The Acute and Phase-Shifting Effects of Artificial Bright Light on Human Physiology, Performance and Symptoms of Jet-Lag

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The following has been excluded at the request of the university

Abstract

The periodic alternation of light and darkness over the solar day has a fundamental role in synchronising human circadian rhythms. Artificial light is known to alter circadian timing depending on time of administration; early morning light can advance circadian timing while late evening light can delay circadian rhythms. Nevertheless, there is a lack of research on how manipulations of the body clock, via bright light, can enhance human performance and/or alleviate the effects of jet-lag after transmeridian travel. The doctoral programme communicated in the present thesis was designed to fill some of these gaps in knowledge.

In the first experiment, the acute effects of light exposure on thermoregulation, pineal function and autonomic nervous system function (indicated by the rate pressure product) were examined. At 20:00 h, eight healthy men were exposed to a baseline period of dim light (< 12 lux) for 45 min followed by 0 lux for another 60 min. Thereafter, participants were exposed to either polychromatic bright light with blue photons (2500 lux), blue photons removed (2,500 lux) or 0 lux for 30 min. Baseline data was altered for all variables in the two light conditions, suggesting an "expectancy effect".. There was evidence that light attenuated the nocturnal fall in core body temperature and rise in melatonin. These data highlight that the circadian system and other areas of the brain which are stimulated by light are still sensitive to short duration exposure and these effects are amplified by the inclusion of blue spectrum light.

In a follow-up study, the effects of light exposure (blue photons included polychromatic bright light vs. no light) on subsequent early morning performance were examined under conditions of thermal stress. Participants were woken at 06:30 h. At 07:00, participants entered an environmental chamber set at 35°C and 60% relative humidity. Three 10-min bouts of exercise (55% $\dot{V}O_{2max}$) were completed on a cycle ergometer with each bout separated with 10 min of passive recovery. Participants then completed a 10-km cycling time-trial. Core body temperature was measured every 30 s throughout the experiment using intestinal thermistors. The time of the sleep-trough in core temperature occurred approximately 1.75 h later following bright light (L) vs. no light (NL) (p = 0.07). Just prior to the time-trial, T_c was 0.27 ± 0.42°C lower in L than NL (p = 0.07). The time trial was completed 1.43 ± 0.63 min quicker in L vs. NL (p = 0.001). Immediately after the time trial, intestinal temperature was 38.21 ± 0.56°C in BL compared to 38.64 ± 0.42°C in NL (p = 0.10). These data provide the first evidence that a 30-min exposure to bright light prior to sleep can delay circadian timing to the extent that exercise performance is improved in hot conditions during the subsequent early morning.

In the third study, the effects of a simulated dawn during the last 30 minutes of sleep on the subsequent dissipation of sleep inertia were examined and it was questioned whether subsequent improvements in simulated work and physical performance were mediated. Eight participants, who reported difficulty with morning waking, were administered in a random order to a control (C) and a dawn simulation (DS) trial (starting 30 minutes prior to waking). Subjective ratings of sleep quality and alertness were obtained alongside measures of cognitive performance (addition and a reaction time tasks measured at 5, 30 and 75 minutes after waking at habitual workday times). Physical performance was also measured 35 minutes after waking using a self-paced cycling protocol. After waking in the DS condition, perceived sleep quality was 1.16 ± 0.89 (p = 0.01) points higher compared with C. Ratings of alertness were significantly higher in DS than C throughout

the testing period (p = 0.04). Cognitive performance improved in both trials as time-awake increased ($p < 0.0005$). On average, participants completed a significantly greater number of additions in DS compared with C (69.5±15.3 vs. 66.9±16.7, p = 0.03). Reaction times were also faster in DS compared with C (0.81±0.07s vs. 0.86±0.06s, $p < 0.0005$). The self-paced trial was, on average, 21.4 s (4.7%) quicker in DS (p = 0.07). These data provide the first evidence that light exposure during the last 30 minutes of habitual sleep can increase subjective alertness and improve both cognitive and physical performance in the morning.

Although light can alter circadian timing, the practical effectiveness of light for reducing jet-lag symptoms in athletes is unclear. Therefore, the doctoral programme also focussed on light intervention studies "in the field". Twenty-two world-class female footballers were randomised to a bright light intervention or control group before a flight from USA to Europe. Intra-aural temperature, grip strength, sleep and various jet-lag symptoms were measured serially. For 4 days, the bright light group were exposed to 2,500 lux of bright light at ≈50 cm for 45-60 min at a time-of-day predicted to accelerate circadian adjustment. On post-flight day 1, light transiently increased intra-aural temperature by 0.38°C (p = 0.001). In parallel, overall jet-lag ratings increased by ≈1 unit. Light had negligible effects on functioning, diet, bowel activity and sleep symptoms, which varied substantially between- and within-subjects over the post-flight days. In conclusion, these data do not support the notion that chronobiologically-timed light is substantially effective for reducing jet-lag symptoms in field conditions after world-class athletes travel from the USA to Europe.

From the previous study, there appeared to be large inter-individual variation in the perception of what constitutes jet-lag. Therefore, in the last study, a descriptive approach was employed to assess the effects of travelling over a numerous time-zones and different directions on perceived jet-lag symptoms. Questionnaire data were collected over the first two post-flight days. An inverted-U relationship between the number of time zones crossed and the severity of perceived jet-lag, function- and sleep-related symptoms was observed. Specifically, the data suggest that crossing over 4-7 time-zones resulted in greater perceptions of jet-lag 4.4 ± 1.0 ($p < 0.0005$) and 2.3 ± 0.8 (p = 0.006) than travelling over ≤3 zones or ≥8 zones, respectively. Within- and between-participant variability in jet lag symptoms was extremely heterogeneous thus further highlighting the rather opaque and difficult-to-study nature of jet-lag.

The studies in this thesis have provided a novel insight into the influence of bright light exposure on physiological function and performance in humans studied in both laboratory and field conditions. While acute and chronic phase-shifting effects of bright light can be detected in the controlled environment of the laboratory, the effectiveness of such light interventions for alleviating actual jet-lag symptoms is difficult to detect against the noise of competing zeitgebers and activities associated with world-class sports competitions.

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Declaration

I declare that the work contained in this thesis is entirely my own. Some of the work has been published in peer-reviewed journals or presented at International conferences, which are listed below. Beyond assistance with data acquisition and input from my supervisors no other individual has examined the findings presented herein.

Publications directly associated with this thesis:

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Thompson A, Batterham AM, Jones H, Gregson W, Scott D, Atkinson G. Randomised controlled trial of a bright light intervention for reducing symptoms of jet-lag in world-class female soccer players. Maribo Medico Sleep Conference (2012). Roskilde, Denmark.

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Other publications completed by candidate during PhD tenure

Verheggen RJHM, Jones H, Nyakayiru J, **Thompson A**, Groothuis JT, Atkinson G, Hopman MTE, Dick H. J. Thijssen DHJ. Complete absence of evening melatonin increase in tetraplegics. *The Journal of the Federation of American Societies for Experimental Biology* (FASEB) 2012; 26 (7): 3059-3064.

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List of Abbreviations

BP	Blood pressure
CI	Confidence intervals
DBP	Diastolic blood pressure
DLMO	Dim light melatonin onset
DLM_{Off}	Dim light melatonin offset
ECG	Electrocardiogram
ELISA	Enzyme-Linked immunosorbant assay
fMRI	Functional magnetic resonance imagining
GEE	Generalised estimating equations
GLM	General linear model
HR	Heart rate
LC	Locus coeruleus
LGN	Lateral geniculate nucleus
MAP	Mean arterial pressure
NIF	Non-image forming system
nm	Nanometre
Opn4	Melanopsin
PRC	Phase response curve
Q	Cardiac output
PET	Photon emission tomography
PVN	Paraventricular nuclei
REM	Rapid eye movement
RHT	Retinalhypothalamic tract
RPE	Rating of perceived exertion
RPP	Rate Pressure Product
SAD	Seasonal affective disorder
SBP	Systolic blood pressure
SCG	Superior cervical ganglia
SCN	Suprachiasmatic nuclei
SD	Standard deviation
SE	Standard error
SNA	Sympathetic nerve activity
SuC	Superior colliculus
T_c	Core body temperature
T_{sk}	Weighted mean skin temperature
VAS	Visual analogue scale
VLPO	Ventrolateral preoptic nucleus
$\dot{V}O_{2max}$	Maximal oxygen consumption

CHAPTER 1

BACKGROUND

1.1 Background

Light is typically considered in terms of the physically visible wavelength of its electromagnetic spectrum. Rods and cones located within the mammalian eye transpose the light signal into specific photons, which the brain can then integrate as meaningful visual information. Over the last three decades, a greater understanding of the importance of light in underlying human physiology has been demonstrated. The eye also has a system to detect environmental intensities or irradiance (Foster and Hankins, 2002). This system is known as the non-image forming system (NIF). A sub-set of retinal ganglion cells, which project directly to the suprachiasmatic nucleus (SCN), have been identified as the photoreceptors responsible for controlling the NIF and circadian systems, by providing photo-entrainment. The identification of the photosensitive pigment, melanopsin, within these specialised retinal ganglion cells was the catalyst that led to the major breakthrough in this area (Hattar et al., 2002, Provencio et al., 2000, Hankins and Lucas, 2002). The exact structure and mechanisms, including rod and cone input of the NIF, is still under intense research; and is discussed later in this thesis.

As in many other species, biological rhythms can be detected in humans. The most important of these rhythms are those that cycle every 24-h; namely "circadian rhythms". In humans, the SCN of the hypothalamus has been identified as the master circadian clock (Moore and Lenn, 1972, Ralph et al., 1990, Moore-Ede et al., 1982). The SCN comprises of a cluster of cells that organise and orchestrate the timing of biological functions; from complete systems and organs, to singular cells. Isolated cells from the SCN continue to show rhythmicity, although the intrinsic period is slightly greater than 24-h (Moore-Ede et al., 1982, Weaver, 1998). *In vivo*, the body clock remains synchronised to a 24-h solar day by entrainment mechanisms involving environmental signals or 'zeitgeibers'. It is

generally accepted that the exposure to ocular bright light is the most predominant zeitgeber and this pathway is known as "photoentrainment" (Roenneberg and Foster, 1997).

Light, either artificial or natural (i.e. sunlight), can not only be used for entrainment but also re-entrainment following circadian misalignment. Disruption to the body clock via shift-work and jet-lag commonly results in a general malaise, which impacts on human functioning. Correctly timed light exposure and/or avoidance has been shown to be useful in ameliorating this disturbance. However, with respect to jet-lag, a dearth of real-world research exists on the use of light to help facilitate realignment of the body clock. Furthermore, amongst the small number of published studies, there appears to be no examinations of light interventions for athletes.

Exposure to bright light can also have profound effects on performance. Neurobehavioral responses to light exposure include improved alertness and cognitive output (Vandewalle et al., 2006, Chellappa et al., 2011), as indexed by specific responses to cognitive tasks in Photon Emission Tomography (PET) (Perrin et al., 2004) and functional Magnetic Resonance Imagining (fMRI) techniques (Vandewalle et al., 2006). The use of bright light to manipulate physical performance is relatively unknown. As mentioned above, bright light can be used to shift circadian timing; including body temperature and melatonin rhythms, which share an inverse relationship (Cagnacci et al., 1992, Dawson and van den Heuvel, 1998). Manipulation of the time-course of these innate rhythms has the potential to improve performance in certain scenarios; for example, delaying the inherent rise in core body temperature (T_c) to elicit a 'pre-cooling' effect prior to exercise in hot conditions. Although such responses exist theoretically, very few researchers have

investigated the use of bright light on physical performance both in an acute and chronic context; thus providing a clear rationale for this thesis.

1.2 Aims and Objectives

The specific aims of this thesis are:

1. To examine the acute and chronic effects of bright light on human physiology and associated performance responses.
2. To investigate the effects of light during sleep and on waking (dawn simulation) on subsequent physical and cognitive performance in humans.
3. To examine the effects of supplementary artificial bright light on jet-lag in elite athletes.
4. To explore the multi-symptomatic nature of jet-lag and how the various constructs, believed to contribute to jet-lag, are perceived by individuals following actual air-travel.

The above aims will be achieved through the following objectives:

1. Through knowledge gathered to formulate phase response curves to light: undertake a study that attempts to 'delay' the body clock, thereby lowering core body temperature prior to exercise in high ambient temperatures. To help address aim 1.
2. To access markers of the human body clock, including core body temperature and melatonin, as well as the assessment of other physiological variables such as blood pressure and heart rate following exposure to light of differing wavelengths. To help address aim 1.

3. Investigate whether gradual increases in luminance during sleep, via dawn simulation device, has effects on subsequent sleep inertia and human functioning in the period immediately post-waking. To address aim 2.
4. Conduct a randomised control trial on the effects of bright light on world-class soccer players following the completion of actual air travel across multiple time-zones. To address aim 3 and help address aim 4.
5. Examine data collected from the *Liverpool jet-lag questionnaire* on intra- and inter-individual perceptions of jet-lag and related symptoms, with particular reference to how these compare with documented magnitude factors (e.g. number of time-zones crossed). To help address aim 4.

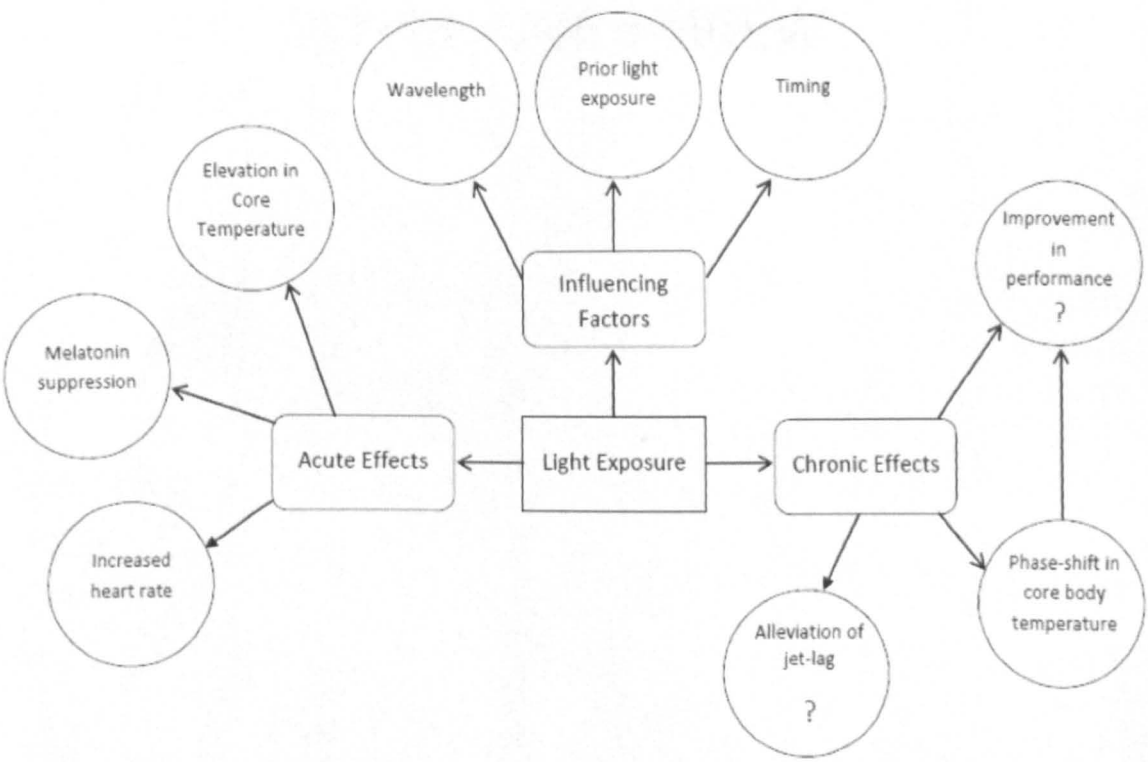

Figure 2.1: Information already known about the effects of bright light exposure and some unknown effects (highlighted by red question marks) which will be addressed in the current thesis.

CHAPTER 2

LITERATURE REVIEW

2.1 The eye, the brain and bright light

In the human brain, light is not only processed to visually represent the external environment but to also detect changes in ambient light level. Irradiance information is transduced from the retina in the eye via the retinohypothalamic tract (RHT) to the site of the master circadian pacemaker. The RHT originates in the retina and is a dedicated monosynaptic pathway projecting directly to various hypothalamic and regulatory structures of the brainstem, including the SCN (Moore and Lenn, 1972). The SCN of the hypothalamus is a paired cluster of ~10,000 neurons and is located above the optic chiasm and laterally to the third ventricle; there is substantial evidence supporting the functional role of the SCN as the master circadian pacemaker (Hastings, 1991, Morin, 2007, Rusak and Boulos, 1981, Meijer and Schwartz, 2003). The temporal oscillations of the SCN organise physiology and behaviour in order to adapt or "fine-tune" to environmental demands (e.g. day and night).

2.1.2 Rods, cones and melanopsin

Until recently it was thought that the classical photosensitive cells, rods and cones, were the only structures within the retina responsible for light transduction (visual and time-keeping). However, elegant studies on rodents disproved this theory and lead to an appreciation of a NIF system. Prior to this breakthrough it was observed that mammals with enucleation did not demonstrate photoentrainment, highlighting the role of ocular photoreceptors in this process (Lockley et al., 1997, Foster, 1998). However, studies performed on blind mutant animals with complete or near-complete degeneration of rods and cones still reported circadian phase shifting (Foster et al., 1991, Freedman et al., 1999, Hankins et al., 2008, Lucas et al., 1999). The discovery of intrinsically

photoresponsive retinal ganglion cells (ipRGCs) expressing the photopigment "melanopsin" (Opn4) was the major catalyst in enhancing our understanding of the NIF system (Hattar et al., 2002, Provencio et al., 2002). Null-Opn4 mice have a severe attenuation in the classical responses to light exposure (e.g. melatonin suppression); interestingly, a residual capacity to phase shift remains, whereas mice lacking functional rods and cones and null melanopsin expression are completely incapable of light-induced phase shifts (Panda et al., 2003, Hattar et al., 2003). Thus, suggesting that rods and cones do actually play a role in circadian entrainment. Indeed, under scotopic conditions it has been postulated that rod input via rhodopsin is the major contributing factor for photoentrainment (Bowmaker, 2008), see Figure 2.2.

Before the discovery of melanopsin and the subsequent research there was suggestion that bright light (13,000 lux) in the popliteal region could phase shift melatonin and T_c (Campbell and Murphy, 1998). However, attempts to duplicate this study and its findings were unsuccessful by other groups (Wright Jr and Czeisler, 2002, Koorengevel et al., 2001); leading to the results generally being discarded as erroneous. Furthermore, the results reported by Campbell and Murphy were compounded by observations of a null effect on phase shifting when the abdomen and chest regions were exclusively exposed to bright light (Lindblom et al., 2000). Therefore, it would appear that the eyes are the only region which detects light for the purpose of phototherapy.

Figure 2.2: Pathways for light-induced activation of non-visual brain areas. Light exposure activates melanopsin containing intrinsically photosensitive retinal ganglion cells and rod and cone-driven classical ganglion cells. Melanopsin-containing ganglion cells (blue) project to a range of 'non-visual' areas of the brain, including the SCN, which then project multisynaptically to the pineal gland, as well as to many areas that share input from the visual photoreceptor system (yellow), such as the lateral geniculate nucleus (LGN), pretectum and superior colliculus (SuC). Through as yet unidentified pathways, light stimulates the ascending arousal system and eventually the cortex to enhance alertness and cognition. Furthermore, light information also reaches sleep-promoting neurons of the ventrolateral preoptic nucleus (VLPO) and the noradrenergic locus coeruleus (LC) system, which is implicated in the circadian regulation of arousal. Taken from Cajochen (2007).

2.2 Phase response curves

Information from the environment (i.e. light) is required to keep the body in-sync, otherwise a state known as "free-running" manifests (Aschoff, 1981). This phenomenon, in humans, is generally categorised by a slight lengthening of the intrinsic day, although the exact duration of tau remains debateable (Smith et al., 2009, Eastman et al., 2012, Czeisler et al., 1999). Although as mentioned previously, zeitgebers can also be used to shift the time of the internal clock. Pioneering studies involving individuals with circadian desynchrony, such as misaligned temperature rhythms (Czeisler et al., 1986),

demonstrated the potency of bright light in phase-shifting the body clock. Since, several groups have attempted to calculate a full phase response curve (PRC) to light (Minors et al., 1991, Honma and Honma, 1988, Khalsa et al., 2003, Lockley et al., 2006b, Czeisler et al., 1989, Revell et al., 2012). A PRC is a figure (see Figure 2.3) which describes how a zeitgeber changes the phase of a circadian rhythm depending on when it is administered. The PRC for light is characterised by a phase delay region in the late biological day/early biological night, a phase advance region in the early biological day, small phase shifts during the middle of the biological day, and a transition point towards the end of the biological night. Although, it should be noted that lighting characteristics (e.g. intensity and wavelength), protocol duration and prior light-dark exposure can all influence results.

Figure 2.3: Example PRC to light. Produced from (Minors et al., 1991).

2.2.1 The importance of light intensity

Early studies on rodents demonstrated that, in addition to the phase-dependent responses , the circadian system showed intensity-dependent responses to light stimuli (Brainard et al., 1983, Nelson and Takahashi, 1991, Bauer, 1992, Sharma et al., 1999). In studies on humans, melatonin (see section 2.3.1 for further information) has been utilised as the predominant marker of circadian rhythmicity. It was found that a greater intensity of light increased levels of melatonin suppression (Brainard et al., 1988, Bojkowski et al., 1987). However, it is important to note that the human circadian system is still sensitive to relatively dim levels of light (~100 lux). Duffy and Czeisler (2009) examined the effects of six different light intensities (0, 12, 180, 600, 1260 and 9500 lux), administered during the early biological day, on human circadian rhythms. The dim light groups (0 and 12 lux) 'free-ran' in terms of their biological timing indicating non-entrainment, whereas, the higher intensities elicited significant phase advances. Zeitzer et al. (2000) examined the effects of single 6.5-h exposures to various light intensities during the late biological day/early night. They reported that the resetting response and melatonin suppression was minimal at irradiance levels < 100 lux and saturated in response to > 1,000 lux. They also postulated that 50% of the maximal phase shift observed at 9,100 lux could be achieved at ~1% (~100 lux) of that intensity.

2.2.2 The importance of wavelength

The magnetic spectrum ranges from gamma rays (~0.1 nm) to long waves (~1 billion nm). In contrast, the human visual spectrum is only ~400-700 nm. Again studies on melatonin suppression have been used to identify the circadian responses to different wavelengths of light. It has been observed that melatonin suppression was much greater at shorter compared with longer wavelengths. For example, monochromatic light at 460 nm

resulted in a two-fold greater phase delay than intensity and duration matched light at 555nm (Lockley et al., 2003). Furthermore, short wavelength light between 436 and 456 nm induced a phase advance similar to that of polychromatic light (i.e. white light) containing 185-fold more photons (Warman et al., 2003). Recently, it has also been found that relatively short wavelengths of light (450-460 nm) have more pronounced effects on body temperature and melatonin rhythms, alertness, reaction times and concentration as well as being superior in increasing electroencephalogram (EEG) high power alpha and reducing EEG delta power than longer wavelengths, which indicates heightened arousal (Cajochen et al., 2005, Lockley et al., 2006a). These results predicate the importance of melanopsin in the resetting of the circadian system, since Opn4 has a maximal sensitivity close to 479 nm (Lucas et al., 2001). Furthermore, these findings demonstrate the superiority of melanopsin in the NIF system compared with cone input. The three cone visual photopic system has a peak sensitivity of approximately 555 nm and is the standard measure of illuminance; thus highlighting that photopic lux (intensity) is an inadequate measure when quantifying the required drive to reset the circadian system. Although as mentioned previously, cones do still have a role to play in the NIF.

In the commercial world, these findings led to a mass-marketing of devices with high levels of short-wavelength light and devices that were 'blue light enriched', targeting individuals with conditions such as seasonal affective disorder (SAD). However, concerns were raised that exposure to such products, which were initially unregulated, could cause long term damage to the retina, exacerbation of age-related macular degeneration, and a photosensitisation hazard with common medications including certain psychotropic drugs (Centre for Environmental Therapeutics). In a short review, Terman (2009) added further weight to the argument, suggesting that serious consideration was needed of the major

conceptual, technological and clinical advancement that blue light "enrichment" provided (Terman, 2009). Conversely, research into short wavelength light has since continued at an intense rate; with the first PRC to blue light recently published (Revell et al., 2012). The analysis of data was particularly sophisticated within this study. Data was collected over a three day free-running period followed by either a control condition or intermittent blue light at a specific time of day. To get a full understanding of the effect size, the phase shift to blue light was corrected for the free-run determined during the control session. The delay portion of their PRC curve was similar to those using white light (e.g.Minors et al., 1991, Czeisler et al., 1989), however, the advance area extended later into the afternoon; suggesting that phototherapy utilising blue spectrum light can facilitate responses over a greater duration of the day, reducing the 'dead-zone'. Overall, the optimal composition of light required to reduce symptoms, specifically of clinical conditions (e.g. SAD), remains debateable.

2.2.3 Duration of light exposure

Much of the early research into the effects of bright light on the human circadian system involved protocols with long continuous periods of light exposure. For example, participants have been exposed to treatment durations of up to 5-h (Czeisler et al., 1989, Boivin et al., 1996). Although results in terms of phase shifting were significant, such protocols are not practically feasible meaning that refinement of methods were needed if phototherapy was going to have clinical implications. More recently, Rimmer *et al.* (2000) demonstrated that an intermittent protocol with a duration totalling 63% of a continuous protocol mediated an effect size which was equivalent to 88% of the continuous exposure. In addition, another study utilised an intermittent protocol amounting to 23% of the total duration of a continuous bright light protocol; this study was conducted during the early

biological night and both protocols significantly phase delayed with no statistical difference between the continuous and intermittent protocols (Gronfier et al., 2004). In a recent study, Zeitzer et al., (2011) postulated that repeated pulses of a moderately bright light stimuli (473 lux) of under 1 second (60 x 2 m-sec) could produce a significant phase adjustment compared with a dark control. In this study the phase-shift for the light pulse group was approximately 15-times greater than the control group. Moreover, subjective alertness was significantly greater and delta and sigma EEG activity was significantly reduced. Collectively, these data indicate that humans are responsive to shorter durations of bright light exposure than had been previously recognised, and that the magnitude of the response is nonlinear to the duration of light exposure.

2.2.4 Prior light-dark exposure

Prior exposure to light has also been found to have a profound effect on the magnitude of response to bright light. Three studies on melatonin suppression have helped to quantify this response (Smith et al., 2004, Hebert et al., 2002, Chang et al., 2011). The first study to demonstrate such effects (Hebert et al., 2002) compared 1 week of exposure to daytime bright light (5000–7000 lux) *vs.* 1 week of daytime dim light (<200 lux); with the amount of melatonin suppression by a 3-h night time light stimulus (500 lux) as the dependent variable. Results of this field study showed significantly greater suppression after the week of dim light compared with the week of bright light. All of the aforementioned studies indicated that a period of dark adaptation prior to light exposure sensitised the biological system, resulting in greater melatonin suppression. Additionally it has been suggested by Wong et al., (2005) that heightened repolarisation of photoreceptors during dark conditions results in greater depolarisation when light is administered.

Taken together, the data above demonstrates the complex interactions that need to be understood when prescribing phototherapy or attempting to phase shift the master clock. The timing, intensity, duration, pattern and wavelength all need to be considered to ensure more comprehensive and efficient protocols are utilised.

2.3 Circadian Rhythms

Chronobiology is the study of cyclic variation in living organisms (Dunlap et al., 2004). Biological rhythms can be found in various time domains, ranging from milliseconds to years. Many biological functions fluctuate between night and day, over a ~24-h period – collectively known as circadian rhythms (Minors and Waterhouse, 1986). These have a large influence on sleep, performance and general health in everyday life. Circadian rhythms can either be exogenous (controlled by external factors) or endogenous (controlled by the internal body clock) in their origin.

The regulation of the mammalian circadian pacemaker derives from a complex interaction of transcription and translation factors. Early in the biological day transcription factors CLOCK and BMAL1 hetrodimerise and activate the expression of Period (Per), Cryptochrome (Cry) (Shearman et al., 1997, Gekakis et al., 1998). These are then translated into protein form in the cytoplasm, where they bind together to form a complex that is transported back to the nucleus. This supresses the production of CLOCK and BMAL1 as Per and Cry proteins negatively feedback on their expression, and it is only when their expression levels drop below a certain threshold that sufficient CLOCK and BMAL1 protein can be produced to turn their expression back on. This feedback cycle provides near 24-hourtiming, and drives the rhythmic expression of several clock-

controlled and clock-modulated genes, which in turn mediate circadian rhythms in behaviour and physiology (Albrecht, 2002, Richter, 1967).

It is important to appreciate to what extent physiological functions are controlled by a self-sustaining pacemaker (i.e. the body clock). Unfortunately, measuring biological rhythms is not easy due to the masking effects of variables such as sleep, physical activity and environmental factors on underlying physiology. This issue has led to the development of constant routine protocols to reduce the potential problems of 'masking'. Such protocols require participants to be kept in a constant posture and environment (temperature, humidity and light), and for small amounts of food to be taken at regular intervals. Two rhythms that have been shown to be regulated by the body clock, at least in part, are melatonin and body temperature.

2.3.1 Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is a secretary hormone that is synthesised in the pineal gland (Huber et al., 1998, Lerner et al., 1958). The SCN controls the secretion of melatonin via a multisynaptic pathway involving the paraventricular nucleus, spinal cord and superior cervical ganglion and is considered a robust marker of the body clock (Klerman et al., 2002). Melatonin levels begin to rise in the evening, prior to sleep, reaching their peak in the early hours of the morning and decreasing to daytime levels after waking (see Figure 2.4). Melatonin is suppressed by light according to a dose response curve (Lewy et al., 1980). However, this response does not require a high irradiance; ordinary indoor lighting (80-160 lux) can reduce secretion and phase shift the body clock (Zeitzer et al., 2000). The pattern of emission conveys information to the rest

of the body regarding the light-dark cycle (Arendt, 1995). This signal is important, as it acts as a humoral messenger aiding the seasonal regulation of appropriate physiology and behavioural changes. Although for humans the technological development of indoor lighting, as alluded to above, is potent enough to affect the 'natural' rhythm of melatonin, meaning the use of such devices at night results in most humans experiencing a summer photoperiod year round (Cole et al., 1995). Moreover, specific groups, such as those with tetraplegia, are unable to secrete melatonin due to the location of the spinal injury, which can lead to disturbed sleep and further medical complications (Verheggen et al., 2012).

Endogenous melatonin is used in many studies as an indirect marker of the body clock and to assess the magnitude of phase shifts induced by a stimulus. Concentrations of melatonin in plasma and saliva have been deemed acceptable for this purpose and melatonin has few 'masking' effects influencing its production (Arendt, 1998, Benloucif et al., 2008b, Lewy et al., 2006, Klerman et al., 2002). Unlike other markers of the circadian phase, it is believed to be minimally masked by exogenous factors such as stress or sleep (Morris et al., 1990, Parfitt and Klein, 1976); although a more recent study proposed that sleep deprivation may influence the amplitude of melatonin (Zeitzer et al., 2007). Light is the only recognised variable which grossly masks melatonin production.

Plasma melatonin levels are typically measured using sensitive radioimmunoassays (Lewy et al., 1999), however, salivary samples may be more practical for assessing circadian phase (Leibenluft et al., 1996). Leibenluft et al. (1996) reported a strong correlation ($r = 0.93$) between plasma and salivary assessment of melatonin as a phase marker. Although, there are large individual differences in peak concentrations and the time that secretion begins (DLMO) and stops (DLM_{Off}) (Burgess and Fogg, 2008). These variations highlight

the importance of assessing the 'time' of the body clock, or at least an individual's chronotype (Roenneberg et al., 2003), prior to undertaking a study which involves phase-shifting. Without such knowledge, the 'windows' in which a phase delay or phase advance can occur might be missed or worse still, the opposite effect than desired could occur.

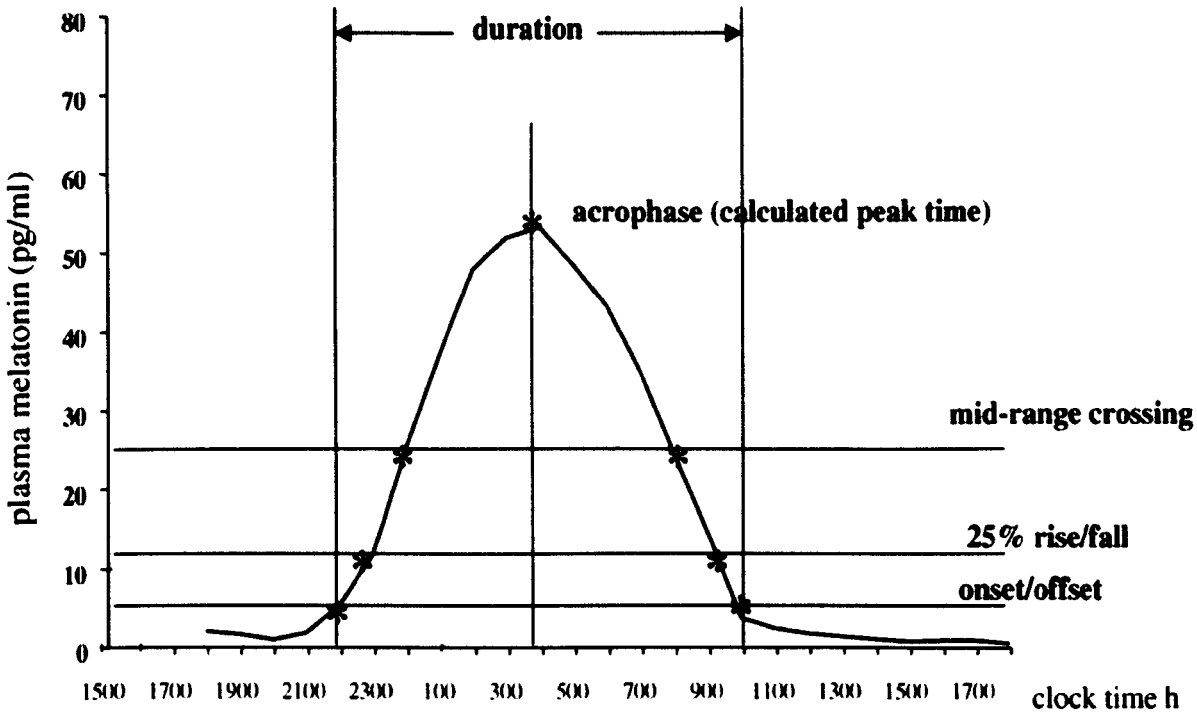

Figure 2.4: The circadian rhythm of melatonin over a 24-h period.

2.3.2 Core Body temperature

The rhythm of core body temperature (T_c), which is also frequently used as a marker of the master pacemaker, is inverse to that of melatonin; possibly inferring that melatonin is a regulatory factor for T_c, or *vice versa* (Cagnacci et al., 1992). The nadir of T_c occurs in the early morning and peaks in the late evening / early night time (see Figure 2.5). During a constant routine protocol these peaks and troughs are easily distinguishable whilst in conventional living a plateau between the hours of 14:00-h and 20:00-h is often reported for the T_c maximum (Krauchi and Wirz-Justice, 1994). The nocturnal decline in body temperature is believed to involve proximal vasoconstriction of the core and distal

vasodilation of the limbs mediated by arteriovenous anastomoses (Waterhouse et al., 2005a). As melatonin is a known vasodilator, this further highlights a possible physiological link between body temperature and the pineal hormone. Indeed, the administration of exogenous melatonin has been shown to transiently reduced T_c (Cagnacci et al., 1994, Cagnacci et al., 1995, Deacon and Arendt, 1995, Hughes and Badia, 1997, Krauchi et al., 1997b). Although the exact mechanisms causing these hypothermic affects remain enigmatic.

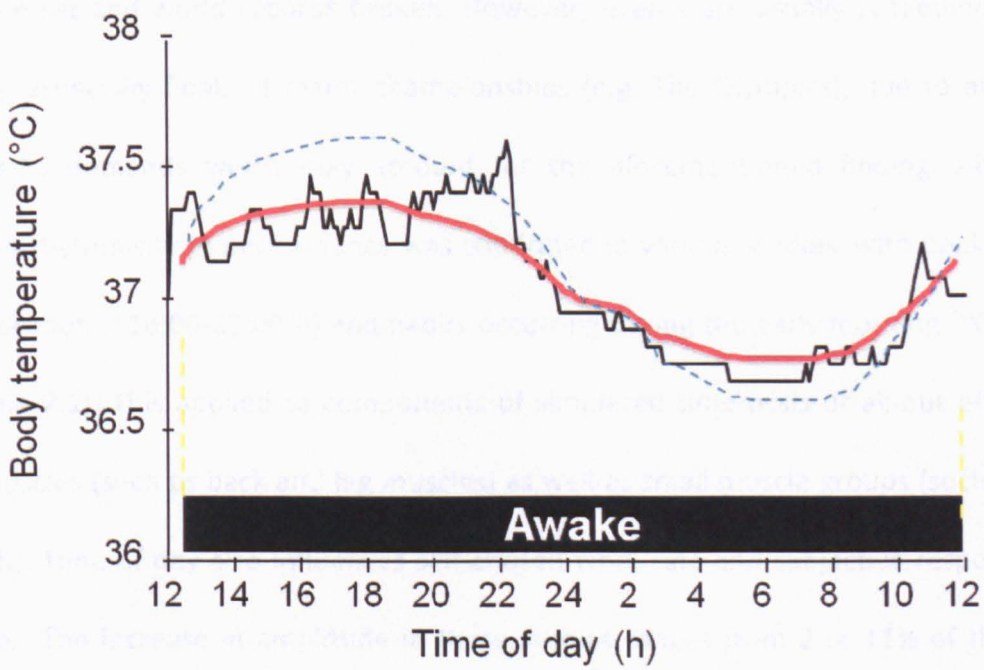

Figure 2.5: The circadian rhythm of T_c over a 24-h period whilst under a constant routine protocol. Adapted from (Edwards et al., 2002).

2.3.3 Effects of light on melatonin and core body temperature

As alluded to previously in this chapter, bright light has direct effects on the body clock and consequently, these robust markers of the circadian pacemaker. Bright light with sufficient characteristics (e.g. spectrum and intensity) has the capacity to suppress melatonin and increase T_c. For example, ten males were exposed to monochromatic light

(460 nm) for 2-h prior to nocturnal sleep. Compared with a control group, melatonin scretion was significantly attenuated by approximately 60% and T_c was ~0.15°C higher at the end of exposure (Cajochen et al., 2005).

2.3.4 Performance and circadian rhythms

The body clock and the innate circadian rhythms appear have a profound effect on both physical and cognitive performance. From just "eyeballing" the available data it would indicate that performance is optimal in the evenings; a time of day when most world lead times are set and world records broken. However, events are usually scheduled in the evening, especially finals of major championships (e.g. The Olympics), due to audience and media demands which may account for the aforementioned finding. Although, circadian rhythmicity in performance was confirmed in various studies, with peaks in the late afternoon (~16:00-20:00-h) and nadirs occurring during the early morning (~04:00-h; see Table 2.1). This applied to components of simulated time-trials or all-out efforts to large muscles (such as back and leg muscles) as well as small muscle groups (such as grip strength). Time of day also influences self-chosen work-rate and subjective responses to exercise. The increase in amplitude in these studies ranges from 2 to 11% of the daily mean (Reilly and Waterhouse, 2009).

This variation in performance closely follows the rhythm in T_c leading to many researchers hypothesising a causal link between the two; however, there are situations where a higher body temperature during exercise is not beneficial (see section 2.4). Moreover, other rhythms have been described as potentially important in the fluctuation of physical performance, including metabolic responses and hormones such as catecholamines. Collectively, the hypothesis that body temperature, or any other rhythm, has direct

perturbations on the rhythmicity of sport/exercise performance is yet to be fully substantiated. In contrast to much of the published research, Youngstedt and O'Connor (1999) argued that there was no evidence for circadian rhythmicity in real-life sporting performance. They suggested that there was no compelling evidence that transmeridan travel impaired athletic performance although the circadian pacemaker was no longer in-sync with the external environment. They cited methodological issues, such as poor protocol design, inappropriate data presentation and unsuitable data analysis techniques as the rationale for their argument. However, as noted by Drust *et al.* (2005), this did not stop the same researchers from providing advice to travelling athletes on alleviating jet-lag using chronobiological concepts.

The sleep/wake cycle also exerts effects on performance. As time awake increases, there is an exponential rise in the homeostatic drive for sleep. This propensity has a greater effect on the tasks which require a high cognitive input (e.g. shooting and archery). Furthermore, performance seems susceptible to the effects of partial or complete sleep loss. Although, skeletal muscle is more resilient to the negative effects of sleep deprivation (Reilly and Edwards, 2007), thus physical performance is less likely to be negatively affected.

Table 2.1: Sample of studies undertaken into the effects of time of day on performance. Highlighting the peak in physical performance occurs in the early evening and that tasks with a higher cognitive load peaks earlier in the day Adapted from (Reilly and Waterhouse, 2009).

Task	Time of optimal performance	Reference
Tennis first serve (speed) (accuracy)	 18:00h 09:00h	(Atkinson and Spiers, 1998)
Simulated time trial cycling	17:30h	(Atkinson et al., 2005b)
Simulated time trial cycling	18:00h	(Bessot et al., 2006)
Major muscle groups	20:00h	(Deschenes et al., 1998)
Badminton serve	14:00h	(Edwards et al., 2005)
Darts (short distance) (long distance)	15:00h 19:00	(Edwards et al., 2007)
Simulated time trial cycling	17:00-19:00h	(Giacomoni et al., 2006)
Elbow flexors, maximal contraction	18:00h	(Nicolas et al., 2008)
Cycling sprints	17:00-19:00h	(Racinais et al., 2005)
Standing broad and vertical jumps	18:00h	(Reilly and Down, 1992)
Soccer specific drills	16:00-20:00h	(Reilly et al., 2007)

In order to get a full appreciation of the role the body clock plays in physiology and performance, effects due to "confounding factors" (environment, social, time-awake etc.) need to be removed or taken into account. These different requirements have led to the development of specific protocols to help facilitate this, each of which has its own benefits and downfalls (see Table 2.2 for summary). One major issue that restricts the development of chronobiology and human performance is time and cost. To fully separate endogenous components and external factors, without the risk of muscle and/or central fatigue, protocols for a single participant would last for at least one week to a month. These methods appear to be too time consuming, costly and onerous for both

subjects and researchers. Moreover, following data collection, extrapolation to real world competition is difficult as athletes are highly motivated and have to overcome advertises such as emotional stress and anxiety.

Table 2.2: Methods used to separate the body clock from influence of the external environment and time since sleep. Taken from (Reilly and Waterhouse, 2009).

Several studies have been conducted in swimmers and these will be used to supplement the information provided within this section. In a study observing diurnal performance, it was found that 100 m and 400 m times were 3.5% and 2.5% quicker, respectively, in the evening (22:00-h), compared with the morning (06:30-h) (Baxter and Reilly, 1983). Another study utilising professional swimmers in the lead up to the 2000 Olympic Games reported quicker times in evening finals than morning heats (1.2% improvement) across three separate competitions. It was also noted that performance had to improve by ~1% within the Olympic year if the athlete was going to be in contention for a medal (Pyne et al., 2004). In a well-controlled study, Kline *et al.* (2007) adopted a 3-h ultra-diurnal sleep/wake cycle, involving 1-h of sleep in complete darkness and 2-h of wakefulness in dim light. Experienced swimmers performed 200 m time-trials every 9-h over a 50-55-h

testing period. Times were quickest 5-7-h before the temperature minimum (~23:00-h) and were worst approximately 1-h before the temperature minimum (~05:00-h). There was a 5.8 s (2.3%) circadian variation between the optimal and worst time. However, contrary data from the XXIX Olympiad in Beijing was recorded. Due to American media demands, the finals were held during the local morning hours. In the majority of events the quickest times were still set in the finals, even though the heats were held in the evenings when performance would be expected to be at its circadian peak. These anomalous results, compared with the published data, could be explained by the sense of occasion and/or pacing strategies or that time of day has minimal effects on performance. However, on balance, there appears to be sufficient data to suggest that performance in events of short or moderate or those which are intermittent in nature show circadian variation. The mechanisms which underpin these findings remain unclear and are further complicated by the effects of sleep loss, time awake, motivation and the environment.

2.4 Exercise in hyperthermic conditions

Cardiovascular physiologist, Loring Rowell, stated *"Perhaps the greatest stress ever imposed on the human cardiovascular system (expect for severe haemorrhage) is the combination of exercise and hyperthermia. Together these stresses can present life-threatening challenges, especially in highly motivated athletes who drive themselves to extremes in hot environments."* (Rowell, 1986). Indeed, the increased metabolic heat production associated with exercise and an impaired ability to dissipate heat to the external environment represents a substantial challenge to human physiology.

In short-lived exercise these conditions are somewhat negligible, however, during prolonged events performance is often diminished and the chance of heat related illness

increased. Empirical evidence has demonstrated that simulated performance is decreased in conditions of high thermal stress compared with equable climates. For example, eight males cycled to exhaustion at 70% $\dot{V}O_{2max}$ in four different ambient temperatures. Time to exhaustion was significantly influenced by ambient temperature, with the worst performance in the hottest condition (30.5°C). However, the optimal performance within this study was not observed in the condition with the lowest temperature (3.6°C); the effect of ambient thermal stress on exercise capacity appeared to follow an inverted U relationship, with the quickest time to completion at 10.5°C. At low temperatures it would appear that alterations in carbohydrate oxidation and oxygen consumption are detrimental to mechanical efficiency (Galloway and Maughan, 1997). Gonzalez-Alonso *et al.* (1999) documented the importance of T_c prior to commencing exercise and the hypothesised a 'critical' threshold of hyperthermic-induced fatigue. They observed an inverse relationship between starting T_c and time to exhaustion; a higher temperature resulted in a lower exercise time. Notwithstanding this, across all conditions, subjects fatigued at the same level of hyperthermia (40.1-40.2°C).

Whilst alterations in metabolism, fluid balance, motor drive and central nervous system function all appear to have a role in exercise induced fatigue in hot conditions, it is evident that an inability to sustain sufficient cardiac output to maintain cutaneous perfusion for heat loss and a critically high T_c, are the two most prominent rate limiting factors. Therefore, methods to reduce the impact of thermal strain on the human body are highly sort-after and researched. Pre-race interventions such as cold showers and cooling vests have been adopted in an attempt to lower body temperature. Such pre-cooling strategies have been shown to be effective in reducing thermal as well as cardiovascular and psychophysical strain in athletes (Booth et al., 1997, Cotter et al., 2001,

Lee and Haymes, 1995). However, these techniques are often uncomfortable for the individual. Exogenous melatonin, as highlighted previously, can transiently reduce T_c. McLellan et al. (1999) examined the effect of 2 x 1mg doses of melatonin on the thermoregulatory responses and tolerance to exercise responses during intermittent treadmill walking at 40°C. Melatonin did not significantly affect T_c or time to exhaustion. McLellan et al. (2000) utilised higher doses of 5 mg melatonin during low exercise intensities. The higher dose of melatonin resulted in a decrease in T_c at rest whilst in environmental conditions of 23°C, and during the first 50 minutes of exercise but this did not result in improvements in exercise tolerance time. At the higher environmental temperature of 40°C no reduction in core temperature occurred. These findings suggest that melatonin does not facilitate exercise performance. However, due to the uncompensatable nature of the heat stress together with the very low intensity of the exercise, it is not possible to generalise these findings to a sporting context. Theoretically, bright light, via phase shifting, could be utilised to lower T_c prior to endurance exercise in the heat. Whether such an intervention is efficacious remains enigmatic and is discussed further in section 2.5.2.

2.5 Applications of phototherapy

Phototherapy is used to treat many mood disorders, especially seasonal affective disorder (SAD). For example, a meta-analysis on bright light treatment for SAD revealed that there was a significant reduction (effect size of 0.84) in depressive symptoms in the 8 studies which matched the inclusion criteria (Golden et al., 2005). Although more research exists on the use of bright light in specific mood and non-mood disorders, this is beyond the scope of the present review. In contrast, the use of phototherapy in both optimising

performance through phase shifting and recovering from major circadian misalignment (e.g. jet lag) in a sporting setting, is relatively poorly researched.

2.5.1 Jet-lag

Athletes often travel over large distances for international events, competitions, training camps, and even to adhere to sponsorship agreements. This travel is often completed rapidly by air, and when multiple time-zones are navigated, the circadian desynchrony commonly known as 'jet-lag' can manifest. Jet-lag results from a misalignment between the internal 'body clock' and the new local time (Graeber, 1982, Nagano et al., 2003, Reilly et al., 1997). The symptoms of 'travel fatigue', which results from any travel over a significant distance, are initially similar to those of jet-lag; however, these generally dissipate following a full night's sleep. The effects of travel fatigue from flights are often due to cramped conditions that offer little opportunity for exercise, a restricted choice of food, dehydration due to dry cabin air (Brown et al., 2001) and cabin hypoxia. Collectively these may increase fatigue and change the daily profiles of some variables (Coste et al., 2005). In a recent review on managing transmeridian flights, focussing on athletes, it was suggested that fatigue and not jet-lag was potentially the bigger issue for sporting professionals. It was argued, that whist problematic, jet-lag was episodic and in the long term was less of a burden; whereas, over the course of a season, travel fatigue was cumulative and required a greater level of monitoring to reduce the risks of illness and injury (Samuels, 2012). When considered this publication makes an interesting point, although greater amounts of epidemiological data are required to validate the observations made.

The severity and duration of jet-lag appears to be dependent upon the number of time-zones crossed, the direction of travel, as well as individual perception (Graeber, 1982, Reilly et al., 1997, Graeber, 1989, Lowden and Åkerstedt, 1998, Waterhouse et al., 2007, Eastman and Burgess, 2009, Forbes-Robertson et al., 2012). Locomotion north-south presents negligible issues in terms of jet-lag as no or very few time zones are crossed, resulting in minimal disruption to the body clock. However, when travelling over the same number of time-zones, eastward flights generally result in a greater severity of jet-lag than westward journeys. This phenomenon is due the ability of the circadian pacemaker to reset more easily to a delay than an advance; delaying the body clock is the common resynchronisation method following westward travel. The underlying physiological rationale for this action is the length of Tau being slightly longer than 24h (see section 2.2). This innate susceptibility to delaying has led to advice of antidromic shifting for eastward flights ≥ 10 time-zones. Such a technique has been demonstrated effectively in simulated laboratory studies (Honma et al., 1995).

In its immediacy, jet-lag can present a plethora of issues including: acute insomnia, daytime sleepiness, impaired performance (cognitive and physical), gastrointestinal complaints, loss of appetite, disorientation and depressed mood (Waterhouse et al., 2007). These symptoms abate as the clock is re-aligned. Exposure to frequent jet-lag has further health complications, which can be long-term. The prevalence of cancer in flight attendants who frequently travel across multiple time-zones is increased (Reynolds et al., 2002, Rafnsson et al., 2001). In addition, female flight attendants are at higher risk of chronic menstrual cycle disturbances. Furthermore, due to the general upheaval of transmeridian travel, meals are often taken at irregular times and intervals; it has been

postulated that this can promote the development of cardiovascular disease and type II diabetes (Hampton et al., 1996).

As highlighted previously, the rhymicity of the body clock may produce variations in performance. Therefore, it is fair to postulate that jet-lag, which causes a disruption between internal and external timing, will impact on athletic performance. Studies have suggested that performance and physiological variables are supressed for a number of days following transmerrdian travel (Lemmer et al., 2002, Reilly et al., 2001). Furthermore, performance has been shown to be reduced in sports such as netball and basketball when a relatively short number of time-zones, 2-3, are negotiated (e.g. Bishop, 2004). Other studies have demonstrated that jet-lag has no effect on performance, however, these studies are minimal and on balance it would be fair to suggest that jet-lag is an issue for travelling athletes. It is only in this thesis, that international level athletes have been studied properly.

The most effective treatment for jet-lag remains elusive with products including melatonin (Edwards et al., 2000) and temazepam (Reilly et al., 2001) studied previously. In the investigation by Edwards and Colleagues (2002b) oral melatonin (5 $mg.day^{-1}$) was administered to thirteen athletes and support staff travelling eastwards over 10 time-zones at a time which was predicted to accelerate realignment of the body clock. They reported a null effect for their treatment, with no alleviation of jet-lag and related components and no influence on the phase shifting process compared with a placebo treatment (n=13). Similarly, Reilly and co-workers (2001) found that a 10 mg (low-dose) of benzodiazepine (temazepam) prior to sleep on the first three nights following a westward flight over 5 time-zones, was ineffective at ameliorating jet-lag symptoms compared with

a placebo. Specific and accurately-timed exercise and meals have also been suggested as methods to aid the alleviation of jet-lag, although the data are limited. Although it has been postulated that exercise itself can mediate a phase-shift in either direction when timed correctly (Buxton et al., 2003, Baehr et al., 1999), there are no studies on the effects of exercise on actual jet-lag symptoms. Furthermore, even highly strenuous exercise has been shown to produce only modest phase-shifts (Buxton et al., 2003). One study that used an "Argonne" diet (alternate days of fasting and feeding on a protein-rich breakfast and carbohydrate-rich evening meal for 4 days before a trans-meridian flight) reported positive effects in soldiers. However, the magnitude of the results was small and, as acknowledged by the authors, the study was impractical for military personnel. Numerous reviewers have advocated the exposure to and/or avoidance of light at specific times as beneficial (Waterhouse et al., 2007, Arendt, 2009, Sack, 2010, Eastman and Burgess, 2009, Forbes-Robertson et al., 2012). Nevertheless, a dearth of research into the use of artificial light currently exists. Boulos et al. (2002) reported that bright light visors (3,000 lux) mediated only modest reentrainment of circadian phase after westward travel over six time-zones (Boulos et al., 2002). Moreover, these shifts were not accompanied by any improvements in sleep, performance, or subjective assessments of jet lag symptoms. Furthermore, Lahti et al. (2007) reported that chronobiologically-timed light exposure did not significantly decrease the subjective symptoms of jet-lag in cabin crew.

Contrary to these findings, laboratory based simulated jet-lag studies have shown a greater amount success when using bright light to re-align the body clock. For example, over a number of separate studies Paul et al. (2009) highlighted the importance (i.e. for direction and magnitude of phase adjustment) of exposure time relative to the body clock. Additionally, Boivin and James (2002) exposed participants to room light (~380 lux) early

in the biological day of the new 'time-zone'. This time was advanced by 1-h each day and was sufficient to produce a phase advance of 5.37 ± 0.25-h in T_c over 7 days. The comparator group were exposed to the same protocol 6-h prior to nocturnal sleep. This group also advanced their rhythm in T_c but the magnitude was much smaller, 1.32 ± 0.9-h.

These paradoxical findings, between laboratory and field studies, demonstrate one of the major issues in extrapolating data to the "real-world", efficacy vs. effectiveness. Efficacy is a measure of the ability of a treatment to improve whatever condition it is indicated for, whereas effectiveness is a measure of how well a treatment works in the "real world" with the target population (Flay et al., 2005). It is unequivocal that appropriately timed light exposure, relative to body clock time, can induce phase shifting. However in laboratory settings, researchers are able to control numerous variables which may impact on their hypothesised outcomes (e.g. social interaction, temperature, unwanted light exposure etc.). In a real world setting this is very rarely the case and individuals, and therefore outcomes, are susceptible to such masking effects. In order to get an idea of a treatments effectiveness, real world data needs to be collected and protocols carefully considered.

Jet-lag is multi-symptomatic and highly subjective which complicates the monitoring and 'treatment' further. As expressed previously this malaise can affect various aspects of physiology including the sleep/wake cycle, digestive system and cognition. The *Liverpool jet-lag questionnaire* and *Columbia jet-lag questionnaire* were developed as more comprehensive approaches to monitoring jet-lag, including questions relating to sleep and fatigue; whereas much of the early subjective work on the topic simply employed a single visual analogue scale (VAS) to measure overall feelings of jet-lag. However, the

development of these 'new' questionnaires has not led to complete understanding of how individuals recover from jet-lag due to individual characteristics and time-course of recovery. It appears that symptoms recover at different rates from each other or jet-lag itself (e.g. Waterhouse et al., 2000, Graeber, 1989). Furthermore, dependent on the time of day, certain symptoms are expressed to a greater or lesser extent. For example, Waterhouse et al. (2000) observed the amount of perceived jet-lag in the morning is predicted by the time of waking from sleep (earlier times predicting more jet-lag) and by a decreased alertness 30 min after waking; and the amount of jet-lag in the daytime is predicted by the fall in the perceived ability to concentrate. Although this study attempted to get an understanding of how different symptoms relate to overall jet-lag, this was done using a simple regression model on each question. Therefore, the contribution of each variable may well have been inaccurately assessed in terms of jet-lag as an overall construct. A more in-depth analysis will be used within this thesis to facilitate a greater appreciation of how symptoms manifest individually and as overall constructs which contribute to jet-lag.

2.5.2 Sport performance

Contrary to the theory 'evening is best', there are data to support the notion that endurance performance, especially in hot conditions, is improved in the morning when core temperature is lower (see section 2.3.2). Hobson et al. (2009) reported that time to exhaustion in high ambient temperatures (35°C) was, on average, 5.3 minutes longer in the morning compared with the afternoon. This observation supports the hypothesis that lower T_c in the morning delays the onset of fatigue due to delaying the onset of a 'critical' T_c (Kräuchi et al., 2006).

As alluded to previously, phototherapy could potentially be used as an ergogenic aid for athletes. Recently, Atkinson et al. (2008a) postulated that bright light exposure could mediate a "natural" state of pre-cooling. They reported that evening bright light attenuated the rise in T_c the subsequent morning and during exercise in six male participants compared with dim light exposure. Although exercise was completed in this study, no performance outcomes were measured. Therefore, deducing a true understanding of how athletic capacity is affected by bright light from this study is difficult. Other previous studies into the effects of bright light exposure on humans at rest and during exercise have produced contradictory findings. Zhang and Tokura (1999) examined the effects of bright light (5,000 lux) exposure from 06:00-12:00-h and during subsequent exercise. They reported that T_c was significantly attenuated compared to dim light. Furthermore, Aizawa and Tokura (1998) reported that T_c was significantly lower after bright light exposure in the morning compared with dim light. These findings are surprising since bright light in the morning (after the temperature nadir) generally increases T_c rather than reducing it. Understanding the effect of bright light on subsequent performance following sleep is one of the fundamental aims of this thesis.

O'Brien and O'Connor (2000) used bright light in a slightly different context. The purpose of their study was to examine the effects of light exposure during 20 minutes of maximal cycling performance. They reported no significant differences between their three light conditions in average total power output: 1,411 lux (274.9 6 21.8 W), 2,788 lux (274.4 6 20.5 W), and 6,434 lux (270.3 6 19.8 W). There was no significant difference in alertness, leg muscle pain, perceived exertion, heart rate, $\dot{V}O_2$, or mood responses to exercise among the trials. This study demonstrates that acute light exposure during exercise has no effect. However, this null response could be due to the relatively high intensities of

light used in each condition and the fact that stimuli above 1000 lux can saturate the circadian system (Zeitzer et al., 2000). Furthermore, no details of environmental conditions or time of day were provided within this investigation, which may well be confounding factors. Paradoxically, a recently published study by Kantermann et al. (2012) reported positive effects of bright light before and during exercise. Participants were exposed to a dose of light, either bright (~4,400 lux) or dim (~230 lux), for a total of 160 minutes. During the last 40 minutes participants completed an exercise test on a cycle ergometer. Total work was significantly greater in the bright light condition, which was paralleled with an increase in individual strain (e.g. heart rate and lactate). It would appear from the results that an augmented activation facilitated by greater illuminance was the catalyst for improved performance. More research is required on this topic before reliable conclusions can be made.

2.5.3 Dawn simulation

Although the exact effects of sleep on human physiology remain equivocal there is evidence to suggest it influences restoration, thermoregulation, tissue repair, immune control and memory processing (Walker, 2008). Perhaps the most intriguing effects of sleep are those that persist over the waking phase. For example, during the period of "sleep inertia" individuals can experience grogginess, disorientation, decreased motor control and lower cognitive and physical performance (Dinges, 1990, Kleitman, 1964, Tassi and Muzet, 2000).

Artificial dawn simulation is considered a method to help reduce the symptoms related to sleep inertia. Dawn simulation was developed by Terman et al. (1989) and essentially involves the gradual increase in illuminance of low intensity light prior to the subject

waking from sleep. Terman et al. (1989) orignally designed their product based on the observations made in SAD patients. They recruited 3 particiapnts with SAD and exposed them to a dawn simulation protocol over 7 days; starting at 0 lux at 2:59 AM rising to 800 lux at 4:53 AM and to a maximum of 1,000 lux a minute later and was maintained until the patient arose. This exposure resulted in promotion of circadian phase adjustments, morning melatonin supression, regularisation of sleep patterns and antidepressent responses. This study, although intially informative had a small sample. The authors openly called for larger controlled studies, to firstly confirm their findings and secondly to help determine the underlying mechanisms involved.

Table 3 presents details of the methods and findings of a large number of studies on dawn simulation. Most of these studies concern the treatment of individuals with SAD. Within these studies dawn simulation has allowed for; significant decreases in depressive symptoms (Avery *et al.,* 2001; Avery *et al.,* 2004; Terman & Terman, 2006), circadian phase advances (Terman *et al.,* 1989 & Terman & Terman, 2010), eased the difficulty in awakening and reduced the severity of tiredness (Avery *et al.,* 2002 & Thorn *et al.,* 2004). In healthy subjects dawn signals have; reduced sleep inertia (Giménez *et al.*, 2010 & Fromm *et al.,* 2011), prevented phase delays of melatonin (Danilenko *et al.,* 2000) and increased post-awakening cortisol levels (Thorn *et al.,* 2004).

From the early studies on dawn simulation it was difficult to get a clear understanding of any treatment effect. For example, Avery et al (1992a) decreased depressive ratings from baseline using a dawn simulation, however, the illuminance of their treatment was relatively high, peaking at 1700 lux. Participants did not tolerate this well, with early morning wakings, headaches, agitation and muscle tension. Furthermore, the lack of a

true placebo within dawn simulation studies makes it difficult to fully appreciate the treatment effects. Indeed, the nature of phototherapy research makes it problematic to separate the treatment effect from chance since it is very difficult to mask the treatment from both the participant and researcher. This is issue is discussed in the review by Duffy and Czeisler (2009).

There are several further limitations of the literature. Many earlier studies only used one bedside light meaning that total light exposure could not be ensured (i.e. if the subject slept facing away from the light), which potentially limits the magnitude of the effects (Terman *et al.,* 1989; Avery *et al.,* 1994; Lingjearde *et al.,* 1998). Moreover there appears to be within study protocol variation, for example; a variable amount of days of exposure to dawn simulation between subjects in the study by Giménez *et al.* (2010) and a variation in light intensity (100-300 lux) between subjects in the study by Lingjaerde *et al.* (1998).

In a recent study on late chronotypes involving 30 min of dawn simulation prior to waking, Van De Werken et al. (2010) reported significantly decreased subjective sleepiness and increased subjective "activation". Nevertheless, cognitive performance, T_c and, contrary to Thorn et al. (2004), awakening cortisol were not influenced by dawn simulation. This was the first study to observe the effects of dawn simulation on performance; although no research group has yet observed the effects of such a protocol on physical performance. This gap in the literature will be addressed within this thesis.

The use of late chronotypes by Van De Werken et al. (2011) is methodically sound. Individuals whom are classed as late chronotypes usually prefer to retire to bed later and

rise later the following morning compared with intermediate and early chronotypes (Roenneberg et al., 2003). This usually means that late chronotypes have to rise earlier than desired on workdays resulting in a large discrepancy between their obligatory and preferred timing of sleep (Roenneberg et al., 2003, Horne and Ostberg, 1976, Zavada et al., 2005). This discrepancy can induce sleep debt, otherwise known as social jetlag (Wittmann et al., 2006). Ultimately, this sleep deprivation may lead to a 'vicious circle' of increasing severity of sleep inertia (Taillard et al., 2003). Therefore, this group is likely to respond positively to a treatment such as dawn simulation.

Table 2.3: Summary of findings from studies involving dawn simulation intervention. DS = Dawn simulation

Study	Participants	Population	DS Intensity (lux)	DS Dose (min)	Duration (days)	Effect
Avery *et al.*, 1992a	7	SAD patients	0.001 – 1700	120	7	Decreases in Hamilton Depression Scale scores were not significant: 18.0 (pre-intervention) 11.3 (post intervention). Frequent early morning awakenings were reported.
Avery *et al.*, 1992b	9	SAD patients	0.001 – 275	150	7	Average Hamilton Depression Scale scores decreased post intervention.
Avery *et al.*, 1993	4 M, 10 F	SAD patients	0.001 – 250	120	7	Average Hamilton Depression Scale scores dropped from 17.1 (pre-intervention) to 5.5 (post-intervention).
Avery *et al.*, 1994	1 M, 9 F	SAD patients	0.001 – 250	90	7	Significant improvement in mean Hamilton Rating for Depression: 20.7(pre-intervention) 8.0 (post intervention).
Avery *et al.*, 2001	4 M, 27 F	SAD patients	0.001 – 250	90	42	84% of participants showed response (decrease of $\geq$50% from baseline SIGH-SAD scores) 61% of participants showed remission (SIGH-SAD score $\leq$8).
Avery *et al.*, 2002	5 M, 23 F	SAD patients	0.001 – 250	120	7	Significant decrease in both difficulty awakening assessment and sleepiness.
Danilenko *et al.*, 2000	9 M	Normal Population	0.001 – 155 average	90	6	Prevented the phase delays of melatonin and temperature that occurred in control conditions.
Fromm *et al.*, 2011	44M, 59F	Young population (7 – 18 year olds)	0.001 – 300	30	7	Participants reported greater ease in getting up and increased alertness.
Gasio *et al.*, 2003	9 F	Elderly Dementia patients	0.001 – 210	34	21	(Dusk and dawn sim) Shortened sleep latency, increased sleep duration, increased nocturnal immobility, decreased nocturnal activity and small advance in circadian rest-activity rhythm.

Study	Participants	Population	DS Intensity (lux)	DS Dose	Duration (days)	Effect
Giménez *et al.*, 2010 (1)	23	Normal population	0.001 - 50/250	30	42	Both studies show beneficial effects on subject ratings of sleep inertia. There were no significant shifts in dim light melatonin onset.
(2)	23	Normal population	0.001 – 264.7 average	30	14	
Leppämäki *et al.*, 2003	30M, 47 F	Normal population	0.001 – 214 average	30	28	Improved quality of sleep (Average 1.7 Increase on quality of sleep scale).
Lingjaerde *et al.*, 1998	27	SAD patients	0.001 – between 100 and 300	60/90	14	Improvements were rated by patients themselves on a visual analogue scale: average improvement of 40%.
Terman *et al.*, 1989	3	SAD patients	0.001 – 800	114	7 – 14	Circadian phase adjustments, morning melatonin suppression, regularised sleep patterns and antidepressant responses.
Terman & Terman, 2006	22 M, 77 F	SAD patients & Bipolar II patients	0.0003 – 250	210	21	Average improvement of 49.5% of participants SIGH-SAD scores.
Terman & Terman, 2010	-	SAD patients	0.001 – 250	93	21	Average phase advance of 34.9 min in dim light melatonin onset.
Thorn *et al.*, 2004	7 M, 5 F	Normal Population	0.001 – 250	30	2	50 – 150% increase in cortisol post-awakening and higher alertness.
Van de Werken *et al.*, 2010	8M, 8 F	Late Chronotypes	0.001 – 300	30	1	Significant decreases in levels of sleepiness and increases in activity. No significant effects on cognitive performance, core body temperature or cortisol.

2.6 Acute effects of light

The majority of this review has so far focused on the effects of light from a phase shifting viewpoint; however, exposure to light also mediates a number of acute responses in human physiology. One effect that has already been documented is the suppression of melatonin. This decline in humoral secretion from the pineal gland has been postulated as the indirect mechanism by which light increases alertness, especially in night time studies. It has been hypothesised that melatonin elicits these effects by attenuating SCN-dependent mechanisms responsible for promoting and maintaining cortical and behavioural arousal at particular times in the circadian cycle (Dijk and Czeisler, 1995). However, more recent studies have reported that bright light exposure mediates an increase in alertness independent of time of day (i.e. during the daytime when melatonin levels are almost undetectable) (Lafrance et al., 1998, Cajochen, 2007). In the brain, it appears that light does not simply affect the SCN and hypothalamic regions but cascades across a large network of neural connections to decrease sleepiness, and improve mood and alertness (Vandewalle et al., 2006). These alterations in brain function and subjective parameters, via bright light, have been shown to increase cognitive performance (Vandewalle et al., 2009). The utilisation of PET and fMRI in the afore-cited studies has finally quantified the altering effects of bright light. Thus, the argument that participants have never been 'blinded' to light treatment is less noteworthy.

Other aspects of human physiology have been shown to be acutely responsive to bright light, including increases in T_c (covered in section 2.5) and heart rate (Scheer et al., 2004). These immediate responses to light also appear to be dependent upon the characteristics of stimulus (e.g. dose and wavelength). Cajochen et al. (2005) observed, that 2-h of monochromatic light at 460 nm mediated superior suppression of melatonin and

sleepiness and increased T_c and heart rate to a greater extent than intensity- and duration-matched light at 550 nm and a no light control. Furthermore, the differences between the second light and control condition for the majority of outcomes were negligible.

2.7 Summary

This literature review summarises some of the key effects that bright light has on human physiology. Whilst it is unequivocal that bright light synchronises the circadian system and has the ability to phase shift the body clock to different 'times', it is unclear to what extent bright light manipulation can have on human performance. Furthermore, the application of common theory, such as the use of bright light to alleviate jet-lag, is poorly researched. The research that does exist in these areas is either inadequately designed or lacks sufficient outcome variables to arrive at valid conclusions. The present thesis aims to address this dearth in research and the shortcomings of previous publications within the topic area relating to bright light exposure and human performance and functioning.

CHAPTER 3

GENERAL METHODS

3.1 Participants

Prior to each laboratory-based study (*Chapters 4, 5 and 6*), participants were provided with details of procedures in writing along with a verbal explanation, before they gave written informed consent. All studies were approved by Liverpool John Moores University Research Ethics Committee and adhered to ethical standards set out in the Declaration of Helsinki. All participants completed a health questionnaire to ensure they met the relevant inclusion criteria. These included; being normotensive (SBP <130 and DBP < 85 mmHg), having no known history of cardiovascular, cerebrovascular, metabolic or respiratory diseases and being free from medication other than the oral contraceptive pill. The participants were non-smokers, they were not involved in shift-work nor had they undertaken travel across multiple time-zones less than 1 month prior to the start of each study. In the laboratory-based studies all participants were recreationally active, typically engaging in moderate intensity (e.g. continuous running/cycling) aerobic activities for at least 3 days/wk; none were competitive semi-professional or professional athletes. With the exception of *Study 3*, participants reported that they had a normal sleep-wake cycle, which was regulated in the week prior to testing commencing. In *Chapters 4 and 5*, all participants were either a moderate or intermediate chronotype according to the questionnaire of Waterhouse et al. (2001). The chronotype questionnaire developed was a composite scale of previous items (Torsvall and Akerstedt, 1980, Horne and Ostberg, 1976). This ensured the exclusion of participants who were extreme morning chronotypes and extreme evening chronotypes since it is known that morning and evening types differ in the phase of their endogenous circadian rhythms (Smith et al., 1989, Waterhouse et al., 2001).

In *Chapter 6,* participants were classed as either intermediate or extreme evening types on the morningness-eveningness questionnaire. This population is shown to be at increased propensity of extended sleep inertia in the post-waking phase. Female participants were tested in the early follicular phase (day 1-7) of the menstrual cycle, determined by the first day of menstruation, or during menstruation of the pill withdrawal phase (~day 2-7).

In *Chapter 8* completion of the questionnaire was taken as implied consent. These participants were not screened for any major medical issues but were asked to give a number of general parameters, including age, gender, height and weight. *Chapter 7* all participants were elite soccer players who had completed travel across multiple time-zones (5-8). All participants were either moderate or intermediate chronotypes and were free from illness in the two weeks prior and during the testing period.

3.2 Familiarisation

Prior to the laboratory-based experiments commencing all participants were familiarised with equipment and the sleep laboratory environment. During this session, anthropometric data were collected; Height (cm) was measured using a stadiometer (Seca, Birmingham, UK) and body mass (kg) was recorded using weighing scales (Seca, Birmingham, UK). Resting blood pressure was measured using a mercury sphygmomanometer (Accoson, Birmingham, UK) following a 10 minute period of seated passive rest.

3.3 Physiological measurements

3.3.1 Salivary melatonin immunoassay ELISA

Saliva is a readily available specimen which can be collected by non-invasive procedures (Hofman, 2001). The samples were collected within a tube before been transferred to storage (-80°C) until analysis took place (< less than 6 weeks after collection). The participants were asked to clear their mouth of any saliva, via swallowing, they were then instructed to allow saliva to slowly build up in their mouths before discharging into the sample tube. Participants were not allowed to consume water at least 10 min prior to collection taking place; food was not consumed 30 min before. Duplicate samples of 100 µl were analysed for melatonin concentration using an Enzyme-Linked Immunosorbant assay Kit (Direct Saliva melatonin ELISA, Buhlmann, Switzerland). The method comparison by the manufacturer with saliva melatonin radioimmunoassay following a linear regression analysis was R^2=0.84. The limits of agreement of saliva melatonin levels to other samples (blood plasma and urine) have been determined as acceptable (Benloucif et al., 2008a).

3.3.2 Intestinal temperature

Ingestible temperature sensors were used to monitor intestinal temperature and act as a surrogate of T_c (HT50002, CorTemp, Human Technologies International, Palmetto, USA). This method of T_c measurement has been shown to be a valid method of measurement (Darwent et al., 2011, Gant et al., 2006, O'Brien et al., 1998, Edwards et al., 2002). Comparisons of the thermometric pill output and rectal temperature (often considered the most practical and accurate site for measuring core temperature) were reported by Gant, Atkinson and Williams (2006). The data presented suggested that though there was a small systematic bias between the recorded intestinal and rectal temperatures it was

consistent and within an acceptable range. The limit of agreement results showed a negligible random error of 0.01°C difference. It was therefore deemed that the thermometric pill gave accurate and reliable readings of core temperature.

The pill is small (22.6 mm x 10.7 mm), ingested orally with water, and measures T_c continuously as it travels through the digestive tract. Sampling rate can be varied between one second to 24 h, in the studies within the current thesis data were recorded every 30 s. Each pill contains a crystal quartz oscillator which vibrates in direct proportion to the temperature of the surrounding area. This vibration then transmits a low frequency radio wave to an external data logger (HT150001, CorTemp, Human Technologies International, USA) attached to a participant's waist.

Six hours prior to the start of the experiments each participant attended the laboratory to ingest the pill. On arrival at the laboratory, participants consumed 100 ml of cold (~11°C) water. If the temperature varied by ≤ 0.1°C it was deemed that the sensor was sufficiently sited in the gastrointestinal tract and the experimental protocol could begin. The duration of this ingestion period varies between studies; however 4-8-h has been shown to be acceptable. The data logger was then placed in a pouch that was fitted around the participant's waist.

3.3.4 Intra-aural temperature

Intra-aural temperature IAT (3000A, FirstTemp, Genius, USA) of the right ear was measured in *Chapter 7*. This technique was utilised due to its ease of use and relative inexpensive compared with other techniques, although, it is considered to have a greater random error than other methods. However, a number of studies consider tympanic

temperature to be acceptably accurate as an equivalent measure of T_c (Bock et al., 2005, Erickson and Kirklin, 1993, Fadzil et al., 2010). Nevertheless, other authors have deemed the use of the technique unacceptable (Lawson et al., 2007, Fulbrook, 1997). In a recent study by Rubia-Rubia et al. (2011) where several methods of temperature measurement were compared, they reported that IAT presented the smallest range of error or variations from their 'gold standard' reading compared with all the other devices tested.

To facilitate valid and reliable measurements, 3 readings were obtained at each data collection point. Furthermore, measures took place in an environment that had standardised ambient temperature and air-flow as each of these can affect readings.

3.3.5 Skin temperature

Skin thermistors (120046, ELAB A/S, Copenhagen, Denmark) were attached to the participants left infraclavicular region, left forearm, mid-medial section of the left thigh and mid-medial section of the left calf (Ramanathan, 1964) using Transpore medical tape (3M, Loughborough, UK). The thermistors were attached to a data logger (TM9616, ELAB A/S, Copenhagen, Denmark) to allow continuous monitoring and recordings at 10 s intervals.

In *Chapter 6*, for data collection during sleep, wireless iButtons (DS1922L, Maxim Integrated Products, Sunnyvale, California, USA; resolution 0.0625°C) were used. The same anatomical positions were used as above and data was collected at 1 min intervals. iButtons have been shown to have a mean random error of – 0.09°C (– 0.4°C at most) with a systematic error of 0.05°C (0.09°C at most). These properties can be improved by using calibration (Van Marken Lichtenbelt et al., 2006). For both techniques (thermistors and iButtons), weighted mean skin temperature (T_{sk}) was then calculated using the

formula 0.3 T_{chest} + 0.3 T_{arm} + 0.2 T_{thigh} + 0.2 T_{calf} (Ramanathan, 1964).

3.3.9 Activity and sleep monitoring

An actiwatch (AW4, Cambridge Neurotechnology Ltd., Cambridge, UK) was placed on the participant's left wrist. This is a light weight electronic device that measures and records physical movements. Activity is measured via a piezo-electric accelerometer that records integration of amount, intensity, and duration of movement. The accelerometer was given to monitor the participants' adherence to the protocols given while not under laboratory supervision and to also monitor objective sleep quality. The 'watch' was programmed to record every minute and later analysed for sleep latency, sleep efficiency and actual sleep time (Actiwatch Activity and Sleep Analysis 5, Neurotechnology Ltd, Cambridge, UK). Whilst this method is widely used and generally considered to be an accurate and reliable measure of activity and sleep levels, some researchers have reported overestimations in sleep quality and time asleep (Verbeek, Klip & Declerck, 2001). Nevertheless, it has been established as effective in determining the effects of various behavioural and medical interventions on sleep–wake patterns (Sadeh and Acebo, 2002).

3.3.10 Dominant hand grip strength

Dominant hand grip strength was recorded in *Chapter 7* using a hand grip dynamometer (Grip-D, Takei Scientific Instruments co. LTD, Japan). Three measures were obtained and then averaged for hand grip strength. A short rest period (~15s) was given between attempts to ensure sufficient recovery. Grip strength is conventionally accepted as a good marker of circadian rhythms in muscle performance (Reilly et al., 2001).

3.4 Liverpool jet-lag questionnaire

This questionnaire was designed to self-assess various components associated with jet-lag and includes measures of fatigue, motivation, hunger, meal satisfaction, sleep quality and bowel movement. Ratings are measured on -5 to +5 scale with 0 representing 'normal' habitual ratings prior to travel. The overarching question pertains to the subjective rating of overall jet-lag on a visual analogue scale (VAS), labelled "0 –no jet-lag" to "10 – very bad jet-lag".

In *Chapter 7* and *Chapter 8* questionnaire data were pooled and summed into related constructs for analysis. Some of the questions were on a linear scale with lower scores meaning worse than normal symptoms and higher scores meaning better than normal symptoms or vice versa. Conversely some variables had no positive response. Thus, data with a negative outcome were given a positive score and data with a positive outcome were given a negative score. These data were then summed and the greater the value of the overall constructs the worse the related symptoms. For example, an overall rating of 10 is worse than a rating of 6; and a rating of -2 is better than "normal" perceived ratings, which is represented by zero. The data were allocated into constructs for function (fatigue, concentration, motivation and irritability), diet (hunger prior to meal, meal palatability and post-meal satisfaction), sleep (sleep latency, quality, inertia, start-time and waking time) and bowel movement (frequency and stool consistency).

CHAPTER 4

STUDY 1

ACUTE RESPONSES OF THE THERMOREGULATORY SYSTEM AND PINEAL GLAND TO LIGHT EXPOSURE OF DIFFERENT WAVELENGTH

4.1 Introduction

In human physiology, light is typically considered most for its visual function or its involvement in circadian control via periodic variation in the light/dark cycle. The development of bright light therapy for people with Seasonal Affective Disorder (SAD) means that individuals were exposed to high concentrations of light (Rosenthal et al., 1984). These acute treatments were reported to improve depressive symptoms as well as general mood and alertness. Nevertheless, it is only recently that the more subtle effects of bright light on human physiology have been appreciated. The rapid suppression of melatonin (Lewy et al., 1980, Brainard et al., 1997) and elevation of T_c (Cajochen et al., 1992, Dijk et al., 1991) are well documented responses to light and are also commonly used as markers of phase shifting effects in Chronobiology.

Understanding the multifaceted actions of bright light has important implications for intervention prescription and industrial design of bright light devices. Previous research into the acute responses to light, as with phase-shifting effects, demonstrates a heightened sensitivity to short wavelength stimuli. Light in the 'blue' section of the electromagnetic spectrum has been shown to be more effective in suppressing melatonin (Brainard et al., 2001a, Brainard et al., 2001b), decreasing subjective sleepiness (Cajochen et al., 2005) and eliciting the strongest reduction in cone ERG β wave–implicit time (Hankins and Lucas, 2002) compared with intensity matched light with a higher wavelength. Therefore, the aim of the present study was to investigate the effects of illuminance-matched polychromatic bright light, one with blue photons removed, and a no light control on the direct response of melatonin and body temperature. It was hypothesised that the acute effect of bright light would be blue-shifted, such that the

blue photon removed light would be less effective at reducing melatonin and elevating T_c than light with blue photons present.

The rationale for conducting this study is two-fold. Ultimately, the acute effects of bright light reported by Cajochen et al. (2005) were desired to be translated into a phase-shifting effect that would be useful for exercise performance in the early morning (Chapter 5). However, there are ethical issues surrounding the use of the monochromatic blue light which Cajochen et al. (2005) selected. The main concern relates to damage of the eye, specifically Photokeratitis (a burn of the cornea) and injury to the retina (Terman, 2009). Therefore, a compromise was reached via a specifically-designed light condition for this thesis in which blue photons were filtered. This light condition was compared with "normal" unfiltered bright light originating from a commercially available product, in order to examine the question of how important is blue light and, in turn, how these results compared with those of Cajochen et al. (2005).

4.2 Methods

4.2.1 Participants

Eight males were recruited for the present study. The mean ± SD age, body mass, height and maximal oxygen uptake ($\dot{V}O_{2max}$) of the participants was 22 ± 2 years, 80.8 ± 10.37 kg, 1.82 ± 0.10 m and 44.9 ± 2.5 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively. All participants were healthy, non-smokers and none reported having a history of major illness or sleeping problems. No participant was involved in nocturnal shift work and none had undertaken transmeridian travel during the 30 days prior to the study commencing. Participants were asked to refrain from alcohol, caffeine, coco-based products, bananas and strenuous exercise 24-h prior to attending the laboratory for each trial. Participants reported

conventional nychthemeral wake-up and retiring to bed times, 06:45-08:30-h and 22:45-00:15-h respectively; reported times were 30-90 min later on free-days (i.e. weekends). Participants were asked to maintain their regular weekday sleep-wake cycle during 48-h prior to testing. Written informed consent was obtained, and all procedures were approved by the ethics committee at Liverpool John Moores University and adhered to the Declaration of Helsinki.

4.2.2 Laboratory protocols

Participants attended the laboratory on four separate occasions, the first for familiarisation purposes, followed by three further visits to complete the main experimental conditions. Data collection for this study took place during the winter and early spring months in the UK, November-April.

During the initial visit, participants became accustomed to all measurement tools and apparatus to be used during the experimental procedures, including the sleep laboratory. Anthropometric measures and resting blood pressure (mercury sphygmomanometer) were recorded during this visit. Resting blood pressure was measured with the participant seated following 10 min of quiet rest.

4.2.3 Experimental trials

Trials were ordered in a counterbalanced fashion and were separated by 5-11 days. Participants arrived at the laboratory at 19:30-h and adopted a semi-supine seated position which was maintained for the whole of the evening testing period, this period allowed for measures to return to resting values prior to the light protocol commencing. Following instrumentation, at 20:00-h, lighting in the laboratory was reduced to <12 lux

for 45 min (Fig. 1). This period was followed by a one hour period of no light. The laboratory lighting was switched off and participants were asked to wear a commercially available eye-mask. This period was followed by a 30 min intervention. Participants were exposed to one of three interventions: 1) Participants remained in the no light environment (no light condition, NL). 2) Participants were exposed to 2,500 lux of polychromatic light from a light box (Zip, Lumie, Cambridge, UK). The light box was placed 50 cm from the participant, they were told not to look directly into the light box for the whole intervention period but instead to gaze at the light for short intermittent periods and to keep the light in their periphery for the remainder of the intervention (bright light, L). 3) The bright light was again used, although on this occasion participants were instructed to wear a pair of filter glasses (Solar3, Eschenbach optik, Ridgefield, Connecticut). These glasses filtered-out light <520nm (Blue-photons removed, NB). Due to the use of this filter, the light box was moved closer to the participant (18.5 cm) to ensure that the light box still produced 2,500 lux at eye-level. Following the intervention period, participants were administered dim light for a further 75 minutes before retiring to bed at 23:45. Participants slept in the laboratory in shorts and t-shirt or vest with a 10.5 tog duvet. Prior to sleep, participants were allowed to use the toilet and to clean their teeth.

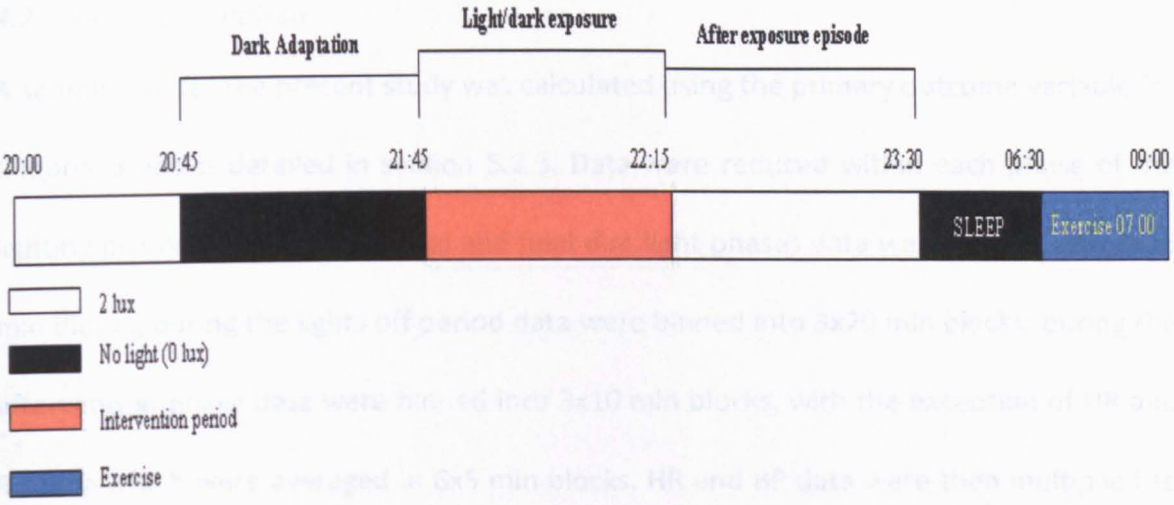

Figure 4.1: Schematic of study design for the lighting protocol used in *Chapter 4* and *Chapter 5* and the sleep and exercise times in *Chapter 5*.

4.2.4 Measurement procedures

At 15:00-h (five hours prior to attending the laboratory), participants were issued with a silicon coated thermometric pill (CorTemp, Human Technologies International, USA) and instructed to swallow it with 50 ml of water. The thermometric pill was used to record Intestinal temperature (T_c). Saliva samples were collected at numerous stages during the lighting protocol and immediately frozen (-80°c). The samples were later analysed for melatonin concentration from duplicate samples using an enzyme linked immunsorbant assay kit (Direct salvia melatonin ELISA, Buhlmann, Schonenbuch, Switzerland). Skin thermistors were attached to the participant's upper chest, mid forearm, upper thigh and medial side of the calf using sweat-proof tape (Transpore, 3M, Loughborough, England). A data logger (TM9616, ELLAB, Copenhagen, Denmark) recorded the temperature of each skin thermistor. Weighted mean skin temperature (T_{sk}) was later calculated (Ramanathan, 1964).

4.2.5 Statistical Analysis

A sample size for the present study was calculated using the primary outcome variable for *Chapter 5* and is detailed in section 5.2.3. Data were reduced within each phase of the lighting protocol. During the initial and final dim light phases data were binned into 3x15 min blocks; during the lights off period data were binned into 3x20 min blocks; during the intervention phase data were binned into 3x10 min blocks, with the exception of HR and BP data which were averaged in 6x5 min blocks. HR and BP data were then multiplied to give RPP. Data were analysed using two-factor within-subjects factors (trial x time) generalized estimation equations (Ballinger, 2004), with order of trial effect controlled within the model. The data were analysed using Statistical Package for Social Sciences (SPSS) for Windows (Version 17, SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD (95% CI). The alpha level of significance was set at $p \leq 0.05$.

4.3 Results

4.3.1 Baseline measurements

Measurements obtained 45 min into the protocol were considered the baseline period and are presented in Table 4.1. The dim-light levels were identical in all conditions although participants were aware which light intervention they were going to receive later in the protocol. There were trends for melatonin and T_c to be higher in the NB and L baseline periods, although the only statistically significant difference was for RPP being higher in NB compared with NL. Mean skin temperature provided reciprocal results to that of T_c; significantly higher temperatures being recorded in the NL baseline period compared with both the NB and L baseline periods.

Table 4.1: Baseline measurements. Taken as the end of the first "low lux" period, 45 minutes into the protocol. * denotes statistical significant difference.

Variable	No blue light	Light	No light	NB vs. L *p*-value	NB vs. NL *p*-value	L vs. NL *p*-value
Melatonin ($pg.ml^{-1}$)	11.7 ± 10.2	15.6 ± 14.1	9.3 ± 12.4	0.48	0.35	0.17
Core body temperature (°C)	37.06 ± 0.12	37.05 ± 0.16	36.97 ± 0.26	0.91	0.38	0.39
Mean skin temperature (°C)	32.58 ± 0.41	32.57 ± 0.48	33.05 ± 0.44	0.96	0.02*	0.04*

4.3.2 No light measurements

In T_c there were significant reductions in all conditions by 0.17 ± 0.14°C (0.09 to 0.24, $p < 0.0005$) in L, 0.19 ± 0.15°C (0.08 to 0.29, $p < 0.0005$) in NL and 0.19 ± 0.09°C (0.12 to 0.26, $p < 0.0005$) in NB. Melatonin and T_{sk} increased in all conditions; however, none of these changes were statistically significant.

4.3.3 Intervention measurements

Between the end of the no light phase and intervention the time course of melatonin changed in the L group compared with NL and NB. Concentrations in both NL and NB significantly increased between these time points, 3.7 ± 4.8 $pg.ml^{-1}$ (-0.05 to -6.9, $p = 0.03$) and 5.6 ± 7.9 $pg.ml^{-1}$ (-0.01 to -11.1, $p = 0.04$), respectively. Whereas concentrations in L decreased, however this did not reach significance -3.3 ± 9.6 $pg.ml^{-1}$ (-10.0 to 3.4, $p = 0.33$; Figure 4.3).

During the intervention phase there were reductions in T_c in all 3 conditions, however, this was slightly attenuated in the two light conditions, with the magnitude of the decrease appearing to be wavelength dependent. After the 30 min intervention

compared with the end of the no light phase, core body temperature was altered by -0.04 ± 0.14°C (-0.14 to 0.05, $p = 0.42$) in L, -0.08 ± 0.11°C (-0.15 to -0.01, $p = 0.04$) in NL and -0.06 ± 0.14°C (-0.15 to 0.04, $p = 0.25$) in NB (see Figure 4.4).

Skin temperature was statistically unaltered by the intervention. However, the direction of the change during the intervention phase was different for the two light conditions (increase) compared with the NL condition (decrease) (see Figure 4.5).

4.3.4 Post intervention measures

Between the end of the intervention and the final post measure taken there were no significant differences observed in T_{sk}. In all conditions T_c reduced further, with a significant decrease of 0.07 ± 0.31°C (0.04 to 0.09, $p < 0.0005$) in NB. Conversely melatonin increased across all groups, with a significant rise of 4.4 ± 19.5 $pg.ml^{-1}$ (1.1 to 7.7, $p = 0.01$) in L.

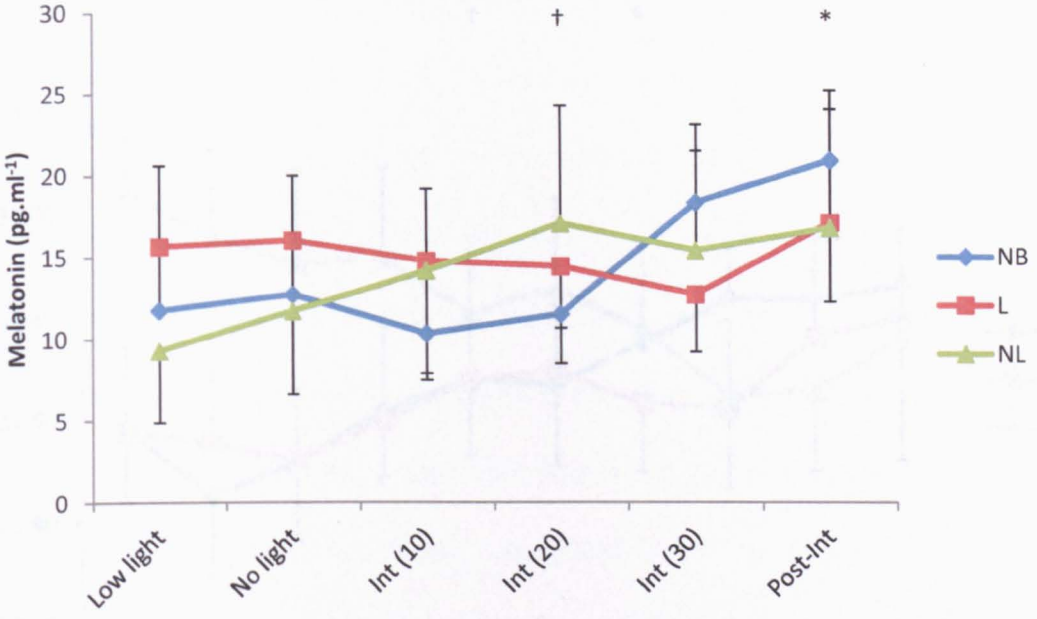

Figure 4.2: Mean±SD Melatonin response during evening light protocol. Parenthesises on the x-axis denote minutes elapsed in each stage of the intervention. * † Denotes significant difference from proceeding value in L and NL, respectively.

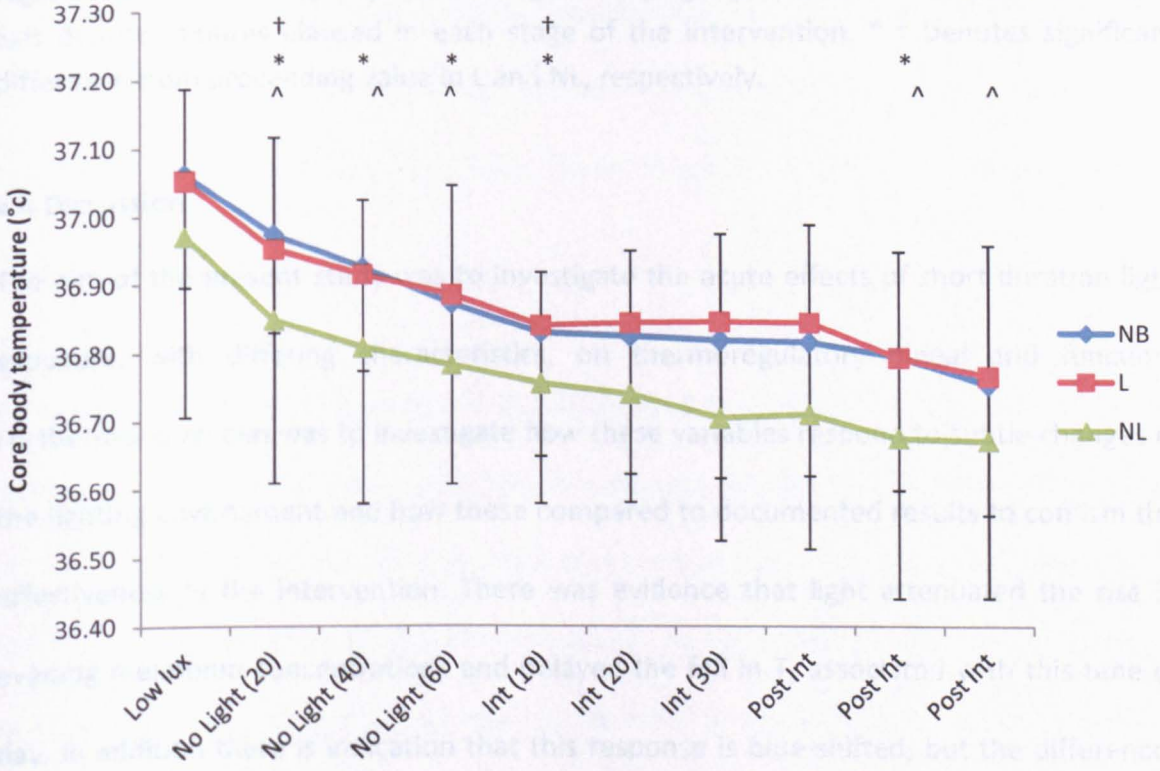

Figure 4.3: Mean±SD T_c in all response during evening light protocol. Parenthesises on the x-axis denote minutes elapsed in each stage of the intervention. ^ * † Denotes significant difference from proceeding value in NB, L and NL, respectively.

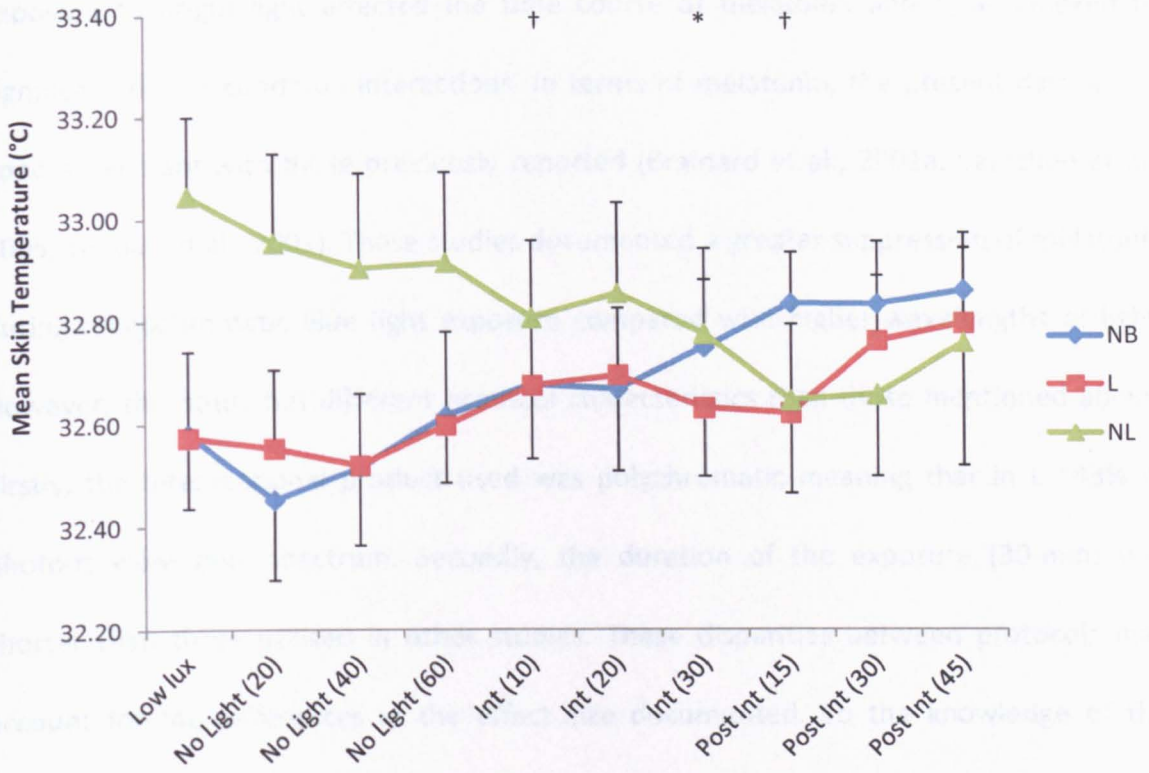

Figure 4.4: Mean±SD T_{sk} response during evening light protocol. Parenthesises on the x-axis denote minutes elapsed in each stage of the intervention. * † Denotes significant difference from proceeding value in L and NL, respectively.

4.4 Discussion

The aim of the present study was to investigate the acute effects of short duration light exposure, with differing characteristics, on thermoregulatory pineal and function. Furthermore, an aim was to investigate how these variables respond to subtle changes in the lighting environment and how these compared to documented results to confirm the effectiveness of the intervention. There was evidence that light attenuated the rise in evening melatonin concentrations and delayed the fall in T_c associated with this time of day, in addition there is indication that this response is blue-shifted, but the differences were not significant.

Exposure to bright light affected the time course of melatonin and T_c as indexed by significant time x condition interactions. In terms of melatonin, the present data are in good agreement with those previously reported (Brainard et al., 2001a, Cajochen et al., 2005, Thapan et al., 2001). These studies documented a greater suppression of melatonin during monochromatic blue light exposure compared with higher wavelengths of light. However, this study has different protocol characteristics than those mentioned above. Firstly, the interventional product used was polychromatic meaning that in L ~43% of photons were blue spectrum. Secondly, the duration of the exposure (30-min) was shorter than those utilised in other studies. These disparities between protocols may account for the differences in the effect size documented. To the knowledge of the author this is the first study to document such observations with a short duration protocol and using polychromatic light. Notwithstanding this it must be noted that the treatment effect may have been more pronounced due to the extended periods of dim and no light; said conditions have been shown to amplify the typical responses to light (Wong et al., 2005).

Although an interaction was noted in T_c responses, the variation between conditions was subtle. Indeed, the magnitude of decline was attenuated in the two light conditions, with this response appearing to be somewhat blue-shifted (i.e. there was a less reduction in the condition which contained blue photons). In L, T_c began to increase from the preceding measurement after 20-min of the intervention until 30-min post exposure. Again, a protocol with longer exposure duration may have augmented the treatment effect. Cajochen and colleagues (2005) only began to note significant effects 45-min into their experimental intervention. Perhaps future studies should alter the duration of exposure across the different phases of the experiment (i.e. increase light exposure and

reduce dim and no light phases). The overarching principle of such experiments should be to reduce durations of protocols whist optimising results to make phototherapy practically relevant (see *Chapter 5*).

A novel finding from this study is the potential anticipatory effect that bright light may have on human physiology. These findings can be used to underpin the design and protocols of phototherapeutic devices and their use. Across the three conditions during the initial dim light and no light phases, with the exception of T_{sk}, all within-variable responses were in the same direction (i.e. increase in melatonin; decreases in body temperature and RPP). However, an interesting observation in the present study was the absolute differences at baseline. It is apparent from simply eyeballing the data that the initial values in the two light conditions, especially in body temperature and RPP, are greater than those in the control condition. This variance could be explained by an expectancy effect as participants were not blinded to conditions on arrival at the laboratory. These types of anticipatory effects have been reported across a range of situations. For example, Zaregarizi et al. (2007) postulated that the most important aspect for inducing the acute reduction in BP prior to a siesta could be the expectancy of sleep itself. Lockley et al. (2006a) stated that the parallel elevation in cortisol prior to light exposure in two trials with differing wavelength characteristics may have been due to subjects' anticipation of the extended novel experimental intervention. This response has also been observed in patients before surgical procedures (Czeisler et al., 1976).

Although this study was conducted under very tightly controlled laboratory conditions (constant posture and room temperature), skin temperatures are still prone to exhibiting large inter- and intra-individual variance (Krauchi and Wirz-Justice, 1994). This

characteristic may go some way to explaining the different time course between conditions, especially during the initial two phases when conditions were matched between trials. Perhaps the use of further measurements sites and the calculation of distal-proximal skin temperature gradients may provide more concise information. Specifically relating to how bright light exposure may alter the evening shifts in core to shell thermodynamics that is associated with sleep onset (Krauchi, 2007) and whether this has any practical or clinical implications.

The data indicate that the protocol used in the present study was sufficiently powerful to acutely manipulate human physiology, as indexed by acute changes in melatonin and core body temperature. However, the magnitude of the effects were reduced compared with those reported by Cajochen et al. (2005), which could be a direct result of differences in the inherent protocol. Nevertheless, the results confirm the effectiveness of the protocol for producing the expected responses. Additionally, this study indicates that the lighting protocol used was sufficiently powerful to evoke acute responses in various physiological systems; whether this effect is magnified via scotopic periods prior to the intervention cannot be deduced from the present study design.

CHAPTER 5

STUDY 2

PRE-SLEEP EXPOSURE TO BRIGHT LIGHT ALTERS THERMOREGULATORY RESPONSES AND PERFORMANCE DURING SUBSEQUENT MORNING EXERCISE

5.1 Introduction

It is widely accepted that exposure to ocular bright light is the most important zeitgeber for circadian entrainment in mammals, including humans (Cajochen et al., 2006, Duffy and Czeisler, 2009). Depending on the time of administration relative to 'body clock time', exposure to bright light can result in phase delays (to later times) or advances (to earlier times) of circadian rhythmicity in many physiological functions. For example, exposure to light in the hours before the nadir of the daily rhythm in T_c induces a phase delay, whereas exposure to light after the nadir advances the rhythm. These responses can be described across a full 24-h cycle to provide a phase response curve to light for humans, which is now well-described (Minors et al., 1991).

In sport, it is common for long distance running and cycling events to be scheduled in the morning, one reason being that athletes might be less prone to thermal stress. For example, during the recent Commonwealth Games in Delhi, the Marathon events started before 07:00-h local time. This scheduling is based, in part, on the knowledge that during competition in high ambient temperatures, a primary objective is to keep T_c low because a high correlation exists between the onset of fatigue and reaching a 'critical' T_c (Gonzalez-Alonso et al., 1999). Consequently, pre-race interventions such as cold showers and cooling vests have been adopted in an attempt to lower T_c. Such pre-cooling interventions have been shown to be effective in reducing thermal as well as cardiovascular and psychophysical strain in athletes (Booth et al., 1997, Cotter et al., 2001, Lee and Haymes, 1995). Furthermore, the naturally lower T_c in the morning hours has been postulated as a reason for improved performance in high ambient temperatures. In support of this notion, Hobson, et al. (2009) reported a greater time to exhaustion whilst cycling at 65% $\dot{V}O_{2peak}$ in the high temperatures by approximately 5 minutes at 06:45-h

(45.8 ± 10.7 min) compared with 18:45-h (40.5 ± 9.0 min), with initial T_c been 0.1°C lower in the morning.

In a recent study, Atkinson, et al. (2008a) hypothesised that manipulation of circadian timing via carefully-timed exposure to bright light could act to 'pre-cool' an athlete prior to an endurance event in hot conditions. In this study exposure to evening bright light delayed the time that T_c fell to its minimum during sleep by 1.46 ± 1.24-h, leading to a lower T_c (0.20°c ± 0.17°c) prior to exercise undertaken the following morning. Nevertheless, no performance-related outcome was measured in this study to confirm that the changes in T_c by the bright-light actually improved athletic capability.

The relationships between bright light, circadian rhythmicity and endurance performance in the heat are still enigmatic. Therefore, the aim of the present study was to manipulate T_c by exposing participants to bright light, with short wavelength characteristics, prior to the nocturnal sleep period. It was hypothesised that evening exposure to bright light results in (i) melatonin suppression, (ii) a delay in the circadian timing of T_c; consequently leading to a lower T_c immediately prior to and during exercise undertaken in the subsequent morning, and (iii) improved endurance performance due to this 'pre-cooling' intervention.

5.2 Methods

Data collection in the present chapter was continued from previous chapter, with the NB condition removed as this present study aimed to observe the effects of light (BL) vs. no intervention (NL); therefore, participants, laboratory procedures and the intervention phase of this chapter can be found within in section 4.2 Furthermore, some of the

measurement procedures are the same as those documented previously. The following section continues where section 4.2.3 concluded.

5.2.1 Experimental trials

At 06:30-h on the following morning, the participants were woken by a researcher and provided a sample of saliva. Participants then got out of bed and drank 568 ml of water and ingested a cereal bar. To recover from any effects of sleep inertia, participants then rested for 30 min (Tassi and Muzet, 2000). Participants were then moved to an environmental chamber, which was pre-set to a temperature of 35°C and a relative humidity of 60%. Thermistors (120046, ELAB A/S, Copenhagen, Denmark) were attached to the participant and then followed a two minute baseline recording period prior to exercise. The initial exercise protocol was intermittent and comprised three 10-min bouts of upright cycling at 55% $\dot{V}O_2$ max on a cycle ergometer. Each bout was interspaced with 10 min of seated passive rest. Two minutes after ceasing each exercise bout participants provided a saliva sample. Participants were then allowed to consume water until five minutes into the rest period. Following the completion of the intermittent protocol, participants undertook a 10-km self-paced time-trial. During this time trial, participants were allowed to drink water ad libitum and adjust the workload on the cycle ergometer to their self-selected pace. Throughout the exercise period (end of each 2-km stage), ratings of perceived exertion (RPE) were recorded using the Borg scale (Borg, 1982).

5.2.2 Measurement procedures

Core body temperature was continuously recorded from the same thermometric pill as ingested in *Chapter* 4 at a sample rate of 30 s. For the present study saliva samples were taken prior to the no light phase and 15 minutes post-intervention, after waking and

during exercise, and immediately frozen (-80°C). The samples were later analysed for melatonin concentration from duplicate samples using an enzyme linked immunsorbant assay kit (Direct salvia melatonin ELISA, Buhlmann, Schonenbuch, Switzerland). Skin thermistors were re-attached to the participant's upper chest, mid forearm, upper thigh and medial side of the calf using sweat-proof tape (Transpore, 3M, Loughborough, England). A data logger (TM9616, ELLAB, Copenhagen, Denmark) recorded the temperature of each skin thermistor. Weighted mean skin temperature was later calculated (Ramanathan, 1964).

5.2.3 Statistical Analysis

The primary outcome was the time to complete the 10-km time trial. Such protocols have been found to be highly reliable with test-retest coefficients of variation (CV) < 2% (Atkinson and Nevill, 2001). Using the NQuery software (Cork, Ireland), it was estimated that 8 subjects would enable the detection of a statistically significant difference between trials of 3.3% assuming a CV of 2%, $P<0.05$ and 80% power with a two-tailed paired t-test.

Data collected prior to the evening light intervention phase were averaged and entered into the hypothesis test as a single covariate (Altman, 1991). Post-intervention and during sleep T_c data was binned into 15 min blocks. Following sleep, during the exercise and time trial phase data (T_c and T_{sk}) were binned depending on phase/stage i.e. each 10 min exercise and rest period and each 2km travelled, respectively.

Data were analysed using two-factor within-subjects factors (trial x time) generalized estimation equations (Ballinger, 2004), with order of trial effect controlled within the model. Post-hoc analysis was used to assess the differences between and within trials.

One important summary statistic was an estimate of the time of minimum T_c during sleep. Core temperature data were averaged into 15-min time periods for data smoothing purposes. The absolute value and the time of the lowest of these 15-min averages as an estimate of the body temperature minimum during sleep was recorded. The data were analysed using Statistical Package for Social Sciences (SPSS) for Windows (Version 17, SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD (95% CI). The alpha level of significance was set at $p \leq 0.05$.

5.3 Results

5.3.1 Light Intervention and Sleep

The exposure to bright light in the early biological night, prior to the exercise day, suppressed the rise in salivary melatonin concentration (Figure 5.1). However, the differences between BL and NL did not reach statistical significance [-9.0 ± 25.4 $pg{\cdot}ml^{-1}$ (-29.3 to 11.3, $p = 0.35$)]. Immediately after waking at 06:30-h, the differences in melatonin concentrations were negligible [BL = 23.2 ± 10.0 $pg{\cdot}ml^{-1}$ (16.3 to 30.1) vs. NL = 24.9 ± 5.9 $pg{\cdot}ml^{-1}$ (20.8 to 29.0), $p = 0.54$].

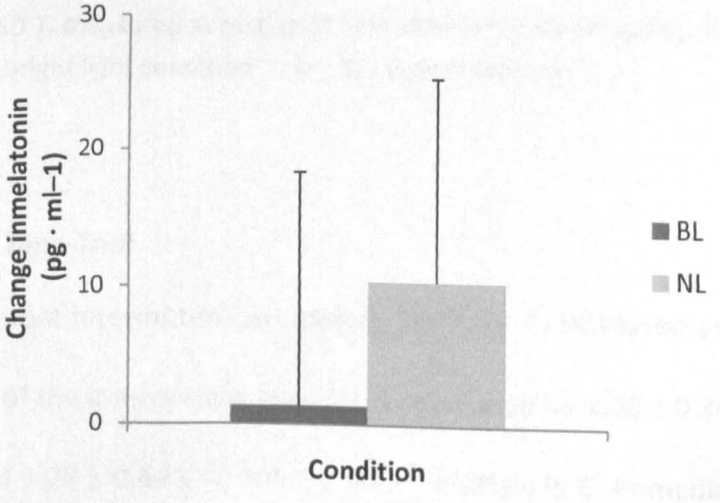

Figure 5.1: Mean ± SD salivary melatonin concentrations changes in response to evening bright light or no light intervention compared with 45-min post dim light.

Evening bright light delayed the T_c minimum during sleep; with the temperature nadir for BL occurring at approximately 04:30-h compared with approximately 02:45-h in NL (P = 0.07) (Figure 5.2). Although the temperature nadirs may have occurred at different times, the absolute values of these minima did not significantly differ [L = 36.36 ± 0.21°C (36.21 to 36.50) vs. NL = 36.36 ± 0.35°C (36.12 to 36.61), p = 0.95]. Nevertheless, T_c was 0.16 ± 0.30°C (-0.37 to 0.05, p = 0.13) lower in BL compared to NL in the 15 minutes prior to waking.

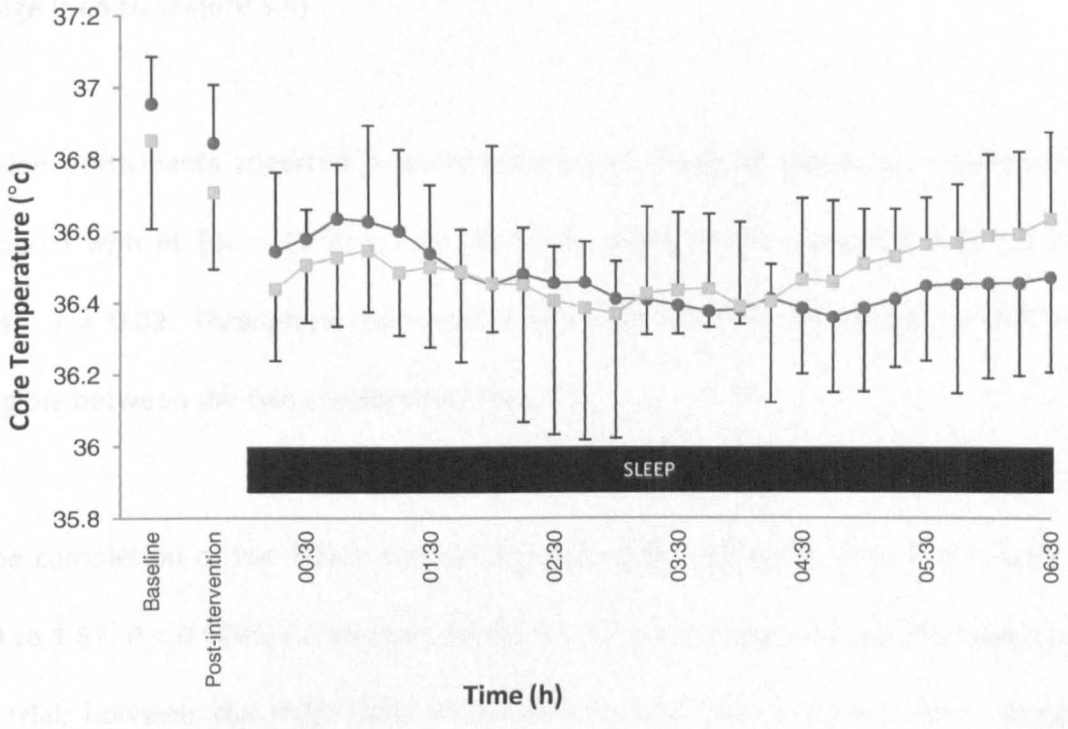

Figure 5.2: Mean ± SD T_c measured at rest, post light intervention and during sleep prior to and during sleep. —●— Bright light condition —■— No light condition.

5.3.2 Exercise and Time-Trial

During the subsequent intermittent exercise in the heat, T_c increased as expected: From waking to the end of the intermittent exercise, T_c increased by 1.08 ± 0.34°C (0.84 to 1.32, P <0.0005) in L and 1.29 ± 0.44°C (0.98 to 1.59, P <0.0005) in C Immediately prior to the initiation of the 10-km time-trial, T_c in the BL condition was 0.27 ± 0.42°C (-0.57 to 0.02, p

= 0.06) lower than NL. There was evidence that the differences between conditions observed in T_c increased as the time-trial progressed ($P < 0.0005$) (Figure 5.3), with a significant difference between conditions ($p = 0.047$). At the end of the time-trial core temperature was 38.21 ± 0.56°C (37.84 to 38.57) in BL compared with 38.64 ± 0.42°C (38.34 to 38.93), $p = 0.10$. Changes in mean T_{sk} were similar to those observed in T_c, with a highly significant increase over time ($p < 0.0005$). There was a significant overall difference between groups, with BL 0.26 ± 0.33°C (0.03 to 0.49, $p = 0.03$) lower on T_c average then NL (Figure 5.4).

At 2-km participants reported a statistically lower rating of perceived exertion in NL compared with BL [BL = 15.00 ± 2.03 (13.59 to 16.41) vs. NL = 13.85 ± 2.15 (12.37 to 15.34), $p = 0.02$. Throughout the rest of the time-trial the differences in RPE were negligible between the two conditions (Figure 5.5).

At the completion of the 10-km time-trial, participants during BL were 1.43 ± 0.63 min (0.98 to 1.87, $P < 0.0005$) faster than during NL. This trend was observed throughout the time-trial; however the magnitude of the difference at 2-km did not reach statistical significance ($p = 0.15$) (Table. 1).

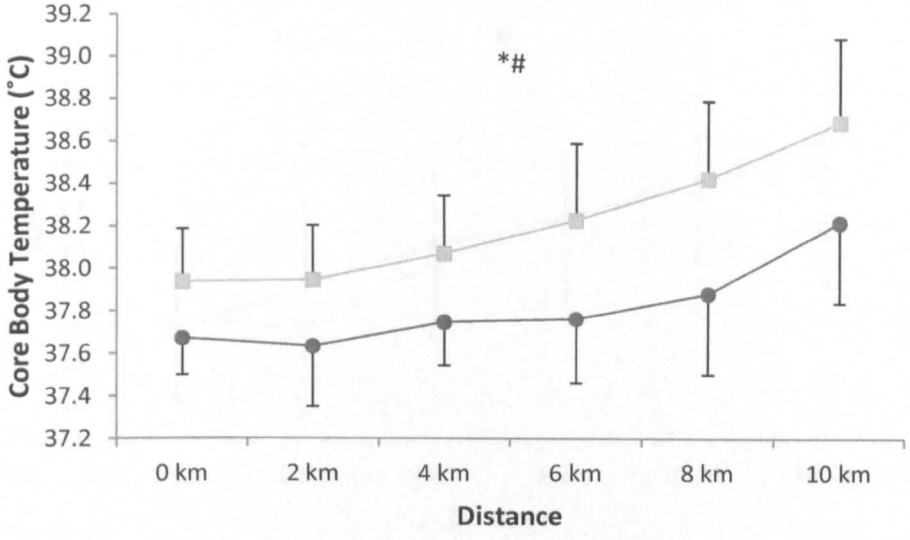

Figure 5.3: Mean ± SD T_c measured prior to and during 10-km time-trial. * denotes significant effect of condition. # denotes significant effect of time. —●— Bright light condition —■— No light condition.

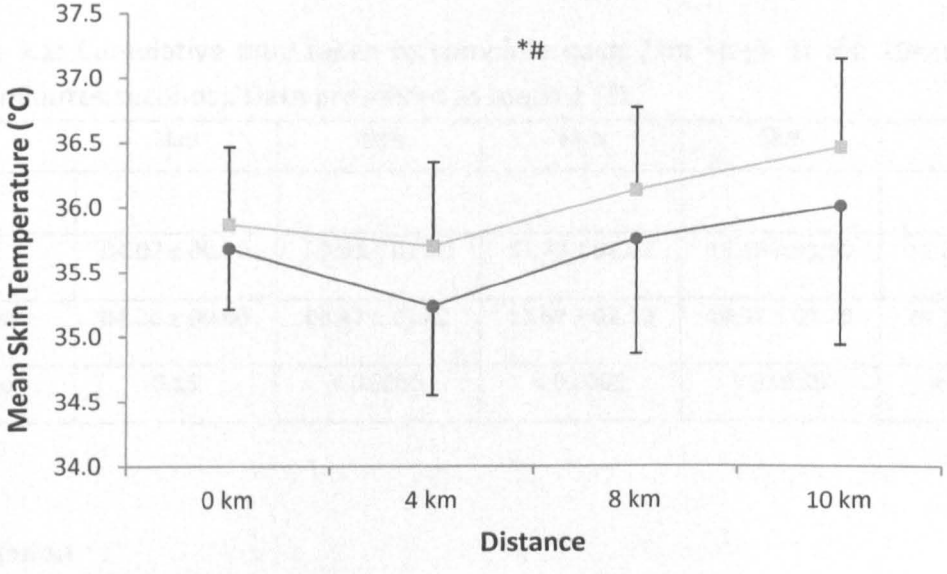

Figure 5.4: Mean ± SD mean T_{sk} measured at rest prior to and during 10-km time-trial. * denotes significant effect of condition. # denotes significant effect of time. —●— Bright light condition —■— No light condition.

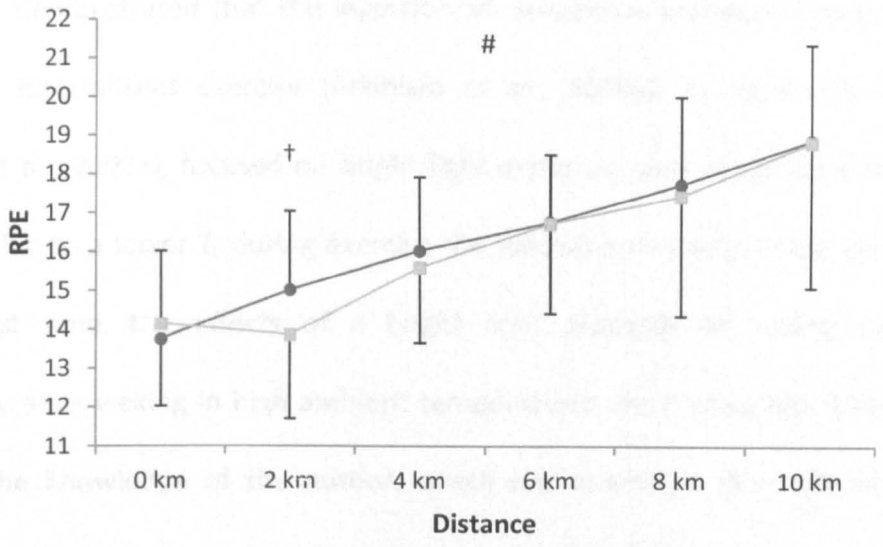

Figure 5.5: Mean ± SD RPE measured at rest prior to and during 10-km time-trial. † denotes significant difference between conditions at time point. # denotes significant effect of time. —●— Bright light condition —■— No light condition.

Table 5.1: Cumulative time taken to complete each 2km stage of the 10km time trial (minutes.seconds). Data presented as mean ± SD

	2km	4km	6km	8km	10km
Trial					
Light	04.02 ± 00.96	07.93 ± 01.60	11.73 ± 02.02	15.15 ± 01.90	18.67 ± 02.15
No Light	04.26 ± 00.60	08.47 ± 01.42	12.67 ± 02.20	16.37 ± 02.20	20.10 ± 02.48
p-value	0.15	< 0.0005	< 0.0005	< 0.0005	< 0.0005

5.4 Discussion

The novel finding in the present study is that 30-min of polychromatic light at an intensity of 2500 lux, 1 hour prior to nocturnal sleep, mediated changes in T_c and improved cycling performance in a 10-km time-trial. These data also exhibit the importance of a lowered T_c before and during exercise in the heat and how light can be used to facilitate this adjustment in the homeostatic set-point of T_c. These findings have implications for athletes competing in early morning events under conditions of high thermal stress.

It has been demonstrated that the ingestion of exogenous melatonin lowers T_c during subsequent intermittent exercise (Atkinson et al., 2005a). In more recent research (Atkinson et al., 2008a), focused on bright light exposure, and established that evening bright light led to a lower T_c during exercise the following morning. In the current study, for the first time, the effects of a bright light protocol on cycling performance immediately after waking in high ambient temperatures were observed. This is the first study, to the knowledge of the author, which has examined the residual effects of evening bright light on subsequent early morning physical performance.

The exposure to ocular bright light in the early biological night transiently attenuated the "normal" rate of decrease in T_c. In parallel, the "normal" rise in melatonin, associated with healthy individuals (Burgess and Fogg, 2008), was suppressed; although it is important to note that light may exert it effects independently from those of melatonin. In agreement with other researchers (Cajochen et al., 2005, Kubota et al., 2002), the transient increase in T_c found in the bright light condition led to delayed timing in T_c minimum. Previous studies observing the effect of bright light interventions on the timing of the T_c nadir have reported different effect sizes. For example, proceeding exposure to ocular bright light in late-evening/early-night; delays in the T_c rhythm of 1.12-h, 0.75-h and 1.46-h were observed by Kubota et al. (2002), Krauchi et al. (1997a) and Atkinson et al. (2008a), respectively.

The inherent differences in methodologies may go some way to explaining the contrasting magnitude of observed effects. The timing ("real world" clock vs. body clock), intensity, duration and spectral distribution of light, as well as prior light-dark exposure, have all been hypothesised as contributing factors which influence phase-shifting effects.

The delay in temperature minimum of approximately 1.75h in the present study resulted in a lower T_c during the final 15 minutes of nocturnal sleep by 0.16°C in BL. It should be noted that in studies which involve attempted circadian manipulation and exercise, directly measuring the effects of the interventional product on any changes in the full circadian rhythm of T_c not feasible. This is due to the inherent study aim of participants having to complete exercise after waking from nocturnal sleep. It is well documented that physical activity produces masking effects on circadian rhythms, making estimates of circadian phase unreliable.

Following a 30 minute period to reduce the effects of transient sleep inertia (Tassi and Muzet, 2000) and three 10 minute bouts of light-moderate exercise, the difference in T_c had increased to ~0.3°C. The lower starting T_c, in these recreationally active participants, resulted in the quickest time to completion in a 10km cycling time-trial in the bright light condition. A lower starting T_c has been shown to improve cycling performance in previous studies conducted in conditions of high thermal stress. Hobson et al. (2009) postulated that a lower rectal temperature at 06:45-h compared to 18:45-h meant that participants were able to cycle for approximately 5 minutes longer until reaching exhaustion in the morning. Atkinson et al. (2008a) reported after evening bright light exposure that T_c was lower prior to morning exercise in the heat compared to dim light. During the subsequent exercise RPE was lower in their evening bright light condition.

High levels of hyperthermia have been associated with the onset of fatigue, which was indexed in the present study by high RPE scores. Gonzalez- Alonso et al. (1999) reported lower starting T_c resulted in a longer time to exhaustion in the heat, although a critical temperature of just above 40°C was observed. It was postulated that higher T_c resulted in

a decline in cardiac output; attributed to a larger reduction in stroke volume via increased skin blood flow and skin blood volume. However, other factors have been hypothesised in the aetiology of hyperthermia-induced fatigue. For example, deviations from the homeostatic set-point in; brain temperature (Nybo, 2012), hydration status, metabolic regulation and/or central nervous system control (Hargreaves, 2008).

Pre-cooling is adopted by many athletes as a method of improving performance. In a recent meta-analysis, which included 27 studies, Wegmann et al. (2012) concluded that firstly, pre-cooling is more effective at improving performance in hot (>26°C; +6.6%) than moderate temperatures (18-26°C, +1.4%). Secondly, pre-cooling prior to time-trials improves performance, on average, by +4.2%. Thirdly, the methods utilised (and changes in performance) in the selected studies were cold drinks (+15.0%), cooling packs (+5.6%), a cooled room (+10.7%), cooling vests (+4.8%) and water application (+1.2%). This compares to a 7.2% improvement using the present interventional product over the 10-km time-trial. Furthermore, the confidence interval highlight that the population mean difference could be as small as 0.98 min and as large as 1.87 min. It is likely that even the lower limit of this confidence interval is practically significant in an event of this duration.

Mean T_{sk} generally followed a similar profile to T_c, with increasing values as the time-trial progressed and higher temperatures, on average, in NL. Given the association with increasing T_c and the up regulation of heat loss mechanisms via the skin, this finding would be expected. High T_{sk} has been proposed as an important rate limiting factor in exercise intensity (Jay and Kenny, 2009, Schlader et al., 2010). Whether T_c or T_{sk} is a better marker of exercise capacity is yet to be fully substantiated.

Ratings of perceived exertion increased over time, although there were no discernible differences between conditions at the end of the time-trial. This finding is not surprising as individuals were asked to performance close to maximal capacity in very high ambient temperatures that they are not accustomed to. Therefore, the lower body temperature noted in BL may have reduced comparative thermal strain leading to increased effort; proportionately improving time to completion during the 10-km time-trial. Indeed, Schlader et al., (2010) highlighted that a reduction in exercise intensity may transpire in order to protect against higher perceived exertion responses in the heat.

Beyond Atkinson et al. (2008), only one other study has observed the effects of bright light on physical performance and subjective responses to exercise. O'Brien and O'Connor (2000) stated that physiological and subjective responses to 20 minutes of stationary cycling were unaffected by light. However, direct comparisons between this and the current study are difficult as light was altered acutely during exercise rather than, chronically, prior to exercise. Furthermore, no information was provided about the ambient conditions in which exercise was performed.

Interestingly, immediately after waking there were minimal differences in the observed salivary melatonin concentrations. This is surprising, especially with the evening suppression of melatonin in the current study and the substantiated inverse relationship between T_c and melatonin (Cagnacci et al., 1992). One possible mechanism that may have influenced the lack of variation between groups post-waking is postural changes. Upon awakening, when the samples were provided, some participants remained in bed for a short time while others got up immediately, this may well have varied across trials within subjects, influencing melatonin concentrations. The effects of posture on melatonin have

been reported by two previous studies (Deacon and Arendt, 1994, Nathan et al., 1998). Deacon and Arendt (1994) were the first to observe such effects and found that levels rise with standing after being supine and fell when the reverse posture was adopted. The mechanism behind this, especially for salivary melatonin, remains unclear. However, it has been postulated that the gravitational forces associated with postural changes (e.g. supine to standing) may influence the human circulatory system (Hagan et al., 1978), and in turn hormone concentrations.

In summary, the results presented in this study demonstrate the potential for evening bright light exposure, prior to sleep, to be used as an ergogenic aid for endurance performance in high ambient temperatures during morning training and/or competition. A lower T_c prior to waking was manipulated, which led to improved 10-km cycling time-trial. This technique has the potential to be more comfortable for the individual than other, more traditional, 'pre-cooling' techniques which often involve cold-packs on the skin on partial/full immersion in ice-baths.

CHAPTER 6

STUDY 3

USING DAWN SIMULATION TO REDUCE SLEEP INERTIA AND IMPROVE POST-WAKING PERFORMANCE IN HUMANS

6.1 Introduction

"Sleep inertia" is a transient state between sleep and feeling fully awake. Individuals can experience grogginess, disorientation, decreased motor control and lower cognitive and physical performance (Dinges, 1990, Kleitman, 1964, Tassi and Muzet, 2000). Sleep inertia can be experienced to some degree after sleep of any duration (Jewett et al., 1999, Brooks and Lack, 2006). Nevertheless, the duration and severity of sleep inertia is influenced by the preceding sleep duration, the presence of prior sleep deprivation and the individual's chronotype. After a typical 8-h sleep period, sleep inertia can persist for up to 2-h after waking (Jewett et al., 1999). In terms of severity, Wertz et al. (2006) reported that cognitive performance is worse immediately after waking (i.e. with sleep inertia) than it is during a state of total sleep deprivation. Furthermore, a nap of any duration, at any time of day, still results in sleep inertia and the magnitude of this effect appears to be most dependent on sleep stage at waking rather than nap duration (Folkard et al., 1976). The severity of sleep inertia was also observed to be more pronounced around the nadir of T_c compared with its circadian peak.

Individuals whom are classed as late chronotypes usually prefer to retire to bed later and rise later the following morning compared with intermediate and early chronotypes (Roenneberg et al., 2003). This usually means that late chronotypes have to rise earlier than desired on workdays resulting in a large discrepancy between their obligatory and preferred timing of sleep (Roenneberg et al., 2003, Horne and Ostberg, 1976, Zavada et al., 2005). This discrepancy leads to a state of sleep debt, which has been termed "social jetlag" (Wittmann et al., 2006). Ultimately, this sleep deprivation may lead to a 'vicious circle' of increasing severity of sleep inertia (Taillard et al., 2003). For elite athletes or recreational exercisers, training in the early morning soon after waking is common. Since

sleep inertia has been shown to reduce physical performance, indexed by hand grip strength (Jeanneret and Wilse, 1963), it would seem reasonable to postulate that if sleep inertia is present, exercise intensity (the training stimulus) and, therefore, training adaptations may be reduced (Reilly and Edwards, 2007). Furthermore, and possibly of greater concern, the risk of injury may also be increased via reduced psychomotor vigilance (Terman et al., 1989). Therefore, methods to ameliorate the effects of sleep inertia are warranted, for the general public and athletes.

Artificial dawn simulation has been proposed to reduce the symptoms related to sleep inertia. Dawn simulation involves the gradual increase in illuminance of low intensity light prior to the waking from sleep. Data from previous studies have indicated that dawn simulation improves sleep quality, increases the awakening cortisol response (Thorn and Hucklebridge, 2004), decreases subjective sleepiness and stimulates subjective "activation" (Van De Werken et al., 2010). Importantly, no researcher has examined the effects of dawn simulation on subsequent exercise performance soon after waking. Therefore, the aim of the present study was to examine the effects of a 30-min dawn simulation protocol on sleep inertia outcomes in subjects who show a late chronotype ("Owls"). It was hypothesised that dawn simulation results in (i) improved subjective alertness post-waking (ii) changes in the rhythms of T_c and melatonin, and (iii) improved cognitive and physical performance in the post-waking period.

6.2 Methods

6.2.1 Participants

Eight young adults were recruited for the present study (four males; four females), with a mean ± SD age 24 ± 9 years. No participant was involved in nocturnal shift work and none

had undertaken transmeridian travel during the 30 days prior to the study commencing. Participants had to live a regular lifestyle, that consisted of at least three 'workdays' a week. Participants had to be classed as moderate evening or evening types (rated on the morningness-eveningness scale (Horne and Ostberg, 1976). They also had to report taking ≥45 minutes to fully wake-up in the morning on 'workdays' (Munich chronotype questionnaire (Roenneberg et al., 2003)). These inclusion criteria resulted in a participant cohort of later chronotypes. All participants were healthy, non-smokers and none reported having a history of major illness nor had any been diagnosed sleeping problems. None of the participants were taking medication, prescribed or otherwise, except oral contraceptives (three women). The one woman not using contraception was always tested at the early follicular phase of the menstrual cycle. Participants were asked to refrain from alcohol, caffeine, coco-based products, bananas and strenuous exercise 24-h prior to attending the laboratory for each trial. Participants were asked to maintain their regular workday sleep-wake cycle during 48-h prior to the start of the testing phase. Participants gave written informed consent to undertake the study, and all procedures were approved by the ethics committee at Liverpool John Moores University and adhered to the Declaration of Helsinki.

6.2.2 Laboratory protocols

Participants attended the chronobiology laboratory at Liverpool John Moores University on three separate occasions; the first for familiarisation purposes, and the second and third to complete the main experimental conditions (intervention condition and control condition). Lighting conditions were standardised to 300 lux, at eye level, when sat 50 cm from the cognitive testing computer screen and when on the exercise bike. Temperature

was controlled at ~21°C. Data collection took place during the British winter and early spring time (December – March).

During the initial visit, participants became accustomed to all measurement tools and apparatus to be used during the experimental procedures, including our sleep laboratory. Anthropometric measures and resting blood pressure (mercury sphygmomanometer) were recorded during this visit. Resting blood pressure was measured with the participant seated following 10 min of quiet rest. All participants were normotensive.

6.2.3 Experimental trials

Trials were ordered in a counterbalanced fashion and were separated by 5-9 days. The night prior to attending the sleep laboratory the experimental phase began and participants were asked to sleep, in their own homes, the exact times they would sleep in the laboratory. To monitor compliance participants were issued with a wrist accelometer (Actiwatch, Neurotechnology Ltd, Cambridge, England). Participants were asked to attend the laboratory two hours prior to the initiation of sleep and slept for a period of 8-h in the laboratory. To calculate sleep time, reported times of wake and sleep from the participants Munich Chronotype Questionnaire were utilised. From these values a mid-sleep point was determined and 4 hours were counted either side of this time (within the nearest 15 min) to calculate sleep onset and awakening.

The experimental trials were identical with the exception of the thirty minutes prior to waking. During this time participants either slept normally in complete darkness (control condition [C]) or were exposed to dawn simulation [DS] (see below for details). Each night's sleep ended with an audible alarm. At the same moment, a researcher entered the

room to ensure the participant was awake. Upon waking participants were allowed to attend the bathroom if required. After waking participants underwent a 75 minute testing protocol which consisted of: three bouts of cognitive assessment, one physical performance test and monitoring of physiological and subjective variables (see measurement procedures for further details).

6.2.4 Dawn Simulation

Two dawn simulation devices (Lumie Bodyclock Active 250, Lumie, Cambridge, UK) were placed at either side of the participants' bed at a distance of 30 cm to ensure participants were exposed to the light. Thirty minutes prior to awakening, dawn simulation was initiated, starting at 0.001 lux rising to 300 lux following the sigmoidal illumination ramp (Figure 6.1). Accuracy was confirmed by measurement of illuminance with a digital photometer (ILM350 ISO-TECH, RS components, Corby, UK).

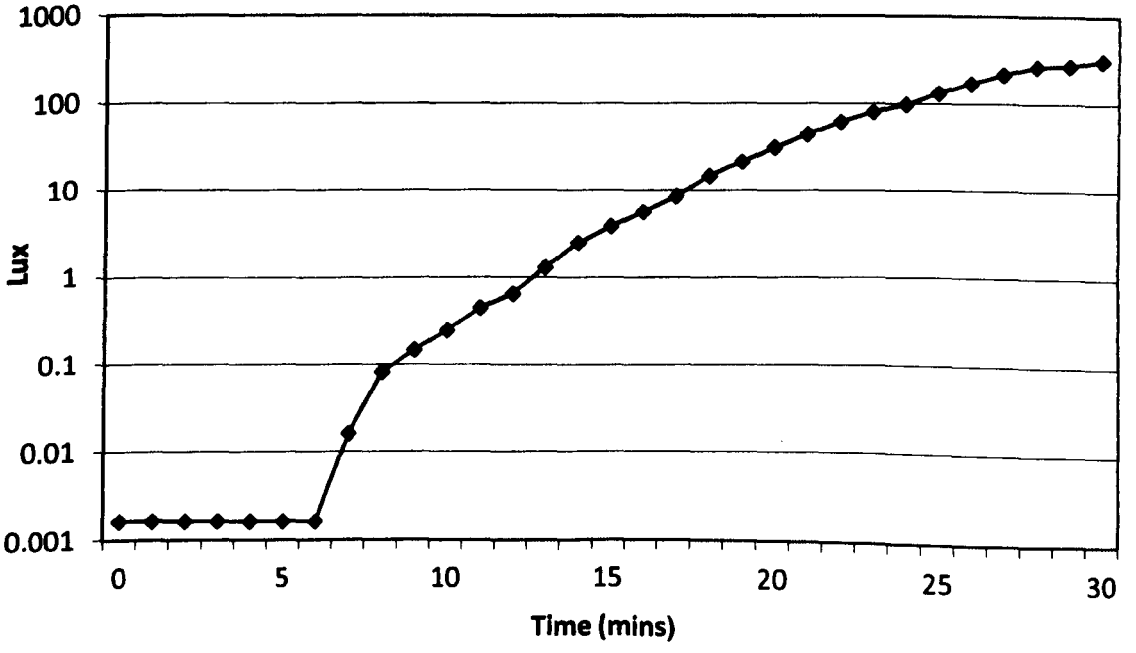

Figure 6.1: Sigmoidal illumination ramp for dawn simulation device.

6.2.5 Measurement Procedures

One hour prior to sleep participants ingested a silicon coated thermometric pill (CorTemp, Human Technologies International, Palmetto, USA) with 50 ml of water. The thermometric pill was used to record Intestinal temperature (T_c). Data was sampled every 30 seconds, beginning 2-h prior to waking. Skin temperature was measured using iButtons (DS1922L, Maxim Integrated Products, Sunnyvale, California, USA; resolution 0.0625°C) that were placed in 4 locations: left infraclavicular region, left forearm, mid-medial section of the left thigh and mid-medial section of the left calf. Data were sampled every minute, beginning 2-h prior to waking. Prior to sleep, participants were also fitted with an accelerometer (Actiwatch, Neurotechnology Ltd, Cambridge, UK) that was worn on the wrist. Data were recorded every minute and later analysed for sleep latency, sleep efficiency and actual sleep time (Actiwatch Activity and Sleep Analysis 5, Neurotechnology Ltd, Cambridge, UK). Saliva samples were collected 60 and 5 min before sleep and at 0, 15, 30, ~45 (post time trial), 60 & 75 min during the experimental protocol and were immediately frozen (-80°C) and later analysed for melatonin concentrations. Upon waking subjective sleep quality was recorded on a 10 cm Visual Analogue Scale ranging from good to poor sleep. Subjective ratings of alertness were obtained using the Karolinska Sleepiness Scale (KSS) (Åkerstedt and Gillberg, 1990) at 1, 5, 15, 30, 45, 60, and 75 min post-waking. Ratings on the KSS range from 1 to 9, with 1 meaning very alert and 9 meaning very sleepy.

On each visit participants were familiarised with the cognitive tests on two occasions prior to sleep. During the post-waking period data was collected on three occasions, at 5, 30 and 75 min. These tests were conducted using the Vienna system (Vienna Test System, A2340, Vienna, Austria). For the first test, a 1.5 min work series preference test,

participants had to mentally calculate additions and enter a single digit response. All additions consisted of two numbers of single units and participants had to enter the last digit of the calculation (e.g. 5 + 7 = 12, the correct entry on the keyboard would be 2). The second test was a determination unit test, which is a complex multi-stimuli reaction unit (Pouw, 1991). The unit allows for the presentation of coloured optical stimuli, which are presented in 10 different locations. Within a 1.5 min time period participants were required to respond to as many of these cptical cues as possible by pressing the corresponding coloured reaction key. In addition to the coloured lamps, an extra 2 white lights were positioned centrally requiring subjects to press a left or right foot pedal when the corresponding side was lit. For both cognitive tests: total, correct and incorrect responses were recorded along with mean reaction time.

Immediately following the second battery of cognitive tests, approximately 35 min post waking, physical performance was assessed using a 4-km self-paced cycling time-trial using a cycle ergometer (Premier 8i Ergo_bike, Daum Electronics, Fürth, Germany). This particular test was chosen as a measure of moderate duration performance as it has been shown to be repeatable under standardised conditions (1.6% coefficient of variation, (Altareki et al., 2009)) and has been shown to be sensitive to interventions. For example in the study by Altereki *et al.* (2009) a significant difference was seen between 4-km time trials in two different temperature conditions. Prior to the time-trial commencing participants were allowed a 2 min warm-up period. During this period participants were instructed to ascertain their preferred resistance (Watts) for the start of the time trial. Participants were blinded to all information expect distance travelled. Changes in power output and ratings of perceived exertion (RPE) (Borg, 1982) were recorded at the end of each km.

6.2.6 Data Analysis

With a sample size of 8 participants, a paired t-test with a 0.05 two-sided significance level would have 80% power to detect a difference in time trial performance of 1.9%, assuming that the within-subjects typical error (coefficient of variation) is 1.6% (Altareki et al., 2009). Temperature data, core and skin, were sampled every 30 s and 1 min, respectively. These data were then binned into single data points represented in the results section below. Data were analysed using two-factor within-subjects factors (trial x time) generalized estimation equations (Ballinger, 2004), with order of trial effect entered into the model. Time-trial and sleep quality data were analysed using paired t-tests. A secondary analysis was also performed on the relationship between melatonin and T_c; data were examined with appropriate within-subjects correlations (Bland and Altman, 1995). The data were analysed using Statistical Package for Social Sciences (SPSS) for Windows (Version 17, SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD (95% CI). The alpha level of significance was set at $p \leq 0.05$.

6.3 Results

6.3.1 Subjective ratings and sleep

Subjective alertness was worse immediately after waking 5.8 ± 1.7 [4.6 to 7.0] and generally improved over the testing period to 2.1 ± 0.6 [1.7 to 2.5], $p < 0.0005$). However, alertness was significantly better in DS (3.5 ± 1.2 [2.6 to 4.4]) compared with C (4.1 ± 1.1 [3.3 to 4.9], $p = 0.04$; Figure 6.2). Ratings of perceived sleep quality on a simple VAS were also higher in DS (6.8 ± 1.6 [5.4 to 8.2]) compared with C (5.6 ± 1.7 [4.2 to 7.0], $p = 0.01$). There were no statistically significant or substantial differences in objective sleep quality data during sleep measured via Actiwatch (i.e. sleep efficiency, sleep latency and total

sleep time; Table 6.1). There were also no significant differences in sleep variables during the last 90 min of sleep between each trial (Table 6.1).

Table 6.1: Mean ± SD objective sleep data.

	Control	Dawn simulation	*p*-value
Sleep efficiency (%)	86.7 ± 4.4	86.8 ± 6.0	0.97
Sleep latency (min)	13:00 ± 12:45	09:08 ± 05:53	0.42
Total sleep time (h)	06:55 ± 00:21	06:56 ± 00:29	0.97
90-min pre-waking sleep efficiency (%)	80.8 ± 11.0	78.2 ± 14.0	0.71
90-min pre-waking total sleep time (h)	01:11 ± 00:09	01:10 ± 00:12	0.79

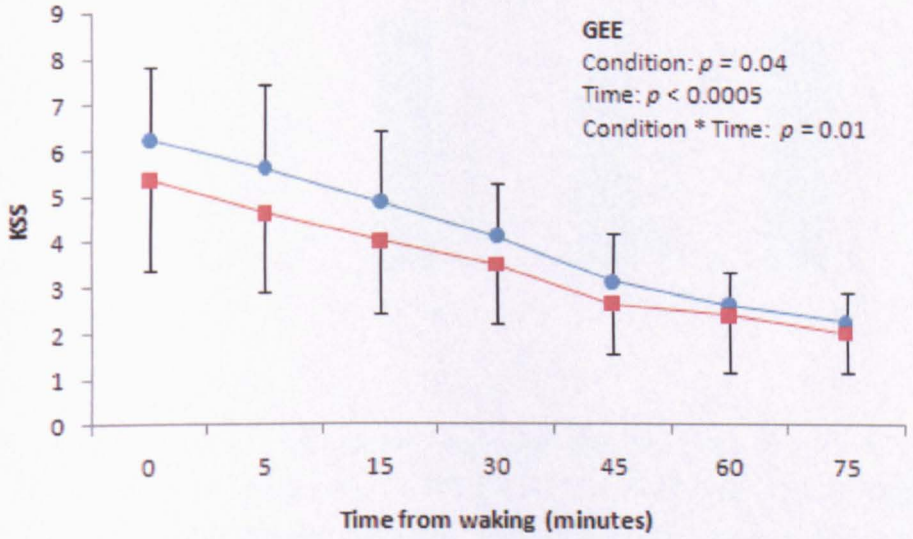

Figure 6.2: Mean ± SD Karolinska Sleepiness Scale. Blue line denotes control condition; red line denotes dawn simulation condition.

6.3.2 Performance

In the cognitive tests, performance generally improved over the testing period (i.e. performance improved as time awake increased in both conditions). In the additions test, the total number of additions made was, on average, greater in DS (69.5 ± 15.3 [56.2 to

77.6]) than C (66.9 ± 16.7 [57.9 to 81.1], $p = 0.03$; Figure 6.3). This corresponds to a 3.9% improvement in performance. The same trend was observed for the number of correct additions made in the 90 s allocation, although this did not quite reach statistical significance (67.7 ± 16.7 [56.1 to 79.2] vs. 65.5 ± 15.0 [55.1 to 76.0], $p = 0.06$). In the multi-choice reaction test performance was significantly better in DS (0.81 ± 0.02 s [0.76 to 0.85 s]) compared with C (0.85 ± 0.03 s [0.79 to 0.90 s], $p < 0.0005$; Figure 6.3). This corresponds to a 4.7% improvement in cognitive performance. Furthermore, the number of correct responses given was significantly greater in DS compared with C.

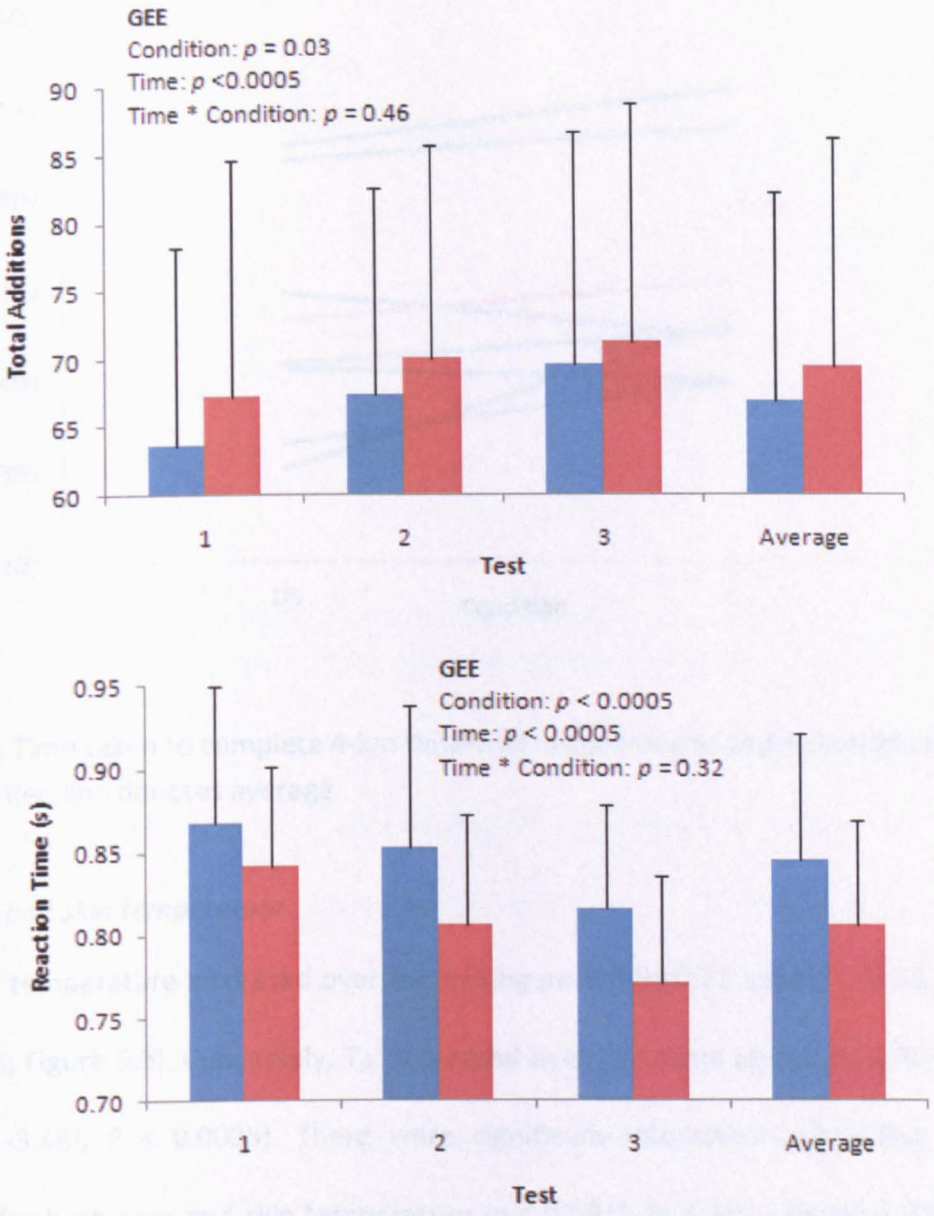

Figure 6.3: Mean ± SD multi-choice reaction time test and total number of additions for each test point post-waking (1 = 5 min, 2 = 30 min and 3 =75 min) and the average of these test. Blue line denotes control condition; red line denotes dawn simulation condition.

The time taken to complete the 4km time-trial was 21.4 s (-1.1 to 44.0 s, p = 0.07; Figure 6.4) faster in DS than C. This corresponds to a 4.7% improvement in time. There were no statistical differences in the selected workload between the two conditions. RPE was slightly greater in DS compared with C at the end of the time-trial, although this did not reach statistical significance.

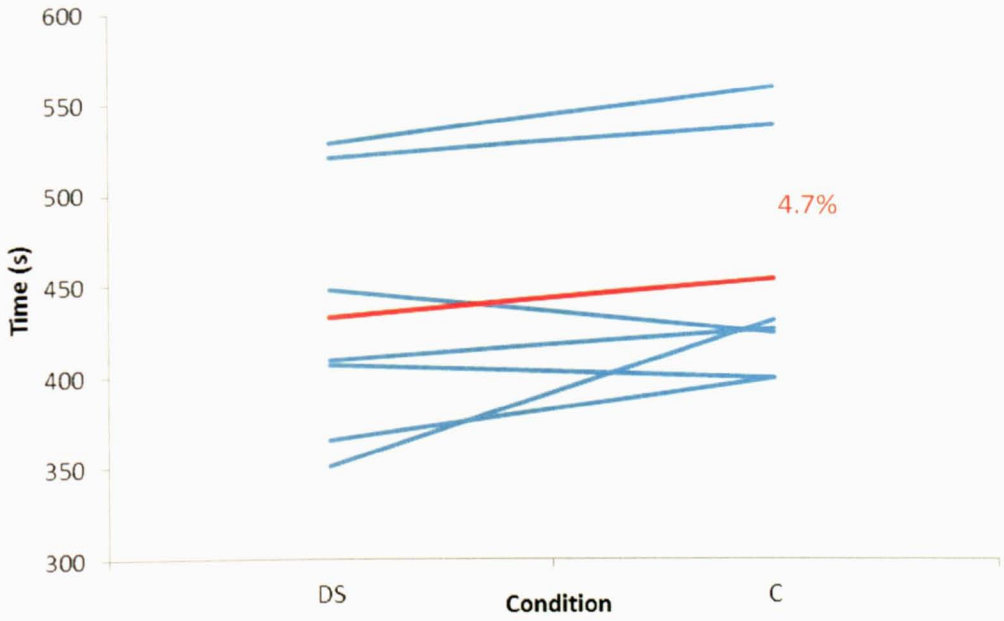

Figure 6.4: Time taken to complete 4-km time-trial in control and dawn simulation condition. Red line denotes average.

6.3.3 Core and skin temperature

Core body temperature increased over the testing period by 0.72 ± 0.20°C [0.58 to 0.86], $P < 0.0005$; Figure 6.5). Conversely, T_{sk} decreased over this same period by 2.83 ± 0.90°C [-2.21 to -3.46], $P < 0.0005$). There were significant interactions (condition x time) observed for both core and skin temperature ($p \leq 0.001$). In T_c the observed differences between conditions manifested post-waking (Figure 6.5), with more pronounced increases in DS. However, none of the time-point post-hoc comparisons reached statistical significance. In T_{sk} the interaction is less clear, with no discernible visual differences between the time-courses of the two conditions and no significant post-hoc comparisons at each time-point between conditions.

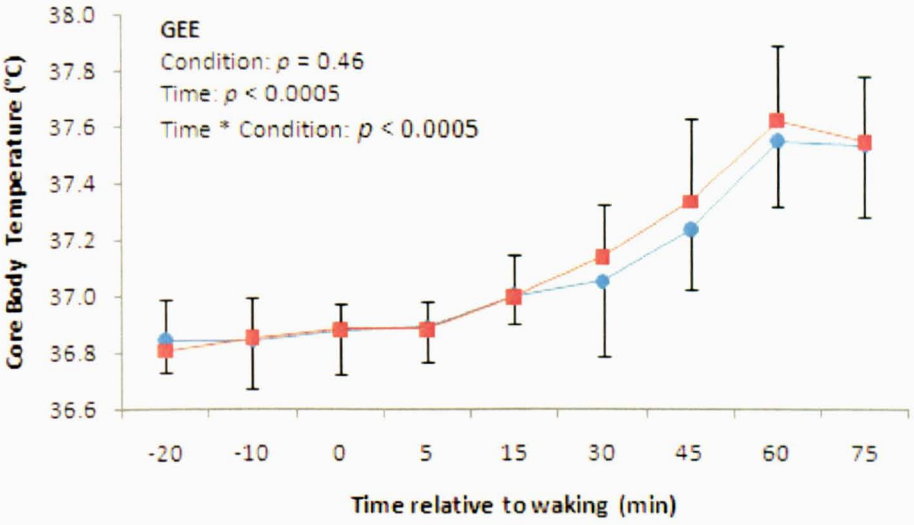

Figure 6.5: Mean ± SD T_c during the last 30 minutes of sleep and waking phase of testing. Blue line denotes control condition; red line denotes dawn simulation condition.

6.3.4 Salivary melatonin

Immediately post-waking there were negligible differences in melatonin between conditions. Nevertheless, melatonin concentrations were significantly lower in DS compared with C at 15 min post-waking (3.9 ± 5.1 [0.3 to 7.5, p = 0.03]) and at 30 min post-waking (5.2 ± 6.2 [0.9 to 9.4, p = 0.02]); Figure 6.6. After exercise, these differences dissipated. The magnitude of responses to exercise were different between conditions; with a negligible increase in pre- to post- exercise melatonin concentrations in C (0.8 ± 6.6 [-3.8 to 5.4], p 0.73), but a significant increase in the DS condition (5.0 ± 2.9 [3.0 to 7.0] p < 0.0005).

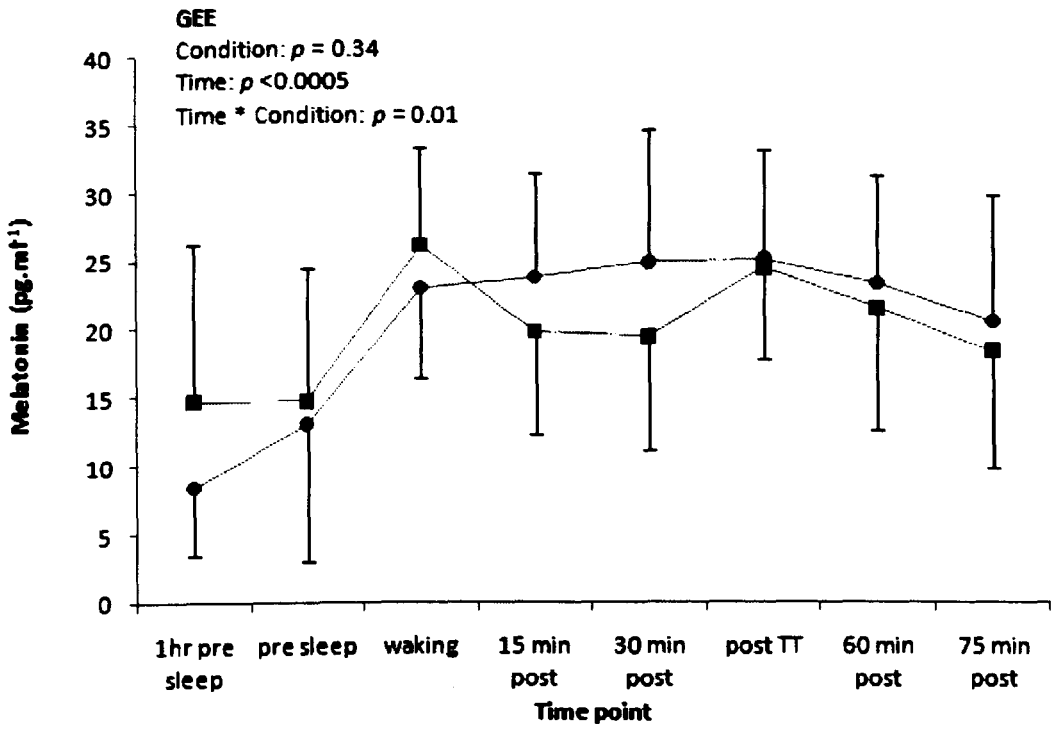

Figure 6.6: Mean ± SD melatonin prior to sleep and during waking phase of testing. Blue line denotes control condition; red line denotes dawn simulation condition.

The correlation between changes in T_c and melatonin was 0.07 in C. In DS, this correlation was negative and strong (r = -0.72). Due to missing-at-random data, T_c from one participants' was not included in this analysis.

6.4 Discussion

The primary aim of the present study was to examine the effects of 30-min dawn simulation on sleep inertia and subsequent early morning exercise performance in recreational exercisers. It was observed that following dawn simulation the time to complete a 4-km time trial improved by 4.7%. Although this difference was not statistically significant, the results may well be practically important. The 95% confidence interval indicates that the population mean difference is likely to be between a slightly negative value (-1.1 s) or as great as a 44.0 s improvement. Although the lower limit indicates a negative outcome, the upper limit suggests that the improvement in

performance would be considered advantageous. These data provide novel insight into the use of phototherapy in sport and exercise as the effects of dawn simulation on physical performance has, to the knowledge of the author, never been investigated previously. The use of dawn simulation prior to waking maybe a useful tool to improve performance in early morning sporting events and also increase training productivity.

It was also observed that cognitive performance outcomes (problem solving [3.9%] and reaction time [4.7%]) were significantly better after dawn simulation exposure compared with the control condition. Conversely, in a study with a similar design, Van de Werken et al. (2010) found no effect of dawn simulation on cognitive performance; however a reduction in sleep inertia severity was reported. This null effect is somewhat paradoxical given the fact that reduced sleep inertia is generally associated with improved cognitive performance. Nonetheless, the differences in reported outcomes may be due the inherent differences with test design (e.g. length and stimuli).

The changes in physiological study outcomes noted in this investigation may go some way to explaining the mechanisms by which these changes in performance manifest. Subjective improvements in alertness paralleled with increased cognitive output; this continuum was observed intra- and inter-condition. Additionally, A significant reduction in melatonin was seen in the dawn simulation condition 15 and 30 minutes post-waking. These reductions may be due to a residual effect of the light exposure on the pineal gland. It is well established that light suppresses the secretion of melatonin (Lewy et al., 1980); however, it is important to note that light may exert it effects independently from those of melatonin.

Due to its soporific effects, the reduction in melatonin may actually be the catalyst for the improvement in alertness. Indeed increased melatonin, whether endogenous or exogenous, is associated with decreased alertness (Cajochen et al., 2003). Previous studies examining the effects of dawn simulation on melatonin have produced contrasting results. Studies by Danilenko et al. (Danilenko et al., 2000) and Terman et al. (Terman et al., 1989) reported shifts in the dim light melatonin onset, whereas Gimenez et al. (Giménez et al., 2010) reported no change. These paradoxical findings could again be due to the inherent differences in study design, sampling rate and intensity and duration of light exposure. In the present study, as with others, involving attempted circadian manipulation and exercise, directly measuring the effects of the interventional product on any changes in the full circadian rhythm of variables (i.e. melatonin and T_c [discussed below]) is not feasible. This is due to the inherent study aim of participants having to complete exercise after waking from nocturnal sleep. It is well documented that physical activity produces masking effects on circadian rhythms, making estimates of circadian phase unreliable.

A greater increase in T_c was observed post-waking in the treatment condition, although this was not statistically significant. This larger rise in T_c may be dependent upon the significant decrease in salivary melatonin in the dawn simulation condition. This is evidenced by highly negative within subjects correlation between melatonin and T_c from waking to 30 min post-waking. The inverse relationship between melatonin and T_c is frequently reported in the field of chronobiology and related disciplines. The greater increase in T_c may actually have influenced both cognitive and physical performance.

Higher body and brain temperatures have been shown to increase synaptic function via increased transmission speed (Masino and Dunwiddie, 1999, Masino and Dunwiddie, 2000). Wright et al. (2002) reported that an increase in body temperature of only ~0.15°c was associated with improved cognitive performance, suggesting that small changes in body temperatures can influence cognitive output. This hypothesis may go some way to explaining the differences in cognitive performance throughout the testing period. Given that T_c and cognitive performance significantly increased throughout the waking period and T_c and cognitive performance were generally higher in the dawn simulation condition. Taken together, changes in cognitive performance may be somewhat dependent upon T_c.

Certain brain functions appear to have different limits of thermal sensitivity (Wilkinson et al., 1964) and if temperature is too high, performance levels decrease (Hancock and Vazmatzidis, 2003). During physical activity, perhaps with the exception of long-duration exercise or conditions of extreme hyperthermia, increased body temperature is linked with improved results, similar to the effects of a warm-up. Increasing overall body temperature is associated with; reductions in muscle and joint stiffness, enhanced nerve transmission, increased anaerobic metabolism and increased oxygen delivery to muscles (Bishop, 2003), all of which have been shown to improve exercise performance. However, compared with some studies (e.g. Bergh and Ekblom, 1979) the difference in T_c between conditions prior to the time-trial in the present was small (~0.16°C). Nonetheless, this mechanism may go some way to explaining the differences in 4-km time-trial performance.

Van de Werken and colleagues (2010) hypothesised that a change in skin blood flow and therefore subsequent alterations in skin temperature upon waking resulted in reduced

sleep inertia. In the present study no change in T_{sk} between conditions was observed, however the technique utilised by Van de Werken (2010) is more sensitive than the current method; meaning that such changes may have not been detectable. As with much research utilising light it is difficult to blind participants to conditions. Although it could be argued that a treatment effect was observed due to no true placebo, the participants were not informed of the experimental rationale.

Whether dawn simulation actually effects the circadian system is equivocal. Appropriately timed light exposure can produce large phase-shifts, in melatonin and body temperature for example. A predominant issue with dawn simulation research is measuring the quantity of light which penetrates through the eyelid; something that remains largely enigmatic in the current literature. Reports vary from around 5% penetration (Ando and Kripke, 1996) to attenuation of circadian-effects of light by approximately two orders of magnitude (Bierman et al., 2011). Recent preliminary data has demonstrated that light through the eyelids can suppress the secretion of melatonin (Figueiro and Rea, 2012). These results are to some extent in agreement with the findings of the present study, in which an acute drop in salivary melatonin levels was noted. Although in terms of dawn simulation, further investigation is required to fully substantiate any affects, both acute and chronic, on the circadian system.

The author acknowledges that extrapolation of results, especially those of physical performance, from recreationally active individuals to professional athletes needs to be done so with caution. The variation between times for elite athletes is likely to be reduced. Furthermore, these results may only be valid for the time at which sleep inertia

is present (i.e. immediately post-waking). Moreover, the use of dawn simulation with early chronotypes *per se* may well be ineffective but this remains to be investigated.

In summary, a single exposure to 30 minutes dawn simulation prior to waking from nocturnal sleep reduces the transient effects of sleep inertia in late chronotypes. This amelioration in sleep inertia was accompanied by improved physical performance and significantly improved cognitive functioning. This in turn, may well be beneficial for athletes who train or compete in the early morning; as well as for individuals who work in an environment that requires high cognitive load. The mechanisms by which dawn simulation exerts its effects remain undetermined and further investigation is required to substantiate this.

CHAPTER 7

STUDY 4

THE PRACTICALITY AND EFFECTIVENESS OF SUPPLEMENTARY BRIGHT LIGHT FOR REDUCING JET-LAG IN ELITE FEMALE ATHLETES

7.1 Introduction

Jet-lag can be experienced when there is misalignment between the internal 'body clock' and the new local time following a transmeridian flight across multiple time zones (Graeber, 1982, Reilly et al., 1997, Nagano et al., 2003). Unlike travel fatigue, the symptoms of jet-lag can vary with time of day and do not tend to abate until circadian rhythms are aligned to the new time zone. Jet-lag is considered a sleep disorder (ICSD, 2005) and its primary symptoms are, but not limited to, acute insomnia and day time sleepiness, impaired physical and neurocognitive performance, poor mood, irritability and gastrointestinal complaints (Waterhouse et al., 2007, Arendt, 2009, Haimov and Arendt, 1999, Arendt et al., 2005).

Although early studies involved the use of a single analogue scale for measurement of the general perception of jet-lag, more recent researchers have employed multi-symptom measurements at numerous times of day for several days after the flight (Edwards et al., 2000). The severity and duration of these symptoms appears to be dependent upon the number of time-zones crossed, the direction of travel, as well as the individual perception (Graeber, 1982, Reilly et al., 1997, Graeber, 1989, Lowden and Åkerstedt, 1998, Waterhouse et al., 2007, Eastman and Burgess, 2009). Since jet-lag is a multi-symptom condition resulting from a 'real-world' problem, it is difficult to research the effectiveness of any treatments for symptoms. This is compounded by the fact that different physiological systems adapt to a new time zone at different rates and individuals perceive the relative importance of each symptom differently (Waterhouse et al., 2007). However, the promotion of accelerated phase shifting of the body clock via careful timing of "zeitgebers" is considered a useful intervention for alleviating jet-lag (Sack, 2010).

While the various important zeitgebers of human circadian rhythms are well-known from laboratory-based studies, there is a lack of well controlled field studies to ascertain whether manipulation of these zeitgebers is effective for reducing jet-lag symptoms (Waterhouse et al., 2007). A difficulty in this research is the separation of any acute effects of a treatment from the circadian phase shifting effects. This problem has been discussed with respect to melatonin, hypnotics and exercise, but not to light. This is surprising given that one of the most commonly-advised treatments for jet lag is to seek or avoid light after arrival at times depending on the phase response curve for light (Minors et al., 1991, Revell and Eastman, 2005, Khalsa et al., 2003), even though light is also known to have acute effects on body temperature and alertness (Cajochen et al., 2005).

To date, there are only two published field studies on the effectiveness of bright light on jet-lag symptoms. Boulos *et al.* (2002) reported that use of bright light visors (3,000 lux) elicited only modest re-entrainment of circadian rhythms after westward travel over six time-zones. These shifts were also not accompanied by any improvement in sleep, performance, or subjective assessments of jet lag symptoms. Lahti *et al.* (2007) reported that chronobiologically-timed light exposure did not significantly decrease the subjective symptoms of jet-lag in cabin crew.

Jet-lag can be merely a self-limiting irritation for many travellers, especially people travelling for vacation (Sack, 2010). Nevertheless, there is a fundamental requirement for international-level athletes to perform well and be physiologically at their optimum soon after travel. International athletes often travel for training camps, international competition and tournaments and their travel schedules and obligations soon after arrival

are considerable. Therefore, the use of jet-lag alleviating methods, such as indoor bright light, may be unacceptable and impracticable during international travel.

Previously, the effects of melatonin (Edwards et al., 2000) and temazepam (Reilly et al., 2001) on various symptoms of jet-lag in athletes have been examined. Neither intervention mediated notable improvements in jet-lag symptoms in this population. However, like the studies on bright light, these studies did not adhere fully to CONSORT recommendations, which guide researchers in good practice for reporting randomised control trials (RCT). No research group has yet investigated the effectiveness of artificial bright light for reducing the jet lag symptoms experienced by world class athletes, especially using a formal RCT approach. Therefore, the aim of the present study was to investigate the practicalities and effects of appropriately timed supplementary light exposure on elite female soccer players following eastward travel over at least five time zones. The primary objective was to evaluate the investigational product on subjective jet-lag, compared to a control group. The secondary objectives included evaluations of circadian phase using body temperature and hand grip strength and the analysis of the manifestation and recovery other subjective symptoms relating to jet-lag.

7.2 Methods

7.2.1 Participants

This was a, parallel-group randomised controlled trial. With respect to the framework of sports performance research (Atkinson et al., 2008b), this was a phase II study of intervention effectiveness in "real-world" conditions. Twenty two elite female soccer players from a national team volunteered for this study. The players were aged 26 ± 4 years, had body mass of 65.1 ± 5.9 kg and a height of 1.71 ± 0.05 m (mean ± SD). The

participants travelled from the east-coast of the USA to Lisbon, Portugal (5 time-zones eastward). Ten players travelled from the west-coast of the USA prior to the final air-travel to Portugal (a total of 8 time-zones eastward), although the number of such players was ultimately balanced across groups (n=5 in each) and this source of variability was partitioned out of all analyses. All travel was undertaken on the same day. Data were collected in February 2010. The time of sunrise and sunset were ~07:30 and ~18:20-h, respectively at this time of year. Participants were provided with electronic and hard copies of the study protocol before supplying informed consent to undertake the study. All procedures were approved by the local ethics committee and conform to the Declaration of Helsinki and the ethical guidelines of IJSM (Harriss and Atkinson, 2011).

7.2.2 Group allocation procedures

During the study, players were accommodated in 11 hotel rooms, two to a room, with rooms coded 1 through 11 in the order of the assigned room number. Therefore, players were randomly assigned, through a blinded individual,to independent control or intervention arms using simple randomisation with no blocking (StatsDirect software v. 2.7.8, Altrincham, Cheshire, UK). Room was the unit of randomisation as the two players accommodated in the same room had to receive the same treatment - intervention or control, as allocated - to avoid contamination. The software uses the Mersenne Twister algorithm for random number generation (Matsumoto and Nishimura, 1998) with the seed taken from the computer clock. Before the arrival of the players, the 11 hotel rooms were labelled as Intervention or Control in accordance with the randomisation schedule, with concealment of the identity of the players to be housed in each room. The reservation details were not known to the person labelling the rooms. Owing to illness,

two participants, one from each group, were obliged to drop out of the study; all their data were discarded from the study. Therefore, a total of 20 participants completed the randomised study (11 Intervention: 9 Control). A small amount (<1%) of the questionnaire data were missing completely at random due to oversight by the participants. All physiological data were collected successfully. Participants' characteristics are shown in Table 1.

7.2.3 Research design

Following a 7-h flight from New Jersey, USA, the players and support staff arrived in Lisbon at around 09:00-h. This was followed by a 2-h journey to their hotel via coach. Immediately upon arrival at the hotel, participants undertook the first battery of tests prior to consuming lunch. The same battery of tests was then completed prior to the evening main meal and retiring to bed on the first day. Also, for the subsequent three post-flight days the tests were undertaken at four times a day, prior to: breakfast, lunch, evening meal and retiring to bed at approximately 08:00-h, 13:30-h, 18:00-h and 22:00-h, respectively. The participants slept between 23:00- and 07:30-h. The measures included in the battery of tests were intra-aural temperature IAT (3000A, FirstTemp, Genius, USA) of the right ear, and dominant hand grip strength (Grip-D, Takei Scientific Instruments co. LTD, Japan). Three measures were obtained and then averaged for hand grip strength. A short rest period (~15s) was given between attempts to ensure sufficient recovery. Participants were familiarised with the hand dynamometer prior to the trip and then again when they arrived in Portugal before the first battery of tests were completed. Subjective ratings of jet-lag were obtained on a visual analogue scale (VAS) with a score 0 = no jet-lag and 10 = very bad jet-lag (Arendt and Deacon, 1997, Reilly et al., 2001). These subjective ratings of jet-lag were obtained as part of the entire Liverpool jet-lag

questionnaire (Waterhouse et al., 2000). These questionnaire data were taken following the physical measurements and after meal consumption, if applicable. Data were collected in either the eating hall during the day or players' rooms at night.

During the intervention periods the control group (C) were instructed to continue with their regular activities; these included reading, general socialising and watching television. During this time, the individuals assigned to C were asked to remain in their rooms and where possible to avoid going outdoors. This group acted as the comparator group. The light group (L) were issued with a portable bright light device (Lumie LED SAD lamp, Cambridge, UK) and were exposed to 2,500 lux of polychromatic bright light at 50 cm at a time of day predicted to accelerate the body clock to the new time-zone (Waterhouse et al., 2007). Participants were exposed to a single dose of artificial light for 45-60 min per day. Optimally participants were asked to use the light boxes for 60 continuous minutes. However, sometimes events such as meetings with coaching staff and other support staff meant that this was reduced to 45 min, although it must be noted that this was only an issue on day 3 and 4, and the full intervention period was completed by all the participants of L on the first two days. Compliance to the intervention period was monitored at least twice per session by the researchers. The time of the interventions were presented was: day 1 15:30-16:30, day 2 14:30-15:30, day 3 10:30-11:30 and day 4 09:45-10:45 (Figure 7.1). Participants in L were given one light box between two people, that is, one per room, meaning that one light box was used by two athletes at the same time. It is recognised that light boxes are designed to be used by individuals, but this compromise was necessary in order to implement the intervention. Participants were instructed how to use the light box and informed that they were free to read and continue to socialise with their roommate. Once the intervention period was complete

the participants continued with their usual activities. Each participant in their pair simultaneously received polychromatic bright light of 2,500 lux at approximately 50 cm for 45-60 minutes on 4 consecutive days. Besides this intervention, no restrictions were placed on any of the players' exposure/avoidance to light, as this would disrupt the players' schedule.

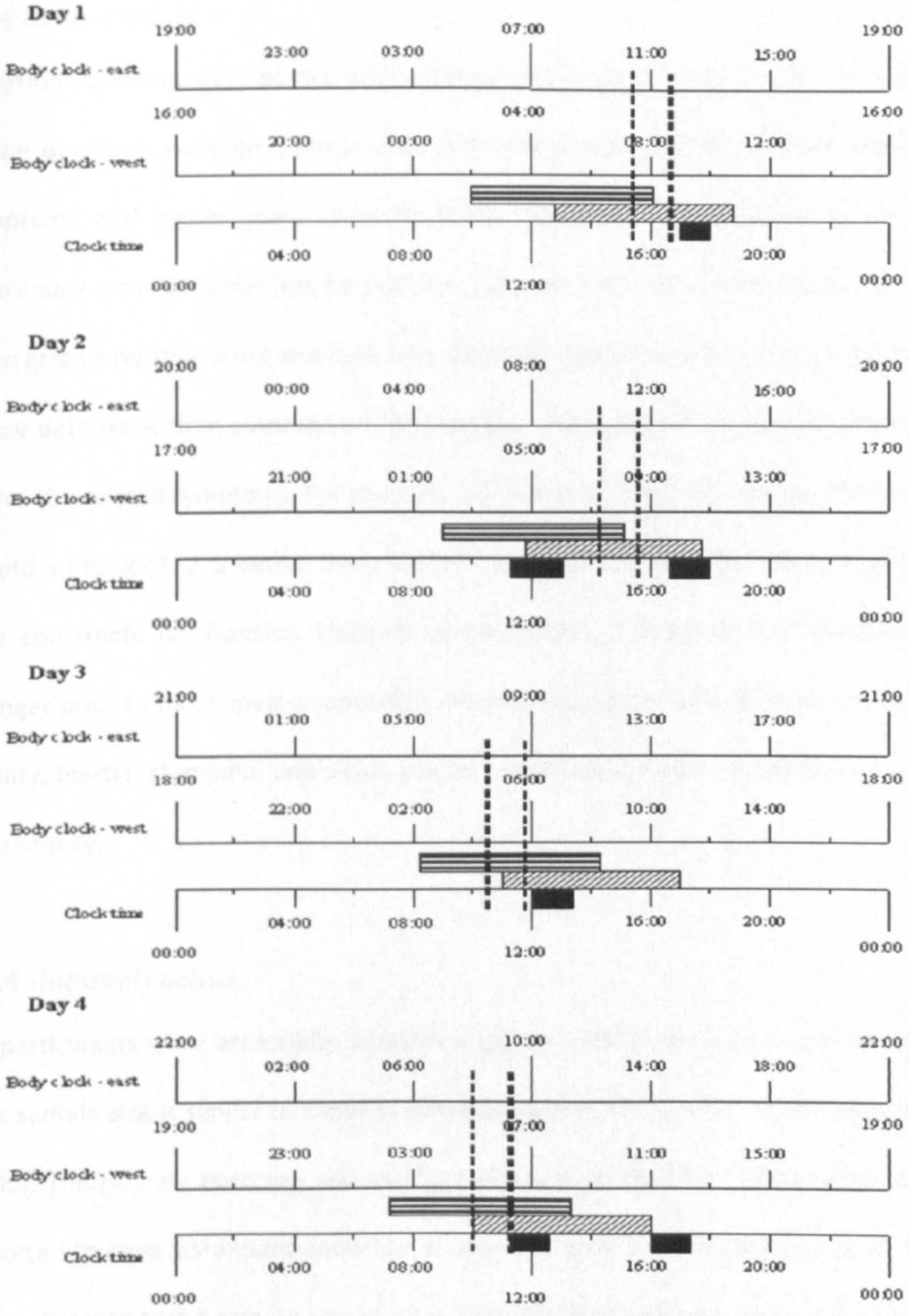

Figure 7.1: Time of light exposure relative to estimated body clock time. ≡ Denotes time light is required to advance the body clock for participants travelling from the East-coast of the USA (5 time-zones). ▨ Denotes time light is required to advance the body clock for participants travelling from the East-coast of the USA (8 time-zones). ¦ ¦ Denotes time that light treatment took place. ■ Denotes time of training (either gym-based or field-based).

7.2.4 Data reduction

Questionnaire data were pooled and summed into related constructs for analysis. Some of the questions were on a linear scale with lower scores meaning worse than normal symptoms and higher score meaning better than normal symptoms or vice versa. Conversely some variables had no positive response. Thus, data with a negative outcome were given a positive score and data with a positive outcome were given a negative score. These data were then summed and the greater the value of the overall constructs the worse the related symptoms. For example, an overall rating of 10 is worse than a rating of 6; and a rating of -2 is better than "normal" perceived ratings. The data were allocated into constructs for function (fatigue, concentration, motivation and irritability), diet (hunger prior to meal, meal palatability and post-meal satisfaction), sleep (sleep latency, quality, inertia, start-time and waking time) and bowel movement (frequency and stool consistency).

7.2.5 Statistical analysis

22 participants were accessible, which is a typical number of players in a soccer squad. This sample size is similar to those in previous studies (Reilly et al., 2001, Edwards et al., 2000). The primary outcome was overall subjective jet-lag (0-10 scale) which has been reported to have a standard deviation of approximately 2 units (Edwards et al., 2000). It was estimated that a sample size of 10 in each group would have 80% power to detect a reduction in jet lag of 2.3 units (standardised effect size of 1.3 using a two group t-test with a 0.05 one-sided significance level).

The data analysts were blinded to group allocation; the coding for each group being revealed only after all primary statistical analyses was completed. Most, but not all (e.g.

sleep-related data), variables were measured at baseline (pre-intervention) soon after arrival in the new time zone and before any intervention was introduced. These pre-intervention data were presented as mean ± SD and entered as a covariate in the statistical model which is considered best practice (Vickers and Altman, 2001). Analysis of data in this way has been found to be a more powerful approach than absolute or percentage change analysis when correlations between repeated measures are low-to-moderate (0.3-0.5) as they were with the present data. Variables without a pre-intervention baseline were analysed in the same model excluding baseline covariate control. The influences of prior travel from west coast of USA or not was explored also in the statistical model and also included as a between-subjects factor in the model. The analysis approach was based on Generalized Estimating Equations, which is considered a powerful and robust approach to the analysis of repeated measures data (Ballinger, 2004). The least significant difference approach to multiple comparisons was adopted in line with current advice not to employ Bonferroni, or similar, approaches to type I error control, especially when data are repeated measures (Rothman, 1990, Perneger, 1998). The data were analysed using Statistical Package for Social Sciences (SPSS) for Windows (Version 17, SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD (95% CI). The alpha level of significance was set at $P \leq 0.05$.

7.3 Results

7.3.1 Pre-intervention

Pre-intervention values for the various outcomes are presented in Table 2. Differences in these variables were found to be negligible (effect size < 0.25) between the study groups, although a substantial difference between means was noted for the diet-related outcome

(effect size = 0.62). However, all these pre-intervention baselines, including those for diet, were entered as covariates in the GEE model.

7.3.2 Overall rating of jet-lag

Overall ratings of jet-lag were greatest during the evening of the first arrival day, especially in the light group (Figure 7.2A). Jet lag was 1.15 ± 3.76 (0.34 to 2.68, P = .13) and 1.09 ± 3.32 (0.22 to 2.40, P = 0.10) points higher (worse) for the light group compared with controls in the early evening and night-time, respectively. Although these differences were observed they did not reach statistical significance. Subjective jet-lag, averaged across the whole of day 2, was 0.93 ± 2.13 (0.09 to 1.77, P = 0.03) points higher in the light compared with controls, this difference was statistically significant. While the control group showed further decrease (improvement) in jet-lag between days 2 and 4 by 0.58 ± 1.02 (-0.25 to 1.41), P = 0.17) points, this did not reach a level of statistical significance. The jet lag ratings for the light group decreased significantly between days 2 and 3 by 1.11 ± 0.86 (-0.61 to 1.62. $P < 0.0005$) points, and by 0.86 ± 0.15 (0.57 to 1.16, $P < 0.0005$) points between days 3 and 4. In summary, overall jet lag ratings were actually higher in the light group for approximately 24-h after the first light exposure, but these ratings decreased more substantially over the remaining post-flight days so that jet lag was rated as lower at the end of the 4-day study in L.

7.3.3 Function-related ratings

Like overall jet-lag, function ratings were worst in the 8-12-h immediately after arrival and showed the most substantial improvement during the 2nd post-flight day. Nevertheless there were some differences between function and overall jet lag ratings in response to the light intervention and in terms of general time course. First, function-related

symptoms were generally much worse in the morning compared with other times of day [lunch = 1.67 ± 1.73 (0.98 to 2.36, $P < 0.0005$); Evening = 1.15 ± 2.28 (0.25 to 2.04, $P = 0.012$); Night = 1.31 ± 2.18 (0.45 to 2.17, $P = 0.01$) points lower compared to morning measures]. The largest difference in function symptoms was observed soon after the first light exposure on day 1 but, unlike jet-lag, function ratings were better in the light group during this period (Figure 7.2B). However, this difference of 2.44 ± 8.02 (-0.73 to 5.61, $P = 0.13$) points at 18:00-h on day 1 did not reach statistical significance.

7.3.4 Diet- and Bowel-related ratings

Bowel-related ratings showed only a shallow decrease from 2.5 to 1.5 units between days 1 and 4 (Figure 7.3). Negligible differences between groups were noted in the time course of diet-related symptoms ($P \geq 0.136$) and time of meal; lunch vs. evening meal ($P \geq 0.238$).

7.3.5 Sleep-related symptoms

The best overall ratings of sleep were reported on the first night (Figure 7.4) in contrast to jet lag ratings being rated as the worst just before this sleep period (Figure 7.2A). Worse sleep was then reported on subsequent post-flight nights; worst scores being reported on the second night. The difference between the first and second night's sleep was 5.97 ± 4.18 (3.50 to 8.43, $P < 0.0005$) points higher in the light group compared with only 2.38 ± 4.08 (-0.28 to 5.03, $P = 0.08$) points higher in the control group.

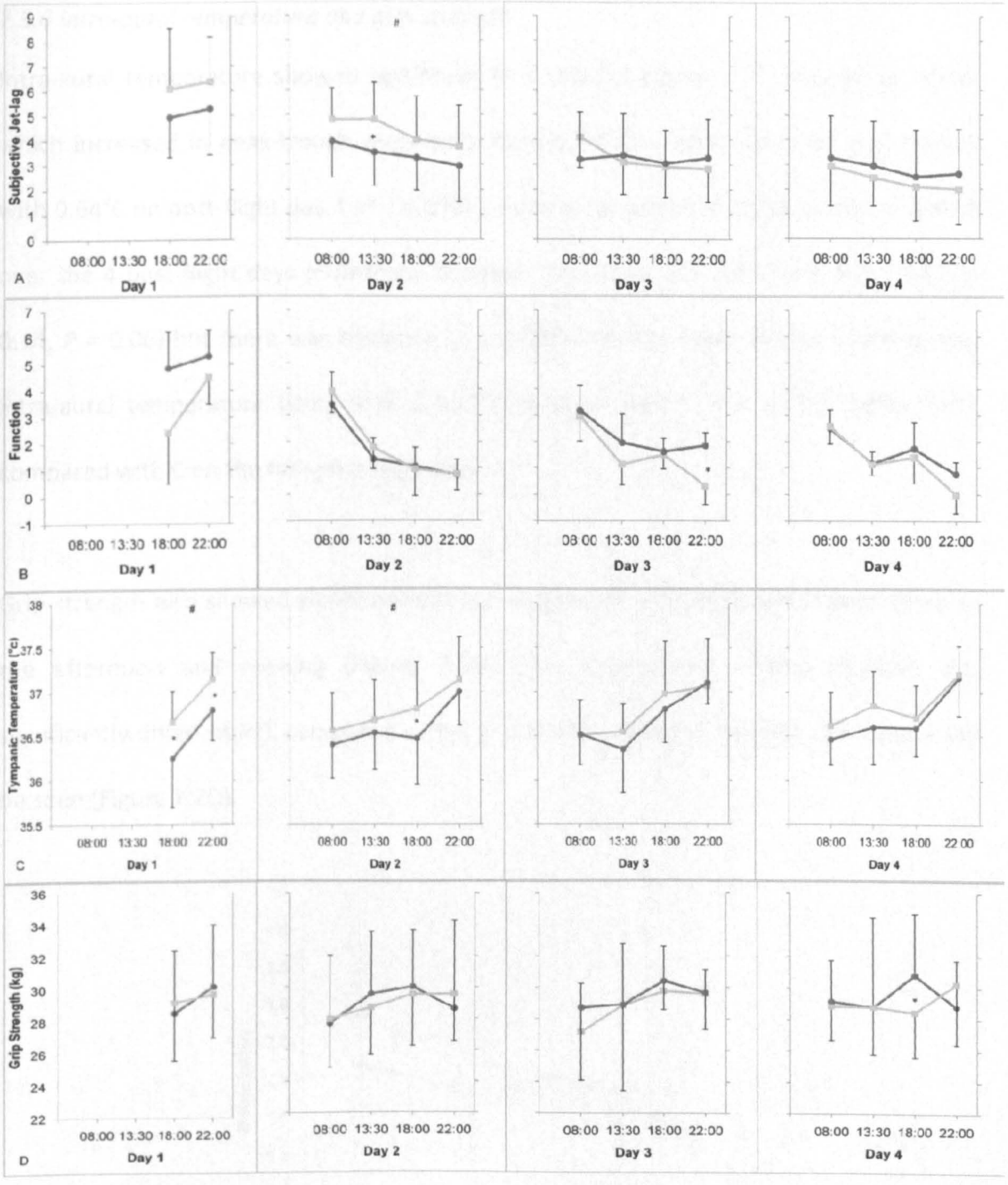

Figure 7.2: Ratings of subjective jet-lag (A), overall subjective function (B), Intra-aural temperature (C) and average grip strength (D) over 4 post-flight days post intervention. * denotes significant difference between groups at time-point. # denotes significant difference between groups on day. (A) and (B) Higher values mean worse symptoms. Presented as Mean ± SD. C – Black line with circles; L – Grey line with squares

7.3.6 Intra-aural temperature and grip strength

Intra-aural temperature showed significant ($P < 0.0005$; Figure 7.2C) diurnal variation, which increased in peak-trough magnitude from 0.58°C on post-flight day 2 compared with 0.64°C on post-flight day 4 ($P < 0.0005$). Intra-aural temperature generally increased over the 4 post-flight days (difference between day 2 and day 4 0.07 ± 0.18°C (0.00 to 0.14, $P = 0.06$) but there was evidence ($P = 0.008$) that this depended on study group; intra-aural temperature being 0.38 ± 0.33°C (0.16 to 0.60°C, $P = 0.001$) higher for L compared with C on the first post-flight day.

Grip strength also showed significant diurnal variation ($P < 0.0005$) with higher values in the afternoon and evening (Figure 7.2D). The time-course of grip strength was significantly different in L compared with C ($P = 0.001$), although no clear differences can be seen (Figure 7.2D).

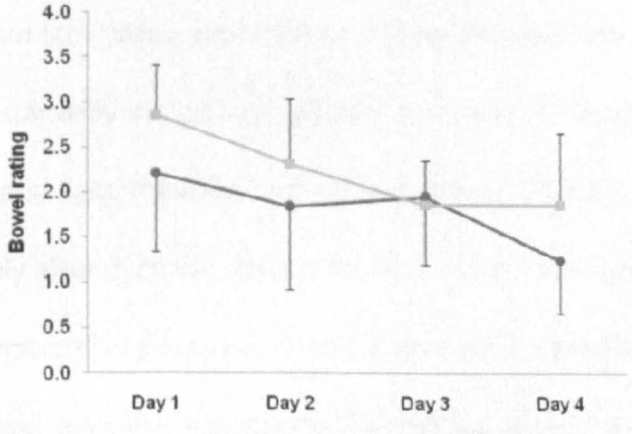

Figure 7.3: Subjective ratings relating to bowel factors over 4 post-flight days. Higher values mean worse symptoms. Presented as Mean ± SD. C – Black line with circles; L – Grey line with squares

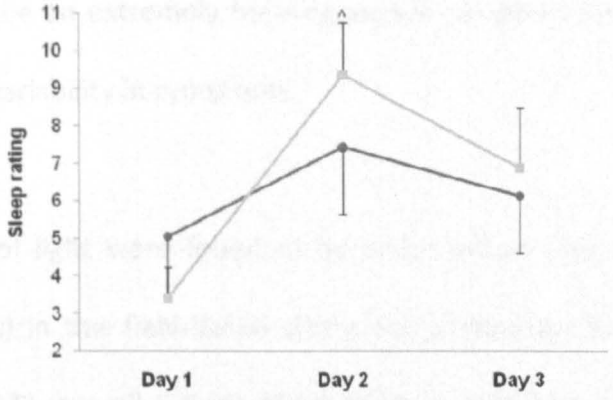

Figure 7.4: Subjective ratings relating to sleep factors over 4 post-flight days. ^ denotes significant difference between current and previous day in L. Higher values means worse symptoms. Presented as Mean ± SD. C – Black line with circles; L – Grey line with squares

7.4 Discussion

This study is the first randomized control trial of a bright light intervention for reducing jet-lag symptoms in world-class athletes. This research is also novel in terms of the consideration of both the immediate and longer-term effects of a supplementary light intervention on a comprehensive suite of jet-lag symptoms measured repeatedly over four post-flight days. Although the bright light protocol was based on chronobiological principles derived from laboratory experiments on the circadian phase shifting effects of light, it did not substantially reduce symptoms of jet-lag. In keeping with data from laboratory experiments, light mediated an acute increase in body temperature, when measured immediately after each light exposure, but this did not translate to any positive effects on jet-lag symptoms in the present field-based study. Nevertheless, considerable compromises were necessary for the intervention to be acceptable to the players and coaching staff, the most significant of which were that players were exposed to the bright light in pairs rather than as individuals Also this particular time-zone transition meant that athletes were required to stay in their rooms during exposure, even though it was light outside, the sample size was naturally small, dictated by the size of the playing squad and

jet-lag was found to be an extremely heterogeneous construct for these athletes, with high inter- and intra-variability in symptoms.

The clearest effects of light were found to be acute rather than chronobiological (i.e. phase shifting effects) in this field-based study. For several symptoms, including intra-aural temperature (IAT), overall ratings of jet-lag and symptoms relating to functioning and performance, the differences between groups were most pronounced following the first exposure to light. This acute photothermic effect of light agrees with data from laboratory studies. For example, Babia et al. (1991) reported transient increases in tympanic temperature whenever participants were exposed to periods of bright light. This apparent masking influence of light is generally under-researched especially in relation to its link with the phase-shifting effect (Postolache and Oren, 2005).

The data indicates that there might be two distinct effects of supplementary bright light in field conditions – an acute psychophysiological effect, which in the current study was not useful to the athletes (possibly due to being restricted to their room during exposure for example) and a more favourable longer-term effect which caused jet-lag symptoms to reduce from their zeniths more rapidly. This latter effect may be explained by more rapid phase-shifting induced by bright light, but this cannot be confirmed by the results of the study. This complicated nature of both the construct of jet-lag as perceived by athletes and the acute and phase-shifting effects of chronobiotics is further highlighted by the finding that sleep on the first night of arrival in the new time zone was the rated as best in quality, while overall jet-lag ratings were highest at this time. All these observations support the research work of Waterhouse et al. (2007) that the symptoms of jet-lag are rated relatively differently at different times of day and do not necessarily vary in parallel

with overall ratings of general jet-lag. The obvious high inter-individual variability in symptoms compounds this problem.

The lack of well-controlled studies on bright light during field conditions is surprising. To the knowledge of the author, there are only two previous field studies that have examined the effects of artificial bright light on jet-lag (Boulos et al., 2002, Lahti et al., 2007). These studies were not randomised controlled trials, but their findings agree with the results from the present study. Boulos, et al., (2002) used 3000 lux visors in an attempt to alleviate jet-lag and described only "modest" circadian phase-shifts. Lahti, *et al.* (2007) concluded that the use of artificial lights boxes mediated no effects on subjective feelings of jet-lag. Based on the overall evidence from these two studies and the present chapter, it cannot be concluded, at present, that artificial bright light is effective in the treatment of actual jet-lag symptoms.

The disparity between these findings and those from experiments involving simulated shifts of the sleep-wake cycle (1997) can be explained by the fact that jet-lag is largely a subjective collection of a wide range of different symptoms which may not vary completely in parallel with changes in circadian phase during adjustment to a new time zone. This classic case of an intervention having efficacy but uncertain effectiveness in the real world is a common problem in exercise science research (Flay et al., 2005). It is possible that, in order to obtain a favourable effect on symptoms, a much more demanding (in terms of time and disturbance to training) protocol was required. However, in a population that needs to be highly active outdoors and already needs to adhere to a busy schedule, an even more demanding intervention protocol would be at least very inconvenient and at worst intolerable to world class athletes. The impact of

participants being exposed to the light intervention in pairs cannot be overlooked. This is contrary to most manufacturers' instructions for use but it was necessary for the study to be undertaken; only a certain number of light boxes could be transported and the players naturally shared rooms.

The objective measure of physical performance (grip strength), IAT and most of the subjective measures demonstrated diurnal variation during this study. Grip strength and IAT were generally lowest in the morning, which agrees with past research (Drust et al., 2005) and serves to illustrate that these outcomes were sensitive to the effects of time of day during this field study. The peak-trough magnitude of the subjective measures was observed to decrease slightly over each post-flight day. This is congruent with the findings of previous studies on jet-lag in athletes (Reilly et al., 2001, Edwards et al., 2000) where greater negative feelings were reported prior to sleep and immediately after waking and these symptoms diminishing over subsequent post-flight days. This is further evidenced by the fluctuations in function-related symptoms. The ratings fall throughout the daytime but then are higher at the start of the subsequent day. It was demonstrated by (Waterhouse et al., 2000) that fatigue and jet-lag are highly correlated. The fact that individuals had just woken, paralleled with circadian desynchrony, may exacerbate the ratings of diminished function and an overall increase in jet-lag. Conversely, physiological measures increased in peak-trough magnitude over the data recording period; a trend that was evident in both groups. This increase in peak-trough variation for strength and temperature indicate attenuation in jet-lag as the body clock, external cues and the sleep-wake cycle become more closely aligned post-flight.

Due to time restraints and being unable to access all the world-class athletes freely over an extended period, only one physical performance outcome (grip strength) was obtained, whereas previous researchers have monitored other performance outcomes including reaction time and back and leg strength (Reilly et al., 2001). On the first day of measurements, grip strength was lowest at pre-intervention and then increased to its highest values prior to sleep, coinciding with a time that would relate to early evening in the disturbed body clock rhythm. On the subsequent days, the diurnal variation of grip strength resembled the normal late afternoon/early evening peaks and early morning nadirs. As originally stated by Reilly, *et al.* (2001) this interaction between post-flight day and time of day highlights the folly with assessing athletic performance across multiple time zones without 1) measuring performance at different times of the day and 2) allowing adequate time for the performance rhythm to be restored before sports competitions are attempted. Although a statistically significant interaction between treatment group, day and time of day for grip strength was produced, a clear trend in phase-shifting is extremely difficult to infer with the current number of measurements (4 per day) that were feasible in this elite group of athletes.

The observation of superior sleep-related variables on the first night after arrival is common and probably explained by general travel fatigue (Waterhouse et al., 2007). The fact that these variables were worst on the second night is consistent with jet-lag beginning to exert its more longer-lasting influence. It was only on day 3 that sleep-related variables began to improve. It is unlikely that any of the results reported are influenced by individual morning-evening types. Each participant recorded scores of either moderate morning or evening types or neutral scores (neither morning nor evening types). This supports the notion of Atkinson and Reilly (1996) that athletes of the same

age and sport show little variation in this factor. Moreover, although not directly controlled for in the random group allocation, morningness-eveningness results were not different between the two groups.

It has been postulated that exercise itself can mediate a phase-shift in either direction when timed correctly (Buxton et al., 2003, Baehr et al., 1999). The players recruited for the current study undertook training, outdoor or gym based, at least once a day. Nevertheless, the timing of this exercise, on the whole, was outside the times predicted to mediate phase advances or delays (Waterhouse et al., 2007). Furthermore, the outdoor training sessions were conducted during daylight hours; the stimulus of bright light is a much more potent zeitgeber than physical activity and would off-set any effect of exercise. Even highly strenuous exercise has been shown to produce only modest phase-shifts (Buxton et al., 2003). Therefore, the potential that exercise could have influenced circadian phase in the current study is highly unlikely.

As already indicated, this study had limitations, which would be expected when one is studying world-class athletes prior to a major international tournament. First, to get a true understanding of circadian phase; temperature should be monitored at more regular intervals, this would enhance profiling and increase the ability to deduce specific within measure variables such as acrophase, amplitude and mesor values. Secondly, a greater range of performance measures (e.g. reaction time tests), including pre-flight baseline measures, would have allowed for a greater understanding of how jet-lag exerts its effects and how, if at all, supplementary bright light effects these variables. Finally, the timing of the treatment period was scheduled in line with suggestions from previous reviews (Waterhouse et al., 2007). This meant that the treatment group were exposed to

the light box during day light hours and the control group had the option to be exposed to the natural light as they were not restricted with activities during the intervention time, although they were asked, if possible, not to go outdoors. Also, restricting individuals to isolated dark conditions, such as the controls during the intervention period, is not realistic to the usual field setting; therefore, a true indication of the effects of supplementary bright light on jet-lag would not have been ascertained.

The use of chronobiologically-timed supplementary bright light in the current study did not alleviate the effects of jet-lag in world-class female football players travelling east across 5-8 time zones. The light was administered on arrival, according to chronobiological principles, in pairs and indoors even though it was light outside at the same time of day. This study also naturally involved a small sample size. In agreement with previous studies on jet-lag, different symptoms of jet-lag were emphasised at different times of day and appeared to recover at different rates between- and within-subjects. This variability in the study outcomes is further compounded by the exposure to potential masking effects, such as sunlight and exercise, which were impossible to control tightly in this field study.

CHAPTER 8

STUDY 5

THE LIVERPOOL JET-LAG QUESTIONNAIRE STUDY: EXPLORING THE RELATIONSHIP BETWEEN DIRECTION OF TRAVEL, TIME-ZONES CROSSED AND SUBJECTIVE SYMPTOMS OF JET-LAG

8.1 Introduction

In study 4 (*Chapter 7*), the effects of bright light on jet-lag symptoms in elite female soccer players were explored. In keeping with other studies in similar populations but testing the different interventions of melatonin and temazepam, no statistically significant reduction in jet-lag was observed. Although the overarching concept of jet-lag is one that many individuals understand, the underlying perceived components of jet-lag are diverse and complex, and it is this "noise" that may have compromised the detection of effects in the previous study.

Jet-lag is multi-symptomatic in nature with a lack of consistency in the type and severity of symptoms reported between individuals. As well as this individuality, jet-lag duration and magnitude appears to be dependent on the number of time-zones crossed and the direction of travel (Graeber, 1982, Reilly et al., 1997, Graeber, 1989, Lowden and Åkerstedt, 1998, Waterhouse et al., 2007, Eastman and Burgess, 2009). However, when travelling over the same number of time-zones, eastward flights generally result in a greater severity of jet-lag than westward journeys.

The highly subjective nature of jet-lag complicates the monitoring and 'treatment'. As expressed previously this malaise can affect various aspects of physiology including the sleep/wake cycle, digestive system and cognition. The *Liverpool jet-lag questionnaire* and *Columbia jet-lag questionnaire* were developed as more comprehensive approaches to monitoring jet-lag, including questions relating to sleep and fatigue. Whereas much of the early subjective work on the area simply employed a single visual analogue scale (VAS) to measure overall feelings of jet-lag. However, the development of these 'new' questionnaires has not lead to full understanding of how individuals recover from jet-lag

due to individual characteristics and time-course of recovery. It appears that symptoms recover at different rates from each other or jet-lag itself (e.g. Waterhouse et al., 2000, Graeber, 1982). Furthermore, dependent on the time of day, certain symptoms are expressed to a greater or lesser extent. For example, Waterhouse et al. (2000) observed the amount of jet-lag in the morning is predicted by the time of waking from sleep (earlier times predicting more jet-lag) and by a decreased alertness 30 min after waking; and the amount of jet-lag in the daytime is predicted by the fall in the perceived ability to concentrate. Although this study attempted to get an understanding of how different symptoms relate to overall jet-lag, this was done using a simple regression model and within individuals who had crossed the same number of time-zones. Indeed, to the knowledge of the author no study has observed the differences in jet-lag perception over multiple time-zones in the same and different directions of travel. This is surprising given the commonly cited theory that more zones crossed results in greater jet-lag. However, it should be acknowledged that several studies have reported empirical data from different flights, although, these have often only compared long- vs. short-haul flights (Bourgeois-Bougrine et al., 2003) or eastward vs. westward travel over a similar number of time-zones (Lemmer et al., 2002).

The overall aim of the present study is to observe the effectiveness of the Liverpool Jet-Lag Questionnaire in:

1. Detecting which construct(s) of symptoms, if any, pertain most closely to the overall ratings of jet-lag.
2. Detecting the severity of jet-lag depending on classical causality factors (i.e. time-zones crossed and direction of travel).

8.2 Methods

8.2.1 Participants

Data from 43 (22 females) participants were used for the present study. Participants were aged 32 ± 11 years had body mass of 69.6 ± 11.8 kg and a height of 1.72 ± 0.10 m (mean ± SD). Travel took place to and from various locations around the globe, between 0 and 11 time-zones; 22 travelled eastward; 19 westward; and 2 did not change time-zone (e.g. Portugal to UK). All procedures were approved by the local ethics committee. Completion of the questionnaire was taken as implied consent.

8.2.2 Research design

Data was collected using the Liverpool jet-lag questionnaire (Waterhouse et al., 2000). Individuals were asked to commence the questionnaire pack on the first full day after travel was undertaken, or as soon as possible thereafter. The pack contained a general section on how to complete the questionnaires; a questionnaire on demographic information and flight details; and 8 jet-lag questionnaires specific to the time of day that completion was requested. The study period was 2 days and on each day participants completed questionnaires <30-min post-rising, immediately post-lunch and -dinner and <30-min prior to retiring to bed.

8.2.3 Data reduction

Data reduction undertaken on questionnaire data in the present chapter was the same as that in *Chapter 7,* for the ease of the reader the details are repeated below.

Questionnaire data were pooled and summed into related constructs for analysis. Some of the questions were on a linear scale with lower scores meaning worse than normal

symptoms and higher score meaning better than normal symptoms or vice versa. Conversely some variables had no positive response. Thus, data with a negative outcome were given a positive score and data with a positive outcome were given a negative score. These data were then summed and the greater the value of the overall constructs the worse the related symptoms. For example, an overall rating of 10 is worse than a rating of 6; and a rating of -2 is better than "normal" perceived ratings. The data were allocated into constructs for function (fatigue, concentration, motivation and irritability), diet (hunger prior to meal, meal palatability and post-meal satisfaction), sleep (sleep latency, quality, inertia, start-time and waking time) and bowel movement (frequency and stool consistency).

8.2.4 Statistical analysis

The number of time-zones crossed (≤3, 4-7 or ≥8) and direction of travel (eastward or westward) was entered into model as between-subject factors; Chronotype was entered as a covariate. The number levels for the within subject-factor was dependent on the number of occasions which the specific question was asked. Significant interactions were explored using appropriate post-hoc analysis. The least significant difference approach to multiple comparisons was adopted in line with current advice not to employ Bonferroni, or similar, approaches to type I error control, especially when data are repeated measures (Rothman, 1990, Perneger, 1998). The data were analysed using Statistical Package for Social Sciences (SPSS) for Windows (Version 17, SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD (95% CI). The alpha level of significance was set at $P \leq 0.05$.

8.3 Results

8.3.1 Jet-lag

Ratings of perceived jet-lag followed an inverted U profile with respect to number of time zones crossed, with significantly worse scores when travelling over 4-7 zones vs. ≤3 zones (difference = 4.4 ± 1.0 CI: 2.4 to 6.4, $p < 0.0005$) or ≥8 zones (difference = 2.3 ± 0.8 CI: 0.7 to 3.9, $p = 0.006$) (see Figure 8.1). This profile was more pronounced for westward ($p \leq 0.004$) vs. eastward travel. There was a trend for jet-lag being worse for eastwards than westwards travel, but this difference did not reach statistical significance (difference = 0.9 ± 0.6 CI: -0.4 to 2.2, $p = 0.18$). There were interactive effects of direction of travel and number of time-zones crossed; with greater jet-lag following westward travel over 4-7 time-zones than eastward (difference = 2.2 ± 1.3 CI: -0.4 to 4.7, $p = 0.09$), and vice-versa over ≥8 time-zones (difference = 1.7 ± 0.9 CI: -0.1 to 3.5, $p = 0.07$).

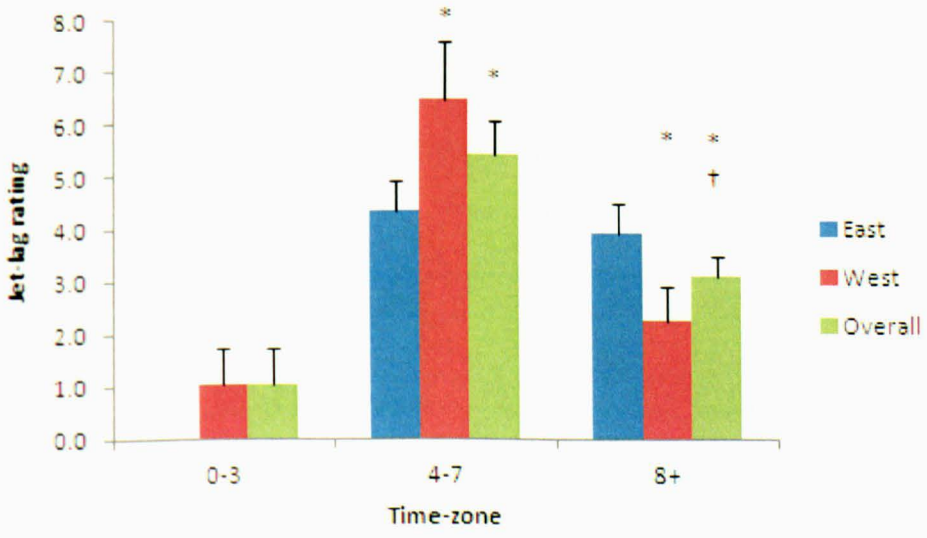

Figure 8.1: Mean±SD jet-lag ratings over the number of time-zones crossed. * Denotes statistically significant difference from preceding measure. † Denotes statistically significant difference between ≤3 and ≥8 zones.

8.3.2 Function

Like the overall jet-lag ratings, functioning symptoms followed an inverted U profile, albeit less pronounced (see Figure 8.2). For 4-7 vs. ≥8 zones crossed the trend was similar as that observed for jet-lag but was not statistically significant (difference = 2.4 ± 1.4 CI: -0.5 to 5.3, p = 0.10); the effect remained significant between 4-7 vs. ≤3 zones crossed (difference = 4.7 ± 1.8 CI: 1.1 to 8.3, p = 0.01). There were negligible effects of travel direction (p = 0.89), although the "inverted U" profile was more prominent after westward vs. eastward travel; jet-lag being worse after travel over 4-7 zones vs. ≤3 zones westward (difference = 6.7 ± 2.4 CI: 1.8 to 11.7, p = 0.009) and 4-7 vs. ≥8 westward (difference = 5.0 ± 2.4 CI: 0.1 to 10.0, p = 0.045). There were no significant differences when comparing results of individual time-zone groups with direction of travel.

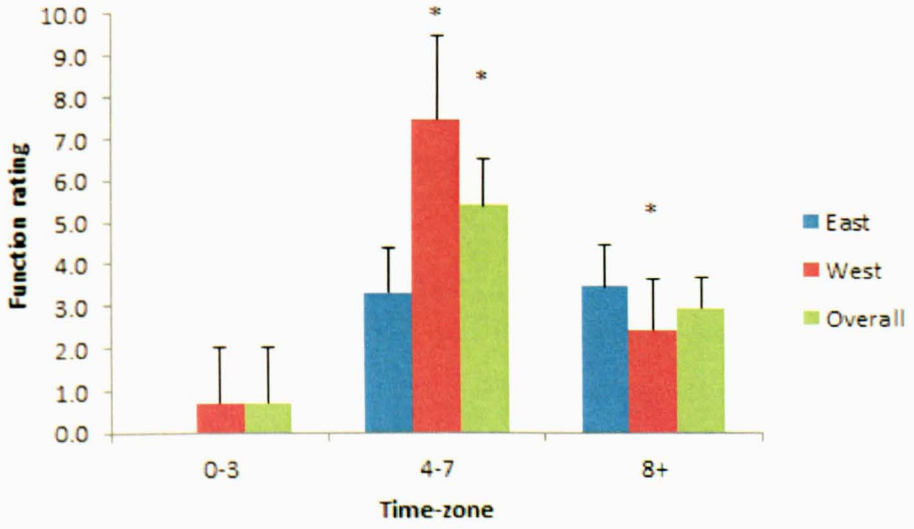

Figure 8.2: Mean±SD jet-lag ratings over the number of time-zones crossed. * Denotes statistically significant difference from preceding measure.

8.3.3 Sleep

As with the previous two variables, perceived sleep symptoms immediately after waking followed an inverted U profile (see Figure 8.3). Symptoms were significantly worse 4-7 vs. ≤3 zones crossed (difference = 7.1 ± 2.5 CI: 2.1 to 12.1, p = 0.006) and were slightly greater than the alpha level of significance for 4-7 vs. ≥8 zones (difference = 3.8 ± 2.0 CI: 0.2 to 7.8, p = 0.062). The differences after westward travel were both significantly lower for ≤3 (difference = 9.0 ± 3.4 CI: 2.1 to 15.8, p = 0.012) and ≥8 (difference = 8.4 ± 3.4 CI: 1.6 to 15.3, p = 0.017) zones crossed compared with 4-7. There was evidence that eastward travel resulted in worse sleep, although this was not statistically significant (difference = 2.5 ± 1.6 CI: 0.7 to 5.7, p = 0.118). Symptoms were worse after travelling 8 or more times-zones east than west (difference = 5.6 ± 2.2 CI: 1.1 to 10.1, p = 0.015).

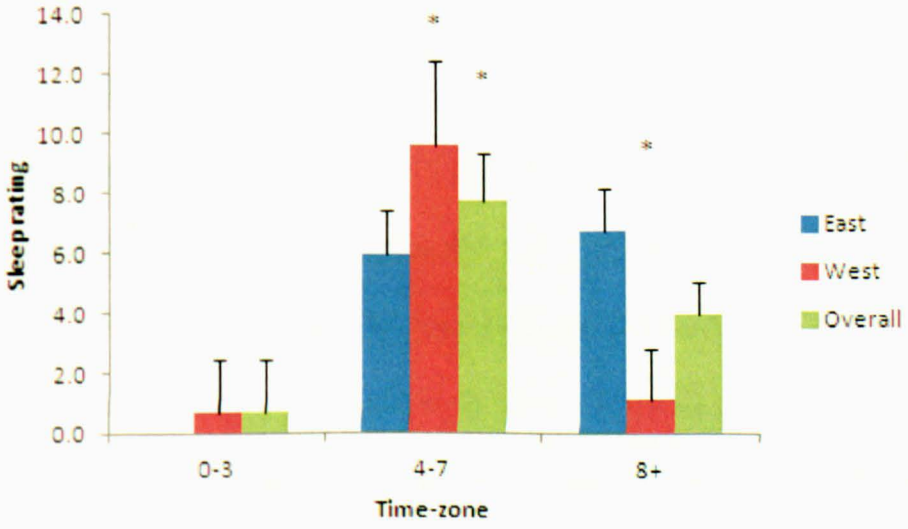

Figure 8.3: Mean±SD sleep ratings over the number of time-zones crossed. * Denotes statistically significant difference from preceding measure.

8.3.4 Diet- and Bowel-related symptoms

Neither diet- nor bowel-related perceived ratings showed any significant effects of travel direction, number of time-zones crossed or interaction between these variables.

8.4 Discussion

The major finding from the present descriptive study was the inverted-U relationship between the number of time zones crossed and the severity of perceived jet-lag, function- and sleep-related symptoms. Specifically, the data suggest that crossing over 4-7 time-zones results in greater perceptions of 'jet-lag' symptoms than travelling over ≤3 zones or ≥8 zones. Although unexpected, this finding illustrates the complexity of jet-lag perception, which may vary not completely in parallel with the magnitude of disturbance of the human circadian clock. Other important factors are probably the time of day of arrival in the new time zone and the more positive effects of travel fatigue on other symptoms of jet-lag, especially those related to sleep on the first night after arrival.

The inverted U relationship between number of time zones crossed and severity of jet-lag symptoms challenges the notion that the more time zones that are crossed the more severe the jet-lag. This is an assumption in the literature which is mainly gleaned from laboratory-based studies and "single-trip" type studies in the field (e.g. Waterhouse et al., 2007, Sack, 2010, Herxheimer and Waterhouse, 2003). Because a disruption to the body clock is considered the underlying causality of jet-lag, it is intuitive to think that the further the distance travelled from 'home' the greater the jet-lag suffered. In agreement with this notion, the mildest jet-lag symptoms were recorded following travel over ≤3 zones. The contradictory data, 4-7 vs. ≥8 zones, is supported somewhat by the previous *Chapter 7* where no difference in jet-lag ratings were reported between individuals who

had crossed 5 zones and those who crossed 8; the control group data is included in the current study analysis. These findings suggest that, at least initially, for those who are suffering any noticeable jet-lag that the effects are of similar magnitude and independent of the number of time-zones crossed. This finding is novel and emphasises the need of real-world studies to examine a time-zone dependent response to jet-lag, if one exists. However, it should be noted that the results observed in the present study are relatively acute and the longer lasting effect in this sample are unknown due to lack of long term follow-up.

Although no formal correlation analysis was performed it would appear from the parallel nature of the time course in symptoms that the constructs of sleep and function are the most influential to the overall changes in jet-lag. As with the rating of jet-lag an inverted U profile was observed; whereas, in diet and bowel-related variables there were no significant changes with the number of time-zones crossed or the direction of travel. This indicates that these later variables may be poor markers of overall perceived jet-lag. However, jet-lag appears to be very heterogeneous in its nature with perception in the severity and components varying between individuals. This is compounded further by a lack of understanding of what jet-lag actually is and whether, for example, being sleepy is associated with jet-lag *per se* or other factors such as travel fatigue or boredom. As mentioned previously the reported effects on sleep and function from air travel were most directly related to that of jet-lag. These findings match those of Waterhouse et al. (2000) who reported perceived jet-lag at any time is predicted by the amount of fatigue at that time (increased fatigue predicting more jet-lag); the amount of jetlag in the morning is predicted by the time of waking from sleep (earlier times predicting more jet-lag) and by a decreased alertness 30 min after waking; and the amount of jet-lag in the

daytime is predicted by the fall in the perceived ability to concentrate. In a further study Waterhouse and colleagues (2005b) reported that falls in alertness and motivation were accurate predictors of increased jet-lag when measured at the same time. Moreover, Lowden and Akerstedt (1998) concluded that jet-lag was most closely related to the amount of sleepiness and the number of awakenings during sleep.

There was evidence that eastward travel was worse than westward for overall jet-lag and sleep, although neither effect reached statistical significance. Nevertheless, phase advancing, as required following eastward, is considered more difficult than delaying the body clock due to the intrinsic length of tau (Aschoff, 1981). This is further compounded by an increased inability for individuals to initiate sleep following travel to the east (Takahashi et al., 2002), an example is provided below. This often means that the recovery from jet-lag is prolonged after eastward travel with the circadian pacemaker resetting at an average of 57 minutes earlier per day compared with 92 minutes later per day following westward travel (Ashcoff, 1975). However, it should be noted that this direction effect wasn't evident for all the variables and it appears that functioning is affected at the same rate independent of travel direction. A possible rationale for why sleep variables are affected less proceeding westward travel could be due to the time of arrival and an associated increase in the homeostatic drive for sleep. For example, flying over 6 time-zones westward and arriving at 16:00-h local time would result in the body clock oscillating at time representative of 22:00-h, which would result in an enhanced need for sleep at night in the new environment. Conversely, this increases the likelihood of waking early from sleep. The use of hypnotics may counter this issue; however, this could lead to increased daytime sleepiness and off-set any jet-lag alleviation methods. The opposite is observed for eastward travel with poor sleep during the new night-time,

including delayed sleep onset, often reported (Waterhouse et al., 2007). Moreover, the issue of sleep in the new environment can be compounded further by inappropriate use of sleeping tablets and napping during the flight at times which are counterproductive to realignment of circadian phase.

As it stands this study has a small sample size. This is highlighted by no participants in the ≤3 category travelling eastward, meaning that analysis is incomplete. Further participants would enable results to be generalised and may allow for the effects of other parameters on jet-lag to be explored such as gender, age and ethnicity. Due to the inherent nature of this study there is a lack of control and the researcher is reliant on the participants' honesty in the completion of the questionnaire. Furthermore, the observation period may need to be extended to get a full understanding of the jet-lag time course.

In summary, from the data presented it appears that the *Liverpool jet-lag questionnaire* is sensitive to changes in time-zone and direction of travel, which on the most part, are consistent with the current understanding of how these factors influence the severity of jet-lag. In the current sample the constructs of sleep and functioning are most predictive of overall jet-lag whereas diet and bowel symptoms provide insufficient information. Identifying effective methods for measuring jet-lag, especially in a field setting, remains an issue and more sensitive models may need to be developed, which in-turn could enhance the reliability when measuring alleviation techniques.

CHAPTER 9

SYNTHESIS OF FINDINGS

9.1 Overview

The research described in the present thesis was designed to investigate the effects of phototherapy on aspects related to sport and exercise; specifically how light can be used to improve physical and cognitive performance and to establish its effectiveness in ameliorating jet-lag. Specifically, these studies examined the responses of the circadian system following light exposure to adjust and realign biological rhythms, respectively; with the objective of optimising physical and cognitive performance in situations which enervate human functioning (e.g. extreme hyperthermia, sleep inertia and jet-lag). Chapter 4 aimed to assess the acute effects of bright light exposure on human physiology and to validate the lighting protocol utilised by comparing the results with published data. Chapters 5 and 6 were completed in laboratory settings where conditions and exposure to competing zeitgeibers could be controlled. These studies explored how phototherapy, through different delivery strategies, can be used to facilitate improvements in performance. Following laboratory studies, a field study was conducted to observe the effects of phototherapy on jet-lag alleviation in world-class athletes, similar physiological measurements were taken in all studies as markers of the body clock, with measures of physical and cognitive collected using various tools and methods.

9.2 Major Findings

Although involving different conditions and protocols, the studies in *Chapters 5* and *6* demonstrated that phototherapy, both in terms of chronobiological and acute effects, can enhance human performance. In *Chapter 5* the use of 30 minutes of bright light prior to habitual nocturnal sleep delayed the circadian rhythm of T_c, which in turn led to a lower temperature at waking. During subsequent exercise in hot conditions, 10-km

cycling time trial performance was improved compared with a no light control condition. The difference in T_c between conditions (0.27°C) prior to commencing the time-trial did not quite reach statistical significance ($p = 0.07$) but was practically meaningful compared with other methods of pre-cooling.

In *Chapter 6*, the more acute effects of light were investigated and a controlled protocol of gradual illumination 30 minutes prior to waking was found to reduce the subjective and objective severity of sleep inertia. Human functioning, as indexed by cognitive and physical performance, significantly improved over the duration of the testing period in both the control and intervention conditions; however, improvements in these variables occurred more rapidly in the dawn simulation condition. Given these results and the desire for "marginal gains" (Stewart and Hopkins, 2000, Atkinson, 2003) in sport, it would not be unreasonable to suggest that phototherapy could be used as an ergogenic aid, in terms of both its phase-delaying properties when administered in the evening and its general more acute effects on human alertness and performance following a period of sleep.

Contrary to the findings from the laboratory-based studies, in *Chapter 7*, a supplementary light intervention was not found to have any substantial effects on the alleviation of jet lag symptoms. A chronobiologically timed intervention did not accelerate the realignment of the body clock over the first 4 post-flight days nor did it aid in reducing the subjective ratings of jet-lag symptoms vs. a comparator group. These results highlight the complex nature of jet-lag, especially the within- and between-subject variability in symptoms making the detection of any effects difficult amongst the background of noise in the real world. In *Chapter 8*, these issues were further explored. Surprisingly the jet-lag

multivariate symptoms reported by individuals whom had travelled over a range of time-zones and in differing directions did not produce the results expected from information derived from published laboratory-based simulations (Paul et al., 2009, Boivin and James, 2002). Taken together these studies demonstrate that our understanding of jet-lag clearly has a sound scientific basis (derived from laboratory studies); however, knowledge about the effectiveness of jet-lag interventions is modest when competing synchronisers are present in the "real world" and individuals' perceptions are not considered.

In *Chapter 4*, it was found that the circadian system and other areas of the brain which are stimulated by light are still sensitive to even relatively short duration exposures. There was some evidence to suggest that these responses were innervated to a greater extent via light which contains blue photons. Furthermore, it appears from the data that participants knowing that they are about to receive bright light produces an anticipatory effect, similar to that seen in the reduction in BP prior to napping (Zaregarizi et al., 2007), which induces a shift from the set-point in the measured variables.

9.3 General Discussion

Effective techniques for enhancing human functioning and performance are highly desired in the world of sports competition and in the ergonomics of performance in occupational contexts. Theoretically, manipulation of the circadian system, via the potent stimulus of bright light, is one method by which this can be obtained. In *Chapter 5* and *Chapter 6* it was demonstrated that phototherapy was indeed efficacious in enhancing physical and cognitive performance.

In *Chapter 5*, it was found that a chronobiological effect of light reduced T_c prior to exercise in the heat and improved physical performance. This improvement may have been mediated by a phase delay in T_c produced by evening exposure to ocular bright light. Prior to the present thesis, only one other study had investigated the effects of light on the circadian rhythm of T_c and physical outcomes (Atkinson et al., 2008a). Although evening bright light facilitated a phase delay and a trend towards lower RPE during subsequent exercise, no actual measures of performance were obtained in this study. Interestingly, in *Chapter 5* no difference in RPE was noted, however, a significant improvement in performance in the evening bright light condition was observed. This combined finding suggests that the participants chose a higher power output for the same level of perceived exertion, which was translated to an improved time to completion over a 10-km time-trial. Mechanistic rationale for this thermal-related reduction in fatigue include attenuating the decline in cardiac output and enhanced homeostatic regulation of brain temperature (Nybo, 2012), hydration status, metabolic and/or central nervous system control (Hargreaves, 2008). However, deducing which mechanism(s) is/are responsible for the delaying the onset of hyperthermia-induced fatigue is unattainable from the present studies. Although, from the data presented it appears that evening bright light could be used to lower pre-exercise temperature in humans.

Pre-cooling strategies have been adopted in sporting situations and occupational settings with the aim of reducing T_c when the body is likely to be subjected to thermal stress. Passive methods such as cold water immersion, ice jackets and resting in hypothermic conditions as well as pharmacological interventions (e.g. melatonin) and the consumption

of cold drinks have been investigated. For example, 60 minutes of cold water immersion was shown to reduce T_c by 0.7°C compared with a control condition which in turn extended the distance covered during 30 min of self-paced treadmill running by ~300 m (Booth et al., 1997). Furthermore, Siegel et al. (2012) reported that exercise tolerance time whilst running in hot conditions was significantly increased following cold water immersion and ice slurry ingestion by approximately 10 and 6 minutes, respectively, compared with hot water ingestion. However, reporting times to exhaustion is not directly transferable to actual sport performance. Moreover, such techniques may well be uncomfortable for the participant and require considerable time for a significant reduction in body temperature to be achieved (Drust et al., 2000, Marino, 2002). Exogenous melatonin is a method which could be beneficial, however the results are equivocal. Atkinson et al (2005a) reported that a 2.5 mg dose of melatonin ingested 75-min prior to a bout of intermittent exercise attenuated the rise in T_c during exercise, however, no performance outcomes were measured. McLellan et al. (2000) utilised higher doses of 5 mg melatonin during low exercise intensities. Melatonin resulted in a decrease in T_c whilst resting in equable conditions (23°C) and during the first 50 minutes of exercise but this did not translate into improvements in exercise tolerance time.

In Chapter 6, the thesis progressed to demonstrate that phototherapy, through dawn simulation, was effective in reducing the transient state of sleep inertia and improved subsequent cognitive and physical performance. Once again the mechanisms underpinning these results are not fully realised from the present study. A significant decrease in melatonin 15- and 30-mins post-waking was observed during the intervention condition. Due to the innate soporific nature of melatonin, such a reduction may have eased the difficulty in awakening. However, this did not translate into any significant

changes in T_{sk} or T_c, which is surprising since these measures often vary in parallel. Van De Werken et al (2010) hypothesised that the dissipation of sleep inertia following dawn simulation involves light sleep and an accelerated skin temperature decline after awakening. However, monitoring sleep stage or depth via EEG was not conducted nor was any significant effect on skin temperature observed. Another potential mechanism for increased arousal following dawn simulation is a superior increase in cortisol compared with a control condition (Thorn and Hucklebridge, 2004), although such findings have disputed (Van De Werken et al., 2010).

The master pacemaker, which orchestrates rhythmicity in surrounding and peripheral cells and organs, is highly sensitive to bright light exposure. Therefore, the results from *Chapters 5* and *6* are not surprising. However, the positive outcomes in performance and arousal in the laboratory-based studies did not translate into any alleviation of jet-lag by phototherapy. A "timed" intervention, informed via chronobiological principles, was ineffective at substantially reducing subjective symptoms of jet-lag. Although similar findings have been reported in other real-world studies (Lahti et al., 2007, Boulos et al., 2002), data from laboratory-based experiments suggest that bright light should be an effective countermeasure for jet-lag. This disparity between field and laboratory studies can be explained by the fact that jet-lag is largely a subjective collection of a wide range of different symptoms which may not vary completely in parallel with changes in circadian phase during adjustment to a new time zone. This classic case of an intervention having efficacy but uncertain effectiveness in the real world is a common problem in exercise science research (Flay et al., 2005). An interesting observation in *Chapter 7* was that light mediated an acute increase in body temperature, when measured immediately after each light exposure, but this did not translate to any positive effects on jet-lag

symptoms. *Chapter 8* revealed further complexities when assessing jet-lag, with deviations from traditional theories with certain aspects of the results. The inverted U relationship between jet-lag and other symptoms such as function- and sleep-related constructs for the number of time-zones crossed contextualises this issue.

Together, these data highlight the need to further our understanding of jet-lag in a real-world setting. It would be beneficial for sport teams and other groups who may require optimal performance soon after air travel to appreciate an individual's phase (i.e. chronotype) and how susceptible they are to jet-lag. Such knowledge may allow for individual programmes to be developed which may enhance the alleviation of jet-lag. However, such courses of action may require considerable time and cost meaning that they are unlikely to be undertaken.

Along with chronic (phase-shifting) effects, light exposure is also capable of producing acute changes in bodily processes. In *Chapter 4* it was demonstrated that the various aspects of physiology are rapidly altered during short duration bright light exposure. There was evidence to suggest that melatonin and T_c were also manipulated by light and that all responses were affected by the characteristics of the stimulus (i.e. wavelength). The effects of light on melatonin and T_c are well documented and the results presented here although not significant follow the respective trends of suppression and augmentation. . It is also evident that these acute responses are required prerequisites for phase shifting to effects to be realised.

Figure 9.1 is a schematic representation of the findings summarised within the present chapter, which has been developed from Figure 2.1. The schematic shows the interaction

between acute and chronic effects and the positive results produced in both cognitive and physical performance. Furthermore, it highlights the confounding factors that may have influenced the results.

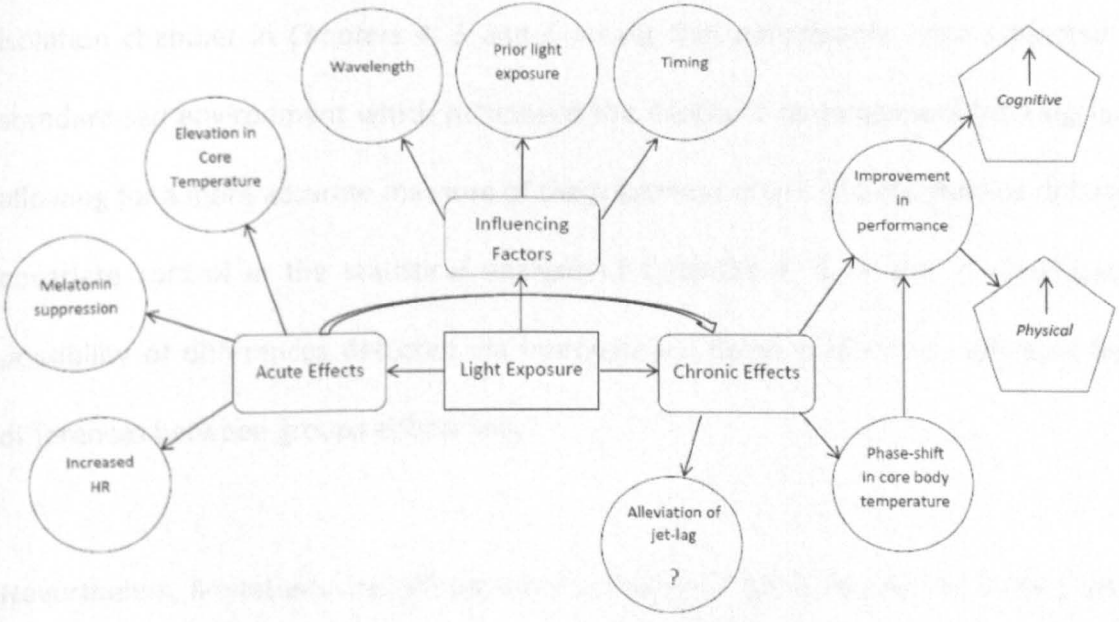

Figure 9.1: Schematic representation of the major findings presented within the present thesis.

9.4 Methodological consideration and limitations

There are several noteworthy strengths in the methods of the current thesis. Firstly, the use of time-trials oppose to time to exhaustion as a measure of physical performance allows for the results to be compared in actual sporting events. Secondly, the use of an isolation chamber in *Chapters 4, 5* and *6* meant that participants were subjected to a standardised environment which minimised the exposure to exogenous masking factors allowing for a more accurate measure of the treatment effect. Thirdly, the use of baseline covariate control in the statistical analysis of *Chapters 4, 5, 6* and *7* eliminates the possibility of differences detected via interventions being masked or enhanced by the differences between groups at baseline.

Nevertheless, limitations are still apparent within this thesis. In *Chapter 7* the timing of the treatment period was scheduled in line with suggestions from previous reviews (Waterhouse et al., 2007). This meant that the treatment group were exposed to the light box during day light hours, during which time the control group had the option to be exposed to the natural light, although they were asked, if possible, not to go outdoors. Also, restricting individuals to isolated dark conditions, such as the controls during the intervention period, is not realistic to the usual field setting; therefore, a true indication of the effects of supplementary bright light on jet-lag would not be ascertained. Furthermore, the use of one light box between two individuals is not ideal; however, logistic restrictions meant that this was inevitable. In *Chapter 8* the sample size is inadequate to generalise the results to the whole population. Data collection is planned beyond this thesis to allow results to be explored in-depth and perhaps a model of jet-lag and its recovery to be formulated. Furthermore, the observation period may need to be extended to get a full understanding of the jet-lag time course.

In *Chapter 6* EEG was not adopted as a measure. This would have allowed for the sleep stage at waking to be deduced, which could explain the differences in sleep inertia at waking. Moreover, it would have allowed quantification of whether the participant was actually asleep during the intervention phase prior to waking. Although the actiwatch data shows no significant difference between conditions, the variables measured are indirect and not as accurate as those produced by EEG. Finally, the issue of blinding participants to interventions during light exposure remains problematic. In dawn simulation studies this could be masked to an extent by using rapid vs. gradual increase in illuminanace, although participants would still be exposed to some degree of light. In studies utilising light boxes a true "control" is difficult to ascertain as well as whether bright light exposure alone is enough to produce changes in performance (i.e. a hawthorn effect).

Placebo effects have long been recognised as an issue in all types of research and have led to the development of sophisticated research designs in an attempt to minimise or eliminate erroneous results. A recent systematic review (Beedie and Foad, 2009) on this topic relating to sport performance reported that administrating a placebo manipulated performance by -1.9% to 50.7% compared with baseline/control data, with the magnitude of the effect usually falling between 1-5%. Therefore, as no true placebo was presented in any of the present intervention studies, the results might be affected. This issue is much discussed within the bright light literature (reference??) and presents a major on-going hurdle in the attempt to produce accurate and reliable results.

Comparing data collected between studies is compounded by the use of different methods of measurement for the same variable. For example, both intestinal and intra-

aural temperature act as surrogate measures for core body temperature within the present thesis. Although the results were not directly compared the ability to do so is limited. A short study to determine the variability between the two methods would negate this issue and allow for reliable comparisons between data. In addition the method of saliva collection undertaken in *Chapters 4, 5 and 6* has not been validated against the manufactures guidelines. Although internal validity was maintained as the collection method used was closely controlled.

9.5 Directions for future research

There are several potential areas of future research that have emerged from the studies reported in this thesis. These are primarily concerned with unravelling the physiological mechanisms pertaining to the interactions between bright light exposure and performance manipulation. Additionally, further work on jet-lag alleviation techniques is required in an applied setting, however, prior to this work methodologically sound and valid techniques may need to be developed.

Firstly, a consensus within the literature needs to be reached on what lighting protocol stimulates the optimal response from the circadian system whilst still been viable for a participant to complete on a regular basis (i.e. not excessively time consuming). Components that need to be assessed, along with duration, are intensity, spectral composition and whether prior dark episodes are a worthwhile addition in light protocols (i.e. is the treatment effect greater after a dark episode or is this time better spent in light exposure?).

Due to the heterogeneous nature of jet-lag developing techniques to aid with its alleviation is difficult. Future research should initially develop a protocol within a laboratory setting, to assess efficacy. Once this is established work in the field can be undertaken using the same model, this would allow for direct comparisons to be made between laboratory findings and real-world effectiveness. Furthermore, in the field to get a true understanding of circadian phase; temperature should be monitored at regular intervals, this would enhance profiling and increase the ability to deduce specific within measure variables such as acrophase, amplitude and mesor values. Secondly, a greater range of performance measures (e.g. reaction time tests), including pre-flight baseline measures, would allow for a greater understanding of how jet-lag exerts its effects and how, if at all, supplementary bright light and/or other interventions effect these variables.

Finally, for the results found in *Chapter 5* and *Chapter 6* to be endorsed as credible interventions for elite sports performance further data needs to be collected using professional athletes. Thus, research investigating phototherapy and its effects on performance need to be assessed in specific target populations.

CHAPTER 10

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