, Weaver et al. 1994; Reppert, Godson et al. 1995). A third melatonin receptor (Mel1c) has been identified in non-mammalian vertebrates (von Gall, Stehle et al. 2002). These receptors belong to the G-protein-coupled receptor superfamily and contain seven transmembrane domains. To date, MT1 and MT2 receptors have been identified in the mammalian brain centrally: in hypothalamic areas, SCN, hippocampus, thalamus, amygdala, cerebellum, ventral tegmental area, nucleus accumbens, cerebral cortex, and medial preoptic area (Reppert, Weaver et al. 1988; Weaver, Stehle et al. 1993; Morgan, Barrett et al. 1994; Williams, Hannah et al. 1995; Weaver and Reppert 1996; Thomas, Purvis et al. 2002) and peripherally including: the retina, the kidney, liver, heart, pancreas, adipocytes, and blood vessels (Brydon, Petit et al. 2001; Ekmekcioglu, Haslmayer et al. 2001; Masana, Doolen et al. 2002; Ekmekcioglu, Thalhammer et al. 2003; Savaskan, Jockers et al. 2007). Binding of melatonin to MT1/Mel1a receptors has been reported to acutely reduce neuronal firing of SCN neurons. The reduction in neuronal firing following MT1 receptor activation has been hypothesized to be associated with a reduction in the wakefulness promoting drive from the SCN. Therefore, melatonin appears to be involved in sleep promotion. Conversely, MT2/Mel1b have been reported to be primarily involved with the phase shifting properties of melatonin (Dubocovich, Yun et al. 1998; Jin, von Gall et al. 2003) since MT2 antagonists block the phase shifting effect, and phase shifting capability is unaffected in MT1 knockout mice. Recently, Fisher and colleagues were the first to use a selective subtype melatonin ligand (IIK7, a MT2 agonist) demonstrating a sleep promoting action with MT2

stimulation in rats, thus challenging the previously held assumption of discrete actions of MT1 and MT2 receptor mediated effects (Fisher and Sugden 2009).

In addition to the membrane-bound melatonin receptors there is cytosolic receptor termed MT3 in mammals. The discovery of this receptor followed the development of specific ligands. This melatonin receptor is not G-protein coupled but instead binds to intracellular quinone reductase (Nosjean, Ferro et al. 2000). The MT3 receptor has been hypothesized to be associated with antioxidant effects of melatonin (Nosjean, Ferro et al. 2000).

Exogenous Melatonin and Melatonin Analogues

Exogenous Melatonin

Exogenous melatonin enters the circulation after absorbance from the small intestine. Both endogenous and exogenous melatonin are then transported by the portal circulation to the liver, where partial metabolism to 6-hydroxymelatonin occurs, followed by renal excretion. The non-metabolized portion of melatonin travels through the systemic circulation to peripheral tissues, as well as crossing the blood brain barrier to bind to receptors in the brain. Exogenous melatonin has been given in doses equivalent to physiological concentrations, as well as pharmacological doses ranging from just above peak endogenous levels to 100X these concentrations. Peak levels of exogenous melatonin are observed in the periphery within about 30-60 minutes post ingestion, with a serum half-life of between 30 and 50 minutes depending on the dose.

Melatonin Analogues

Synthetic compounds that closely resemble the chemical structure of melatonin and consequently act on MT1 and MT2 receptors have been developed and approved for the treatment of insomnia. Due to increased receptor specificity and half-life, melatonin analogues such as ramelteon and tasimelteon might be advantageous to melatonin in the treatment of certain types of insomnia such as those associated with low melatonin secretion, jet lag, and shift work sleep disorder (Pandi-Perumal, Srinivasan et al. 2007). Melatonin analogs are distinct from other sleep medications by their mechanism of action. Most over the counter (OTC) sleep aids act by antagonizing the effects of the wakefulness promoting actions of histamine on the VLPO. Most prescribed sleep medications interact with the GABAergic system, e.g., benzodiazepine and non-benzodiazepine hypnotics. From a public health perspective, melatonin analogues may offer a safer alternative to these drugs since OTC and prescribed sleep medications have been reported to impair cognition (Srinivasan, Pandi-Perumal et al. 2009) and prescribed medications have been reported to be associated with a number of adverse behaviors (Dolder, Lacro et al. 2002).

Thermoregulation

Thermoregulation can be thought of as the balance between heat production and heat loss at any given moment in time (Van Someren 2006). The range of internal or core body temperature (CBT) is tightly regulated by nuclei in the preoptic area of the anterior hypothalamus within a narrow range ~1 degree C. These nuclei coordinate appropriate efferent responses based on sensory afferent information from core (internal) and peripheral (skin) temperature sensitive neurons in order to maintain heat balance (Gilbert, van den Heuvel et al.

1999; Harris, Burgess et al. 2001; Charkoudian 2003). In cold stress conditions, activation of sympathetic nerve terminals results in increased binding of norepinephrine on blood vessels causing vasoconstriction. Vasoconstriction leads to the narrowing of blood vessel diameter and thus a reduction of blood flow to the skin. This minimizes heat loss to the environment. Conversely, during heat stress, the peripheral blood vessels in glabrous and non-glabrous skin undergo small increases in vessel diameter (vasodilation) that facilitate heat exchange between the body and the environment by redistributing blood flow towards the skin. This is accomplished by two mechanisms: an increase in the cutaneous active vasodilator system and a reduction in tonic vasoconstrictor activity (Charkoudian 2003).

Body temperatures (core and peripheral) display a circadian rhythm that is regulated by the master circadian clock located in the SCN. CBT, as assessed through tympanic and rectal thermistors has been shown to have a peak that occurs prior to habitual bedtime, decreases through the night, and a nadir that occurs approximately two hours prior to habitual waketime (Krauchi, Cajochen et al. 2006). The circadian rhythm of peripheral temperature measured at distal skin sites e.g. the hands and feet, have been shown to be phase advanced with respect to CBT (Krauchi, Cajochen et al. 1999; Krauchi, Cajochen et al. 2000). In accordance with the circadian rhythm of peripheral temperature, skin blood flow measured at the finger is approximately 4 hrs phase advanced relative to CBT (Smolander, Harma et al. 1993) with a peak occurring near the nadir in CBT. Modulation of blood flow to the distal skin in turn, modulates peripheral heat loss. Thus, it has been shown that the circadian variation in peripheral heat loss contributes to ~75% of the oscillation in CBT (Aschoff 1972). Another method by which peripheral heat loss is routinely quantified is by calculation of the distal-to-proximal skin temperature gradient (DPG) (Rubinstein and Sessler 1990). An increase in distal temperature

narrows the gradient between the two skin sites, indicating peripheral heat loss at distal sites. The DPG is a standard method to measure heat loss since skin blood flow varies greatly depending on ambient temperature conditions. The use of a reference temperature from a proximal skin region exposed to the same ambient temperature as the distal skin region provides an important internal control standard (Rubinstein and Sessler 1990; Krauchi, Cajochen et al. 2000). Further, the sensitivity of this method has been tested in a number of thermoregulatory challenges (Frank, Higgins et al. 1997).

In addition to heat loss, heat production will also affect CBT via changes in metabolic rate, (or the energy expended in a given amount of time) (Landsberg, Young et al. 2009). Energy expenditure is a result of the caloric cost of cellular, chemical, and mechanical reactions that occur in the body. Since ~70% of metabolism of substrate is lost as heat, this is an effective way to increase CBT. Conversely, decreases in energy expenditure would lower heat production and alter CBT (Landsberg, Young et al. 2009). During cold stress, increases in sympathetic nerve activity (SNA) redistribute blood flow away from the periphery, and may also increase basal metabolic rate (BMR) through β -adrenergic receptor activation on brown and white adipocytes (Shimizu, Nikami et al. 1991). This is especially true in rodents that possess large amounts of brown adipose tissue. In this way, SNA has been linked to alterations in metabolism and heat production. Additionally, there is a circadian rhythm to energy expenditure and sleep has the effect of lowering oxygen consumption as measured by indirect calorimetry when compared to a condition of continued wakefulness while lying in bed (Fraser, Trinder et al. 1989; Jung, Melanson et al. in press)

Thermoregulation and Sleep

Findings from early studies in humans revealed a close temporal relationship between changes in CBT (Czeisler, Weitzman et al. 1980; Zulley, Wever et al. 1981) and peripheral heat loss and sleep onset (Haskell, Palca et al. 1981; van den Heuvel, Noone et al. 1998). Due to the time lag needed for heat loss from the periphery to reach the core (called thermal inertia), the increase in peripheral heat loss precedes CBT changes by approximately 25 to 100 min (Gilbert, van den Heuvel et al. 2004). Peripheral heat loss has been correlated with a faster sleep onset latency (SOL) (Krauchi, Cajochen et al. 1997; Krauchi, Cajochen et al. 1999; Krauchi, Cajochen et al. 2000). Further, skin warming through the use of a liquid filled, temperature controlled whole body suit has been shown to decrease SOL by 27% (Raymann, Swaab et al. 2005). Additionally, Raymann and colleagues used this skin thermosuit with healthy sleeping young and older subjects and found that proximal warming resulted in deeper sleep and suppressed wakefulness, whereas distal skin warming enhanced REM sleep and suppressed light sleep (Raymann 2008). Based on these study findings and work from Krauchi and colleagues (Krauchi, Cajochen et al. 1997; Krauchi, Cajochen et al. 2000; Krauchi and Wirz-Justice 2001), it is now thought that distal skin temperature (peripheral heat loss) is a better predictor of sleepiness and sleep onset than CBT (Krauchi, Cajochen et al. 1999; Krauchi, Cajochen et al. 2000). Therefore, it has been proposed that body temperature might act to provide a signal to sleep-related neurons in somnogenic brain areas such as the medial preoptic area (McGinty, Alam et al. 2001; Szymusiak, Steininger et al. 2001; Gilbert, van den Heuvel et al. 2004) and the VLPO (Saper, Scammell et al. 2005).

It is possible that initiation of sleep could occur via changes in CBT leading to changed spinal cord and brain temperatures (Boulant 1981; Van Someren 2000). A subpopulation of

warm-sensitive POAH neurons spontaneously increases its firing rate at sleep onset and experimental warming of the POAH induces a similar increase in this firing rate, and ultimately facilitates sleep in animals (McGinty and Szymusiak 1990; Alam, Szymusiak et al. 1995; McGinty, Alam et al. 2001). Selective neuronal activation was observed with c-Fos expression during non-rapid eye movement sleep in the medial preoptic nucleus in cats (Tortorello, Benedetto et al. 2009). Additionally, experimental warming of a subset of serotonergic neurons in the interfascicular part of the dorsal raphe nucleus (DRI) results in thermoregulatory cooling responses such as peripheral heat loss in cats (Cronin and Baker 1977). Stimulation of the DRI alters neural activity in the POAH (Werner and Bienek 1985) and direct projections between the two nuclei have been confirmed (Tillet, Batailler et al. 1993). Additionally, medial preoptic inactivation with a GABA_A receptor agonist increased c-Fos expression in serotonergic neurons in the dorsal raphe nucleus (DRN), suggesting an inhibitory role for the medial preoptic area on the wake-promoting activity of the DRN (Kumar, Szymusiak et al. 2008). Therefore, it is possible that a neural network between the POAH and DRN neurons may play a role in the link between sleep and temperature regulation. Sleep, however is not usually attempted during the circadian peak of CBT when brain temperature is highest and sleep propensity is at its lowest. Therefore, if sleep and thermoregulatory processes are functionally coupled, the peak in CBT may trigger a sequence of neural events in preparation for sleep. Alternatively, it is possible that a signal may arise elsewhere to turn on the sleep-promoting firing patterns of POAH neurons.

There is emerging evidence that temperature activated transient receptor potential ion channels 3 and 4 (TRPV3 and TRPV4), may play a role in the sensory relay of peripheral temperature to the brain (Lowry, Lightman et al. 2009). These temperature sensitive proteins have been located on primary sensory neurons in the skin and respond to increases in

temperature within the range of 22 - 42°C (Patapoutian, Peier et al. 2003). As stated earlier, there is a circadian rise in distal skin blood flow (and thus temperature) which precedes CBT by ~4 hrs (Smolander, Harma et al. 1993). This increase in distal skin temperature is amplified by postural change (Tikuisis and Ducharme 1996; Krauchi, Cajochen et al. 1997), a warm sleep environment (Goldsmith and Hampton 1968; Muzet, Libert et al. 1984; Okamoto, Iizuka et al. 1997), and lights off (Krauchi and Wirz-Justice 2001). Therefore, it seems plausible that via increases in distal skin temperature, skin thermosensory afferents may become activated and alter the firing rates of POAH and DRI neurons. Finally, melatonin via central and peripheral effects might act, in part, as a link between the thermoregulatory system and sleep/wakefulness regulation.

The Role of Melatonin and Melatonin Analogues in Sleep

The Effect of Exogenous Melatonin During the Biological Day

Exogenous melatonin has been administered during the biological day in physiological and pharmacological doses to examine its effect on sleep in humans. The advantage of this model is that acute physiological effects of melatonin can be observed when endogenous melatonin levels are low.

Findings from several studies suggest that melatonin has a soporific effect when the circadian rhythm of endogenous melatonin level is low. Melatonin has been shown to affect subjective and objective parameters of sleep and sleepiness. Dollins et al administered (0.1-10mg) doses of melatonin at 11:45AM and found that all doses significantly increased sleep duration, as well as self-reported sleepiness and fatigue, relative to placebo. Moreover, all of the

doses significantly decreased SOL (Dollins, Zhdanova et al. 1994). Wyatt et al., reported that (0.5mg or 5mg) of melatonin given during the daytime increased sleep efficiency in healthy adults (Wyatt, Dijk DJ et al. 1999). When three different doses of exogenous melatonin were given during the daytime after a seven hour sleep opportunity, all three doses resulted in a decrease in SOL, an increase in total sleep time (TST), and a decrease in wake after sleep onset (WASO) (Hughes and Badia 1997), additionally, when a 5mg preparation of melatonin was given at 1400 to healthy participants there was a 40% decrease in SOL to stage 1 sleep (Reid, Van den Heuvel et al. 1996). Further, Gilbert and colleagues compared melatonin (5mg) to the commonly prescribed benzodiazepine, temazepam during the daytime and found a similar reduction in SOL as compared to placebo (Gilbert, van den Heuvel et al. 1999). This finding also occurred when 5mg melatonin was compared to the hypnotic Zopiclone during the daytime (Holmes, Gilbert et al. 2002). The repeated finding that exogenous melatonin improves sleep when endogenous levels are low makes it a prime candidate for the treatment of circadian rhythm sleep disorders such as jet lag.

The Effect of Exogenous Melatonin During the Biological Night

There has been extensive research in humans on the use of exogenous melatonin during the biological night with varying results on sleep. Early studies investigated the effects of high doses of melatonin administered intravenously. Cramer and colleagues reported sleep inducing effects of 50 mg iv melatonin (Cramer, Rudolph et al. 1974), and Anton-Tay et al, reported that melatonin administration of between 0.25-1.25 mg/kg reduced sleep latency (Anton-Tay, Diaz et al. 1971). Additionally, melatonin (0.1-0.3mg) decreased SOL and latency to stage 2 sleep when

compared to placebo, with no significant effect on other sleep parameters (Zhdanova, Wurtman et al. 1996). In summary, most findings from nighttime melatonin administration in healthy sleepers have only observed significant decreases in SOL.

Melatonin Analogues and Sleep

There are several melatonin analogues currently on the market or undergoing clinical trials. It should be acknowledged that most studies were designed to examine the effects of using a melatonergic analogue on circadian phase shifting. Only the studies that pertain to the acute, soporific effects of melatonin analogues will be discussed in this section.

Ramelteon, (S)-N-[2-(1,6,7,8-tetrahydro-2H-indeno-[5,4-b]furan-8-yl)ethyl]propionamide (TAK-375), is a high affinity selective MT1/MT2 receptor agonist that shows approximately four times greater potency at the MT1 receptor and 17 times greater potency at the MT2 receptor compared to melatonin, with very low affinity for binding to the MT3 (non membrane-bound) receptor or other neurotransmitter receptor systems (Kato, Hirai et al. 2005). It has been shown that ramelteon, unlike other hypnotics, acts preferentially on the MT1 and MT2 receptors of the SCN (Hirai, Kato et al. 2003).

Two randomized, placebo controlled single dose trials were conducted to determine the efficacy of ramelteon in a transient model of insomnia in which healthy volunteers were required to sleep in a novel environment. In the first study, 375 participants received either placebo or ramelteon 16 or 64 mg administered 30 minutes prior to habitual bedtime. Ramelteon improved latency to persistent sleep by approximately 10 minutes, and total sleep time increased by roughly 12 minutes, largely due to shortened sleep latencies (Roth, Stubbs et al. 2005). Using a

similar methodology, 289 adults were randomized to placebo or ramelteon 8 or 16 mg. Compared to placebo, ramelteon 8 mg decreased sleep latency by 7.5 minutes (Zammit, Schwartz et al. 2009). The effects of ramelteon on a daytime sleep paradigm have not been assessed.

Tasimelteon (VEC-162, previously known as BMS-214778) ((1R-trans)-N-[[2-(2,3-dihydro-4-benzofuranyl)cyclopropyl] methyl] propanamide) is a melatonin receptor agonist with high affinity for MT1 and MT2 receptors (Rajaratnam, Polymeropoulos et al. 2009). Two recent randomized controlled trials (phase II and III) demonstrated that tasimelteon dose-dependently improves the initiation and maintenance of sleep after a 5-hour advance of the sleep-wake and light-dark cycle, and that these effects occurred simultaneously with a phase advance of the plasma melatonin rhythm (Rajaratnam, Polymeropoulos et al. 2009). Since tasimelteon improved sleep at a scheduled time when melatonin levels are low the authors suggest that the compound has potential for treatment of circadian rhythm sleep disorders.

The Role of Melatonin in Thermoregulation

Melatonin and Thermoregulation during the Biological Day

Administration of exogenous melatonin during the biological daytime, when endogenous melatonin levels are low, has been shown to reduce CBT (Dawson, Gibbon et al. 1996; Reid, Van den Heuvel et al. 1996; Hughes and Badia 1997; Satoh and Mishima 2001), and increase peripheral heat loss (Krauchi, Cajochen et al. 2000; Aoki, Stephens et al. 2003; Aoki, Stephens et al. 2006; Krauchi, Cajochen et al. 2006). This melatonin-induced increase in peripheral heat loss is reported to be correlated with a decrease in SOL (Krauchi, Cajochen et al. 1999; Krauchi,

Cajochen et al. 2000; Lack and Gradisar 2002). Heat loss following exogenous melatonin administration has been primarily determined by measuring skin temperature levels, at the hands and feet (Aoki, Yokoi et al. 2005; Krauchi, Cajochen et al. 2006; Aoki, Zhao et al. 2008), and by calculating the distal-proximal gradient (DPG) in skin temperature as described earlier (Krauchi, Cajochen et al. 2000). This hypothermic effect observed during the daytime mimics the natural progression of thermoregulatory events that occur at nighttime when endogenous melatonin levels begin to rise. Increases in peripheral heat loss are highly correlated with increases in blood flow to the skin (Rubinstein and Sessler 1990). Aoki and colleagues have used the laser Doppler flowmetry technique to assess the thermoregulatory effects of daytime melatonin administration by measuring changes in skin blood flow. Using a cold thermic challenge test to reduce CBT, it was shown that skin blood flow was higher in the melatonin (3mg) condition than placebo condition. Melatonin-induced sleepiness in participants occurred in parallel with a reduction in the threshold for vasoconstrictor activity. This change in thermoregulatory set-point might indicate that melatonin acts as a circadian modulator of the thermoregulatory set-point (Aoki, Zhao et al. 2008). Additionally, exogenous melatonin caused a shift to lower temperatures in the blood flow response to internal temperature during whole body heating (Aoki, Stephens et al. 2006) and this was most likely accomplished through the cutaneous active vasodilator system (JM 1996). Thus, neural control of both the cutaneous vasoconstrictor and active vasodilator systems are affected by exogenous melatonin administration, suggesting that secretion of melatonin at night might play an important role in nocturnal thermoregulation via the sympathetic nervous system.

Melatonin and Thermoregulation during the Biological Night

The skin blood flow promoting property of melatonin may account for ~40% of the amplitude of the circadian rhythm in CBT due to heat loss under resting conditions (Cagnacci, Elliott et al. 1992). As mentioned earlier sleep is usually attempted ~2 hrs after endogenous melatonin levels begin to rise. It is possible that endogenous melatonin may affect sleep/wakefulness regulation through potential central and peripheral effects on blood flow and skin temperature. Exogenous melatonin administration to healthy sleepers when endogenous levels are already high has not been reported to have any further effect on thermoregulatory variables.

Energy Expenditure

Melatonin has been shown to play a role in energy expenditure and body mass regulation in hibernating mammals (Bartness, Demas et al. 2002). In nocturnal animals, melatonin has been proposed to increase the thermogenic capacity of the animal by increasing sympathetic activity to white and brown adipose tissue. Additionally, there is correlational evidence from studies of rats that daily melatonin administration suppressed the age-related gain in visceral fat (Rasmussen, Boldt et al. 1999; Prunet-Marcassus, Desbazeille et al. 2003) and prevented the increase in body fat normally observed in ovariectomized rats (Ladizesky, Boggio et al. 2003). A reduction in CBT (0.3°C) has also been shown with a MT₂ melatonin analogue in rats (Fisher and Sugden 2009). As stated earlier, melatonin has an acute hypothermic effect on CBT in humans that is partly mediated through changes in peripheral heat loss. It has long been recognized that the drop in core temperature that occurs during sleep is mainly the result of an increase in heat dissipation, and to a lesser extent the result of a decrease in metabolic heat

production (Van Someren 2006). In humans, it remains to be determined if melatonin or a melatonin analogue can acutely modulate energy expenditure and thus contribute to the observed thermoregulatory effects of melatonin. This question will be addressed in chapter 3 of this dissertation.

Melatonin analogues and thermoregulation

Given the extensive evidence that melatonin alters thermoregulatory physiology through its hypothermic effect on CBT, and increase in heat loss, it seems logical that a melatonin analogue such as ramelteon might have the same effect. Additionally, given the temporal association (Czeisler, Weitzman et al. 1980; Zulley, Wever et al. 1981) and recent evidence for a functional, mechanistic link between body temperature and sleep (Krauchi, Cajochen et al. 2000; Gilbert, van den Heuvel et al. 2004; Krauchi, Cajochen et al. 2006; Aoki, Zhao et al. 2008) it will be important to assess temperature changes with melatonin analogues and will be addressed in chapter 2 of this dissertation.

The Effect of Caffeine on Sleep and Wakefulness

Caffeine is a methylxanthine and an adenosine antagonist that is widely used to promote wakefulness. There have been three identified mechanisms of action by caffeine: intracellular mobilization of calcium, inhibition of phosphodiesterases, and antagonism at the level of adenosine receptors (Nehlig, Daval et al. 1992). It is the latter of these three mechanisms that is most likely to mediate caffeine's effects in the central nervous system (Nehlig, Daval et al. 1992). Adenosine is a byproduct of cellular metabolism that binds to adenosine A_1 and A_{2A} receptors throughout the central nervous system (Dunwiddie 1985), and in the periphery

(Schindler, Karcz-Kubicha et al. 2005). In the brain, adenosine accumulates with time spent awake from the previous sleep episode and dissipates during sleep. Consequently, it is considered a marker of sleep homeostatic pressure and regarded as a sleep promoting substance. As adenosine accumulates with time awake it may exert its sleep promoting effect via binding to adenosine receptors in the VLPO (Saper, Scammell et al. 2005), other sleep/wakefulness associated nuclei, i.e. activation of noradrenaline neurons (Nehlig, Daval et al. 1992), reduction in serotonin availability at postsynaptic receptor sites (Hirsh 1984)), or through its effect on pineal melatonin levels (Sarda, Cespuglio et al. 1989). There is evidence that adenosine binds to adenosine receptors on the pineal gland (Sarda, Cespuglio et al. 1989), and increases melatonin levels in a dose dependent manner (Gharib, Reynaud et al. 1989). Further, caffeine blocked the increase in pineal melatonin after the adenosine agonist 5'-N-ethylcarboxamidoadenosin was given (Babey, Palmour et al. 1994). Specifically, there is some evidence that caffeine's arousal effect is mediated via the adenosine A_{2A} receptor since A_{2A} receptor knockout mice do not show a strong sleep rebound after sleep deprivation and caffeine does not induce wakefulness in these mice (Huang Zhi-Li 2005). It has been suggested that the effect of caffeine on sleep/wakefulness may also be mediated, in part, by caffeine's action on melatonin levels during the biological night, and in turn, modulation of melatonin's effects on temperature (Wright KP 1997). This has not yet been studied. Chapter four of this dissertation will address this question.

The Effect of Caffeine on Thermoregulation

Previous studies have reported that caffeine attenuates the nocturnal decrease in CBT when compared to dim light exposure in humans, (Wright, Badia et al. 1997; Wright, Myers et al. 2000), and increases brain temperature in rats (Pechlivanova, Tchekalarova et al.) Further,

caffeine in doses ranging from 2 to 8 mg/kg of body weight has been shown to increase resting metabolic rate (RMR) in humans (Acheson, Zahorska-Markiewicz et al. 1980; Arciero, Gardner et al. 1995; Arciero, Bougopoulos et al. 2000; Acheson, Gremaud et al. 2004). Astrup and colleagues found a dose response relationship for RMR to 100, 200, and 400 mg caffeine that resulted in 9.2 ± 5.7 , 7.2 ± 6.0 , and 32.4 ± 8.2 kcal/hour above the placebo responses, respectively (Astrup, Toubro et al. 1990). This increase in RMR, if uncompensated for, would increase CBT since ~70% of energy is lost as heat during metabolic processes (Cagnacci, Elliott et al. 1992). There is minimal evidence as to caffeine's effect on skin temperatures in a controlled laboratory setting. Past studies have shown a decrease in finger temperature (France and Ditto 1992), while others have shown no change in forearm blood flow (Umemura, Ueda et al. 2006). The effect of caffeine on the DPG has never been looked at in humans and is addressed in chapter four of this dissertation.

Bright Light and Thermoregulation

It was first discovered 30 years ago that bright light exerts a strong influence on the circadian system when Lewy and colleagues noticed that after exposure to bright light, melatonin levels in the blood were suppressed (Lewy et al. 1980). It was further discovered the amount of suppression is intensity dependent (McIntyre et al. 1989), and response time is rapid (Lewy et al. 1984). Additionally, bright light given during the biological night prevents the normal decline in the CBT rhythm compared to a dim light placebo condition (Badia, Myers et al. 1991; Dijk, Cajochen et al. 1991; Wright, Badia et al. 1997; Burgess, Sletten et al. 2001; Cajochen, Munch et al. 2005). Further, bright light of two different spectral frequencies (460 nm and 550 nm) decreased the DPG to the same extent when light exposure occurred in the late evening

(Cajochen, Munch et al. 2005). The effect of bright light on thermoregulatory physiology is mediated through the SCN. This may be a direct effect as findings from animal studies have identified neural projections from the SCN to the subparaventricular hypothalamic nucleus (SPZ) and efferents from the SPZ to temperature regulating neurons in the POAH (Saper, Scammell et al. 2005). It may also be a reflection of endogenous melatonin suppression, thus removing the ability of the neurohormone to bind to its receptors in the brain (i.e. SCN and POAH), and exert thermoregulatory effects. The effect of bright light in combination with caffeine has never been examined for skin temperatures (i.e., the DPG) in humans and will be addressed in chapter four of this dissertation.

Summary

This literature review has highlighted several research studies in animals and humans aimed at understanding the relationship between the sleep and thermoregulatory processes. In general, there is evidence that endogenous melatonin plays a role in the integration of these two systems. Further that caffeine and bright light (two common behavioral and environmental factors) impact each system directly, or via their impact on endogenous melatonin. The complex relationships that exist between sleep, thermoregulatory, and melatonin physiology make it difficult to elucidate which factors preceding sleep at night are causally related to one another. Therefore, several questions remain pertaining to the nature of these relationships and if answered, may aid in the development of treatment interventions for the diverse number of sleep problems that affect the population. For example, it has been suggested that it is the peripheral heat loss (change in DPG) that occurs during the nocturnal increase in melatonin levels that is most predictive of SOL. This may be of relevance, but SOL may also be impacted by a potential

role for melatonin on metabolic physiology, a question that has not yet been examined in humans. Further, it is necessary to recognize the potential impact of caffeine consumption and bright light at night on the thermoregulatory variables (i.e. DPG) that have been shown to be predictive of SOL. Finally, with the development of melatonin analogues, coupled with the increased number of individuals attempting to sleep during the biological day (i.e. night shift workers), it will be important to understand if these medications are effective at promoting sleep during the biological day because they mimic the nocturnal thermoregulatory pattern that occurs when endogenous melatonin levels are high.

Dissertation Aims

The aim of this dissertorial work was to further explore the relationship between the sleep and thermoregulatory processes. The following questions were addressed: 1) Does a melatonin analogue, ramelteon, improve daytime sleep in part, due to thermoregulatory effects on DPG and CBT similar to what is observed during the biological night? 2) Is a daytime dose of exogenous melatonin capable of reducing resting energy expenditure thereby affecting heat production and CBT? 3) Does a commonly consumed dose of caffeine in the early evening affect the DPG, as well as the pattern of other thermoregulatory variables? Is this dose of caffeine disruptive to sleep scheduled six hours later and is it associated with changes in the DPG. Further, is there a compound effect of caffeine and bright light on the DPG and sleep quality during the biological night? Specifically, the following hypotheses were tested: 1) Ramelteon (a melatonin analogue) would reduce core temperature, increase the DPG, as well as shorten SOL, reduce WASO and increase TST during a daytime sleep opportunity 2) Daytime exogenous melatonin administration (5 mg) would reduce resting metabolic rate and calculated energy expenditure compared to placebo 3) caffeine would act individually and in combination with bright light to attenuate the circadian fall in CBT, attenuate the circadian rise in the DPG during the time of habitual bedtime, as well as increase SOL and decrease slow wave sleep during a nighttime sleep opportunity.

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CHAPTER 2

EFFECTS OF THE MELATONIN MT-1/MT-2 AGONIST

RAMELTEON ON DAYTIME BODY TEMPERATURE AND SLEEP

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Abstract

Study Objectives: A reduction in core temperature and an increase in the distal-proximal skin gradient (DPG) are reported to be associated with shorter sleep onset latencies (SOL) and better sleep quality. Ramelteon is a melatonin MT-1/MT-2 agonist approved for the treatment of insomnia. At night, ramelteon has been reported to shorten SOL. In the present study we tested the hypothesis that ramelteon would reduce core temperature, increase the DPG, as well as shorten SOL, reduce wakefulness after sleep onset (WASO) and increase total sleep time (TST) during a daytime sleep opportunity.

Design: Randomized, double-blind, placebo-controlled, cross-over design. 8 mg ramelteon or placebo was administered 2 h prior to a 4 h daytime sleep opportunity.

Setting: Sleep and chronobiology laboratory.

Participants: Fourteen healthy adults (5 females), aged (23.2 ± 4.2 yrs).

Results: Primary outcome measures included core body temperature, the DPG and sleep physiology [minutes of TST, WASO and SOL]. We also assessed as secondary outcomes, proximal and distal skin temperatures, sleep staging and subjective TST. Repeated measures ANOVA revealed ramelteon significantly reduced core temperature and increased the DPG (both $P < 0.05$). Furthermore, ramelteon reduced WASO and increased TST, stages 1, & 2 sleep (all $P < 0.05$). The change in the DPG was negatively correlated with SOL in the ramelteon condition.

Conclusions: Ramelteon improved daytime sleep, perhaps mechanistically in part by reducing core temperature and modulating skin temperature. These findings suggest that ramelteon may have promise for the treatment of insomnia associated with circadian misalignment due to circadian sleep disorders.

Introduction

Insomnia is a common symptom of circadian sleep disorders (Sack, Auckley et al. 2007). Circadian sleep disorders are a special class of sleep disorders that are often associated with misalignment between internal biological time and the desired or imposed sleep-wakefulness schedule (Sack, Hughes et al. 1997; Sack, Auckley et al. 2007; Sack, Auckley et al. 2007). The resulting circadian misalignment leads to sleep occurring at an internal biological time when the circadian clock is promoting wakefulness. Humans usually initiate sleep on the downward slope of the circadian body temperature rhythm (Czeisler, Weitzman et al. 1980), when the rate of change of core temperature and peripheral heat loss are maximal and when endogenous levels of melatonin begin to rise (Zulley, Wever et al. 1981; Campbell and Broughton 1994). A close temporal relationship between sleep and core body temperature (CBT) has been found, such that sleep is optimal during the biological night when CBT is low and also when endogenous melatonin levels are high (Czeisler, Weitzman et al. 1980; Zulley, Wever et al. 1981; Dijk and von Schantz 2005).

Administration of exogenous melatonin during the biological day, when endogenous melatonin levels are low, has been shown to reduce CBT (Dawson, Gibbon et al. 1996; Reid, Van den Heuvel et al. 1996; Hughes and Badia 1997; Satoh and Mishima 2001), increase peripheral heat loss (Krauchi, Cajochen et al. 1997; Krauchi, Pache et al. 2002; Aoki, Stephens et al. 2006; Aoki, Zhao et al. 2008), and improve sleep (Wright KP Jr. 2007). The increase in peripheral heat loss is reported to be negatively correlated with sleep onset latency (SOL) (Krauchi, Cajochen et al. 1999; Krauchi, Cajochen et al. 2000; Lack and Gradisar 2002). Heat loss following exogenous melatonin administration has been primarily determined by measuring skin temperature levels at the hands and feet (Aoki, Yokoi et al. 2005; Krauchi, Cajochen et al.

2006; Aoki, Zhao et al. 2008) and by calculating the distal-proximal gradient (DPG) in skin temperature (Krauchi, Cajochen et al. 2000). The DPG was first developed by subtracting fingertip temperature from a site on the forearm (Rubinstein and Sessler 1990), and has been modified by others (Krauchi, Cajochen et al. 2000; van der Helm-van Mil, van Someren et al. 2003) to be calculated as the difference in distal skin temperature sites on the hands and feet minus skin temperature levels at proximal sites such as the chest. The sensitivity of the DPG method has been tested in a number of thermoregulatory challenges (Frank, Higgins et al. 1997). Heat loss has also been assessed through thermoimaging of the palmar and dorsal surfaces of the hand and fingers (Krauchi, Cajochen et al. 2006) and measurement of cutaneous blood flow (Aoki, Stephens et al. 2003; Aoki, Stephens et al. 2006; Aoki, Zhao et al. 2008). During the biological night, exogenous melatonin administration decreases SOL with little effect on sleep architecture (Zhdanova, Wurtman et al. 1996); whereas during the biological day, exogenous melatonin administration decreases SOL (Dollins, Zhdanova et al. 1994; Reid, Van den Heuvel et al. 1996; Gilbert, van den Heuvel et al. 1999; Holmes, Gilbert et al. 2002) and wakefulness after sleep onset (WASO) (Hughes and Badia 1997) and increases total sleep time (TST) (Dollins, Zhdanova et al. 1994; Hughes and Badia 1997; Wyatt, Dijk et al. 2006).

Ramelteon is a sleep promoting therapeutic approved for the treatment of insomnia that acts specifically on melatonin MT-1 and MT-2 receptors with a greater affinity and a longer half-life than melatonin at these receptors (Pandi-Perumal, Srinivasan et al. 2007). Additionally, ramelteon has no affinity for the cytosolic MT-3 receptor (Pandi-Perumal, Srinivasan et al. 2007). At night, ramelteon has been reported to decrease SOL and thereby increase TST in healthy sleepers on the first night sleeping in a novel environment (Zammit, Schwartz et al. 2009). Ramelteon was also reported to decrease SOL in older adults with chronic insomnia over

five weeks of treatment (Roth, Seiden et al. 2006). Further, a 6 month study showed that nightly ramelteon administration reduced both electroencephalograph (EEG) determined latency to persistent sleep as well as subjective SOL in adults with chronic primary insomnia (Mayer, Wang-Weigand et al. 2009).

The influence of ramelteon on thermoregulatory physiology and daytime sleep in humans is unknown. If improvements in daytime sleep following ramelteon are associated with decreases in CBT and increases in peripheral heat loss, such findings may provide information regarding possible physiological mechanisms by which ramelteon improves sleep during the biological day. Furthermore, showing the efficacy of ramelteon for improving sleep during the biological day would provide support for testing the application of ramelteon in the treatment of insomnia due to circadian sleep disorders (e.g., jet lag, shift work, delayed sleep phase). Therefore, we tested the hypothesis that ramelteon (8mg) would reduce CBT and increase the DPG under controlled bed rest conditions, as well as decrease SOL and WASO and increase TST in a daytime sleep opportunity. We also hypothesized that a greater change in DPG would be associated with a shorter SOL.

Methods

Subjects

Fourteen healthy adults (5 females), aged (23.2 ± 4.2 yrs; mean $\pm$ SD), body-mass index (22.5 ± 2.7 kg/m²) participated. Women were studied during the follicular phase of their menstrual cycle to control for effects of decreased responsiveness to daytime melatonin administration during the luteal phase (Cagnacci, Soldani et al. 1996). Participants underwent a

medical evaluation at the Clinical and Translational Research Center (CTRC) at the University of Colorado – Boulder. Participants were admitted into the study if they were free of any medical conditions as determined by medical and psychiatric history, physical exam, blood chemistries, 12-lead clinical electrocardiogram, and urine toxicology for drug use. Exclusion criteria were: current smoker, not lived at Denver/Boulder altitude (1600 m) for at least one year, any medical, psychiatric, or sleep disorder, habitual sleep duration <6 or >9 h, and taking any medication. Study procedures were approved by the University of Colorado - Boulder IRB and the Scientific Advisory and Review Committee of the CTRC. Participants gave written-informed consent, and all procedures were performed according to the Declaration of Helsinki.

Study Design

A randomized, within-subject, crossover, placebo-controlled research design was used in which participants spent two separate days in the laboratory separated by one week. Both visits occurred on the same day of the week. Each laboratory visit was preceded by a one week consistent sleep-wakefulness schedule verified by call-ins to a time stamped recorder, actigraphy recordings (Actiwatch-L, Mini Mitter Respironics, Bend, OR) and sleep logs. Participants refrained from caffeine, nicotine, and over-the-counter drugs. Urine toxicology and breath alcohol testing (Lifeloc Technologies Model FC10, Wheat Ridge, CO) verified participants were free of drugs each visit.

Ramelteon and placebo administration were double-blind. Computerized implementation of treatment allocation was performed by the CTRC pharmacist who provided pills identical in appearance in numbered containers. Participants received either ramelteon or rice-powder filled placebo pills on the first visit with the alternate condition administered on the second visit. The

allocation sequence was concealed until interventions were assigned and data prepared for statistical analysis.

Experimental Procedures

Participants were admitted ~1 h 50 min after their habitual wake time to control for circadian phase within individuals between visits. Shortly after arrival to the lab, participants were prepared for polysomnography (PSG) and temperature physiology recordings. Participants were studied using a modified constant routine protocol in which they remained awake, seated in a semi-recumbent position in a hospital bed with the head raised at ~35 degrees. Participants were studied in an environment free of time cues in dim light (< 8 lux maximum), sound attenuated and temperature controlled rooms. One exception to the latter was that participants were informed in the consent form that they would be asked to sit in bed awake for ~4 h out of each ~10 h study visit. Throughout the protocol participants wore a light tee shirt and a blanket covered them up to their waistline to provide for similar microclimate conditions at the skin temperature recording sites. A prepared lunch was given of the exact same quantity and dietary composition for both visits to control for the thermoregulatory effects of digestion and absorption. The lunch was served at the same time each visit and occurred 1 h and 45 min prior to pill administration. The timing of the meal was designed so that ramelteon would be rapidly absorbed and to reduce effects of hunger on the subsequent sleep episode. Core and skin temperature physiology and daytime sleep measurements were performed during the biological day on the circadian rise of the CBT rhythm. Core and skin temperature recordings began ~2.5 h following habitual waketime. Ramelteon or placebo was administered 2 h after sitting in bed.

At 2 h after drug administration, the bed was lowered to the supine position and the lights were turned off for a 4 h sleep opportunity (Fig 1). If participants awoke before the end of the sleep opportunity they were instructed to continue lying in bed and try to sleep.

Temperature Recording

Core temperature was measured via an ingested temperature pill that transmitted internal temperature by radio frequency to an external unit placed around the participant's waist (Vital Sense, Mini Mitter Respironics, Bend, OR). Proximal and distal skin temperatures were measured via thermopatches placed inferior to the left clavicle, at the location of the subclavian vein (T_{sub}), and the left dorsal foot (T_{ft}) respectively. The DPG was calculated as the difference between the (T_{ft}) and the (T_{sub}) thermopatches. Core and skin body temperatures were analyzed for 2.67h prior to the PSG recorded sleep opportunity.

Polysomography Recording

Sleep recordings were obtained with Siesta digital sleep recorders (Compumedics USA Ltd, Charlotte, NC) using monopolar EEGs referenced to contra-lateral mastoids according to the International 10-20 system (C3-A2, C4-A1, O1-A2 and F3-A2), right and left electrooculograms (EOG), chin electromyogram (EMG), and electrocardiogram (ECG). Impedances were below 5 kohms. Data were stored and sampled at a rate of 256 samples per second per channel with a 12-bit A-D board. High and low pass digital filters for EEG and EOG were set at 0.10 and 30 Hz, respectively. High and low pass digital filters for EMG were set at 10 and 100 Hz, respectively.

Relative Clock Hour

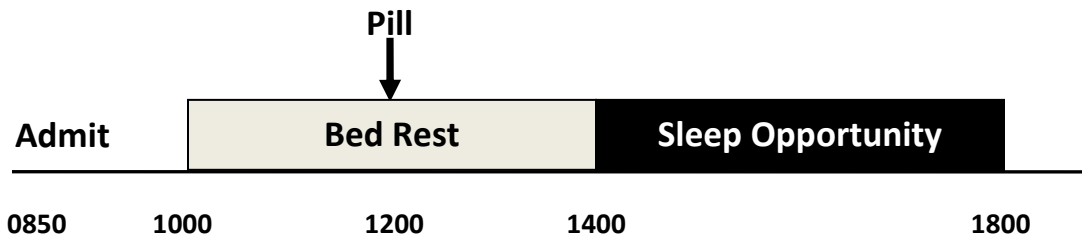


Figure 1: Protocol Timeline

Protocol events were scheduled according to habitual bed and wake times. The example depicts a protocol for a participant with a habitual wake time of 0700 h. Participants were admitted to the laboratory ~1 h 50 min after their scheduled wake time. Pill administration is denoted by the arrow and occurred 2 h after the admit and 2 h prior to the 4 h sleep opportunity.

Sleep was scored according to standard guidelines from brain region C3-A2 (Rechtschaffen A. 1968). SOL was defined as the time from lights out to the onset of three continuous epochs of EEG defined sleep. Subjective TST was assessed immediately after each sleep opportunity. One participant did not provide a subjective assessment of TST and was therefore not included in that analysis.

Data Analysis

Actigraphic analysis of sleep the week prior to each visit verified a similar amount of sleep between conditions with an average difference in TST of 0.01 h (± 0.30 SD).

The primary outcome variables for body temperature were CBT and the DPG. Post hoc analyses were also performed on T_{sub} and T_{ft} . Data for each body temperature measure were averaged into 10-minute bins. Differences from pill administration were calculated for DPG measures to control for between subject differences in the baseline distal skin temperature level. Data from two individuals were not used for the CBT analysis due to temperature pill malfunction. There was missing data for one individual for T_{sub} , and four individuals for T_{ft} due to technical difficulties. The four individuals who did not have T_{ft} data were consequently not included in the DPG analysis. The allocation sequence resulted in a balanced design despite these missing data. The primary outcome variables for sleep were TST, WASO and SOL.

The effects of ramelteon versus placebo on each of the above variables were compared using repeated-measures analyses of variance (ANOVA) with Hunyh Feldt degree of freedom correction factors to address heterogeneity of variance. Planned comparisons were conducted between each condition for every time bin for temperature and summary measures for sleep

episode. Multiple comparisons were examined with Fishers least significant difference tests combined with a modified Bonferroni correction to reduce type I error. Data are expressed as mean $\pm$ standard error of the mean (SEM). A priori Pearson's correlation coefficients were computed between DPG and SOL measures because findings from prior studies indicated relationships among these measures.(Krauchi, Cajochen et al. 2000) In addition, post hoc Pearson's correlation coefficients were computed for the change from baseline in CBT, T_{sub} , T_{ft} or DPG and sleep measures. Baseline temperature level was defined as the 40 minutes prior to pill administration and was compared to the average change in temperature for the 110 minutes following pill administration for the correlation analyses.

Results

Body Temperature

Ramelteon (8 mg) significantly attenuated the circadian rise in CBT (Fig 2A) and significantly increased the DPG (Fig 2B) compared to placebo. There was a significant main effect of time and interaction of drug by time for CBT (Table 1). Additionally, there was a significant main effect of time for the DPG. Planned comparisons revealed a significant difference in the ramelteon condition for the DPG beginning 50 min prior to the sleep opportunity. There was a significant interaction of condition by time (Table 1) for T_{sub} with significant differences between conditions at 50 min and 110 min after drug intake. There was a higher proximal temperature at 50 min and a lower proximal temperature at 110 min in the ramelteon condition (Figure 2C). We found a significant main effect of time for T_{ft} (Figure 2D).

Table 1: ANOVA Results for thermoregulatory variables

Measure	Drug	Time	DxT
CBT	F(1,11)=4.12, p=0.067	F(10,110)=2.88, p=0.003*	F(10,110)=3.94, p=0.00013*
DPG	F(1,9)=.89, p=0.370	F(10,90)=11.09, p=.00000*	F(10,90)=1.49, p=0.154
T _{sub}	F(1,12)=.078 P=0.79	F(10,120)=1.04 P=0.41	F(10,120)=3.09 P=.002*
T _{ft}	F(1,9)=.28 P=0.61	F(10,90)=10.84 P=0.00000*	F(10,90)=.89 P=0.55

*denotes a significant value.

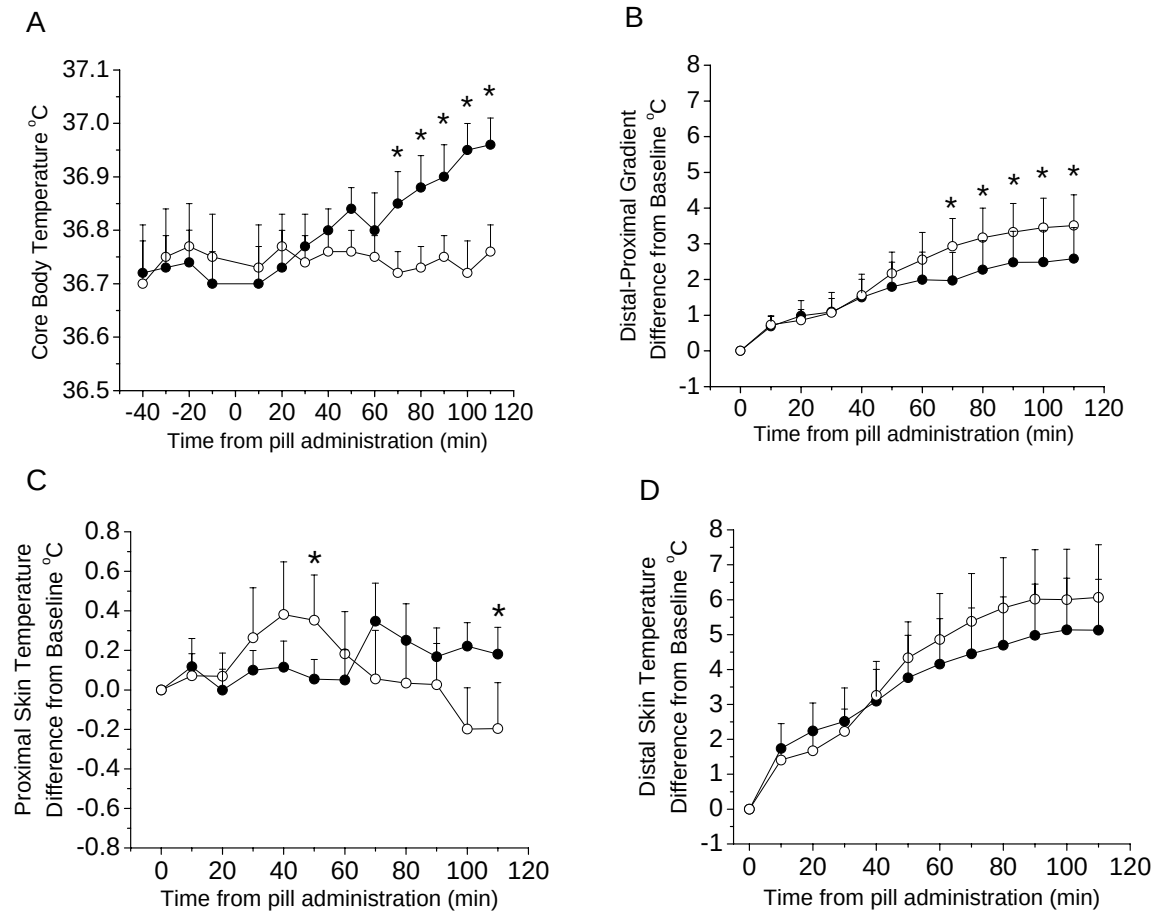


Figure 2: Thermoregulatory effects of ramelteon on core body temperature, the DPG, proximal and distal skin temperature sites.

Values expressed as means $\pm$ SEM. The open circles represent the ramelteon condition and closed circles represent the placebo condition. The 0 point denotes time that ramelteon or placebo were administered. (A) The melatonin agonist, ramelteon, significantly reduced core temperature by attenuating the normal daytime circadian rise as seen in placebo. (B) The DPG was increased after ramelteon ingestion relative to placebo. (C) Proximal skin temperature measured at the subclavian vein (T_{sub}) (D) Distal skin temperature measured at the dorsal foot (T_{ft}).

Sleep Physiology

Ramelteon significantly reduced % wakefulness and WASO and significantly increased PSG recorded and subjective TST (Table 2). Increased sleep duration was primarily due to significant increases in the percentage of stages 1 and 2 sleep, with non-significant changes in slow wave sleep (SWS), rapid eye movement sleep (REM) and SOL (Table 2).

Correlations Between Temperature and Sleep Physiology

In the placebo condition, there were no significant correlations for the DPG (Figure 3A, $p=0.28$), CBT, T_{sub} , T_{ft} temperatures with any sleep measure. Whereas, in the ramelteon condition there were significant negative correlations for the DPG with SOL (Figure 3B, $p=0.013$) and with percent of stage 1 sleep ($r = -.73$, $p = .02$). Similarly, T_{ft} was negatively correlated with SOL ($r = .68$, $p = .03$) and with percent of stage 1 sleep ($r = -.71$, $p = .02$). Additionally, T_{ft} was positively correlated with percent of stage 3/4 sleep ($r = .69$, $p = .03$). There were no significant correlations with CBT or T_{sub} and any sleep measure in the ramelteon condition. It appears in Figure 3A that two participants were outliers for the change in DPG during the placebo condition. Calculating the interquartile range (IQR) identified one subject as an extreme ($\text{IQR} \times 3$) and the other subject as a mild outlier ($\text{IQR} \times 1.5$). When these two individuals are removed from the placebo analysis, then a significant negative correlation of -0.83 ($p=0.01$) is observed between SOL and DPG, but not between DPG and any other sleep measure.

Table 2: Results of EEG and subjective sleep measures

Parameter	Placebo	Ramelteon	P Value
Stage 1 (% RT)	6.0 ± 0.9	8.5 ± 1.1	0.035*
Stage 2 (% RT)	34.4 ± 4.0	50.4 ± 3.4	0.0004*
Stage 3/4 (% RT)	12.9 ± 2.3	11.5 ± 1.8	0.57
REM (% RT)	9.6 ± 1.7	8.5 ± 1.4	0.45
Wakefulness (% RT)	37.2 ± 6.8	21.0 ± 4.3	0.02*
SOL (min)	8.1 ± 1.2	6.5 ± 1.0	0.33
WASO (min)	82.2 ± 15.7	45.7 ± 9.6	0.01*
TST (min)	150.7 ± 16.2	193.9 ± 10.8	0.02*
Subjective TST (min)	101.5 ± 15.4	177.5 ± 20.5	0.002*

Sleep stages and % wakefulness are expressed relative to the 240 minute sleep opportunity recording time (RT). *denotes a significant value using the modified Bonferonni correction ($p < 0.047$).

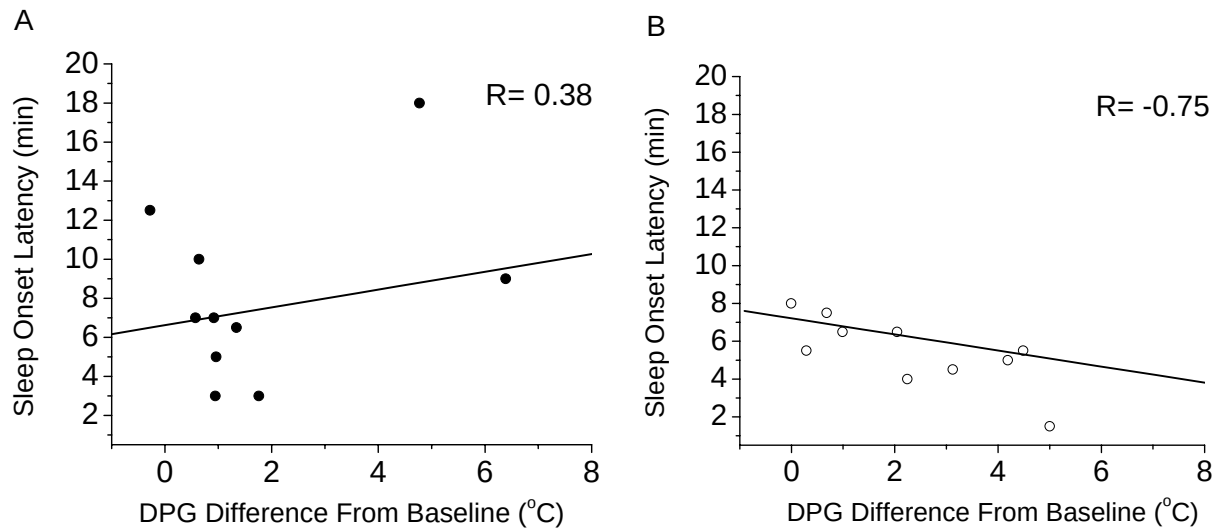


Figure 3: Correlation between SOL and the DPG

SOL (3 continuous epochs to sleep) and the DPG were correlated such that a greater change in DPG was associated with a shorter SOL in the ramelteon condition (B) compared to placebo condition (A). The lines represent the best linear fit through the data.

Discussion

Administration of the melatonin MT-1/MT-2 agonist ramelteon (8mg) 2 h prior to a daytime sleep opportunity reduced CBT, increased the DPG, initially increased and then decreased proximal skin temperature, and improved daytime sleep relative to placebo. Ramelteon improved daytime sleep as measured by increases in TST, stages 1 and 2 sleep, and a decrease in WASO. Ramelteon has been reported to significantly reduce SOL at night (Zammit, Schwartz et al. 2009). We did not find a significant reduction in SOL in the ramelteon condition, perhaps due to a floor effect as we tested healthy young individuals without sleep problems during an afternoon sleep opportunity. The attenuation of the circadian rise in CBT following ramelteon administration was accompanied by changes in skin temperature as determined by proximal skin temperature and the DPG. Finally, the magnitude of the increase in the DPG and distal skin temperature were correlated with a faster latency to sleep in the ramelteon condition.

Together with findings from previous studies that have demonstrated a hypothermic effect of melatonin (Dawson, Gibbon et al. 1996; Reid, Van den Heuvel et al. 1996; Hughes and Badia 1997; Satoh and Mishima 2001), the current findings suggest that the sleep promoting effect of ramelteon may be mediated in part through its ability to lower CBT. The magnitude of change in CBT observed with ramelteon administration was similar to what has been reported with exogenous melatonin (Hughes and Badia 1997). Perhaps more important to the initiation of sleep is the finding that ramelteon caused an increase in distal skin temperature and the DPG, as the latter has been shown to predict SOL (Krauchi, Cajochen et al. 2000). We also found a greater change in the DPG and distal skin temperature to be associated with less stage 1 sleep and a greater change in distal skin temperature to be associated with more stage 3/4 sleep. Previous research findings have demonstrated an increase in slow wave sleep in young and older

individuals following skin warming (Raymann, Swaab et al. 2008). The present study provides further evidence that changes in skin temperature are associated with changes in sleep staging.

Melatonin and melatonin analogues appear to primarily influence skin blood flow as compared to other circulatory beds such as cerebral blood flow (van der Helm-van Mil, van Someren et al. 2003). Heat loss after the administration of exogenous melatonin has been reported to occur through vasodilation of peripheral skin blood vessels, presumably arterial-venous anastomoses in the hands and feet (Aoki, Stephens et al. 2006; Aoki, Zhao et al. 2008). The opening of these important thermoregulatory sites facilitates a high level of blood flow to the skin, thus creating a positive thermal gradient with the environment and allowing for the convective exchange of heat to occur (Charkoudian 2003). It is unclear mechanistically how melatonin or melatonin analogues are able to modulate skin temperature. It is possible that both the sympathetic noradrenergic vasoconstrictor and non-adrenergic active vasodilator systems that are involved in temperature regulation during cold and heat stress, respectively, contribute to the thermoregulatory changes observed (Charkoudian 2003). For example, it has been reported that daytime exogenous melatonin administration reduces the thermoregulatory set-point for vasoconstriction during whole body cooling (Aoki, Zhao et al. 2008). Additionally, whole body warming has been reported to result in a lower internal threshold for activation of the active cutaneous vasodilator system following melatonin administration (Aoki, Stephens et al. 2006). Further, in the rat caudal artery (an important site for thermoregulation), melatonin potentiated the vasoconstrictor response to an α -adrenergic agonist through MT-1 receptor activation, while causing vasodilation through MT-2 receptor activation (Masana, Doolen et al. 2002). Taken together, these data suggest melatonin and/or melatonergic analogues may modulate temperature through central mediated changes in sympathetic outflow as well as peripheral activation of the

membrane bound MT-1 and MT-2 receptors located on the smooth muscle cells of the vasculature.

The time course of the effects of ramelteon on CBT and proximal skin temperature suggests that thermoregulatory effects are first observed near the reported time of peak ramelteon concentration, approximately 45 minutes following administration (Kato, Hirai et al. 2005). It is unknown specifically how changes in skin and core temperature are mechanistically involved with initiating and/or maintaining sleep. An increase in skin temperature may stimulate thermosensitive nerve endings located in the skin (Van Someren 2000; Lowry, Lightman et al. 2009), the preoptic anterior hypothalamus (POAH) (Van Someren 2000), interfascicular part of the dorsal raphe nucleus (Cronin and Baker 1977; Werner and Bienek 1985; Lowry, Lightman et al. 2009), or other thermosensitive brain centers. Stimulation of these temperature sensitive neural circuits may affect sleep promoting brain areas and/or inhibit wakefulness-promoting brain areas. It has been demonstrated that cutaneous warming of proximal skin via a liquid filled, temperature controlled whole body thermosuit resulted in a decrease in SOL by 27% (Raymann, Swaab et al. 2005). Additionally, an effect of temperature manipulation on the neural activity of brain centers thought to be involved in sleep and wakefulness has been reported. Specifically, a subpopulation of warm-sensitive POAH neurons spontaneously increases its firing rate at sleep onset and experimental warming of the POAH induces a similar increase in this firing rate (McGinty and Szymusiak 1990; Alam, Szymusiak et al. 1995; McGinty, Alam et al. 2001). Further, POAH thermosensitive neurons have been shown to affect the discharge rate of neurons located in areas known to regulate sleep and wakefulness (Alam, Szymusiak et al. 1995). It has been proposed that a positive feedback loop exists where changes in CBT and heat loss both trigger and reinforce sleep onset (Van Someren 2000; Gilbert, van den Heuvel et al.

2004). In this model, a reduction in the thermoregulatory set point that occurs during the transition from wakefulness to sleep results in further heat loss and a sustained reduction in CBT. Our findings are in agreement with previous research showing that changes in CBT may be less associated with decreases in SOL as compared to changes in skin temperature in younger and older adults (Raymann, Swaab et al. 2005; Raymann and Van Someren 2008).

Ramelteon may also influence sleep physiology by binding to melatonin receptors in the central nervous system. As noted, melatonin's actions are mediated by two G-protein coupled receptor subtypes termed the MT-1 and MT-2 receptors (Dubocovich 2007). It has been hypothesized that the sleep promoting response is mediated by the MT-1 receptor subtype (Dubocovich 2007). Binding of melatonin to MT-1 receptors on the suprachiasmatic nucleus (SCN) is reported to inhibit SCN electrical activity (Liu, Weaver et al. 1997; Jin, von Gall et al. 2003). The SCN projects to wakefulness and sleep promoting brain regions and thus ramelteon may promote sleep during the biological daytime by inhibiting the circadian arousal signal (Hughes and Badia 1997; Sack, Hughes et al. 1997). In contrast, when endogenous melatonin levels are high, ramelteon has less influence on sleep physiology, except for reductions in sleep latency (Roth, Stubbs et al. 2005). It is also possible that ramelteon promotes sleep during the biological day via binding to MT-2 receptors. The selective MT-2 analogue IIK7 is reported to promote NREM sleep in rats (Fisher and Sugden 2009), thus challenging the previously held assumption of discrete MT-1 and MT-2 receptor mediated effects.

To our knowledge, there have been no published studies in which ramelteon has been compared to melatonin on any outcome measure. Such a comparison between melatonin and its analogues could be useful. We believe that the future development of selective melatonin receptor agonists may provide more precise determination of the influence of melatonin receptor

subtypes on human physiology. This ultimately may lead to more targeted therapies.

It is unclear why two of the participants when receiving placebo had a large DPG. Removing these individuals resulted in a significant correlation between DPG and SOL for the placebo condition. It is worth noting that in the placebo condition, only two participants had a DPG greater than 2 degrees Celsius whereas more than half of participants had a DPG greater than 2 degrees Celsius in the ramelteon condition. Thus, ramelteon appears to have increased the magnitude and variance in the change of the DPG, increasing the likelihood of observing a significant relationship between the DPG and SOL (Zammit, Schwartz et al. 2009).

In conclusion, ramelteon improved objective and subjective markers of sleep during a daytime sleep episode. These effects were associated with thermoregulatory adjustments observed in response to ramelteon administration. The findings that ramelteon improves sleep during the biological day suggests that ramelteon may have potential as a therapeutic target for the treatment of insomnia associated with circadian rhythm sleep disorders in which sleep occurs at inappropriate biological times.

Acknowledgements

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CHAPTER 3

THE ACUTE EFFECT OF EXOGENOUS MELATONIN ON RESTING METABOLIC RATE IN HUMANS

Rachel. R. Markwald, Kenneth P. Wright Jr.

Abstract

Exogenous melatonin has been shown to improve daytime sleep. These improvements in sleep are thought to be partly mediated by melatonin induced thermoregulatory changes in core body temperature (CBT) and peripheral heat loss. It is also possible that melatonin may facilitate daytime sleep by reducing resting metabolic rate (RMR). Therefore, we tested the hypothesis that exogenous melatonin (5mg) would acutely decrease RMR as assessed by volume of oxygen consumption (VO₂), and calculated energy expenditure (REE) when compared to placebo. We studied eighteen healthy adults (7 females), BMI ($22.5 \pm 1.7 \text{ kg/m}^2$) aged ($22 \pm 5 \text{ yrs}$) in a randomized, double-blind, placebo-controlled study. Participants were studied under modified constant routine conditions to control for influences of activity, posture, and lighting. Melatonin/placebo was administered 1.25h after awakening from a 5h daytime sleep opportunity while in the fasted state. Resting VO₂ was assessed using indirect calorimetry (Truemax, Parvomedics) just prior to pill administration, and twice after (60 and 90 min). Additionally, REE expressed as kilocalories per minute (kcal/min) was calculated for each test. Changes in metabolic measures were calculated as differences from baseline by repeated measures ANOVA. As hypothesized, exogenous melatonin significantly decreased VO₂ (ml/kg/min) and REE (kcal/min) ($P < 0.05$). Together with previous research findings that have shown thermoregulatory effects of melatonin on CBT and skin temperatures these findings suggest that melatonin, may also facilitate sleep through reductions in RMR.

Introduction

The sleep and thermoregulatory systems have evolved together (Campbell 1984; Zepelin 2000) and are temporally associated such that sleep is best when initiated on the circadian decline of core body temperature (CBT) and following the nocturnal increase in peripheral heat loss (Zulley, Wever et al. 1981; Campbell and Broughton 1994; Murphy and Campbell 1997; Krauchi, Cajochen et al. 2000). Temperature signals from the periphery and the core are integrated in the preoptic area of the anterior hypothalamus (POAH). Activation of warm sensitive neurons in the POAH by central and peripheral afferents evoke thermoregulatory responses similar to that which is normally observed during sleep onset at night.

Sleep/wakefulness active neurons are also located in areas of the POAH and their circuitry overlap with the thermoregulatory system (Kumar, Vetrivelan et al. 2007) and have been shown to be responsive to changes in temperature. Specifically, McGinty and Szymusiak examined warm sensitive neurons in the POAH of rats and found that their activity increased at sleep onset (McGinty and Szymusiak 2001). Additionally, preoptic warming in the cat promoted EEG slow-wave activity during sleep (Roberts and Robinson 1969), decreased the discharge rate of wake active neurons in the lateral hypothalamus (Methippara, Alam et al. 2003) serotonergic neurons in the dorsal raphe nucleus (DRN) (Guzman-Marin, Alam et al. 2000), and evoked behavioral and electrographic signs of sleep in rats (Benedek, Obal et al. 1982). These findings suggest that temperature afferent signals may influence the timing and quality of sleep.

Melatonin is a hormone that is controlled by the master circadian clock in the suprachiasmatic nuclei (SCN) and is released during the biological night with the onset occurring on average 2 hours prior to sleep (Wright, Gronfier et al. 2005). Melatonin receptors have been identified in several brain areas involved with sleep and thermoregulation including: the SCN,

POAH, and throughout the cortex (Stankov, Cozzi et al. 1991) as well as the peripheral vasculature (Ekmekcioglu, Haslmayer et al. 2001; Masana, Doolen et al. 2002; Ekmekcioglu, Thalhammer et al. 2003). In humans, the nocturnal increase in endogenous melatonin is associated with an increase in sleep propensity, a decrease in CBT, and an increase in peripheral heat loss (Krauchi, Cajochen et al. 1999). During the daytime when endogenous levels are low, the administration of exogenous melatonin or a melatonin analogue produces similar physiological changes (Markwald, Lee-Chiong et al. ; Reid, Van den Heuvel et al. 1996; Gilbert, van den Heuvel et al. 1999; Harris, Burgess et al. 2001). In fact, improved daytime sleep with exogenous melatonin or an analogue is thought to be partly mediated by the increase in peripheral heat loss and reduction in CBT (Markwald, Lee-Chiong et al. ; Krauchi, Cajochen et al. 1999). The mechanism by which melatonin affects the thermoregulatory system is not known but may be a result of central binding to MT1 and MT2 receptors in the brain, in the peripheral circulation, or both. Findings from these research studies suggest that melatonin may be functionally important to the nocturnal regulation of sleep and body temperature.

In addition to its ability to increase peripheral heat loss, it is also possible that melatonin may facilitate daytime sleep by reducing resting metabolic rate (RMR) and arousal. A reduction in RMR, if uncompensated for would reduce heat production and consequently lower CBT. Further, previous research findings have demonstrated that under controlled constant routine conditions, there is a circadian rhythm to heat production, with the trough occurring between 4-5 hours (Krauchi and Wirz-Justice 1994), and ~8 hours (Spengler, Czeisler et al. 2000) prior to the CBT minimum. It is possible that the rhythm of endogenous melatonin contributes to the observed circadian variation in REE. Therefore, we tested the hypothesis that exogenous melatonin (5mg) would acutely decrease RMR as assessed by volume of oxygen consumption

(VO₂), and calculated resting energy expenditure (REE) when compared to placebo during the biological daytime when endogenous melatonin levels are low and CBT is high.

Methods

Subjects

A total of eighteen healthy adults, BMI (23 ± 2 kg/m²) aged (22 ± 5 yrs) completed the study. Potential subjects underwent a comprehensive medical evaluation at the Clinical and Translational Research Center (CTRC) at the University of Colorado – Boulder. Participants were admitted into the study once they were considered free of any medical conditions as determined by medical history, physical exam, blood and urine chemistries, 12-lead clinical electrocardiogram, and toxicology screens for drug use. Screening procedures also involved sleep disorder questionnaires and psychological testing to ensure participants were free from such problems. A prior diagnosis of a sleep or psychiatric disorder was also exclusionary. Potential participants were excluded if they were smokers, had not lived at Denver altitude for at least 3 months, had a habitual sleep duration outside of <7 and >9 range, or took any medications. This study was approved by the University of Colorado - Boulder Institutional Review Board and the Scientific Advisory & Review Committee of the Colorado Clinical and Translational Sciences Institute. Participants gave written-informed consent, and all procedures were performed according to the Declaration of Helsinki.

Study Design

The study was a randomized, double-blind and placebo-controlled research design. This study was part of a larger research protocol examining the phase resetting response to exogenous melatonin. The protocol required participants to live for 3.7 days in the Sleep and Chronobiology Laboratory. The laboratory visit was preceded by a one week ~8h consistent sleep-wakefulness schedule verified by call-ins to a time stamped recorder, actigraphy (Mini Mitter, Respironics, Bend, OR) and sleep logs. Additionally, participants refrained from caffeine, nicotine, and over-the-counter drugs for the duration of the study. An alcohol breath tester and urine drug screen verified that subjects were free of drugs upon admission to the laboratory. Pill administration was double-blind and randomized such that, participants received either melatonin (5 mg) or a rice-filled placebo.

Experimental Procedures

Participants were studied under modified constant routine conditions to control for influences of activity, posture, and lighting. Ambient temperature was held constant within the thermoneutral range of (22-24°C). Participants were awoken from a 5 hour daytime sleep opportunity and remained in a fasted state until all metabolic measurements were complete. Additionally, participants were not allowed to get up from the bed during metabolic testing and kept a constant supine position. The pill, either melatonin or placebo was administered 1.25h upon awakening in the late afternoon. See Figure 1 for a more detailed protocol timeline. Metabolic testing was performed once before the pill and two times post pill ingestion.

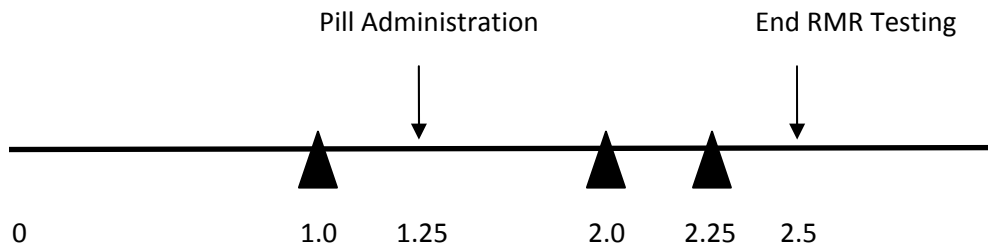


Figure 1: Protocol Timeline

Following a 5 h daytime sleep opportunity resting metabolic rate (RMR) was assessed once at baseline, and twice more following either the ingestion of melatonin (5 mg) or placebo. The 0 point denotes end of sleep opportunity. The pill was given 1 h and 15 minutes after awakening. Filled triangles represent RMR assessments. Post pill assessments began at 45 and 60 minutes after pill intake, or (2 h, and 2 h and 15 min after awakening, respectively).

Metabolic Assessment

Resting metabolic rate (RMR) was examined by the measurement of the volume of oxygen consumed (VO_2) via the indirect calorimetry technique (Frankenfield and Coleman 2009). A metabolic cart (Parvo Medics Truemax 2400, Salt Lake City, UT) was used to assess the percentage of fractional expired oxygen (O_2) via paramagnetic analyzers and percentage of fractional expired carbon dioxide (CO_2) via infrared analyzers. Gas analyzers compare the sampled air to the known concentrations of the reference air. This technique is the gold standard for assessing changes in metabolism through changes in cellular oxygen consumption instead of heat production (a more expensive and impractical technique). A clear plastic ventilated hood, or alternatively, a mouthpiece connected to a 3-way valve in conjunction with nose clips were used to collect expired air from the participants. The method of collection was kept consistent for each participant. A hose connected the hood (or mouthpiece) to oxygen and carbon dioxide analyzers. The metabolic cart was always calibrated ~30 min prior to the baseline assessment. The gas analyzers were calibrated with known concentrations of CO_2 and O_2 and the flow rate was calibrated using a 3.0 L syringe. One 15 minute baseline RMR was conducted with two more assessments beginning at 45 and 60 minutes post pill administration. VO_2 relative to body weight in milliliters per kilogram per minute (ml/kg/min) and the respiratory quotient (RQ) were assessed on a breath by breath basis for 15 minutes. The first five minutes of data collected were discarded to account for initial changes in ventilation as the participants became accustomed to either the ventilated hood or mouthpiece and nose clips. The last 10 min of the RMR assessment was averaged and used for the analysis. Resting energy expenditure (REE) in kilocalories per min (kcal/min) was then calculated from VO_2 and the RQ based on the equations of (Jequier and Felber 1987).

Statistical Analysis

The effects of melatonin and placebo on the metabolic variables measured were compared using repeated-measures analyses of variance (ANOVA). A difference from baseline was calculated to account for inter-individual differences in baseline RMR. Data are expressed as means $\pm$ S.E.M.

Results

Metabolic Data

Repeated measures ANOVA revealed that exogenous melatonin (5 mg) significantly reduced VO_2 (Figure 2a) and calculated REE (Figure 2b) as compared to baseline. VO_2 ($F(1, 16)=8.98$, $p = 0.009$) and REE were significantly lower in the final assessment than in the second assessment for the melatonin group. No significant difference was observed within the placebo group or between the melatonin and placebo groups. Additionally, there were no differences in the respiratory quotient (RQ) between groups or assessments (Figure 3).

Discussion

The finding from this study is that exogenous melatonin (5 mg) acutely lowered RMR during the late afternoon when endogenous melatonin levels are low. These data suggest that the onset of the circadian melatonin rhythm at night may promote a state of reduced arousal during this circadian time in part by reducing energy expenditure.

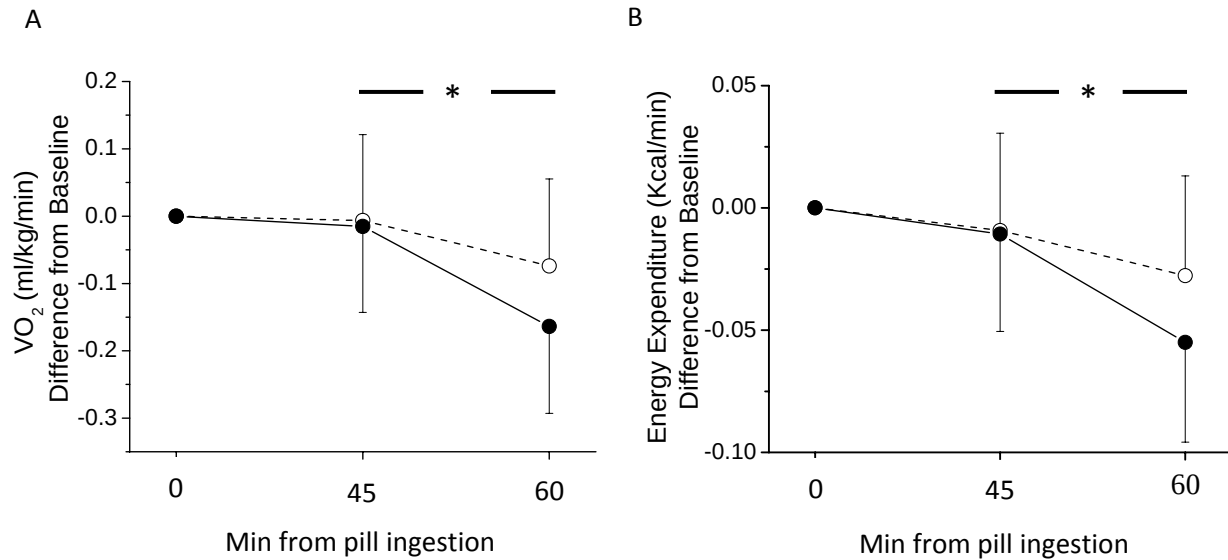


Figure 2: Effect of melatonin on VO₂ and REE

Values expressed as means $\pm$ SEM. Open circles represent the placebo group and closed circles represent the melatonin group. The 0 point denotes the time that melatonin or placebo was administered. Each metabolic assessment lasted for 15 minutes (beginning at 45 and 60 min post pill). (A) Melatonin (5 mg) significantly reduced VO₂ relative to baseline. (B) Melatonin (5 mg) significantly reduced REE relative to baseline. The * denotes a significant difference from the second metabolic assessment.

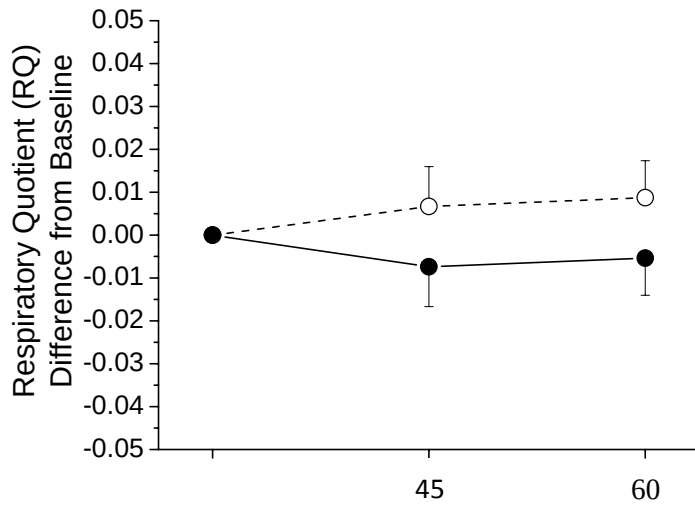


Figure 3: Effect of melatonin on RQ

Values expressed as means $\pm$ SEM. Open circles represent the placebo group and closed circles represent the melatonin group. The 0 point denotes the time that melatonin or placebo were administered. Each metabolic assessment lasted for 15 minutes (beginning at 45 and 60 min post pill).

These data also demonstrate another mechanism by which changes in temperature may be interpreted by our brain in order to initiate and/or maintain sleep. It is well known that sleep is best when initiated on the downward arm of the core body temperature (CBT) rhythm (Czeisler, Weitzman et al. 1980; Zulley, Wever et al. 1981). Furthermore, it is known that both heat loss and heat production contribute to the circadian rhythm in CBT, with heat loss more of a contributing factor to the evening circadian reduction in CBT than heat production (Krauchi and Wirz-Justice 1994). This does not preclude endogenous melatonin from a potential role in CBT regulation during the night however, via small changes in REE. A melatonin-initiated reduction in heat production may contribute to the observed nocturnal decline in CBT and serve as an additional thermoregulatory signal in the regulation of sleep and wakefulness. The observed acute reduction in metabolic rate in the current study may be a reflection of a central effect of melatonin to reduce arousal, or a direct effect on thermoregulatory processes to reduce CBT. Future research protocols will need to focus on the contribution of the observed reduction in RMR to melatonin in conjunction with core and skin thermoregulatory variables.

The magnitude of the effect of melatonin on RMR is relatively small; however this change may still be meaningful with regards to the physiology preceding sleep at night. Preliminary data from our laboratory demonstrate a circadian variation in 24 hour REE with a magnitude of ~ 0.3 Kilocalories/minute from peak to trough (Jung CM, Chaker Z et al. 2010). Furthermore, the trough occurred ~ 5.5 hours before the CBT minimum around the time of habitual bedtime. The results from the current study suggest that the onset of the circadian endogenous melatonin rhythm may account for $\sim 1/3^{\text{rd}}$ of the circadian variation in 24 hour REE. This may promote a state of reduced arousal during habitual bedtime in part by reducing REE.

There were several limitations associated with this study. Melatonin did not elicit a significant change in REE from the placebo group. In the future, the use of a within subjects experimental design may be a more sensitive approach for detecting small changes in REE. Further, this was part of a larger research protocol that involved a level of prior sleep deprivation. The effect of prior sleep deprivation on metabolic physiology is not known and may have impacted these data, however all subjects underwent the same experimental conditions. Future studies should also examine thermoregulatory variables (i.e. CBT and skin temperatures) in conjunction with the effect of daytime exogenous melatonin on metabolic physiology.

In conclusion, exogenous melatonin (5mg) decreased resting VO_2 and REE when compared to placebo. Together with previous research findings that have shown thermoregulatory effects of exogenous melatonin on CBT and skin temperatures, these findings suggest that melatonin may also facilitate sleep through reductions in REE.

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CHAPTER 4

THE INFLUENCE OF BRIGHT LIGHT AND CAFFEINE ON THERMOREGULATION AND SLEEP

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Abstract

The sleep and thermoregulatory systems are temporally connected such that a decrease in core body temperature (CBT) and an increase in peripheral heat loss occur prior to sleep onset in individuals entrained to the light/dark cycle. Further, sleep quality is best when sleep is initiated under these thermoregulatory conditions. Caffeine and bright light exposure are reported to disrupt sleep. Bright light has been reported to impact skin temperature physiology yet it is unknown if caffeine impacts skin temperature physiology during the time of habitual sleep onset. Also unknown is whether the combination of bright light and caffeine impacts skin temperature physiology more than does either bright light or caffeine alone. Therefore, we investigated the effects of caffeine and bright light (two common pharmacological and environmental factors) on sleep and thermoregulatory physiology during a modified constant routine protocol. Subjects completed four laboratory visits in which they were randomly assigned to receive either bright light (~3000 lux) or dim light (~1.5 lux) during the time of habitual sleep onset. Within each lighting condition subjects ingested either caffeine (2.9mg/kg) or placebo in a double blind fashion, three hours before light exposure began. Results showed that caffeine attenuated the nocturnal fall in CBT, delayed the circadian increase in peripheral heat loss as assessed by distal temperature and the distal to proximal skin gradient. Furthermore, the combination of bright light and caffeine reduced the amount of slow wave sleep and increased sleep onset latency. These data demonstrate that the sleep and thermoregulatory systems are impacted individually and in combination by caffeine and bright light.

Introduction

From an evolutionary perspective, the processes of sleep and thermoregulation have co-evolved in endotherms (Zepelin 2000). The primary nuclei controlling sleep and thermoregulation sit in close proximity to one another in the anterior hypothalamus and have overlapping neural circuitry (for reviews see (Van Someren 2000; Saper, Scammell et al. 2005; Kumar, Vetrivelan et al. 2007; Szymusiak, Gvilia et al. 2007). Physiologically, the two processes are temporally associated such that a decrease in core body temperature (CBT) and an increase in peripheral heat loss occur prior to sleep onset in individuals entrained to the light/dark cycle (Dijk and von Schantz 2005). Sleep quality is best when sleep is initiated under these thermoregulatory conditions. Research findings have demonstrated that the nightly increase in peripheral heat loss is a predictor variable for how quickly an individual will fall asleep (Krauchi, Cajochen et al. 2000). People often maintain wakefulness several hours into the night in response to work, family and social demands. This behavior is facilitated with the emergence of artificial lighting, and the development of readily available central nervous stimulants such as caffeine.

Caffeine is the world's most widely consumed stimulant (Nawrot, Jordan et al. 2003), and it has been estimated that the mean daily intake is 4 mg/kg body weight or approximately 280 mg for a 155 pound individual (Barone and Roberts 1996). Caffeine has been shown to have a negative effect on sleep as evidenced by a reduction in slow wave sleep, longer sleep onset, shortened total sleep time, decreased sleep efficiency and decreased stage 2 sleep (Okuma, Matsuoka et al. 1982; Landolt, Dijk et al. 1995; Drapeau, Hamel-Hebert et al. 2006). Caffeine is an adenosine receptor antagonist and is thought to increase arousal via binding to adenosine A_{2A} receptors (Huang, Qu et al. 2005). Adenosine is a byproduct of energy metabolism; breakdown

of ATP, and adenosine accumulates in the brain with time spent awake since the previous sleep episode (Porkka-Heiskanen, Strecker et al. 1997). Additionally, adenosine has been shown to increase the firing rate of sleep promoting neurons in the ventral lateral preoptic area (VLPO), a key neural center in the regulation of sleep/wakefulness (Morairty, Rainnie et al. 2004). Caffeine may therefore increase arousal by inhibiting A_1 and A_{2a} receptors on the VLPO, thereby inhibiting the gradual increase in sleep pressure normally evoked as adenosine levels rise. Additionally, caffeine may affect sleep by reducing adenosine-mediated increases in nightly melatonin levels in the pineal gland (Gharib, Reynaud et al. 1989). Finally, caffeine has been shown to attenuate the circadian fall in core body temperature (CBT) observed during the biological night (Wright, Badia et al. 1997; Wright, Myers et al. 2000), and this may be one more mechanism by which caffeine interacts with the regulation of sleep/wakefulness.

Bright light at night has been shown to suppress endogenous levels of melatonin (Lewy, Wehr et al. 1980; McIntyre, Norman et al. 1989), attenuate the circadian fall in the CBT rhythm compared to dim light (Badia, Myers et al. 1991; Dijk, Cajochen et al. 1991; Wright, Badia et al. 1997; Burgess, Sletten et al. 2001; Cajochen, Munch et al. 2005) and decrease peripheral heat loss (Cajochen, Munch et al. 2005). In humans, during the biological night, the increase in melatonin is associated with a decrease in CBT, an increase in distal skin temperature, and a decrease in the distal to proximal skin temperature gradient (DPG) (Krauchi, Cajochen et al. 1999; Krauchi, Cajochen et al. 2000). The DPG is considered a marker of peripheral blood vessel tone where a smaller temperature difference measured between distal skin sites to proximal skin sites indicate heat loss via an increase in blood flow to the peripheral cutaneous circulation (Rubinstein and Sessler 1990).

The impact of caffeine on skin temperature physiology during the time of habitual sleep onset and the compound effect of bright light and caffeine together on sleep and thermoregulatory physiology is unknown. Therefore the present study sought to investigate the individual and combined effects of caffeine and bright light on skin temperature regulation and sleep in individuals during a modified constant routine protocol. We hypothesized that caffeine would act individually and in combination with bright light to attenuate the circadian fall in core temperature, attenuate the circadian rise in the DPG during the time of habitual bedtime, as well as increase SOL and decrease slow wave sleep during a nighttime sleep opportunity.

Methods

Subjects

Five (3 women, 2 men) young (mean age $\pm$ SE) = 24 ± 4 years and with normal body mass indexes ($24.0 \pm 1.0 \text{ kg/m}^2$) participated. Subjects underwent a medical evaluation at the Clinical and Translational Research Center (CTRC) at the University of Colorado – Boulder. Participants were admitted into the study once they were considered free of any medical conditions as determined by medical history, physical exam, blood and urine chemistries, 12-lead clinical electrocardiogram, and toxicology screens for drug use. Screening procedures also involved sleep disorder questionnaires and psychological testing to ensure subjects were free from such problems. A prior diagnosis of a sleep or psychiatric disorder was also exclusionary. Potential subjects were excluded if they were smokers, had not lived at Denver altitude for at least 3 months, had a habitual sleep duration outside of <7 and >9 range, or need to take or were taking any medications. This study was approved by the University of Colorado - Boulder Institutional Review Board and the Scientific Advisory & Review Committee of the Colorado

Clinical and Translational Sciences Institute. Subjects gave written-informed consent, and all procedures were performed according to the Declaration of Helsinki.

Design

A randomized, within-subject, crossover, and placebo-controlled research design was used in which participants came to the Sleep and Chronobiology Laboratory for 4 separate visits, each separated by one week. Each laboratory visit was preceded by a one week consistent sleep-wakefulness schedule verified by call-ins to a time stamped recorder, actigraphy recordings (Actiwatch-L, Mini Mitter Respironics, Bend, OR) and sleep logs. Subjects refrained from caffeine, nicotine, and over-the-counter drugs for two weeks preceding the first laboratory visit and throughout the duration of the study. Compliance was checked with urine toxicology and breath alcohol testing (Lifeloc Technologies Model FC10, Wheat Ridge, CO) at the beginning of each lab visit.

Subjects visited the laboratory for each of the following four conditions: (dim-light + placebo, dim-light + caffeine, bright-light + placebo, bright-light + caffeine). Caffeine and placebo administration were double-blind. Computerized implementation of treatment allocation was performed by the CTRC pharmacist who provided pills identical in appearance in numbered containers. Subjects received either caffeine (2.9 mg/kg of body weight), or rice-powder filled placebo pills. For dim-light conditions, subjects continued to receive the same dim light exposure as the rest of the experimental protocol (~1.5 lux in the angle of gaze, < 8 lux maximum) and for the bright-light conditions, light exposure was ~3000 lux in the angle of gaze.

Procedures

This study was part of a larger research protocol examining the phase resetting response to bright light and caffeine. Subjects lived in the Sleep and Chronobiology Laboratory in a special sleep suite that was an environment free from time cues. As part of the larger research protocol, subjects were sleep deprived for 40 hours and were then given an 8 h sleep opportunity at their habitual bedtime. Subjects were then awoken at their habitual wake-time for the experimental day. Exercise was prohibited for the entire protocol. Beginning at 12 hours of scheduled wakefulness on the experimental day, subjects were seated in bed with the head raised to ~35 degrees. Ambient temperature was kept stable in the thermoneutral range (22-24°C). Continuous electroencephalography (EEG) recordings verified wakefulness. At 13 hours of scheduled wakefulness the pill (either caffeine or placebo) was administered. Pill administration was followed 3 hours later by continued dim light exposure or overhead bright light exposure for an additional 3 hours. See Figure 1 for a more detailed depiction of the experimental protocol. During light exposure, subjects were instructed to lie in the semi-recumbent supine position in bed, and look directly at the ceiling mounted fluorescent light source (Sylvania T8, 6500 K). Clear polycarbonate lenses filtered light in the UV range. Subjects fixed their gaze in the direction of the light source for 6 min and then were allowed free gaze for 6 minutes; this cycle continued for the entire 3 hours of light exposure. Once light exposure was complete, the lights were turned off for a 5 hour sleep opportunity. Thus, sleep was attempted 3 hours after habitual

Overview of Experimental Day

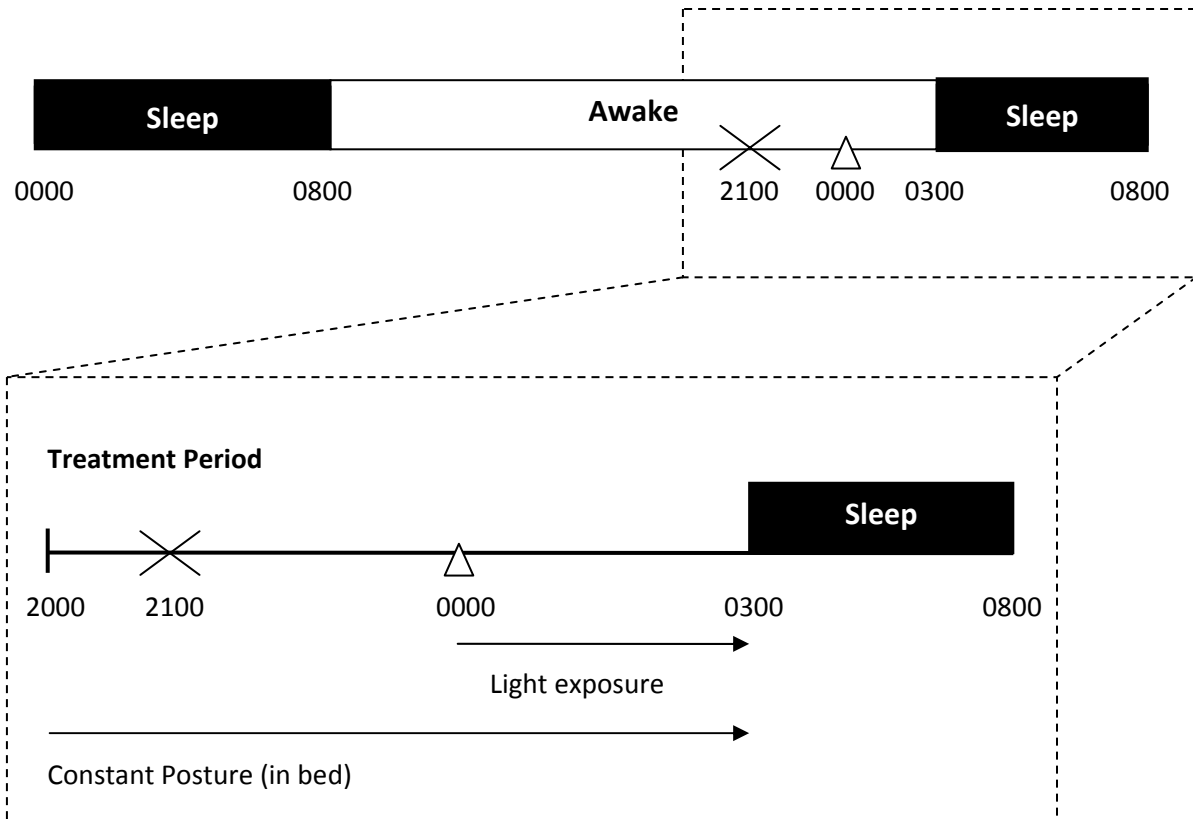


Figure 1: Protocol Timeline

A schematic depiction of the entire experimental day with an additional specific focus on the pill and light treatment period. Representative timeline based on a 12AM – 8AM sleep/wake schedule. Subjects were awoken at habitual wake time and spent the day sitting or lying in bed until one hour prior to the pill was administered. At this point, subjects were told to remain in bed, in a semi-recumbent supine position for the remainder of the experimental day (constant posture). The X denotes pill administration (caffeine or placebo), and the triangle denotes beginning of light exposure. Light exposure began 3 hours after pill administration and occurred for 3 hours before the lights were turned off for a 5 hour sleep opportunity. Subjects were then awoken at their habitual wake time.

bedtime. Sleep laboratory staff continually monitored subjects to ensure that the protocol was followed.

Assessment of core and skin temperatures

Core body temperature (CBT) was measured with a temperature pill that was ingested and transmitted internal temperature via radio frequency to an external unit placed around the participant's waist (Vital Sense, Mini Mitter Respironics, Bend OR). Proximal and distal skin temperatures were measured via thermopatches placed inferior to the left clavicle, at the location of the subclavian vein (Sub) and the top of the left dorsal foot (Foot) respectively. The DPG was calculated as the difference between the Foot and Sub thermopatches. Core and skin body temperatures were analyzed for 7 continuous hours beginning 30 minutes before the pill was ingested and ending 30 minutes after the lights were turned off for the sleep opportunity.

Assessment of EEG recordings

Sleep recordings were obtained with Siesta digital sleep recorders (Compumedics USA Ltd, Charlotte, NC) using monopolar EEGs referenced to contra-lateral mastoids according to the International 10-20 system (C3-A2, C4-A1, O1-A2, F3-A2), right and left electrooculograms (EOG), chin electromyograms (EMG) and electrocardiogram (ECG). Impedences were below 5 kohms. Data were stored and sampled at a rate of 256 samples per second per channel with a 12-bit A-D board. High and low pass digital filters for EEG and EOG were set at 0.10 and 30 Hz, respectively. High and low pass digital filters for EMG were set at 10 and 100 Hz, respectively. Sleep was scored according to standard guidelines from brain region C3-A2 (insert

Rechtschaffen A 1968). Sleep onset latency (SOL) was defined as the time from lights out to the onset of three continuous epochs of EEG defined sleep.

Assessment of subjective sleepiness

Subjective sleepiness was assessed using the Karolinska Sleepiness Scale (KSS). A baseline KSS was given at the time of pill administration and every hour post pill administration for 5 hours.

Data analysis

The primary outcome variables for body temperature were CBT and the DPG. Data for each body temperature measure were averaged into 10-minute bins. A two stage mixed model analysis was performed on each of the measures. Differences from an average 10 minute baseline preceding pill administration and light exposure were calculated for distal and proximal skin temperature sites as well as the DPG and CBT to control for differences in baseline body temperature levels between laboratory visits. To determine the initial effect of caffeine on thermoregulatory variables, the two caffeine and the two placebo conditions were averaged together for the first 3 hours of assessment. Data from two individuals for one condition were not available for the foot, subclavian, or DPG analysis due to equipment malfunction. The primary outcome variables for sleep were SOL and slow wave sleep. Data are expressed as mean $\pm$ standard error of the mean (SEM)

Results

Core and skin temperatures

The caffeine conditions significantly attenuated the circadian fall in CBT (Fig 2A) and significantly delayed the circadian increase in DPG (Fig 2D) compared to the placebo conditions. The mixed model ANOVA revealed a significant main effect of time and interaction of drug by time for foot and DPG measures, and a drug by time interaction for CBT (Table 1). For bright light treatment, the mixed model ANOVA revealed a significant main effect of time for CBT, Sub, and the DPG (Table 2). For difference from pill administration baseline, planned comparisons revealed a significant difference for the core temperature measure between the caffeine and placebo conditions at 165 minutes post pill ingestion, see Figure 3a. For difference from light exposure baseline, planned comparisons revealed a significant difference for the Sub temperature measure between the bright light-placebo condition and both caffeine conditions at 15 minutes after light exposure began and for Sub between bright light placebo and dim light-caffeine conditions for a majority of the light exposure session (Figure 3b) ($P < 0.05$).

Sleep physiology

There was a significant effect for condition in the SOL measure to 3 continuous epochs of sleep in the 5 h sleep opportunity ($p = 0.03$). There was also a significant condition effect for the percentage of PSG recorded slow wave sleep in the 5 h sleep opportunity ($p = 0.03$). Planned comparisons revealed a significant difference in SOL from the bright light-placebo and dim light-placebo conditions from the bright light-caffeine condition. There was a significant difference for stage 3/4 sleep for the bright light-caffeine condition from the bright light-placebo

Table 1: ANOVA Results for thermoregulatory effects of caffeine on body temperatures following pill administration

Measure	Caffeine	Time	CxT
CBT	F(1,6)=4.13, p=0.11	F(5,180)=1.36, p=0.28	F(5,180)=6.64, p=0.001*
DPG	F(1,6)=0.18, p=0.69	F(5,180)=13.14, p<0.001*	F(5,180)=7.21, p=0.001*
Sub	F(1,6)=0.07 P=0.80	F(5,180)=2.33 P=0.09	F(5,180)=0.16 P=0.98
Foot	F(1,6)=0.07 P=0.81	F(5,180)=19.80 P<0.0001	F(5,180)=6.12 P=0.002*

*denotes a significant value from placebo conditions.

Table 2: ANOVA results for thermoregulatory effects of caffeine and bright light following Light exposure.

Measure	Condition	Time	CxT
CBT	F(3,6)=1.16, p=0.37	F(5,180)=15.91, p<0.0001*	F(15,180)=0.77, p=0.71
DPG	F(3,6)=0.41, p=0.75	F(5,180)=1.69, p=0.18	F(15,180)=0.80, p=0.67
Sub	F(3,6)=3.15 P=0.06	F(5,180)=1.56 P=0.22	F(15,180)=0.89 P=0.58
Foot	F(3,6)=0.20 P=0.89	F(5,180)=1.42 P=0.26	F(15,180)=0.85 P=0.62

*denotes a significant main effect of time

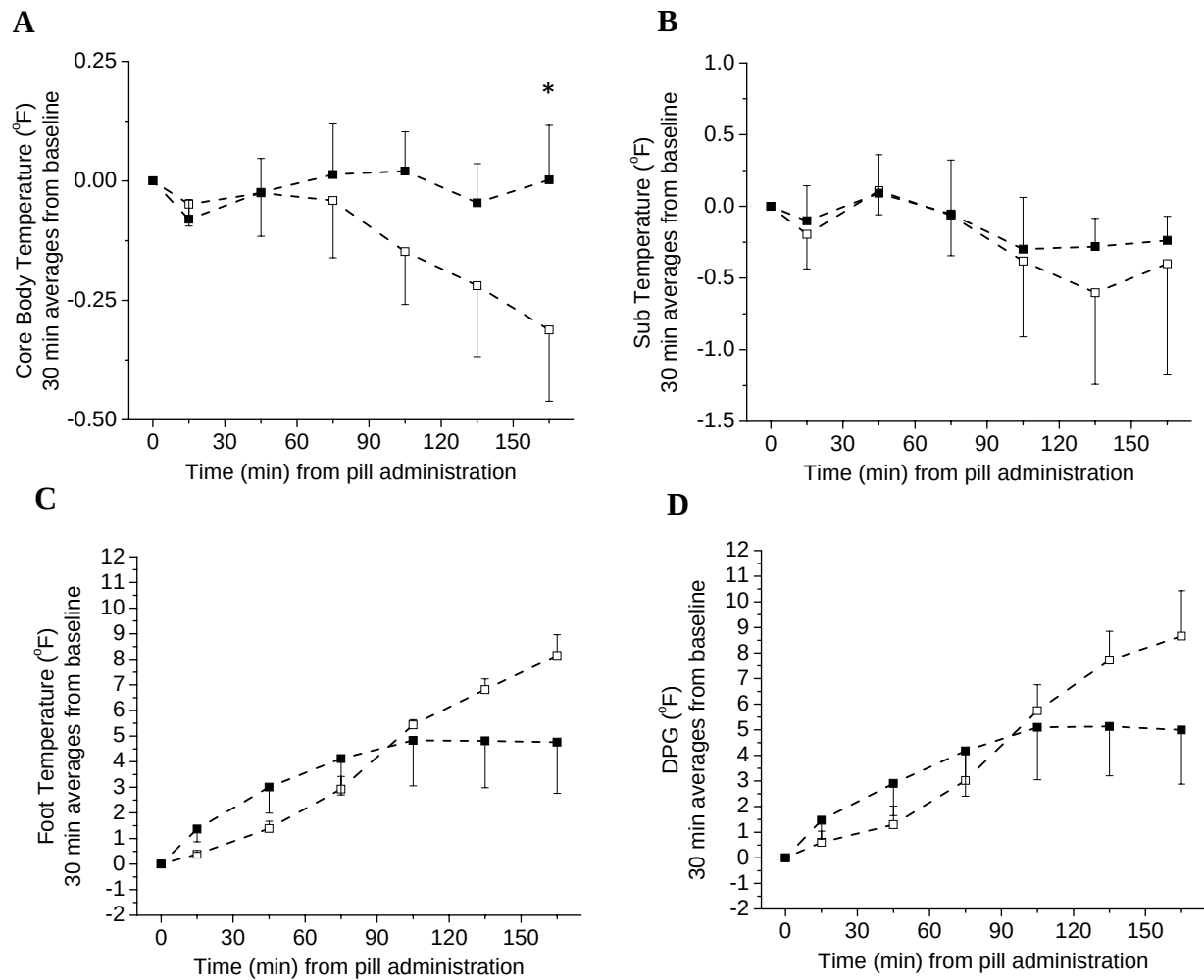


Figure 2: Thermoregulatory effects of caffeine on CBT, proximal and distal skin temperature sites, and the DPG following pill administration.

Values expressed as means $\pm$ SEM. The open squares represent the placebo conditions and closed squares represent the caffeine conditions. The 0 point denotes time that caffeine or placebo were administered. (A) Caffeine significantly reduced core temperature by attenuating the normal nighttime circadian fall as seen in placebo. (B) Proximal skin temperature measured at the subclavian vein (C) Distal skin temperature measured at the dorsal foot (D) The circadian increase in DPG was significantly attenuated after caffeine ingestion relative to placebo. * denotes a significant difference for caffeine from placebo conditions.

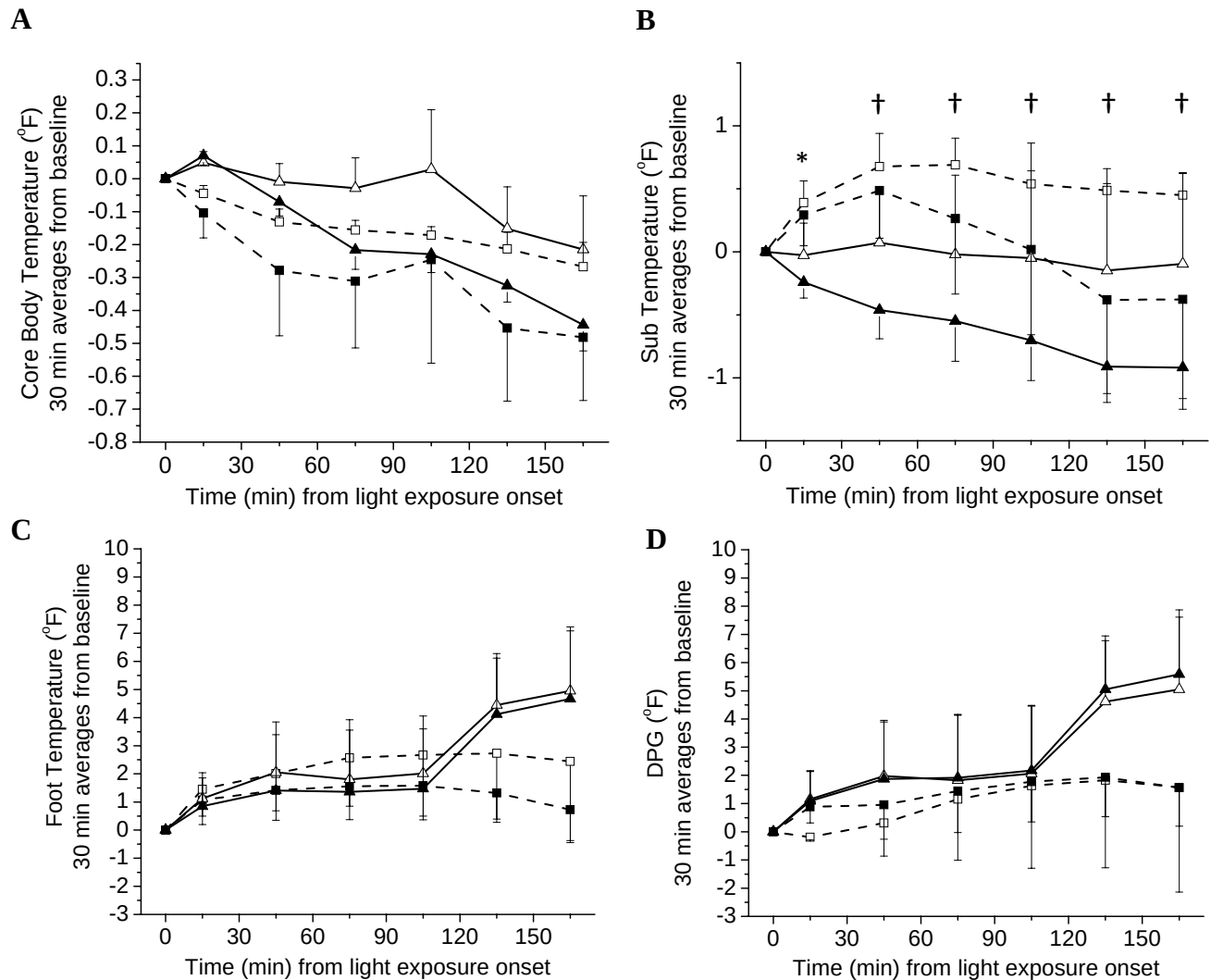


Figure 3: Thermoregulatory effects of caffeine and bright light on CBT, proximal and distal skin temperature sites, and the DPG following light exposure.

Values expressed as means $\pm$ SEM. The squares represent the placebo condition, triangles represent the caffeine condition, filled symbols represent the dim light condition and open symbols represent the bright light condition. The 0 point denotes time light exposure began. (A) Bright light significantly attenuated the circadian fall in CBT as seen in placebo drug and dim light condition. (B) Proximal skin temperature measured at the subclavian vein (C) Distal skin temperature measured at the dorsal foot (D) The circadian increase in DPG was significantly delayed after caffeine ingestion and bright light had no effect on this measure relative to placebo conditions. * denotes a significant difference for bright-light condition from dim light-caffeine and bright light-caffeine conditions. † denotes a significant difference for bright light-placebo from dim light-placebo.

and dim light-placebo conditions. There was also a significant difference in the stage 2 sleep for the dim-light caffeine condition from the dim light-placebo condition, and a significant difference in stage REM for the bright light-caffeine condition from the dim light-caffeine condition. No other significant effects were observed in the mixed model for the remaining sleep measures (see table 3).

Subjective sleep assessment

The mixed model ANOVA revealed a significant main effect for time ($F=8.61$, $p<0.0001$), a time by condition interaction ($F=1.86$, $p=0.05$), a time by subject interaction ($F=1.83$, $p=0.05$), and a condition by subject interaction ($F=5.91$, $p<0.0001$) on the KSS. Planned comparisons revealed a significant difference at hour 2 between the bright light-placebo and dim light-placebo conditions (Figure 4).

Discussion

The primary findings from this study are as follows: Caffeine administration 6 h prior to a delayed nighttime sleep opportunity attenuated the circadian fall in CBT, delayed the circadian increase in distal skin temperature measured at the foot, and the DPG. While the interaction of bright light and caffeine suppressed slow wave sleep and significantly increased SOL as compared to either condition alone.

To our knowledge, this is the first study in humans to demonstrate that caffeine affects peripheral heat loss near habitual sleep time. Krauchi and colleagues showed that individuals

Table 3: Results of EEG sleep measures

Parameter	BL + Placebo	BL + Caffeine	DL + Placebo	DL + Caffeine
Stage 1 (% RT)	3.4 ± 1.2	3.5 ± 1.4	3.0 ± 2.4	4.7 ± 2.1
Stage 2 (% RT)	39.4 ± 4.7	39.4 ± 9.5	40.9 ± 9.1	46.9 ± 6.2 [†]
Stage 3/4 (% RT)	29.3 ± 6.4*	22.7 ± 3.8	27.4 ± 6.3*	24.5 ± 6.5
REM (% RT)	22.8 ± 5.8	25.4 ± 2.9 ⁺⁺	22.9 ± 6.6	17.8 ± 6.0
Wakefulness (% RT)	5.1 ± 2.7	9.2 ± 8.0	5.8 ± 1.4	6.0 ± 1.6
SOL (min)	-3.56 ± 3.7*	11.25 ± 6.7	-3.0 ± 1.0*	0.19 ± 2.4

* denotes a significant difference from bright light-caffeine condition

† denotes a significant difference from dim light-placebo condition

++ denotes a significant difference from dim light-caffeine condition

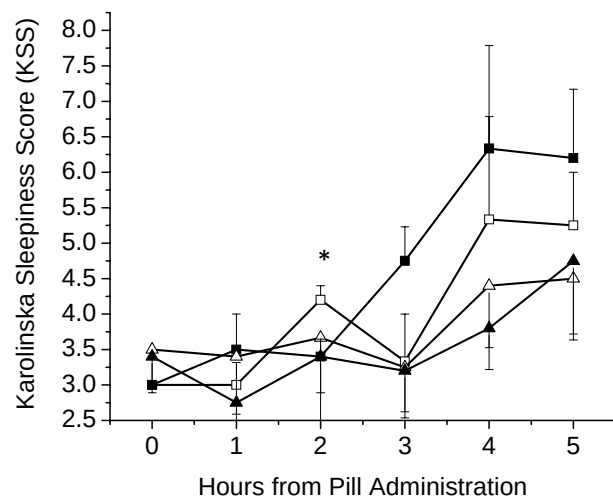


Figure 4: The effect of caffeine and bright light on subjective sleepiness.

Values expressed as means $\pm$ SEM. The squares represent the placebo condition, triangles represent the caffeine condition, filled symbols represent the dim light conditions and open symbols represent the bright light conditions. Bright light significantly increased scores on the KSS as compared to the dim light conditions. * denotes a difference between bright light-placebo and dim light-placebo conditions.

who had a larger magnitude of change in DPG fell asleep faster in a nighttime sleep opportunity compared to those that did not have much change in this thermoregulatory parameter (Krauchi, Cajochen et al. 2000). We did not find a significant individual effect of caffeine on SOL in the current study despite the delay in peripheral heat loss in the caffeine conditions. This may suggest that SOL would have been longer in the caffeine condition if the experimental design had scheduled sleep to occur earlier than 6 hours post pill administration, (prior to the observed increase in the DPG). We did not find an effect for bright light on the DPG in the placebo condition whereas previous research findings have shown a decrease in the DPG with bright light exposure that occurred 2.5 hours prior to habitual sleep time (Cajochen, Munch et al. 2005). This discrepancy may be explained by the circadian timing of which bright light exposure occurred in both studies. There is a circadian increase in peripheral heat loss that occurs around the time of melatonin onset (Krauchi and Wirz-Justice 1994). In fact, the increase in the magnitude change in the DPG that occurs 90 minutes prior to habitual sleep is a good predictor for faster sleep onset (Krauchi, Cajochen et al. 1999). Caffeine in the current study was given at 3 hours prior to habitual sleep onset and attenuated the increase in DPG similar to what was observed with bright light (Cajochen 2005). Data from the placebo condition in the study by Cajochen et al., demonstrate that the circadian rise in DPG begins to taper off around the time of habitual sleep onset (Cajochen, Munch et al. 2005) and this is when bright light exposure began in the current study. It may be that caffeine maximally affected the DPG and bright light exposure was not able to further affect this variable at a circadian time when levels begin to taper off. This may also explain why bright light alone did not affect SOL but that the interaction of caffeine and bright light did have a significant effect on SOL.

Caffeine with bright light, 6 hours prior to bedtime reduced slow wave sleep in the current study. Previous research findings have shown a reduction in slow wave sleep and EEG delta band spectral activity when caffeine (100 mg) was given at bedtime (Landolt, Dijk et al. 1995). To our knowledge this is the first study to show that caffeine in conjunction with bright light affects the amount of slow wave sleep 6 hours preceding a night time sleep opportunity. The reduction in slow wave sleep occurred despite the increase in sleep homeostatic drive from the 3 hours of extended wakefulness. Previous research findings have shown that slow wave sleep and delta band spectral activity are markers of sleep homeostatic pressure, with increased time and intensity reflecting time awake from the previous sleep episode (Paterson, Nutt et al. 2009). Further, adenosine has been shown to build up throughout the day in the basal forebrain of cats, and dissipate with sleep (Porkka-Heiskanen, Strecker et al. 1997). It has therefore been proposed that adenosine acts on adenosine A₁ and A_{2A} receptors located on the ventral lateral preoptic area (VLPO) of the hypothalamus and cortex to promote sleep (Saper, Scammell et al. 2005). Results from the current study are consistent with the physiological model that caffeine increases arousal through its action as an adenosine receptor antagonist.

It has been shown that nighttime caffeine (200 mg) administration can reduce endogenous melatonin levels in young men (Wright, Badia et al. 1997) and young women during the luteal phase (Wright, Myers et al. 2000). These research findings suggest that the effect of caffeine on core temperature and peripheral heat loss in the current study may be mediated in part by a reduction of melatonin levels. The result of which may be a decreased ability of melatonin to evoke cutaneous vasodilation and peripheral heat loss as has been shown with daytime exogenous melatonin administration (Krauchi, Cajochen et al. 2000; Aoki, Stephens et

al. 2003; Aoki, Stephens et al. 2006; Krauchi, Cajochen et al. 2006). Future studies will need to examine this possibility.

Bright light of ~3000 lux did not significantly impact core temperature or the DPG as has been previously shown (Badia, Myers et al. 1991; Dijk, Cajochen et al. 1991; Wright, Badia et al. 1997; Burgess, Sletten et al. 2001; Cajochen, Munch et al. 2005). This may be explained in part by the effect of caffeine on these variables when given 3 hours earlier. As mentioned above, caffeine may have maximally affected these variables via adenosine antagonism of melatonin levels. It may also reflect a lack of statistical power to detect significant differences in these variables at this experimental time period.

In summary, the current study findings show that in young healthy individuals caffeine not only impacts the thermoregulatory system around the time of habitual sleep onset by preventing the circadian fall in CBT but also by delaying the circadian increase in peripheral heat loss. Further, the combination of caffeine and bright light reduced the amount of slow wave sleep compared to the other conditions and significantly increased sleep onset latency. The effects of caffeine on thermoregulatory and sleep physiology occurred despite an increase in sleep homeostatic drive from the delayed bedtime. This may have implications for individuals who commonly consume caffeine and have diseases and/or conditions that affect the thermoregulatory system (i.e diabetes, vasospastic syndrome, menopause). Future studies may want to examine the effect of caffeine on sleep and thermoregulatory physiology in these specific populations.

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CHAPTER 5

CONCLUSION

Rachel R. Markwald

Summary of results

The purpose of this dissertation was to address the following deficiencies in our present state of knowledge about the relationship between sleep and thermoregulatory physiology. First, it was unknown whether a melatonin receptor analogue ramelteon would impact daytime sleep and body temperature, and if there is a relationship between the two processes with this pharmaceutical compound. Therefore, we tested the hypothesis that ramelteon (8mg) would reduce CBT and increase the DPG under controlled bed rest conditions, as well as decrease SOL and WASO and increase TST in a daytime sleep opportunity. We also hypothesized that a greater change in DPG would be associated with a shorter SOL. We found that ramelteon significantly improved daytime sleep, lowered CBT, increased peripheral heat loss, and that the magnitude of increase in the DPG and distal skin temperature were correlated with a faster latency to sleep in the ramelteon condition.

Secondly, it was unknown if exogenous melatonin affects metabolic rate as this would decrease heat production and might act as an additional thermoregulatory signal in the regulation of sleep/wakefulness. We tested the hypothesis that exogenous melatonin (5mg) would acutely decrease resting energy expenditure as assessed by volume of oxygen consumption (VO_2) when compared to placebo during the biological daytime when endogenous melatonin levels are low and CBT is high. We showed that exogenous melatonin acutely lowered resting energy expenditure and VO_2 during the late afternoon when endogenous melatonin levels are low.

Finally, it was not known how caffeine and bright light may individually, or in a combined fashion, impact the sleep and thermoregulatory systems at the time of habitual sleep onset. Specifically, to our knowledge, no study has examined the effect of caffeine taken 6 hours prior to a delayed bedtime on skin temperature physiology. Thus, we tested the hypothesis that

caffeine would act individually and in combination with bright light to attenuate the circadian fall in core temperature, attenuate the circadian rise in the DPG near the habitual bedtime, as well as increase SOL and decrease the amount of slow wave sleep during a nighttime sleep opportunity. We found that caffeine administration 6 h prior to a delayed nighttime sleep opportunity attenuated the circadian fall in CBT, delayed the circadian increase in distal skin temperature measured at the foot, and the DPG, while the interaction of bright light and caffeine significantly reduced the percentage of slow wave sleep and significantly increased SOL as compared to either condition alone.

In summary, this dissertation aimed to improve our understanding of the relationship between the sleep and thermoregulatory systems. We showed that a melatonin analogue improved daytime sleep and produced a thermoregulatory pattern similar to what is observed at night when endogenous melatonin levels are high. Furthermore, daytime exogenous melatonin decreased metabolic rate and may contribute to the circadian variation in resting energy expenditure with a trough occurring around the time of habitual sleep onset. Lastly, caffeine and bright light (two common pharmaceutical and environmental factors) negatively impacted both the sleep and thermoregulatory systems near bedtime.

Future directions

Possible extensions of the research from this dissertation include the following. First, it has been reported that exogenous melatonin also improves daytime sleep and produces a thermoregulatory pattern similar to what is observed during the biological night when endogenous melatonin levels are high (Hughes and Badia 1997; Krauchi, Cajochen et al. 2006).

Exogenous melatonin however, is not currently regulated by the food and drug administration in the United States and may not be a safe option for the treatment of insomnia. Furthermore, the development of melatonin analogues (i.e. ramelteon) with longer half lives and greater binding specificity to the G-protein coupled melatonin MT1 and MT2 receptors may provide an advantage over melatonin in improving sleep. As we learn more about the discrete actions of these receptors, the development of even more specific analogues may be a viable treatment option for individuals who suffer from insomnia related to circadian sleep disorders.

Second, although this dissertation demonstrated that daytime exogenous melatonin can acutely reduce resting energy expenditure; future research will need to verify these findings in the presence of thermoregulatory variables. The use of a within-subjects research design may be advantageous in detecting small but physiologically meaningful changes in these metabolic variables. The relationship between thermoregulatory (i.e. the DPG) and metabolic variables have not been examined simultaneously with acute daytime exogenous melatonin administration.

Finally, our results show that in young healthy individuals, caffeine and bright light negatively impact nocturnal sleep via slow wave sleep reduction and an increase in SOL, and this may be due in part to caffeine-induced changes in thermoregulatory physiology. This may have implications for individuals who commonly consume caffeine and have diseases and/or conditions that affect the thermoregulatory system (i.e diabetes, vasospastic syndrome, menopause). Future studies may want to examine the effect of caffeine on sleep and thermoregulatory physiology in these specific populations. Additionally, future studies may want to examine the effect of caffeine on EEG spectral power throughout the sleep period to see if delta band activity is also reduced.

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