Examining the Interplay of Sleep, Exercise, and Nutrition

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Examining the Interplay of Sleep, Exercise, and Nutrition

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Statement of Authorship and Sources

This thesis contains no material that has been extracted in whole or in part from a thesis that I have submitted towards the award of any other degree or diploma in any other tertiary institution.

No other person's work has been used without due acknowledgement in the main text of the thesis.

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players: tests adopted, characteristics reported and recommendations for practice. *Sports Medicine*, *52*(7), 1491-1532.

7. Weakley, J., Munteanu, G., Cowley, N., Johnston, R., **Morrison, M.**, Gardiner, C., ... & García-Ramos, A. (2023). The Criterion Validity and Between-Day Reliability of the Perch for Measuring Barbell Velocity During Commonly Used Resistance Training Exercises. *Journal of Strength and Conditioning Research*, *37*(4), 787-792.
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List of abbreviations used within this thesis

AASM – American Academy of Sleep Medicine

ALS – autophagy lysosome system

AMPK – AMP-dependent protein kinase

BDNF – brain-derived neurotrophic factor

BMAL1 – brain and muscle ant-like protein-1

CBT – Core Body Temperature

CLOCK – circadian locomotor output cycles kaput

CRY - cryptochrome

CV – coefficient of variation

DLMO – Dim-light melatonin onset

DNA – deoxyribonucleic acid

EEG – electroencephalogram

EMG – electromyography

ERK – extracellular signal-regulated kinase

FNDC5 – fibronectin type III domain containing 5

FOX – forkhead box

FSR – fractional synthetic rate

GABA – gamma-aminobutyric acid

GH – growth hormone

HIF-1α – hypoxia-inducible factor 1α

HPA – hypothalamic-pituitary-adrenal

IGF-1 – insulin-like growth factor one

IL-1α – interleukin-1α

IL-1β – interleukin-1β

IL-6 – interleukin-6

IQR – interquartile range

IRR – inter-rater reliability

KSS – Karolinska Sleepiness Scale

MCMC – Markov Chain Monte Carlo

MCTQ – Munich Chronotype Questionnaire

MEQ – Horne-Ostberg's Morningness-Eveningness Questionnaire

MPS – muscle protein synthesis

MPB – muscle protein breakdown

mTOR – mammalian target of rapamycin

NREM – non-rapid eye movement

PER – period

PGC-1α – peroxisome proliferator-activated receptor *y* coactivator 1α

PPARδ – proliferator-activated receptor δ

PPG – photoplethysmography

PSG – polysomnography

PSQI – Pittsburgh Sleep Quality Index

PVT – psychomotor vigilance task

REM – rapid eye movement

ROL – REM onset latency

RPE – Rating of Perceived Effort

SCN – suprachiasmatic nucleus

SOL – Sleep onset latency

SpO_2 – peripheral blood oxygen saturation

SQSTM1 – p62/sequestosome-1

SWS – slow wave sleep

TIB – time in bed

TNF-α – tumour necrosis factor alpha

TST – total sleep time

UPS – ubiquitin proteasome system

VAS – Visual Analogue Scale

WASO – Wake after sleep onset

Thesis abstract

Sleep is a vital component of health. When sufficient sleep durations are not obtained, health may be negatively affected in numerous ways, including an increased propensity for metabolic disease, cardiovascular disease, decreases in mood and cognitive performance, and reductions in physical performance. With approximately 30% of adults not achieving sufficient sleep durations (7h – 9h), developing and implementing effective strategies to augment sleep is prudent. Therefore, the overall aims of this thesis are to: 1) review the relationship between sleep, circadian biology, skeletal muscle, and metabolic health; 2) investigate how exercise and nutritional interventions interact with subsequent sleep; 3) investigate whether exercise-induced peripheral factors are potential mechanisms for changes in sleep observed after exercise; 4) determine the efficacy of a pre-sleep nutritional intervention for improving sleep outcomes, and next-morning cognitive function or physical performance.

The aim of study one was to review the literature pertaining to the relationship between sleep, circadian biology, and skeletal muscle. From this review, it became clear that insufficient sleep has a negative effect on metabolic health, with adverse effects observed on various cells, tissues, and organs. However, the underpinning mechanisms of these deleterious effects are often complex and not well understood. Additionally, exercise and nutrition emerged as potential strategies that show promise in mitigating some of the negative health consequences resulting from insufficient sleep durations.

The aim of study two was to investigate the effect of afternoon moderate-intensity (70% heart rate maximum) cycling exercise on objective and subjective sleep in healthy adult males. No statistically significant differences were observed in objective or subjective sleep quality or quantity assessed via polysomnography and subjective sleep questionnaires after exercise compared to no exercise. Wide inter-quartile ranges were observed for total sleep time (exercise: 51.50 min vs no exercise: 13.38 min) and sleep efficiency (exercise: 9.53% vs no exercise: 2.45%) which suggest there was more individual variability in subsequent sleep responses after afternoon exercise compared to no exercise. Consequently, healthy adult males can complete afternoon moderate-intensity exercise without compromising their subsequent sleep, but it is important to acknowledge and consider individual responses in sleep outcomes after exercise.

Study three explored components of a novel hypothesis suggesting exercise-induced peripheral factors may be potential mechanisms that explain exercise-induced changes in subsequent sleep. The aims of study three were: 1) to determine the effect of afternoon, moderate-intensity exercise (70% heart rate maximum) on metabolic factors suggested to be associated with sleep, tumour necrosis factor alpha (TNF-α), interleukin-1α, interleukin-1β, interleukin-6 (IL-6), and brain-derived neurotrophic factor (BDNF); and 2) to investigate the relationship between exercise-induced peripheral factors and subsequent sleep in healthy, adult males measured using polysomnography. There was no effect of exercise on BDNF, interleukin-1α, and interleukin-1β, but a small amount of evidence for exercise increasing IL-6 (posterior probability = 0.650) and TNF-α (posterior probability = 0.866). Additionally, there were no clear relationships between TNF-α, interleukin-1α, interleukin-1β, IL-6, and BDNF levels measured at bedtime and sleep. Consequently, our findings do not support components of the proposed hypothesis that exercise-induced peripheral factors explain changes in sleep after exercise.

Study four extended the findings of study two and three by examining the effects of exercise performed in conjunction with a pre-sleep nutritional intervention on sleep. The aim of study four was to determine the effect two different doses of a formulated nutritional intervention, compared against a volume and colour-matched placebo consisting of non-nutritive sweetener, on objective and subjective sleep and next-morning physical performance (measure using a 10-minute maximal effort cycling time trial), cognitive function (measured using the psychomotor vigilance task), and balance (measured using a force plate) in trained adult males. The nutritional interventions consisted of high glycaemic index carbohydrate, whey, tryptophan, theanine, and 5'AMP. No improvements in objective or subjective sleep were observed with either dose of the nutritional intervention. Some aspects of sleep appeared to be affected by the supplement, the low-dose appeared to reduce N3 sleep duration compared to placebo (-13.6 min) and reduce the number of arousals experienced during non-rapid eye movement sleep (-7.6 count). The high-dose intervention appeared to increase N1 sleep duration compared to placebo (+7.4 min). No differences in subjective sleep, physical performance, cognitive function, or postural sway were observed after either dose. Whilst some components of sleep appeared to be negatively influenced by the

interventions, the magnitude of changes observed were not likely to cause any meaningful reductions in sleep quality and quantity.

This thesis has contributed new knowledge elucidating the relationship between sleep, exercise, and nutrition. Four main outcomes were identified from this program of research: 1) sleep is consistent when measured using gold-standard techniques in highly controlled environments; 2) individual sleep responses after exercise vary but afternoon exercise does not appear to interrupt sleep; 3) certain peripheral exercise-induced metabolic factors may not be the underlying mechanism for changes in sleep following exercise; and 4) a proprietary nutritional intervention consumed in conjunction with afternoon exercise does not enhance sleep. These findings highlight the importance of considering an individualised approach to developing interventions to augment sleep and health.

Chapter 1: Introduction

Sleep is essential for psychological and physiological restoration and recuperation from activities of daily living (1-4). Subsequently, the National Sleep Foundation recommend adults achieve seven to nine hours of sleep per night to maintain health (5). However, only 20-45% of adults are currently achieving the recommended sleep durations (6, 7). This sleep loss epidemic may in part be exacerbated by the modern 21st century lifestyle, with excess caffeine consumption, increases in screen time, and round-the-clock access to light and energy dense food all posing challenges to obtaining sufficient sleep (8, 9).

Insufficient sleep durations can cause a myriad of deleterious effects on the body. Acute bouts of insufficient sleep have been shown to have a detrimental effect on metabolic, cognitive, psychological, and physiological processes (10, 11). For instance, disrupted periods of sleep (i.e., five nights of four hours of sleep) have been shown to disrupt skeletal muscle metabolism (12). Chronic periods of insufficient sleep (i.e., > 1 – 2 weeks) may also incur negative health consequences. For example, extended periods of insufficient sleep promote an environment that may facilitate an increased risk of metabolic conditions such as diabetes and obesity, due to alterations in glucose kinetics, changes in appetite, and a reduction in net energy expenditure (13). To counteract the negative effects of insufficient sleep durations, strategies such as pharmacological interventions (14), sleep hygiene strategies (15), pre-sleep nutritional interventions (16), and physical activity and exercise (17) are often implemented in an attempt to enhance sleep.

Sleep hygiene recommendations commonly suggest that exercise should not be performed in close proximity to bedtime (e.g., 90 mins before bedtime), as exercise can disrupt sleep (15). To date, research has resulted in equivocal findings regarding this commonly held opinion (18-20), with exercise in close-proximity to bedtime resulting in improved sleep (18), no changes to sleep (19), or impaired sleep (20). For instance, 40 minutes of high-intensity running exercise performed between 21:20h and 22:00h (with a bedtime of 23:00h), delayed sleep onset (14 mins, $p < 0.05$), increased HR (25.7 bpm, $p < 0.01$), and a lower high-frequency heart rate variability (-590 ms^2, $p < 0.01$) compared to rest or moderate-intensity running (20). Whereas, moderate-intensity resistance training and

aerobic exercise that finished 90 minutes before bedtime had no influence on sleep (19). In contrast, a quantitative synthesis of evidence identified high-intensity exercise ending 30 minutes to 4h before bedtime decreased rapid eye movement sleep by 2.34% ($p = 0.002$) compared to no exercise (18). Whilst exercise performed in the evening has equivocal results on sleep, determining how exercise performed further from bedtime effects sleep is largely unknown and given the prevalence of afternoon exercise, is prudent to examine.

When high-intensity exercise is performed 2h – 4h from bedtime, sleep is not disrupted in health adults (18). However, the relationship between sleep and exercise is complex, and improvements in sleep as a result of exercise are likely multifaceted (17, 21). The beneficial effects of exercise on sleep appear to be modulated by a range of factors. These include individual characteristics such as age, sex, and fitness level, but also the mode and intensity of exercise, whether the stimulus is aerobic or anaerobic, the time of day, environment the task is performed in, and if the exercise is completed over an acute (e.g., someone who does not normally exercise) or chronic (e.g., someone who exercises regularly) manner (17). After acute bouts of exercise, improvements in total sleep time (TST), slow wave sleep (SWS), and rapid eye movement onset latency (ROL) have been reported (21, 22). Additionally, periods of chronic exercise have been associated with increased SWS and TST, decreased REM sleep, sleep onset latency (SOL), and wake after sleep onset (WASO) in people with regular sleep/wake patterns (22). However, contrasting findings have also been reported, with acute bouts of evening resistance and aerobic exercise being shown to have no effect on sleep architecture in healthy adult males (19). The inconsistent findings emphasise the benefits of exercise on sleep are multifactorial and influenced by numerous moderating factors. Therefore, the mechanisms that underpin improvements in sleep warrant investigation to establish appropriate interventions and guidelines to improve sleep-wake behaviours through exercise.

The relationship between sleep and exercise has many complexities and intricacies. Consequently, understanding the mechanisms that underpin changes in sleep in response to an intervention is challenging. Although unclear, it is likely a combination of circadian rhythm, metabolic, immune, thermoregulatory, endocrine effects influence sleep-wake behaviour (17). As such, there are several hypotheses that attempt to explain the mechanism by which exercise enhances sleep. Three commonly reported hypotheses include improved sleep via exercise-induced changes in thermoregulation, energy

conservation, and body restoration (23). However, recently, the notion of exercise-induced peripheral factors as a potential catalyst for improved sleep has been suggested (24). Rodent models have shown the potential for peripheral factors such as irisin, brain-derived neurotrophic factor (BDNF), peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α), tumor necrosis factor alpha (TNF- α), and interleukin-6 (IL-6) to improve sleep (24). Although, research supporting this hypothesis in humans is lacking.

Nutritional strategies are commonly employed as an alternative to pharmacological interventions to enhance sleep/wake patterns (16, 25). Often, ingredients such as melatonin, valerian, tryptophan, and high glycaemic index carbohydrates are consumed prior to sleep in an attempt to improve sleep outcomes (16). Numerous neurotransmitters, including gamma-aminobutyric acid (GABA), 5-HT, and melanin-concentrating hormone are involved in the sleep-wake cycle, and subsequently, are the target of nutritional interventions (16, 26). For example, levels of tryptophan in the brain are important for the regulation of 5-HT synthesis, which is a precursor to melatonin (27), which plays a critical role in the onset of sleep, by signalling a cascade of downstream responses that direct the body to initiate sleep (28). Whilst there is evidence to support the use of various non-pharmacological interventions to enhance sleep, the optimal dosage, timing, and combination of ingredients is unclear. Therefore, developing robust and effective guidelines for nutritional interventions to enhance sleep would be prudent.

The overall aim of this thesis is to investigate how exercise and nutritional interventions interact with subsequent sleep. The relationship between sleep, skeletal muscle, and circadian biology will be examined to determine how the interaction of these factors may influence metabolic and skeletal muscle health. Additionally, the effect of afternoon exercise on subsequent sleep will be determined. Furthermore, determining whether exercise-induced peripheral factors secreted from skeletal muscle during moderate-intensity aerobic exercise are a potential mechanism for improved sleep will be investigated. Finally, examining the efficacy of a pre-sleep nutritional intervention for improving sleep outcomes, without impairing next-morning cognitive function or physical performance will also be explored.

Chapter 2: Literature review

2.1 Sleep

Sleep is a complex phenomenon that must be undertaken by every living being (6, 29). Several physiological, psychological, and cognitive processes are regulated during periods of sleep (1, 9, 30-33). For instance, procedural and declarative memory consolidation appear to be facilitated during specific stages of sleep (33). The National Sleep Foundation recommends adults should attempt to achieve a sleep duration of seven to nine hours per night to maintain health (5). However, approximately a third of adults are not currently meeting these guidelines (34). Furthermore, a study of ~67,000 adults, highlighted that even when recommended sleep durations are being met, there is often a lack of sleep regularity, with only ~15% of participants sleeping between seven and nine hours at least five nights per week (34). Collectively, these findings highlight the prevalence of poor sleep/wake behaviours exist in our modern society.

The combination of insufficient sleep and sleep irregularity are concerning. When recommended sleep durations are not being met, a host of negative health consequences such as metabolic disease (35), cardiovascular disease (29), cerebrovascular disease (36), hypertension, (10) and an increase in all-cause mortality (29, 37) may arise. Likewise, sleep irregularity may manifest health concerns such as hypertension (38). Whilst the need to obtain sufficient durations of sleep is clear, it is apparent numerous populations such as shift-workers and athletes are not currently achieving these recommendations (6, 39). This may be due to the accessibility of technology that may disrupt sleep (e.g., mobile phones and tablets), societal expectations, and work demands, which are all pressures of the modern lifestyle (40, 41). Nonetheless, the prevalence of insufficient sleep quality and quantity is concerning and has resulted in current sleep/wake behaviours being labelled a ‘public health epidemic’ (10).

2.1.1 Sleep stages

2.1.1.1 REM sleep

There are two disparate stages that comprise sleep, rapid eye movement (REM), and non-rapid eye movement (NREM) sleep. Brain activity measured using electroencephalogram (EEG) during REM sleep identifies the presence of low-voltage, high-frequency beta brain

waves which occur with periods of rapid, saccadic eye-movements. The neuronal activity observed during REM sleep is similar to the activity observed during periods of wakefulness (42). Furthermore, the high levels of neurological activity that occurs during REM sleep is purported to facilitate dreaming (43). Additionally, muscle atonia occurs during REM sleep, and is interspersed with brief moments of muscle contraction and jerk-like movements. Thus, REM sleep is reflective of reduced muscle activity when compared to wakefulness when measured using electromyography (EMG). REM sleep is also suggested to be when various cognitive and emotional processes such as learning consolidation and emotional regulation occur (33, 44).

2.1.1.2 NREM sleep

During NREM sleep, the parasympathetic nervous system dominates autonomic nervous system activity. While in NREM sleep, there are three distinct stages that occur. Stage one (N1) is a transitional stage where alpha brain wave activity declines and low voltage, mixed frequency Theta waves are observed (45). There is an absence of rapid saccadic eye movement during N1, rather, slow rolling eye movements are often observed. Stage 2 (N2) also comprises low voltage but mixed frequency EEG activity (45). During N2 the appearance of sleep spindles, which are short periods of high frequency rhythmic activity lasting approximately half a second, are observed. Additionally, the emergence of K-complexes, which are low frequency high-voltage negative waves followed by positive waves, occurs during N2 (45). The slow rolling eye movements observed during N1 are no longer observed in N2. After sleep progresses from N2, stage 3 (N3) sleep occurs, and the onset of high magnitude, slow frequency, delta brain waves emerge. N3 sleep, is also commonly referred to as delta wave sleep or slow-wave sleep (SWS). Figure 2.1 shows an EEG trace of the neuronal activity observed during periods of wakefulness, NREM, and REM sleep.

2.1.1.3 Wake

Whilst it is clear there are differences between NREM and REM sleep, it is important to discern the differences between sleep and wake. During periods of wakefulness, there is often a high level of arousal, which exhibit low amplitude and high frequency EEG activity (46). Furthermore, the rhythmic or saccadic patterns of eye movement that are present whilst awake, are no longer observed and the ability to coordinate and initiate

movement return. During wakefulness, neuronal activity reflects the body's continual observation of information and detection of various stimuli from the environment (46). Periods of wakefulness typically coincide with the active (i.e., presence of light or daytime hours) phase of the light/dark cycle, while periods of sleep (i.e., rest) usually align with the dark (i.e., presence of darkness or nighttime hours) phase. However, there are often exceptions to this pattern, with activities like shift-work altering the alignment of the typical sleep/wake behaviours of the active/rest cycle toward the rest/active cycle which coincides with the dark/light cycle (47).

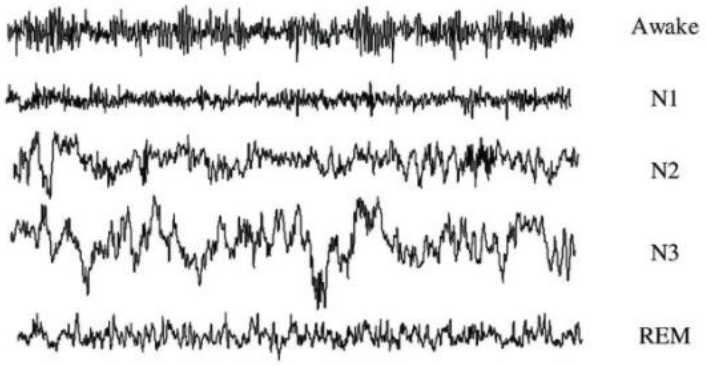

Figure 2.1. EEG recording of neuronal activity during periods of Awake, N1, N2, N3, of NREM, and REM sleep (48).

2.1.2 Sleep architecture

The 24-hour sleep/wake cycle is comprised of states of wakefulness and sleep (46). During which, a cyclic pattern transitioning from stage N1 to N2, stage N2 to N3, and then stage N3 to REM occurs over approximately 90 minutes and repeats between four to six times per night (30). However, the distribution of time spent in each sleep stage changes throughout the sleep bout. For example, the initial NREM component of a sleep cycle is primarily comprised of SWS with relatively small amounts of REM sleep. Throughout subsequent sleep cycles, there is a shift in the contribution of each stage of sleep, with REM durations increasing and NREM durations decreasing. Figure 2.2 depicts the potential changes in sleep staging across a typical night of sleep.

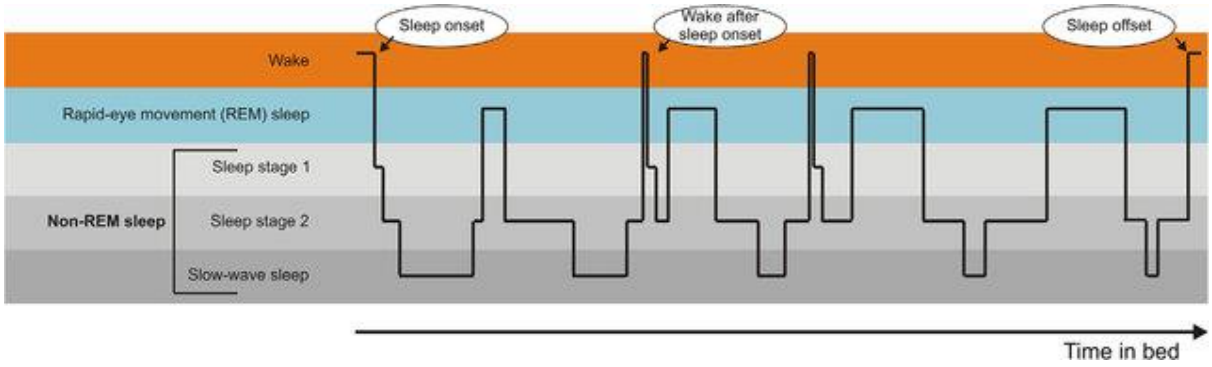

Figure 2.2 A hypnogram depicting the composition of sleep and visualising the changes in sleep architecture over a night of sleep (24).

2.1.3 Circadian rhythm

Circadian rhythms are daily endogenous oscillations in biological and metabolic pathways that occur approximately every 24 hours which are typically controlled by various molecular clocks (49). The circadian clock is governed in a hierarchical manner, by the hypothalamic suprachiasmatic nucleus (SCN), commonly referred to as the 'central clock'. The SCN is located in the anterior hypothalamus, superior to the optic chiasm. The SCN is influenced by various photic and non-photic stimuli in the environment, with cues such as light and dark, time of feeding, temperature, and exercise which are each able to regulate and/or influence endogenous circadian rhythms (50). Molecular clocks are ubiquitous across all tissues in the body, with each cell regulated by the expression of the sum of all clocks within that cell. Light is a common 'zeitgeber,' or 'time cue', that helps to align endogenous rhythms in humans with the external environmental conditions. Considering the human active/rest cycle is aligned with the light/dark cycle, as the external light fades and darkness increases, clocks in the body begin to align with the dark phase and initiate various processes, some of which prepare the body for sleep.

The molecular clock is composed of two primary core transcription factors, Circadian Locomotor Output Cycles Kaput (CLOCK), and Brain and Muscle Arnt-like Protein-1 (BMAL1). CLOCK and BMAL1 form the positive arm of the molecular clock and regulate the transcription of numerous core clock-controlled genes. Period1 (PER1), Period2 (PER2), and cryptochrome (CRY) are induced by CLOCK and BMAL1, and form the negative arm of the molecular clock and function to repress the transcriptional activity of CLOCK and BMAL1 (51). These Period and Cryptochrome proteins act as a negative feedback loop and oppose the positive arm of the molecular clock. This process takes

approximately 24-hours to complete and drives the circadian rhythm of cells. A schematic of the core molecular clock is provided in Figure 2.3.

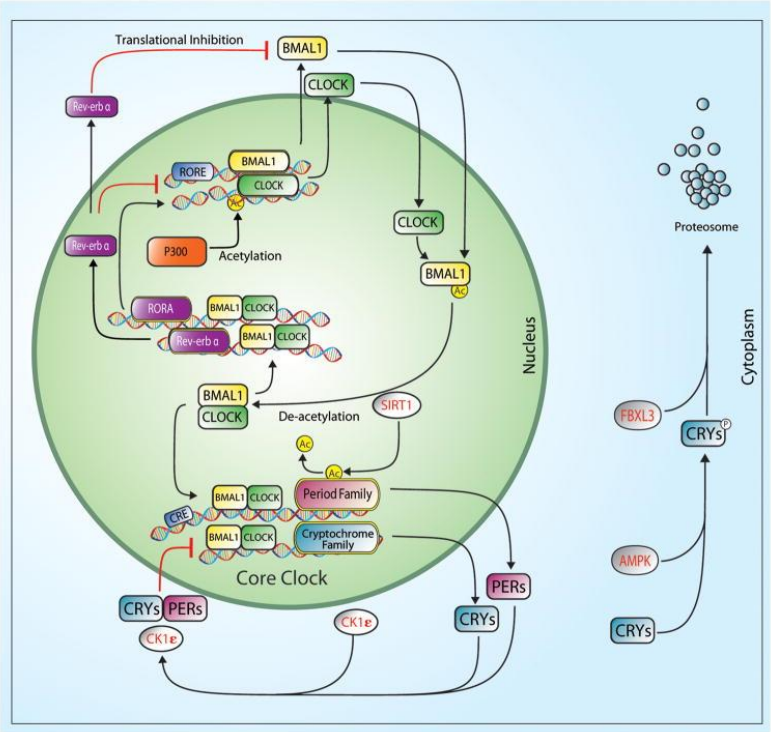

Figure 2.3. A schematic of the molecular clock mechanism (52).

2.1.4 Two-process model of sleep

The process of transitioning from a state of wakefulness to sleep occurs from two driving factors. The first component of the two-process model is homeostatic drive, which hypothesises the accumulation of 'sleep pressure' throughout the day, which is representative of the metabolic cost associated with wakefulness. The drive to sleep gradually builds throughout the day, and subsequently, the accumulation of adenosine in the brain occurs (53). As adenosine levels accrue, there is an increase in the magnitude of 'sleep pressure', and an elevated desire to sleep (54). This 'sleep pressure' is directly influenced by the duration of prior wakefulness (53). The second component of the two-process model of sleep is the input of the circadian rhythm. Various external cues, such as the onset of darkness and reduction of light, will orchestrate a cascade of hormonal and physiological processes which aid in initiating the sleep component of the sleep-wake cycle. For example, the release of the hormone melatonin from the pineal gland is highly synchronised with an individual's sleep/wake behaviours and plays a vital role in initiating sleep onset (55). Thus, the two-process model of sleep relies on both the increase in 'sleep pressure' (i.e., the accumulation of adenosine) as a consequence of wakefulness, coupled

with the onset of hormonal and physical processes regulated by the body's endogenous rhythms and information detected from the environment (i.e., the reduction of light).

2.2 Methods used to measure sleep and sleepiness

2.2.1 Polysomnography

Polysomnography (PSG) is considered the 'gold-standard' in sleep measurement and consists of electrodes attached to the body measuring EEG, EMG, and electrooculogram (EOG) activity while sleeping. Often, a finger-tip oximeter is also placed on the distal end of the second finger to measure peripheral blood oxygen saturation (SpO_2). By measuring the complex neuronal activity that occurs during sleep, in conjunction with muscle activity, eye-movements, and heart rate, a trained polysomnographic technician can score sleep bouts and determine (typically in 30-second epochs) the sleep architecture of an individual's night sleep. Pre-defined rules outlining the criteria that must be observed for each respective stage of sleep are used to score sleep. Guidelines such as those put forward by American Academy of Sleep Medicine (AASM) provide robust criteria to be followed by polysomnography technicians in order to produce standardised and reproducible assessments of sleep (56). Typical inter-rater reliability (IRR) of manual overall sleep scoring suggests substantial agreement exists between scorers (Cohen's kappa (κ) = 0.76; 95% CI, 0.71-0.81), with IRR varying between specific sleep stages, wake (κ = 0.70), N1 (κ = 0.24), N2 (κ = 0.57), N3 (κ = 0.57), and REM (κ = 0.69) (57).

Laboratory-based PSG is considered the 'gold-standard' and often used as the criterion measure when assessing other sleep measuring devices accuracy. However, there are inherent limitations around the use of PSG. For instance, the financial cost associated with using PSG to analyse sleep is significant, with staff required to prepare the montage of electrodes and 'wire-up' the participant prior to sleep (i.e., wages), a sleep technician is usually required to be present to observe and monitor the entire sleep overnight, and the data collected during the sleep is required to be scored by an appropriately trained PSG technician (which can typically take ~2 hours per sleep recording). Further, this can cause a delay in the time it takes to access the results of the sleep. Another potential limitation of PSG is whether the assessment is replicative of a regular night's sleep, as the assessment of PSG takes place in a laboratory setting, rather than in the individual's own home. A potential solution which may address the limitations around the ecological validity of PSG,

along with the reduced need for such extensive labour and extensive analysis, is the use of wearable devices to monitor sleep.

2.2.2 Wearable devices

Wearable devices that use various microtechnology, such as accelerometers and photoplethysmography (PPG) have become a practical alternative to laboratory-based PSG sleep assessment. Activity monitors often use a triaxial accelerometer that measure movement over time to determine sleep/wake activity. However, wearable devices have shown varying levels of agreement when compared to concurrent PSG sleep measurements (58-60). The differences in performance between wearables and polysomnography become apparent when attempting to measure specific sleep stages. For instance, compared with polysomnography, agreement (%) and Cohen's kappa (κ) for multi-state categorisation of sleep stages or wake was 60% (κ = 0.44) for the WHOOP, 53% (κ = 0.20) for the Apple Watch, and 61% (κ = 0.43) for the Oura ring (60). Thus, when looking to accurately measure sleep quantity and quantity, polysomnography may be preferable and comparison between different sleep measurement methods should be avoided.

Wearable sleep tracking devices provide an alternative and ecologically valid method for measuring sleep in an individual's own free-living environment. However, it is important to acknowledge that data reported by wearables may be confounded by calculations performed by each device's own proprietary algorithms that assess, score, and present the data. Therefore, it is recommended to not compare sleep measurements obtained using different methods due to the inherent variability and limitations present within each approach. Nonetheless, wearable devices have shown promise when assessing two-state sleep compared with polysomnography, with agreement of 86% (κ = 0.44) for the WHOOP, 88% (κ = 0.30) for the Apple Watch, and 89% (κ = 0.51) for the Oura ring observed (60). Similarly, the use of research-grade activity monitoring (e.g., Philips ActiCal watch) has been shown to be a valid and reliable source of data when quantifying two state sleep (i.e., sleep or wake) when the appropriate sensitivity of the device is set (61, 62). For example, the Philips ActiCal device has shown a mean bias of 8.5 minutes in total sleep time compared with PSG, when the watch is set to a medium threshold (61). While the use of activity monitoring to assess sleep is widely used to measure sleep/wake

activity, self-reported measures of sleep (i.e., sleep diaries) are often provided in conjunction with activity monitoring to gain further insight into the individual's sleep (62).

2.2.3 Sleep diaries and questionnaires

Subjective measures of sleep can be used to complement the objective measures of sleep. Sleep diaries provide additional context around the environmental and behavioural factors that may influence sleep. For example, a sleep diary may record important information regarding sleep timing, perceived fatigue, quality of sleep, pre-sleep screen time exposure, and dietary considerations. However, a limitation of sleep diaries is that they are reliant on an individual's ability to recall information in a timely and accurate manner. Furthermore, subjective assessments of sleep are susceptible to recall bias, with the potential for responses to be altered in line with social expectations (62). Various questionnaires can be used to gather additional information regarding sleep/wake behaviours and feelings of sleepiness. For instance, the Pittsburgh Sleep Quality Index (PSQI) (63) (a global PSQI score of > 5 has a diagnostic sensitivity of 89.6% and specificity of 86.5%; $\kappa = 0.75$) and Karolinska Sleepiness Scale (KSS) (64) (EEG alpha and theta activity correlation: $r = 0.70$) are two commonly implemented assessments that have been shown to provide valid and reliable data regarding sleep/wake behaviours of individuals as well as feelings of sleepiness. Whilst there are inherent limitations around subjective assessments of sleep, their use in conjunction with objective methods can help to provide a holistic overview of an individual's sleep.

2.3 Sleep and skeletal muscle interactions

2.3.1 Sleep and muscle

Over the lifespan, age related physiological changes can predispose muscle tissue to deterioration and atrophy (65). For instance, one study has demonstrated anabolic signalling pathways (e.g., IGF1-PI3K-Akt) in skeletal muscle are downregulated when sufficient sleep durations are not obtained, particularly in older adults (66). However, nutritional and exercise interventions may be able to mitigate the negative effects associated with ageing on muscle tissue. Amino acid availability (67, 68) and the mechanical loading of muscle tissue (69, 70) are two mechanisms that are likely to increase rates of protein synthesis, a mechanism that underpins muscle protein balance (muscle protein synthesis – muscle protein breakdown) (71). Furthermore, maintaining

muscle mass and function with ageing may provide a protective effect against chronic disease and metabolic pathologies (65). In addition to the health benefits associated with skeletal muscle, performance-based outcomes such as greater force production, muscular endurance, and faster time-course recovery from exercise have been identified (72, 73). Considering the numerous health benefits associated with healthy muscle tissue, it is clear the ability to augment muscle mass over the lifespan is important for general and athletic populations. However, whether there is an optimal strategy to do so is unclear. Further, how sleep interacts with the muscular environment is still not fully understood.

2.3.2 Insufficient sleep and implications for muscle

Insufficient sleep has been shown to contribute to muscle atrophy (74-77). In rodent models, restricted sleep (48h – 96h) has consistently been linked to atrophy of muscle tissue (75, 76, 78, 79). Reductions in muscle tissue weight and cross-sectional area of the plantaris muscle were observed in 75-day-old Wistar rats after 96 hours of paradoxical sleep deprivation (i.e., deliberate prevention of obtaining REM sleep) compared a control group (79). Additionally, decreases in body mass, along with reductions in tibialis anterior mass were reported after 96 hours of paradoxical sleep deprivation in three-month-old Wistar rats (75). The proposed mechanism responsible for sleep-deprived muscle atrophy in rodents has been suggested to be due to alterations to the hormonal environment, with increased levels of corticosterone and reduced levels of testosterone as a response to reductions in sleep (75, 76, 78, 79).

In humans, the relationship between sleep restriction and muscle atrophy is not as extensively researched as it is in rodents (12, 80, 81). Population data suggests chronic (i.e., four weeks or longer) insufficient or poor-quality sleep (scores of >5 measured using the PSQI) is correlated to lower muscle mass (31). However, the mechanisms that underpin these changes are not well understood. To date, research has shown that acute (one night) and chronic (five nights) bouts of insufficient sleep negatively affect rates of muscle protein synthesis (12, 74, 80). Saner et al. (12) found reduced sleep (four hours of time in bed (TIB), each night) over five nights impaired myofibrillar protein synthesis in the vastus lateralis (fractional synthetic rate (FSR): 1.24 ± 0.21% day^{-1}) in healthy male adults compared to a normal ~8 hours of TIB (FSR: 1.53 ± 0.09 % day^{-1}). Additionally, Lamon et al. (80) reported one night of total sleep deprivation was sufficient to decrease

muscle protein synthesis in the vastus lateralis by 18% (FSR: 0.059 ± 0.014%·h^{-1}) compared to a night of normal sleep (FSR: 0.072 ± 0.015%·h^{-1}) in a group of healthy male and female participants. However, females (n = 6) in the same study did not experience a unanimous decrease in muscle protein synthesis after a night of sleep deprivation like the males, which authors suggest may be a sex-specific response requiring further investigation to further elucidate the variable responses observed in female participants. Nonetheless, the trend of a reduction in FSR of muscle protein synthesis was similar across interventions (12, 80). In both studies (12, 80), authors suggest that a shift in the hormonal environment toward a more catabolic state contributed to the decreased rates of protein synthesis.

To explore the mechanistic underpinnings of sleep restriction and muscle atrophy, investigating changes in gene expression in response to insufficient sleep and disrupted circadian biology is prudent. During the aforementioned study by Lamon et al. (80), a night of complete acute sleep deprivation did not induce changes in the mRNA expression of clock genes *BMAL1*, *CRY1*, or *PER1* when samples were taken between 13:00h and 15:00h from the vastus lateralis. Additionally, indicators of protein degradation, assessed by the protein markers Atrogin-1, MURF1, forkhead box protein O1 (FoxO1), p62/sequestosome-1 (SQSTM1) and forkhead box protein O3 (FoxO3) did not appear to be affected by insufficient sleep (80). These findings contrast earlier work that observed a single night of sleep deprivation altered the epigenetic and transcriptional expression of core clock genes such as *BMAL1* (-18%) and *CRY1* (-22%) in skeletal muscle (82). However, the tissue biopsy samples were collected at different time points, and different nutritional intakes were observed, possibly contributing to the variation observed between studies. It is important to note, biopsies represent a static snapshot of gene expression at a single timepoint in a dynamic and rapidly changing environment. However, biopsies can detect changes at the gene level which can provide insight into the molecular underpinnings of protein expression and muscle metabolism. Nevertheless, the timing of muscle biopsies and nutritional state of the individual must be carefully considered when assessing gene expression as the information that is elucidated from the tissue is limited to the activity at a singular time point.

2.4 The bi-directional relationship of sleep and exercise

2.4.1 The effect of exercise on sleep

Exercise is frequently suggested to improve sleep (17, 21). However, there are equivocal findings in the literature regarding the effects of exercise on sleep, with exercise suggested to improve (17, 21), impair (22, 83), and at times, not influence (84,) sleep. Numerous moderating factors related to the individual and the configuration of the exercise performed underpin exercise-induced effects on sleep (23). For instance, individual factors including age, sex, fitness level, health status, and sleep habits may all influence the relationship between exercise and sleep (17). Additionally, the configuration of the exercise prescription also influences exercise-induced effects on sleep, with the mode, intensity, frequency, duration, and proximity to bedtime all potentially influencing subsequent sleep (23). However, due to the equivocal findings in the literature, and lack of consensus pertaining to the effects of exercise on sleep, developing robust guidelines and recommendations regarding sleep and exercise is challenging (23). Therefore, greater insight into the mechanisms that underpin exercise-related changes in sleep may help to elucidate the relationship that exists between the two.

The complex relationship between exercise and sleep makes discerning the mechanisms that are responsible for improvements in sleep challenging. There are several hypotheses that attempt to explain the relationship between exercise and sleep, however, the exact mechanisms remain unknown. Commonly supported hypotheses for the improvements in sleep after exercise include changes in thermoregulation, energy conservation, and body restoration (23). A catalyst for sleep onset is the diurnal decline in core body temperature, which is primarily mediated by increased peripheral blood flow and heat dissipation. These thermoregulatory processes are a likely catalyst for the reduction in core body temperature and lowered metabolic rate observed during sleep (85). Alternate hypotheses such as the energy conservation and body restoration theories propose that during sleep, as a result of the reduced metabolic demands while asleep, there may be a less metabolic energy required and subsequently, an increase of restorative activity in various tissues occurs. Furthermore, these hypotheses suggest that improvements in total sleep time and SWS after exercise are due to the greater energy expenditure incurred by exercise and the larger metabolic demands encountered due to the exercise task. Recently, it has been suggested that exercise-induced peripheral factors may be a potential mechanism for improved sleep

after exercise (24). For instance, elevated levels of brain-derived neurotrophic factor (BDNF) have been linked with increased slow wave activity during sleep in rodent models (86). The BDNF-induced improvements in sleep architecture are purportedly underpinned by increases in the synaptic strength of corticocortical connections as a response to the elevated brain BDNF (24). However, evidence in humans is lacking and exploring this novel hypothesis may provide valuable insight into potential mechanisms for exercise-related changes in sleep.

Exercise may also have the potential to impair sleep. Consequently, common sleep hygiene strategies recommend against exercising in the evening, due to the potential for disturbed sleep (15). Increases in core body temperature in response to exercise and increased post-exercise psychological arousal are suggested to underpin these disruptions to sleep. The onset of sleep is associated with a diurnal decline in core body temperature (23), whereas periods of activity and wake typically correspond with higher core body temperatures (87). Thus, the thermogenic effect of exercise may interfere with the decline in core body temperature and impair sleep. However, sleep hygiene recommendations regarding exercise timing are being challenged, with the emergence of a body of literature highlighting exercise in close-proximity to bedtime is unlikely to disturb sleep (19, 21, 22). For instance, moderate-intensity resistance exercise and moderate-intensity aerobic exercise ending 90-minutes before bedtime did not influence sleep (19).

The relationship between sleep and exercise may also be influenced by the chronotype of the individual. Chronotype, which reflects an individual's circadian entrainment to the 24-hour day and accompanying preferential sleep/wake cycle can be classified into various groups, ranging from the early chronotypes (i.e., 'morning larks') to late chronotypes (i.e., 'night owls') (88). The timing of exercise can affect certain chronotypes in different ways and have implications for subsequent sleep. For example, evening exercise delays the circadian phase of morning chronotypes, but phase-advances evening chronotypes (89). Additionally, athletes who train at opposing times to their circadian preference encounter higher levels of perceived exertion compared to training at a time that aligns with their circadian preference (90). Collectively, these findings suggest chronotype and exercise timing may have the potential to influence sleep via disrupted circadian phases and increased psychophysiological arousal after exercise (91). Thus, it is important to consider

individual chronotype when scheduling exercise and the potential consequences that the timing of exercise may have on sleep.

2.4.2 The effect of sleep on exercise

The relationship between sleep and exercise performance has been well documented (92-94). Whilst discussing the effect of sleep on all measures of physical performance is outside the scope of this thesis, it is important to acknowledge the relationship between sleep and the muscular system from a practical performance standpoint. A review by Knowles et al. (95) found multiple nights of insufficient sleep hindered maximal strength in compound resistance exercises when appropriate motivational strategies were not implemented. However, the supplementation of caffeine was able to mitigate the effects of acute sleep loss on maximal strength during resistance training (96). The extent to which submaximal or strength-endurance efforts are affected by insufficient sleep is not yet clear. Furthermore, the physiological mechanisms responsible for the apparent decrease in performance are not overtly apparent. Rather, psychological changes associated with sleep loss such as increased perception of effort and decreased mood may reduce enthusiasm and motivation to execute training with maximal intent (97).

The psychological influence that insufficient sleep may have on skeletal muscle function should also be considered. It is well known that sleep restriction and sleep deprivation negatively affect mood, motivation, and perception of effort (32). Furthermore, when psychological wellness is impaired, there may be an array of subsequent physiological consequences. For instance, athletes have been shown to have an increased perception of effort during resistance and endurance exercise tasks when sleep deprived (98). Additionally, if motivation is low and perception of effort is magnified, the desire to exercise with maximal intent, perform exercise at the prescribed training loads, and complete the total amount of required work may be lacking in athletic populations. This may result in lower training intensity and blunted physiological adaptations to training. Furthermore, these decrements in mood may reduce the amount of physical activity undertaken in non-athletic populations as well.

2.4.3 Sleep extension, muscle performance, and recovery

The effects of insufficient sleep on muscle have been previously documented (74-77, 79, 95, 99). However, the effects of extended sleep on the muscular environment are far less well understood. This may be a result of challenges around performing rigorously controlled sleep intervention studies where sleep durations are extended (i.e., increased from habitual sleep durations). From research in athletic populations, extending habitual sleep appears to facilitate improvements in reaction time (100, 101), sprint times (100), sport-specific skill (e.g., basketball free throw shooting percentage and tennis serving accuracy) (100, 102), and possible reductions in cortisol levels (101). Further sleep extension research is warranted to elucidate the potential mechanisms that underpin the improvements in performance. This may be particularly prudent for populations who experience high physical demands (e.g., shift-workers, military personnel, athletes). Additionally, exploring the possible interaction between extended sleep and the hormonal environment warrant further investigation as these findings may provide insight into using sleep as an ergogenic aid for recovery, and to attenuate greater adaptations to training, particularly in times of high training loads or psychological stress.

2.4.4 Influence of diurnal variation and endogenous rhythms on exercise

Circadian rhythm and exercise performance have been suggested to have a time-of-day interaction, primarily due to daily fluctuations in body temperature (103). Greater physical performance has been suggested to occur at peak core body temperature, which typically coincides with the afternoon (103). Several physiological mechanisms have been suggested to underpin these changes; the body's preferential utilisation of carbohydrates for energy (104), the potential for enhanced function of contractile elements within the sarcomere (e.g., myosin-actin cross-bridges) (105), and greater calcium release and excitability by the sarcoplasmic reticulum and contractile proteins, respectively (106). Maximal aerobic exercise has been suggested to exhibit a time-of-day effect, with performance declining in the evening compared to the afternoon (107). Findings suggest that aerobic performance may be greater in the afternoon when peak body temperature occurs, compared to the morning or late at night. However, equivocal observations of time-of-day aerobic performance have been reported (108). A recent meta-analysis did not provide evidence for or against a specific time of day to exercise for optimal results, but did provide evidence that larger effects may occur when performance testing occurs at a

similar time to the individual's typical exercise (109). Thus, the timing of physical performance testing should be considered and where possible, standardised to mitigate any potential time-of-day effects.

Resistance and strength-based exercise have reported potential time-of-day effects (103, 108). Force expression has been suggested to be enhanced during afternoon bouts of exercise compared to morning (103), possibly due to greater neuromuscular drive, and increased coordination and motor-unit synchroneity between agonist-antagonist muscle contractions (103). Time-of-day improvements in performance also support the notion of optimal core body temperature having a beneficial effect on exercise performance, which may be related to the diurnal variation and rhythmic fluctuations in body temperature that occur across a 24-hour period. Time-of-day should be considered when implementing exercise programmes as multiple physical characteristics display diurnal variation. Furthermore, undertaking regular exercise at the same time of day may allow for greater adaptations to training compared to exercising without a regular routine (108).

2.5 Nutritional interventions and sleep

2.5.1 Nutritional interventions to enhance sleep

Pre-sleep nutritional strategies have been used as an alternative to pharmacological interventions for enhancing sleep. Non-pharmacological methods are popular for improving sleep over prescription medications, due to the potential side-effects such as reduced cognitive function, addiction, tolerance issues, and rebound insomnia upon cessation, often occurring in response to various pharmacological interventions (110). Furthermore, next-morning cognitive impairment may manifest after the consumption of medication known to affect sleep (111). For example, when driving a car, the risk of causing a traffic accident and sustaining an injury was 3.9 times greater in sleep medication (i.e., benzodiazepine hypnotics) users, likely due to residual cognitive impairment from the medication (112) compared to healthy control subjects. Consequently, several populations may benefit from nutritional interventions that are able to improve sleep without subsequent cognitive impairment.

The consumption of several nutritional ingredients prior to sleep have shown promise for improving sleep. In particular, the use of high glycaemic index (GI) carbohydrates,

tryptophan, tart cherry, kiwifruit, whey protein, and melatonin have shown promise for promoting sleep, for extensive reviews see Gratwick et al. (113) and Halson (26). High GI carbohydrates have been shown to improve sleep in healthy adults by decreasing sleep onset latency when consumed four hours prior to bed (114). This improvement in sleep may be due to an increased ratio of free tryptophan to branched chain amino acids and downstream increases in brain free tryptophan and serotonin (114, 115). Tryptophan ingestion prior to sleep has been shown to improve WASO in dosages of 1g (25). Improvements in sleep as a result of tryptophan supplementation may be due to tryptophan being converted to 5-hydroxytryptamine (5-HT) in the brain, which causes a cascade of reactions resulting in increased serotonin and subsequently, melatonin (115). Additionally, whey protein consumption prior to bed has also been suggested to enhance recovery and sleep (116). Whilst protein supplements are typically consumed with the aim of enhancing skeletal muscle, body composition, and promoting recovery, sleep may also be augmented by the ingestion of protein high in α-Lactalbumin (117). The ability of whey protein consumed prior to bed to influence the sleep/wake cycle is likely due to the presence of increased tryptophan, which is a highly concentrated component of α-Lactalbumin (113).

Tart cherries have shown promise in augmenting sleep (118). For instance, tart cherries have shown anti-inflammatory properties, with reductions in circulatory inflammatory markers observed after consumption in cyclists and runners (119, 120). Individuals with sleep disorders may present with increased levels of oxidative stress (121), and the consumption of an antioxidant such as cherry tart may help facilitate improvements in sleep due to the presence of melatonin, which has an essential role in facilitating the onset of sleep. The consumption of kiwifruit (a moderate-GI food), prior to bed has also been reported to have positive effects on total sleep time (361.8 ± 14.9 mins vs 416.6 ± 16.2 mins) in healthy adults who self-reported that they suffer from sleep disturbances, when measured using actigraphy (122). However, the utility of kiwifruit to improve sleep in healthy populations who are not experiencing sleep disturbances would be important to determine as kiwifruit could be used as a nutritional strategy to augment sleep when individuals may be aware of periods of upcoming sleep disturbances. Whilst there is an array of nutritional interventions available to enhance sleep, guidelines detailing the exact dosage and timing of how to effectively implement nutritional strategies are lacking.

Furthermore, whether ingredients can be consumed concurrently for a more efficacious effect is an important area to elucidate further.

2.6 Summary

Across this literature review, a range of topics and literature pertinent to this thesis have been critically reviewed. The bullet points below help provide a summary of the key findings:

- Approximately one third of adults are not achieving the recommended 7 – 9h of sleep per night.
- The relationship between sleep, skeletal muscle, and circadian rhythms is complex. However, when sleep and endogenous rhythms are disrupted, there are negative downstream implications for skeletal muscle and metabolic health.
- Exercise can have both positive and negative effects on sleep depending on the timing and configuration of exercise.
- The underlying mechanisms explaining exercise-induced improvements in sleep are not clear.
- Peripheral metabolic factors have been suggested to be potential mechanisms responsible for improvements in sleep after exercise but evidence in humans is lacking.
- Nutritional interventions appear to be effective at improving the sleep/wake cycle but optimal doses and timings for consumption are unclear.

2.7 Rationale for research studies

The proposed studies aim to review the relationship between sleep, circadian biology, and skeletal muscle as well examine the effects of modifiable behaviours such as exercise and nutrition on sleep quality and quantity.

Chapter 4 – Study 1: Sleep, Circadian Biology and Skeletal Muscle: Implications for Metabolic Health

The relationship between sleep, circadian biology, and skeletal muscle is complex and not well understood. Insufficient or disturbed sleep, coupled with disrupted circadian rhythms may elicit negative effects on numerous cells and tissues. Consequently, it is important to review potential strategies that may be able to mitigate the negative effects of insufficient

sleep and disturbed endogenous rhythms. This review aims to outline the importance of obtaining sufficient sleep durations (7h to 9h), provides a synopsis of circadian biology, and highlights the complex relationship between sleep, circadian biology, and skeletal muscle.

Chapter 5 – Study 2: – Quantifying the effect of afternoon moderate-intensity exercise on sleep quality and quantity in healthy adult males using polysomnography.
Exercise can influence sleep, depending on the configuration and timing of the exercise bout. Given recommended sleep durations are not currently being achieved, determining whether a typical moderate-intensity exercise session undertaken at a popular time to exercise, ~15:30h in the afternoon, may be used to induce improvements in subsequent sleep is valuable. Therefore, the aim of study this study was to investigate the effect of afternoon moderate-intensity cycling exercise on objective and subjective sleep in healthy adult males.

Chapter 6 – Study 3: Relationship between sleep quality and quantity and exercise-induced peripheral factors in healthy adult males.
The mechanisms underpinning exercise-related improvements in sleep are not well understood. Recently, exercise-induced peripheral factors have been suggested to be potential mechanisms for exercise-related improvements in sleep. However, there is no evidence in humans to support this novel hypothesis. Consequently, the aims of this study were twofold: 1) to determine the effect of afternoon, moderate-intensity exercise on TNF-α, interleukin-1α (IL-1α), interleukin-1β (IL-1β), IL-6, and BDNF as these are markers of an exercise-induced inflammatory response associated with improvements in sleep in rodents; and 2) to investigate the relationship between exercise-induced peripheral factors and subsequent sleep in healthy, adult males measured using polysomnography.

Chapter 7 – Study 4: The influence of a formulated nutritional intervention on subsequent sleep and next-morning physical performance, cognitive function, and postural sway in adult males
It is well established that a third of adults are not currently achieving sufficient sleep durations. Consequently, pharmacological interventions are often used to assist falling asleep. However, sleep medications may incur next day 'hangover' type effects such as

drowsiness and impaired psychomotor performance. Nutritional interventions offer a potential alternative to sleep medications. There is evidence supporting various nutritional interventions that can act on neurotransmitters in the brain and can subsequently, influence sleep. However, the exact dose and combination of ingredients for improving sleep is unknown. Given the clear benefits of both exercise and nutritional interventions on health, it is important to examine how these two modifiable behaviours interact and if they influence sleep. Consequently, the aim of this study was to determine the effect two different doses of a formulated nutritional intervention compared with placebo on objective and subjective sleep and next-morning physical performance, cognitive function, and balance in trained adult males.

Chapter 3: General Methodology

This chapter outlines the methods for variables that were collected multiple times during the course of this thesis. For protocols that were only implemented once, specific details of each methodology are provided in the corresponding chapter where that method was used. The methods provided in each chapter adhere to the formatting guidelines required of the publishing journal where each manuscript is published or being prepared for publication. Additionally, an overview of the experimental designs, participant information, methodologies, and statistical analyses used are outlined below.

3.1 Experimental design

One review and three studies were conducted to a) review the complex relationship between sleep, circadian biology, and skeletal muscle; b) determine how 40 minutes of moderate-intensity (70% heart rate maximum) cycling exercise influences subsequent objective and subjective sleep; c) investigate whether peripheral metabolic factors secreted from skeletal muscle after exercise may be a potential molecular mechanism for improving sleep; and d) determine the effects of a formulated pre-sleep nutritional intervention on sleep and next-morning cognitive and physical performance. All intervention studies (i.e., studies 2 - 4) were completed at Central Queensland University's Appleton Institute, Australia. Specific experimental approaches for each study within this thesis are provided in Table 3.1. Of note, data for studies two and three (i.e., Chapters five and six) were

collected concurrently, as a part of a larger study, using the same participants. All data were collected between 3/01/2022 and 9/08/2022.

Studies 2 – 4 employed repeated-measures, counter-balanced, crossover study designs. Repeated-measures designs allowed each participant to act as their own control, allowing for more precise detection of intervention effects. Further, within-participant designs typically require lower numbers of participants compared to between-subject study designs to achieve statistical power. Studies were counter-balanced to mitigate potential order effects that may arise, ensuring the sequence of conditions administered do not bias the results.

Table 3.1. Overview of experimental approaches used for each study within this thesis

Study	Chapter	Experimental approach
Study 1	Four	Narrative review of the literature
Study 2	Five	Repeated-measures, counter-balanced, crossover design
Study 3	Six	Same data collection as study two
Study 4	Seven	Repeated-measures, double-blinded, placebo controlled, counter-balanced, crossover design

3.2 Participants

All participants recruited to undertake the study protocols outlined in this thesis were healthy, adult males, without a diagnosed sleep disorder. Specific inclusion/exclusion criteria for each study are provided in their respective study/chapter. However, descriptive statistics of the healthy, male, participants who completed the studies are provided in Table 3.2. The physical activity levels of participants from study two and three (mean ± SD; weighted average physical activity/exercise per week: 247.5 ± 170.7 mins) and study four (weighted physical activity/exercise per week: 502.6 ± 8.4 mins) were collected using a pre-exercise screening tool by Exercise and Sports Science Australia (123). The weighted physical activity scores indicate the participants for studies two and three were recreationally active and for study four were highly active.

Healthy adult males between 18 – 40 years old were recruited within this thesis. This demographic was selected for examination as there are changes in sleep physiology that typically occur with ageing (124) yet between 18 – 40 years, remain relatively stable.

Males were selected as participants due to the exploratory nature of study three, involving the measurement of metabolic peripheral factors where the influence of the menstrual cycle is unknown. Additionally, study four is an extension of previous work investigating the effects of a nutritional intervention on sleep in healthy adult males (125), thus a similar cohort of participants were required. A power calculation was conducted to identify the number of participants required to be recruited (see Chapter seven for specific calculations). It must be acknowledged that although an a-priori power calculation was conducted for studies two and three, the final sample of 12 participants recruited was limited by resource constraints (i.e., cost associated with the laboratory and analysing blood samples) (126).

Table 3.2. Descriptive statistics of participants recruited throughout this thesis

Study	Chapter	*N*	Age	Height	Mass
Study 2 and 3	Five and six	12	24 ± 4.7 years	177.3 ± 9.4cm	76.4 ± 14.9kg
Study 4	Seven	17	25.4 ± 6.5 years	179.3 ± 7.2cm	74.2 ± 10kg

Note: All data are mean ± standard deviation.

3.3 Objective sleep measurement

3.3.1 Polysomnography

Polysomnography (PSG) provides a comprehensive physiological assessment of sleep quality and quantity, typically measured in a clinical laboratory setting. Throughout this thesis, objective sleep outcomes were measured using polysomnography (Grael, Compumedics; Victoria, Australia) recordings that were completed between 23:00h – 08:00h (Chapter five and six) and 22:30h – 08:00h (Chapter seven). The sleep times differed between studies due to Chapter seven being an extension of previous work, where the sleep opportunity needed to be replicated (125) and Chapter five and six conforming to the host laboratory's standard operating procedure. During the 60-minutes prior to sleep, participants were fitted with a standard montage of electrodes. Two electroencephalography electrodes (i.e., C4-M1, C3-M2), two electro-oculograms (i.e., left/right outer canthus), and one submental electromyogram electrodes attached to the face and scalp of participants recorded brain, eye, and muscle activity were fitted by laboratory technicians. PSG recordings were manually scored in 30-second epochs by a registered and experienced polysomnographic technician in compliance with standard criteria (56, 127). Outcome variables recorded include total sleep time (min), which

reflects the time spent in any stage of sleep (i.e., N1, N2, N3, (REM)) during time in bed; time spent in stages N1, N2, N3 and REM sleep (min); sleep onset latency (min), which represents the time between lights-out to the first epoch of any stage of sleep (i.e., N1, N2, N3, REM); wake after sleep onset (min), which reports the time spent in bed awake minus sleep onset latency; sleep efficiency (%), which represents total sleep time divided by time in bed x 100; arousals (count); arousals in NREM (count); arousals in REM (count); awakenings (count); stage shifts (count); stage REM onset latency (min); and stage N3 onset latency. All PSG variables and their definitions are provided in Table 3.3. Additionally, two electrocardiogram (ECG) electrodes (left-positive and right-negative) were attached to each participant to assess cardiac activity. The positive electrode was secured to the left side of the torso, parallel to the left hip and leg, between the fifth, sixth, or seventh intercostal spaces on the lower left side of the rib cage. The negative electrode was placed three centimetres below the right clavicle, positioned on the torso parallel to the right leg. An oximeter was placed on the distal phalanx of the second digit of the non-dominant hand of each participant to measure peripheral oxygen saturation (SpO_2). Both ECG and SpO_2 were recorded using the aforementioned Grael PSG system. Scoring of sleep stages was done in accordance with the American Academy of Sleep Medicine (AASM) Manual for the Score of Sleep and Associated Events criteria and has an average inter-scorer agreement of 82.6% across a large sample of experienced scorers (n = >2,500) and scoring decisions (n = 3,200,000) (128).

Table 3.3 Definitions of sleep outcome measures obtained from polysomnography

Polysomnography variable	**Definition**
Total sleep time (min)	Total duration of sleep obtained during a sleep period.
Sleep efficiency (%)	Total sleep time divided by time in bed, multiplied by 100.
Sleep onset latency (min)	Period of time between going to bed and the onset of sleep.
Rapid Eye Movement Latency (min)	Period of time between sleep onset and the first epoch of REM sleep.
Stage three latency (min)	Period of time between going to bed and the first epoch of stage three sleep
Stage one (N1) duration (min)	Period of time spent in stage one sleep over the entire sleep opportunity.
Stage two (N2) duration (min)	Period of time spent in stage two sleep over the entire sleep opportunity.
Stage three (N3) duration (min)	Period of time spent in stage three sleep over the entire sleep opportunity.

Wake after sleep onset (min)	Total duration of time spent awake between sleep onset and lights on.
Rapid Eye Movement duration (min)	Period of time spent in rapid eye movement sleep over the entire sleep opportunity.
Arousal (count)	Number of abrupt shifts in EEG activity lasting at least three seconds.
Awakening (count)	A period of wakefulness that exceeds 15 seconds.

3.4 Subjective sleep measurement

3.4.1 Subjective sleep quality, sleep quantity, and sleep latency

The subjective assessment of sleep allows for an individual to self-report how they slept. Additionally, subjective sleep assessment can provide complementary data to provide further insight alongside an objective assessment of sleep. Throughout this thesis, subjective sleep quality, sleep duration, and sleep latency were collected each morning, in the 30 minutes after waking. Subjective sleep quality was assessed using a 7-point scale, where 1 = "extremely poor", 2 = "very poor", 3 = "poor", 4 = "average", 5 = "good", 6 = "very good", and 7 = "extremely good". Subjective sleep quantity and subjective sleep onset latency were assessed verbally by asking participants "how much sleep do you think you got?" and "how long did it take you to fall asleep?". These standardised questionnaires have been frequently used in the literature as a method of determining self-ratings of sleep quality, quantity, and latency (125, 129, 130).

3.5 Sleep questionnaires

3.5.1 Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) is a self-rated questionnaire that assesses quality of sleep and sleep disturbances over a one-month period (131). The PSQI comprises 19 individual self-rated questions from seven equally weighted components; subjective sleep quality, sleep latency, duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. Each question is presented using a 7-point Likert scale including 0 – not during the past month, 1 – less than once a week, 2 – once or twice a week, to 3 – three or more times a week. The seven components are then scored from 0-21. A global PSQI score of >5 has demonstrated a diagnostic sensitivity of 89.6% and specificity of 86.5% (kappa = 0.75, $p < 0.001$) in distinguishing good and poor sleepers (131). Participants were required to complete the

PSQI in the week prior to the commencement of each study and were excluded from participating if their PSQI score exceeded five. Good sleepers were selected as participants for this thesis to mitigate any potential interference of pre-existing sleep disorders or irregularities as well as allowing for more generalisable findings to a healthy population.

3.5.2 Munich Chronotype Questionnaire

The Munich Chronotype Questionnaire (MCTQ) is a self-reported tool used to collect information around habitual sleep patterns (132). Specifically, the MCQT focuses on details pertaining to sleep and wake times, sleep latency, and sleep inertia. The MCTQ includes 17 items across four categories: work schedule, work day sleep schedule, free day sleep schedule, and self-assessment of chronotype. The MCTQ assesses individual chronotype based upon the midpoint between sleep onset and offset on work-free days. Participants completed the MCTQ in the week prior to each study and was used to identify if participants were of an 'extreme' chronotype, such as *extremely early* or *extremely late*. If participants were identified as 'extreme' chronotypes, they were excluded from participating in the studies within this thesis. The MCTQ has been shown to be a valid tool to discern the midpoint of sleep on free days when compared to wrist-worn actigraphy (r = 0.73, $p < 0.001$; 95% limits of agreement (LoA) = - 1:37:19 and 2:14:38) (133). Additionally, the MCTQ has been shown to correlate with Horne-Ostberg's Morningness-Eveningness Questionnaire's (MEQ) assessment of mid-sleep on free days (r = - 0.73) and work-days (r = - 0.61) (134). The MCTQ was used in studies 2 – 3 to determine participant chronotype.

3.5.3 Horne-Ostberg Morningness-Eveningness Questionnaire

The Horne-Ostberg MEQ is a self-rated scale used to assess individual circadian phase preferences (135). The MEQ uses the sum of 19 questions to determine an individual's 'morningness' or 'eveningness' phenotype. A score of 41 and below reflects "evening types", whereas scores of 42-58 are deemed "intermediate types" and scores of 59 and above identify "morning types". Participants completed the MEQ in the week prior to each study. The MEQ was originally validated in a cohort of students using oral temperature curves, which highlighted that "morning types" displayed earlier circadian peaks in body temperature when compared to "evening types" (135). Furthermore, the MEQ shows agreement with objective sleep data gathered via actigraphy (bedtime = r: -0.56; rise time

= *r*: -0.53) (136). Additionally, in a cohort of healthy middle-aged workers, the thresholds for the "evening types", and "morning types" were identified as scores of less than 53, and above 64, respectively (137). These new thresholds may be reflective of changes in sleep/wake patterns that occur across the lifespan. As such, they highlight the utility of using population-dependent cut-offs to accurately determine chronotype via the MEQ. The MEQ was used in study four due to the ease of adapting the questionnaire to the online format of the participant screening process.

3.6 Subjective Sleepiness Questionnaire

3.6.1 Karolinska Sleepiness Scale

The Karolinska Sleepiness Scale (KSS) is a 9-point scale used to assess an individual's state of sleepiness (138). The KSS scoring ranges from one – 'extremely alert' to nine – "very sleepy, great effort to keep alert, fighting sleep" and requires the participant to self-identify their individual perceived level of sleepiness. The KSS may be implemented multiple times prior to sleep or after waking to gauge the progressive increase or decline of sleepiness. Throughout this thesis the KSS was recorded prior to sleep and within 30-minutes of waking. In Chapters five and six, KSS were completed at 22:30h, whereas KSS data were collected every 30-minutes between 20:00h and 22:00h in Chapter seven.

The KSS is a valid tool for identifying subjective sleepiness. The KSS can detect changes in an individual's "state" of sleepiness in response to various tasks, such as partial repeated sleep deprivation and extended wakefulness in a laboratory. During which, these tasks have shown large effects on KSS scores (Cohen's *d*: 1.34 – 3.00) (139). Additionally, the KSS has shown substantial convergent validity for measuring sleepiness, with significant correlations observed between electroencephalographic (EEG) variables, such as eye-open alpha (averaged Pearson's product-moment correlation coefficient (*r*) *r*: 0.40) and theta (*r*: 0.38) power density and psychomotor vigilance task (PVT) performance (lapses *r*: 0.56; mean reaction time *r*: 0.57) (140).

3.7 Physical performance assessment

3.7.1 Stationary cycle ergometer

Throughout this thesis, exercise tasks varying in intensity and duration were implemented using a stationary cycle ergometer (Wattbike Trainer, Wattbike Ltd; Nottingham, UK) to

assess physical performance. During studies two and three (Chapters four and five), a submaximal graded exercise test and standardised cycling exercise protocol were implemented, whereas in study four (Chapter seven), a maximal effort time trial and a simulated training session were used. Specific details of each protocol are provided in their respective chapters. Cycling was chosen as the exercise modality for this thesis due to the ease of use for participants (e.g., lower impact exercise than running) and the ability to control and refine the stimuli being delivered to the participant (e.g., reducing wind resistance at a fixed cadence to lower the participant's heart rate).

The Wattbike is a valid and reliable instrument for measuring cycling performance across a range of power outputs and distances (e.g., mean difference of 0.6W when cycling at 100W and 25.5W when cycling at 1000W, respectively) (141). Furthermore, the Wattbike has shown acceptable agreement with a mechanically driven dynamic calibration rig between power outputs of 50 and 700W at 70 $rev.min^{-1}$ (± 1.7%) and power outputs of 100 and 1250W at 90 $rev.min^{-1}$ (± 1.4%) (142). The reliability of the Wattbike differed between trained (coefficient of variation (CV) across power outputs between 150 to 300W: 1.8 to 3.2%) and untrained participants (CV across power outputs ranging from 100 and 200W: 3.8 to 6.2%) (142).

Data collected during each exercise task performed on the Wattbike were stored using individualised, de-identified profiles created on the stationary ergometer head unit. Raw data were retrieved from the Wattbike head unit via USB and downloaded using the Wattbike Expert Software package (Wattbike Expert, v2.60.20, Wattbike, Birmingham, UK). The rating of perceived exertion (RPE) was recorded using either the 6-20 Borg Scale (Table 3.4) (143) or the Borg CR-10 scale (Table 3.5) (144) and was recorded during each physical performance task.

The aerobic cycling exercise configuration for studies two and three was designed to provide a sufficient stimulus to elicit a secretion of metabolic peripheral factors (see Chapter six – study three) without incurring delayed-onset muscle soreness that would impair next-day performance. A 40-minute moderate intensity (70% heart rate max) afternoon cycling session was selected as this configuration would likely elicit an increase in metabolic factors (145) and was also judged to be an exercise session that would commonly be completed by healthy adults. Study four used a morning 10-minute maximal

effort time trial to assess differences in physical performance the morning after consuming a nutritional supplement (see Chapter seven – study four). Further, similar configurations of cycling exercise (146) have been shown to be affected by the previous night sleep. The high-intensity afternoon cycling interval session was configured to replicate a typical exercise session that a well-trained athlete may complete.

Table 3.4. The 15-grade scale for ratings of perceived exertion.

6	
7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

Table 3.5. The Borg CR10 scale for ratings of perceived exertion.

0	Nothing at all
0.5	Extremely weak (just noticeable)
1	Very weak
2	Weak (light)
3	Moderate
4	Somewhat strong
5	Strong (heavy)
6	
7	Very strong
8	
9	
10	Extremely strong (almost max)

3.8 Statistical analysis

Throughout this thesis a range of statistical approaches were used. Detailed explanations of each statistical approach are provided within each respective chapter. However, below is a brief overview of the approaches used in each study.

Chapter four – study one

No statistical analysis was required.

Chapter five – study two

All statistical analyses were performed in RStudio (Version 2022.12.0+353) using the R programming language (Version 4.2.1). Before statistical analysis, data were examined for normality through visual inspection of density plots using ggplot2 (Version 3.3.6) and objectively via the Shapiro Wilk test. For variables that were significantly different from a normal distribution ($p < 0.05$), non-parametric tests were used. For variables that were normally distributed, parametric tests were used. For nonparametric variables, the Wilcoxon Rank-Sum test and for parametric variables Welch Two Sample T-Test were used to assess differences between conditions. Statistical significance was set with an alpha of $p < 0.05$. For data that were not normally distributed, the magnitude of differences was assessed by calculating effect sizes (r) and 95% confidence intervals as trivial ≤ 0.10 small, ≤ 0.3; medium ≤ 0.5; and large, > 0.5 using the *rcompanion* package (Version 2.4.16). Cohen's *d* effect sizes and corresponding 95% confidence intervals were calculated and interpreted as small > 0.2, medium > 0.5, and large ≥ 0.8 for normally distributed data (147). Effect sizes were calculated to standardise the measurement of the magnitude of effects. Data are presented as mean ± standard deviation (SD). Interquartile ranges (IQR) were calculated to present the dispersion of the data, allowing for clearer observation of potential variability within each outcome variable.

Individualised objective sleep data points were plotted and visualised using the *raincloudplots* package (Version 0.2.0) (148). Sleep stage distributions during each condition were plotted using histograms. Sleep hypnogram data (recorded in 30-second epochs) were binned into five-minute intervals, and the percentages of each stage (i.e., wake, stage N1, stage N2, stage N3, and REM) were calculated. The percentages were

plotted on the y-axis, with each stage stacked on top of each other to represent the overall distribution, while the x-axis shows plots of the five-minute bins.

Chapter six – study three

A Bayesian framework was employed to model the changes in peripheral metabolic factors following exercise and their relationship to objective and subjective sleep outcomes. Specifically, Bayesian mixed effects models were fit using the *brms* package and the *brm* function in R. These models are more flexible in modelling complex relationships than classic frequentist multi-level approaches. Furthermore, unlike classical frequentist theory, they do not rely on sample size to control type I and type II error rates – small samples will just result in more posterior uncertainty (the uncertainty in the parameter estimates after incorporating data and priors).

First, to understand changes in peripheral metabolic factors following exercise, models were fit with a condition (Exercise *vs.* Control) by time interaction. Subject ID was included as a random intercept term. Separate models were fitted for each BDNF, TNF-α, IL-1α, IL-1β, and IL-6; a Gamma log link function was stipulated in the model due to the positively skewed nature of the peripheral metabolic factors. Models were fit with default, non-informative priors due to the exploratory nature of the study. All posterior distributions were estimated by running four separate Markov Chain Monte Carlo (MCMC) chains, with a standard 2000 iterations over each chain (149). Any convergence issues among the chains were assessed by visually inspecting the plots, ensuring the Gelman-Rubin statistic was close to one, with one indicating perfect convergence, and assessing the effective sample size used after accounting for autocorrelation. Where any divergence issues arose, the adapt delta (parameter controlling the target acceptance probability) and maximum tree depth (allows algorithm to explore the parameter space to a greater depth) were increased (to make the model more robust) and the model was rerun. Pareto estimates were examined to determine if there were any problematic observations within the data. The influence of exercise on peripheral metabolic factors was interpreted using the *hypothesis* function within *brms*, an evidence ratio less than one suggests that the data supports the null hypothesis and an evidence ratio greater than one shows the data is in support of the alternative hypothesis.

Second, the relationship between pre-bed peripheral metabolic factors and objective and subjective sleep outcomes were modelled using a multivariate Bayesian mixed effects model, with pre-bed peripheral metabolic factors as fixed effects, subject ID as a random intercept term and objective and subjective sleep outcomes as the outcome variable. A separate model was built for each outcome variable.

Chapter six – study three

All data were analysed with linear mixed effects models using separate models built for each outcome variable of interest. Condition and time were included as fixed effects and participant ID included as a random effect using the R package *lme4* (150, 151). All models were estimated using Restricted Estimated Maximum Likelihoods from the *lme4* package. *P*-values will be obtained using Type III analysis of variance with Satterthwaite's tests with Kenward-Roger degrees of freedom as implemented in the R package *CAR* (152). Results were reported as mean estimates with alpha set at $p < 0.05$. The magnitude of differences were assessed using Cohen's *d* effect size statistic and 95% confidence intervals using the *t_to_d* function in the *effectsize* package, where the t value from the linear mixed model is divided by the square root of the degrees of freedom error from the same model and interpreted as trivial, <0.20; small, 0.20-0.49; moderate, 0.50-0.79, and large ≥0.80 (153).

Chapter 4: Study 1 – Sleep, Circadian Biology and Skeletal Muscle: Implications for Metabolic Health

4.1 Linking paragraph

In Chapter two, the review of the literature highlighted the complex relationships that exist between sleep, exercise, and nutrition, establishing a comprehensive foundation for further exploration. Chapter four builds upon these insights and provides a focused narrative review of the relationship between sleep, circadian biology, and skeletal muscle. The preceding review will serve as a springboard for this thesis, transitioning from reviewing the current state of evidence and identifying gaps in the literature to implementing original research investigations. This approach aims to help enrich our understanding and elucidate in further detail the relationship between sleep, exercise, and nutrition.

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Sleep, Circadian Biology and Skeletal Muscle Interactions:
Implications for Metabolic Health

4.2 Summary

There currently exists a modern epidemic of sleep loss, triggered by the changing demands of our 21st century lifestyle that embrace 'round-the-clock' remote working hours, access to energy-dense food, prolonged periods of inactivity, and on-line social activities. Disturbances to sleep patterns impart widespread and adverse effects on numerous cells, tissues, and organs. Insufficient sleep causes circadian misalignment in humans, including perturbed peripheral clocks, leading to disrupted skeletal muscle and liver metabolism, and whole-body energy homeostasis. Fragmented or insufficient sleep also perturbs the hormonal milieu, shifting it towards a catabolic state, resulting in reduced rates of skeletal muscle protein synthesis. The interaction between disrupted sleep and skeletal muscle metabolic health is complex, with the mechanisms underpinning sleep-related disturbances on this tissue often multifaceted. Strategies to promote sufficient sleep duration combined with the appropriate timing of meals and physical activity to maintain circadian rhythmicity are important to mitigate the adverse effects of inadequate sleep on whole-body and skeletal muscle metabolic health. This review summarises the complex relationship between sleep, circadian biology, and skeletal muscle, and discusses the effectiveness of several strategies to mitigate the negative effects of disturbed sleep or circadian rhythms on skeletal muscle health.

4.3 Introduction

Sleep is essential for sustaining life, with humans spending approximately one third of their existence asleep (37, 154). Guidelines from the National Sleep Foundation advocate that 7-9 hours of sleep each night is required for maintaining health in adults (5), but roughly one third of Americans aged 18 and over fail to meet these recommendations (39). This modern epidemic of sleep loss coincides with the shifting demands of our 21st century lifestyle (9, 155) that place a premium on 'round-the-clock' remote working hours, made possible by 24 hour access to light, food, and internet-based social activities. In the face of low levels of habitual physical activity and the ease of acquiring unrestricted energy-dense snacks, normal circadian rhythms are disrupted, carrying profound implications for many physiological and metabolic processes (156). For example, adults who fail to meet the

recommended quantity of sleep have an increased risk of all-cause mortality (29), with chronic sleep insufficiency underpinning numerous negative health and performance-related outcomes (47, 157-160). During periods of short-term and chronic sleep restriction there are disruptions to skeletal muscle and whole-body glucose homeostasis that predispose individuals to several disease states including obesity, insulin resistance, and type 2 diabetes (4, 35, 161, 162). Endocrine function is also sensitive to reductions in sleep duration, provoking alterations in the concentrations of appetite hormones such as leptin and ghrelin, which influence feelings of hunger and satiety (4, 159). Additionally, the secretion of steroid hormones are affected by sleep, with higher concentrations of plasma cortisol reported in healthy adult males the evening following one night of acute sleep deprivation (163). These alterations to the hormonal milieu induced by insufficient sleep may be a catalyst underpinning disturbances to skeletal muscle metabolism. Indeed, disturbed sleep results in decreased rates of muscle protein synthesis in healthy male adults (80) that, over time, may result in a loss of lean mass and concomitant reductions in muscle strength and functional outcomes (95).

To rescue some of the deleterious effects of sleep loss on health and wellbeing triggered by our modern-day lifestyle, there is a need to understand the mechanisms that underpin the perturbations to metabolic and hormonal homeostasis incurred by insufficient sleep, and their impact on skeletal muscle and other organs/tissues. Identifying these mechanisms is important to develop and implement preventative public health strategies to combat the detrimental effect of sleep loss at both the individual and population level. As the light/dark cycle is the dominant ‘zeitgeber’ (time giver) for the endogenous molecular clock and has a major influence on the sleep/wake cycle, an understanding of the interactions between sleep disturbances and circadian biology and how these impact on peripheral tissues is critical to develop the most efficacious interventions to tackle our modern-day sleep epidemic. This review outlines the importance of adequate sleep for human function, provides a synopsis of circadian biology, and highlights the complex interactions between sleep, circadian biology, and skeletal muscle. A discussion of several strategies to help overcome the negative effects of disturbed sleep or circadian rhythms on skeletal muscle health are also summarised.

4.4 Discussion

4.4.1 Sleep architecture and the role of sleep in human function

There are two distinct stages of sleep, consisting of Rapid eye movement (REM) and non-rapid eye movement sleep (NREM) (30, 46). REM sleep is characterised by episodic bursts of rapid eye movements, reductions in core and skin temperature, an irregular respiratory rate, and muscle atonia (164, 165). REM sleep, measured using electroencephalography (EEG), reveals low-amplitude, high-frequency brain waves, similar to those observed during periods of wakefulness (166). The increased brain activity during REM sleep is associated with dreaming and has been proposed to play a critical role in memory consolidation (33). NREM sleep is comprised of three phases: the first phase (N1) is considered a transitional stage of sleep and is characterised by the reduction of alpha brain waves and cessation of saccadic eye movements (167). During stage two (N2), the emergence of short bursts of mixed-frequency sleep spindles and high-amplitude K-complexes are observed (167). While these first two phases are considered 'light sleep', stage three (N3) is referred to as 'deep sleep' or slow wave sleep (SWS) due to the presence of high-amplitude, low-frequency delta brain waves (168). Although a fourth stage of NREM sleep has been reported (169), this category has since been combined with stage three, collectively referred to as N3, mainly due to the difficulties associated with discerning the complex composite neuronal activity.

Sleep plays a critical role in maintaining whole-body homeostasis and preserving normal physiological and psychological function (3, 30, 33, 157-159). Sleep has a complex and often bi-directional relationship with many organs and tissues in the body that impact metabolic (1), immune (158), endocrine (3), musculoskeletal (95), and cognitive processes (33). Adequate sleep facilitates complex higher cognitive processes such as memory consolidation and learning (33), whereas bouts of insufficient sleep act as a catalyst for decreased cognitive performance, manifested by increased reaction times, lapses in attention, and cognitive dysfunction (157). An association between patterns of reduced sleep and decreased performance and function is also observed in other physiological processes (3, 4, 99, 159). However, the complex nature of sleep coupled with difficulties in controlling epigenetic factors has made it challenging to determine the precise mechanisms that underpin declines in performance and physiological function.

4.4.2 Circadian biology: Keeping time

Circadian rhythms are defined as roughly 24-hour oscillations in biological and metabolic pathways, with a large number of these daily cycles dependent on endogenous molecular clocks that control a significant portion of the genome. While many of the molecular and physiological oscillations can vary in amplitude and even phase, they share a 24-hour periodicity, which temporally follows the earth's rotation around its axis. Circadian rhythmicity can be observed in many physiological processes including sleep, core body temperature, glucose metabolism, heart rate, blood pressure, and hormonal and neurotransmitter secretion (170). The circadian clock is cell autonomous and present in most human tissues/organs, with each tissue containing clocks exhibiting properties based on the sum of all the cell clocks in that tissue. The circadian clock is organised in a hierarchical manner with the hypothalamic suprachiasmatic nucleus (SCN) functioning as the 'central clock' (171, 172). Examples of SCN-directed circadian rhythms include the sleep-wake cycle, meal timing, glucose metabolism, insulin secretion, and learning and memory (170).

At the epicentre of the molecular complex that constitutes the circadian clock are the core transcription factors Circadian locomotor output cycles kaput (CLOCK) and brain and muscle arnt-like protein-1 (BMAL1) that collectively drive the transcription of a large array of clock-controlled genes. CLOCK and BMAL1 also orchestrate the transcription of their own repressors, period (PER) and cryptochrome (CRY), forming a self-regulated feedback loop. In humans, during the active phase of the day, which typically corresponds with daylight hours, increases in the transcription of *per* and *cry* genes results in the accumulation of the PER and CRY circadian repressors: these sequentially inhibit CLOCK-/BMAL1-driven transcription of *per*, *cry* and other clock-activated genes. While rodents express similar patterns of core clock genes in skeletal muscle, their expression is aligned with the night-time hours of the rest/active cycle (173). The regulated degradation of PER and CRY alleviates transcriptional repression and permits CLOCK-/BMAL1-mediated transcription to proceed once again, thus underpinning the recurring and rhythmic cycles in circadian gene expression (170-172).

'Zeitgebers' are external time cues that function to align and 'fine tune' the body's endogenous clock mechanisms with the prevailing external environmental conditions.

Light exposure is the dominant zeitgeber for the SCN oscillator, which then orchestrates rhythms in the peripheral organs/tissues at appropriate phases. Circadian cycles can also be reprogrammed or phase-shifted by cues from peripheral tissues/organs as they adjust to changing environmental or epigenetic signals. Such fine tuning to the SCN comes from a variety of inputs including the sleep-wake cycle, food intake (both the timing of meals and meal composition), and patterns of physical activity/inactivity. In this regard, skeletal muscle is a major peripheral tissue capable of recalibrating circadian oscillations by virtue of its central role in maintaining whole-body and cellular homeostasis. Accounting for about 45% of total body mass, skeletal muscle is the major insulin sensitive tissue for post-prandial glucose disposal, while exerting a major impact on core body temperature (via shivering or sweating) and driving metabolic rate via patterns of physical activity/inactivity (174). Physical activity modulates the molecular clock in skeletal muscle, affecting both the amplitude and phase of circadian oscillations (49). Over 2,300 genes governed by circadian oscillations have been identified in skeletal muscle with crucial roles in myogenesis, transcription and metabolism (51). Recent attention has focussed on the timing of exercise bouts to coordinate with an individual's circadian rhythms as an efficacious strategy to maximize the health benefits of exercise (175). Synchrony between the SCN and peripheral clocks is important, as several deleterious outcomes arise if phases of the clocks become misaligned. For example, altered sarcomere structure, mitochondrial pathologies, and impaired muscle function have been observed in skeletal muscle of rodents with circadian misalignment (176), while disturbed metabolism and peripheral insulin resistance have been reported in humans (173). Factors that disrupt synchronicity between the SCN and peripheral clocks include insufficient sleep, perturbations to the normal timing of meals that alter individual feeding-fasting cycles, and variations to patterns of physical activity and inactivity (173, 177), all of which have been exacerbated by the ongoing global pandemic (178).

4.4.3 Skeletal muscle and sleep cross-talk

The human sleep/wake cycle can be described by the two-process model of sleep, involving the interaction of circadian rhythms and homeostatic drive (30). While awake, levels of adenosine accumulate in the brain causing a build-up of 'sleep pressure' throughout the day. This increase in adenosine concentrations has been proposed to be responsible for the inhibition of excitatory neurons and suppression of CNS activity, which

decreases wakefulness, subsequently inducing feelings of sleepiness and a desire to sleep (30). In conjunction with the homeostatic drive, the SCN detects multiple zeitgebers from the environment that synchronise the body's internal clock and signal the release of hormones (e.g., melatonin) that facilitate the process of falling asleep. Thus, the interaction of homeostatic drive and normal circadian oscillations largely regulate the sleep/wake cycle. However, over the lifespan, there are age-related changes to sleep patterns (both architecture and duration) influenced by intrinsic and epigenetic factors that, in turn, are associated with alterations to the function of several major tissues, including skeletal muscle. For instance, the composition of sleep architecture changes with age, with infants and children obtaining longer sleep durations compared to adolescents, adults, and the elderly (179). In older age, when regular sleep patterns are not attained, anabolic signalling pathways in skeletal muscle are down-regulated, contributing to a loss of lean mass and a predisposition to sarcopenia (66). In this regard, peak skeletal muscle mass is attained within the first three decades of life and thereafter begins to decline with the incidence and severity of sarcopenia progressively increasing over the remaining lifespan. Muscle mass is lost in the course of healthy ageing from approximately age 30 years, a loss reaching a rate of ~1% per year after the age of 65, and associated with a corresponding 2–3 fold loss in strength (180). The coexistence of diminished muscle mass coupled with increased fat mass, so-called 'sarcobesity', is ultimately manifested by impaired mobility and/or development of many chronic life-style-related diseases (180). Accordingly, achieving appropriate sleep durations is important for maintaining the integrity of muscle mass across the lifespan.

At the cellular level, the maintenance of muscle tissue is regulated throughout the day by a series of cyclic metabolic processes that coordinate rates of protein synthesis and protein breakdown (181). Such processes are influenced by habitual levels of physical activity, age, and dietary protein availability. If the rate of muscle protein synthesis is greater than the rate of protein breakdown over a sustained period (i.e., several weeks and months), there is a net increase in protein accretion and muscle hypertrophy (182). When rates of muscle protein breakdown exceed the rate of protein synthesis for sustained periods, there is a loss of muscle mass (71). Rates of muscle protein synthesis can be augmented by several factors including resistance-based exercise, protein intake and meal timing, the hormonal milieu, and sleep (47, 71, 80, 81, 182, 183). Exactly how protein signalling pathways interact with fragmented or insufficient sleep durations is unknown. For

instance, a night of complete sleep deprivation induces a catabolic environment, possibly leading to a subsequent increase in the rate of muscle protein breakdown (184). In contrast, there have been reports of no changes in the expression of proteolytic genes after a night of sleep deprivation (80). These differences may be attributed to the varying timepoints (07:30h (184) vs 13:00h (80)) at which muscle biopsies were collected and differences in nutrition status (fasted (184) vs postprandial (80)). While there are direct links between sleep quality and duration, and skeletal muscle homeostasis, our understanding of how the critical nodes that control muscle bioenergetics are disrupted by disordered sleep are incomplete. How systemic inflammation in response to disturbed sleep influences muscle health and functional outcomes is an important consideration, as inflammation may contribute to muscle protein breakdown and impaired myogenesis (185). The influence of circadian biology in these processes may also be a factor determining the magnitude of responses.

4.4.4 Circadian rhythm and skeletal muscle

The interactions between circadian rhythms, peripheral clocks, and skeletal muscle function have been reviewed previously (52, 176, 186). Peripheral clocks, located in skeletal muscle tissue are predominantly regulated by BMAL1, CLOCK, PER, and CRY genes (52, 186), with their expression largely regulated by the prevailing muscular environment (i.e., contractile state) and the timing of meals (187). Zambon et al. (187) used DNA microarrays to determine the effects of a single bout of resistance exercise on gene regulation in human muscle biopsy samples obtained six and 18 hours after an acute bout of isotonic unilateral knee extensions. A comparison of gene expression profiles of the exercised and non-exercised legs revealed 704 genes were differentially regulated after six hours, and 1,479 genes at 18 hours post exercise, whereas in the non-exercised 'control' leg, only 608 genes were differentially regulated at comparable time points. The bout of resistance exercise upregulated circadian clock genes (*Per2*, *Cry1*, and *Bmal1*) and circadian output genes, demonstrating that peripheral clocks are regulated independently of the SCN (187). In support of this contention, Dyar et al. (188) report that contractile activity controls the oscillation of around 15% of skeletal muscle circadian genes independently of the core muscle clock, thereby providing direct evidence that circadian locomotor activity rhythms drive circadian rhythms of selected nuclear translocation and target gene expression.

The local ‘muscular environment’ appears to be sensitive to modifications to the expression of circadian clock genes, with circadian rhythms influencing rates of skeletal muscle protein synthesis (186). Chang et al. (189) reported that circadian oscillations occur in the phosphorylation of the mammalian target of rapamycin (mTOR)/p70S6K and extracellular signal-regulated kinase (ERK) pathways in different tissue (cardiac versus skeletal muscle) and muscle fibre types (oxidative vs glycolytic). These findings suggest that the circadian oscillation in the activities of protein synthesis-related intracellular signalling pathways are tissue-specific (189). While the precise mechanism underpinning the circadian oscillation of mTOR/p70S6K remain to be determined, the importance of activating the mTOR pathway and its downstream effector, p70S6K, and their roles in the regulation of muscle protein synthesis is well established (190). Determining the interaction between circadian oscillations and the molecular pathways that underpin skeletal muscle protein balance is an important avenue for future research, as preservation of muscle mass is critical to maintain metabolic health and function.

4.4.5 The effects of disturbed circadian rhythms on skeletal muscle physiology

There are numerous mediators that act to disrupt the normal rhythm of the molecular clock. Insufficient or fragmented sleep is a common cause of disruption to daily biological rhythms and metabolic homeostasis, with just one night of total sleep deprivation suppressing BMAL1 expression in human peripheral leukocytes (191). In BMAL1 global knock-out Macaque monkeys, higher nocturnal locomotion and reduced sleep are observed with physiological circadian disruption reflected by the markedly dampened and arrhythmic levels of blood hormones and disturbances to blood cortisol concentrations (192). While suppression of BMAL1 inhibits sleep, this ‘master’ clock protein also plays a vital role in regulating sleep patterns (193). Rodent models support the relationship between core clock genes and sleep/wake behaviour, with BMAL1 activity in skeletal muscle identified as a critical regulator of NREM sleep duration (193). However, the mechanisms of how skeletal muscle BMAL1 activity influences sleep architecture in humans are not well established.

Disrupted biological rhythms negatively impact several physiological processes, including muscle atrophy (186, 194) and disturbances to normal mitochondrial function (195),

combining to exacerbate metabolic conditions such as sarcopenia and type 2 diabetes (196). Mitochondrial health and function are critical for skeletal muscle physiology, with over a third of the proteins in the mitochondrial proteome exhibiting circadian patterns (186, 197). Rhythmicity of the mitochondria are also observed on a functional level, with rates of muscle mitochondrial respiration exhibiting daily oscillations in both rodent (198) and human (195) skeletal muscle. When the daily oscillation of protein expression in the mitochondria is disturbed, there are reductions in the quantity of mitochondria, elevated apoptosis, and detrimental effects on exercise capacity (197). When biological rhythms are disturbed by periods of insufficient sleep, there are marked reductions in the amplitude of the diurnal rhythm of peripheral skin temperature along with impaired mitochondrial function. In healthy young males, five nights of sleep restriction reduced glucose tolerance and decreased mitochondrial respiratory function (81). The relationship between disturbed sleep and impaired glucose metabolism is highlighted by the sensitivity of postprandial glycaemic control to disrupted sleep/wake behaviours (199). It appears that glucose kinetics are significantly influenced by sleep efficiency, with postprandial glycaemic control impaired in adults when delayed bedtimes were implemented and poor sleep efficiency was present (199). Additionally, disruptions in skeletal muscle core-clock genes, lower magnitude and quantity of cycling genes, and altered patterns of oxygen consumption are observed in individuals with type 2 diabetes (195). These findings highlight the sensitivity of the interplay between endogenous rhythms and metabolic health (Figure 4.1).

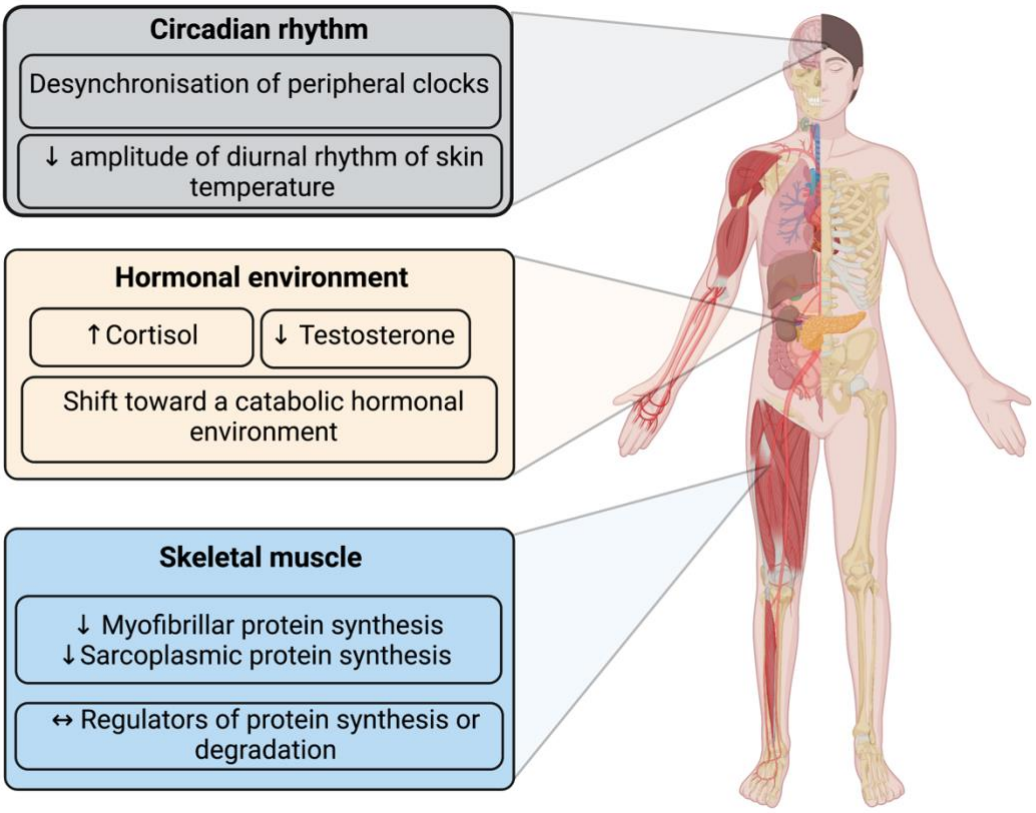

Figure 4.1. Effects of insufficient sleep on the muscular environment.

4.4.6 The effect of insufficient sleep on skeletal muscle physiology

Insufficient sleep has consistently been shown to contribute to muscle atrophy (74-77) and in rodent models, restricted sleep is linked to atrophy of muscle tissue (75, 76, 78, 79). Reductions in muscle tissue weight and cross-sectional area of the plantaris muscle were observed in 75-day-old Wistar rats after 96 hours of paradoxical sleep deprivation compared to a control group of animals with normal sleep patterns (79). Additionally, decreases in body mass, along with reductions in tibialis anterior mass were reported after 96 hours of paradoxical sleep deprivation in three-month-old Wistar rats (75). The proposed mechanism responsible for sleep-deprivation induced muscle atrophy in rodents may be due to alterations to the local hormonal environment, with increased levels of corticosterone and reduced levels of testosterone a response to reductions in sleep (75, 76, 78, 79) (Figure 4.2). Additionally, the catabolic environment may act as a trigger for increased activity of the glucocorticoid signalling pathway which may have downstream effects on protein synthetic rates and protein degradation in skeletal muscle (200).

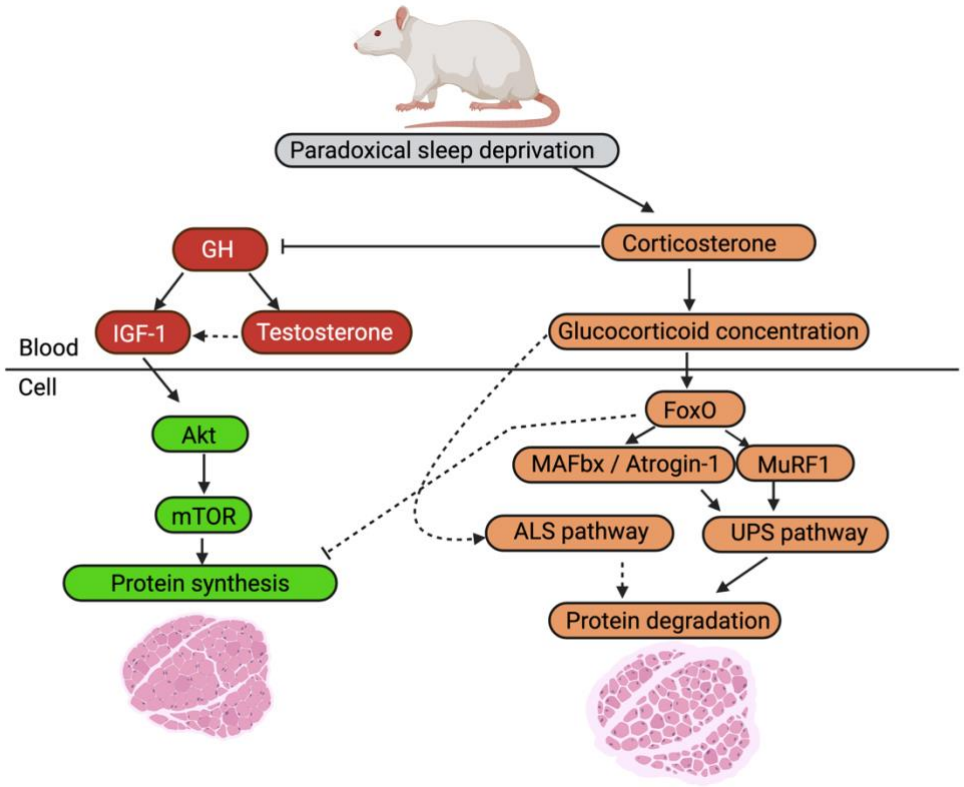

Figure 4.2. Potential mechanisms that affect rates of skeletal muscle protein turnover after paradoxical sleep deprivation in rodents. Elevated levels of corticosterone in response to paradoxical sleep deprivation can cause stimulation of the Forkhead box O (FoxO) family, MAFbx1, and muscle RING finger protein (MuRF1) which can amplify the activity of the ubiquitin proteasome system (UPS) and autophagy lysosome system (ALS) protein degradation pathways (201). Additionally, the elevation of catabolic hormones may down-regulate the phosphorylation of proteins involved in the Akt-mTOR pathway (76), inhibiting rate of protein synthesis. GH: Growth hormone, IGF-1: Insulin-like growth factor one, mTOR: Mammilian target of rapamycin.

While sleep restriction induces muscle atrophy in rats, it is important to place such findings in context: there is a large degree of homogeneity within individual skeletal muscles from rodents, but this is not the case for humans (174). For example, muscle atrophy induced by restricted sleep is preferentially confined to type IIa and IIb muscle fibres of the gastrocnemius when three-month-old Wistar rats were subjected to 96 hours of paradoxical sleep deprivation (76). Furthermore, oxidative muscle tissue of the soleus from rodents was resistant to sleep restricted muscle atrophy compared to glycolytic muscle tissue of the flexor digitorum longus and tibialis anterior, and mixed fibre types of the gastrocnemius in adult male Wistar rats (78). It appears that insufficient sleep has the most marked effect on glycolytic muscle fibres, with muscles comprised of predominantly

fast twitch fibres being more sensitive to sleep deprivation than slow-twitch, oxidative type I fibres.

Compared to rodent models, the relationship between sleep restriction and muscle atrophy in humans has received less investigation (12, 80, 81). Population data has suggested chronic insufficient or poor-quality sleep is correlated to lower muscle mass (31), although the precise mechanisms that underpin these observations are not well understood. To date, studies reveal that acute and chronic (one to five nights) bouts of insufficient sleep negatively affect rates of muscle protein synthesis in healthy adults (12, 74, 80). Saner et al. (12) found reduced sleep (four hour sleep opportunity each night) over five nights impaired myofibrillar protein synthesis in the vastus lateralis (fractional synthetic rate (FSR): 1.24 ± 0.21% d^{-1}) in healthy male adults compared to an eight hour sleep opportunity (FSR: 1.53 ± 0.09% d^{-1}). Additionally, Lamon et al. (80) reported one night of total sleep deprivation was sufficient to decrease muscle protein synthesis in the vastus lateralis by 18% (FSR: 0.059 ± 0.014%·h^{-1}) compared to a night of normal sleep (FSR: 0.072 ± 0.015%·h^{-1}). This reduction in FSR after sleep deprivation is of a similar magnitude to the decreases in muscle protein synthesis observed after short-term energy restriction (202).

4.4.7 The interplay between sleep, circadian rhythms, the hormonal environment, and skeletal muscle

The endocrine system and sleep have an intricate bi-directional relationship (203), with sleep affecting the secretion of hormones and in turn, sleep being affected by their secretion. Hormones commonly reported when assessing the interaction between sleep and muscle tissue include cortisol, testosterone, insulin-like growth factor 1 (IGF-1) and growth hormone (GH) (74, 80, 103), all of which are subject to diurnal rhythms. However, there are marked differences in the hormonal profiles between males and females, without consideration of the normal hormonal fluctuations encountered during the menstrual cycle (204). Cortisol is secreted by the adrenal glands in a pulsatile cycle, with the highest concentrations typically measured in the early morning upon waking, and declining throughout the afternoon and evening (203). In contrast, low concentrations of testosterone are observed in the late evening, followed by a rise shortly after the onset of sleep, reaching their highest levels during the early morning in healthy adult males (205). In

addition, GH, secreted by the pituitary gland, occurs during SWS, with the majority (~ 80%) of the total 24-hour GH release occurring during the first 90 min of sleep (206). Sleep is a critical regulator of these endocrine secretions, with each hormone showing regular oscillations in their expression and often being sensitive to disrupted or fragmented sleep, altering their patterns of expression (203).

In rodents, chronic sleep deprivation markedly affects the expression of a several hormones, with prolactin, leptin, GH, and IGF-1 suppressed by paradoxical sleep deprivation (207). Additionally, the pulsatile nature of GH appears to be shunted by sleep deprivation, with the high-amplitude pulses which normally occur during the first episode of SWS not observed when sleep is disrupted (208). In contrast, catabolic 'stress' hormones such as corticosterone are elevated in response to paradoxical sleep deprivation (75, 76). In response to sleep deprivation, there may be an emergence of a potential 'anabolic resistant' phenotype, as observed in elderly humans, causing a decrease in the rate of muscle protein synthesis even in the presence of adequate amino acid availability (76, 209). It has also been suggested that the elevation of catabolic hormones down-regulates the phosphorylation of proteins involved in the Akt-mTOR pathway (76), further inhibiting rates of protein synthesis. This pattern of a sleep-restricted catabolic hormonal environment is thought to be similar in humans.

Disrupted sleep phases lead to shorter periods of REM sleep, which may induce increases in cortisol levels due to disruption of the hypothalamic-pituitary-adrenal (HPA) axis (160). As a result of increased HPA activity, corticotropin-releasing hormone is secreted from the hypothalamus causing a downstream effect on both the anterior pituitary gland and subsequently, the adrenal glands, resulting in elevated levels of cortisol released (210), promoting a more catabolic environment. One night of complete sleep deprivation was sufficient to elevate plasma cortisol levels by 21% in healthy adult male and females (80). Additionally, plasma cortisol was reported to be elevated by 37% the evening after acute partial sleep restriction and by 45% after complete sleep deprivation (163). Considering the role of cortisol and glucocorticoids in stimulating muscle protein degradation pathways such as the ubiquitin proteasome system (UPS) and the autophagy lysosome system (ALS) (211), insufficient sleep is likely to be a catalyst for a catabolic and proteolytic muscular environment, potentially promoting anabolic resistance in humans.

Anabolic hormones such as testosterone, GH, and IGF-1 are regulators of muscle tissue growth and repair through their effect on rates of muscle protein synthesis (212). However, during periods of insufficient sleep, anabolic hormone concentrations in healthy adult males decrease (213). For example, Lamon et al. (80) observed a 24% decrease in testosterone concentration following a single night of sleep restriction in a cohort of healthy young adult males and females. Although the study was not powered to detect between-sex differences, male participants encountered greater declines in testosterone during periods of sleep deprivation compared to females. In contrast, there were no observable changes in total testosterone, but a higher ratio of cortisol:testosterone after 48 hours of sleep deprivation followed by a 12-hour sleep in a group of healthy males (74). However, both studies reported a similar pattern of perturbed testosterone secretion across the day (74, 80). It is important to acknowledge the physiological differences in hormonal profiles between males and females (214) as findings and recommendations from research are likely to be sex-specific. Therefore, further investigation into sex-specific differences in hormonal changes, as well as how they interact with sleep and the muscular environment is warranted (215). Moreover, accounting for the variation in hormone concentrations during the menstrual cycle may be prudent, as hormonal fluctuations with varying stages of the menstrual cycle have been observed and how these changes interact with sleep are largely unknown.

4.4.8 Mitigating the effects of insufficient sleep

Considering the detrimental effects of sleep deprivation on skeletal muscle, several strategies to attenuate muscle tissue atrophy induced by insufficient sleep have been proposed (76, 77, 79, 81). In rodent models, high-intensity resistance-based exercise or leucine supplementation may provide a protective mechanism against sleep-restricted muscle atrophy by stimulating muscle protein synthesis via the Akt/mTORC1/p70S6K signalling pathway. An overview of strategies used to mitigate the detrimental effects of insufficient sleep on muscle protein synthesis in rodents are displayed in Figure 4.3.

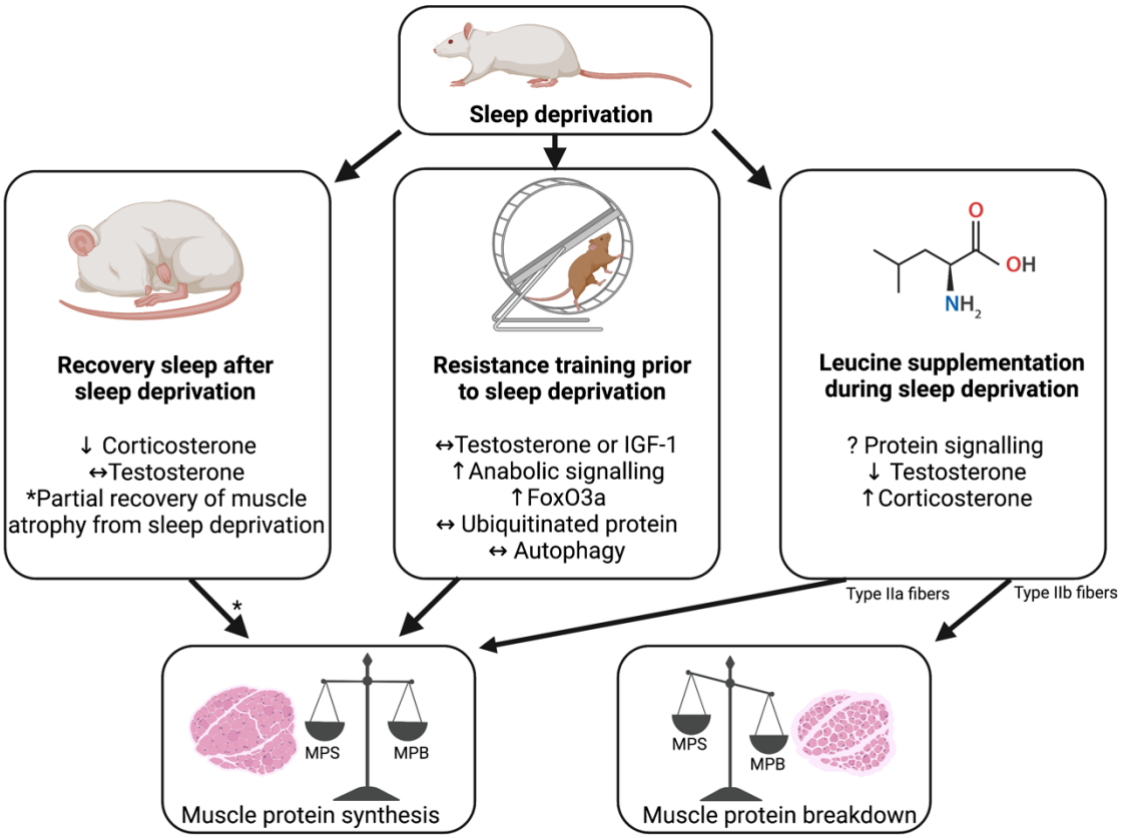

Figure 4.3. Effects of sleep deprivation on muscle protein turnover in rodents and potential strategies to mitigate these effects. In rodents, high-intensity resistance training can mitigate the catabolic effects of sleep restriction on skeletal muscle, and promote a more 'anabolic environment' with increases in insulin-like growth factor (IGF-1) and testosterone (79). Such increases in anabolic hormones result in similar levels as observed in rodents who obtain normal sleep patterns. Supplementing rodents with leucine (1.35g/kg of body mass per day) provides a protective effect on type IIb muscle fiber atrophy during 96 hours of paradoxical sleep deprivation (76). However, intermediary (i.e., Type IIa) fibers atrophied even with leucine supplementation (76). MPS: Muscle protein synthesis, MPB: Muscle protein breakdown, Fox03a: Forkhead box O3 a.

To date, the precise mechanisms responsible for insufficient sleep-related muscle atrophy in humans are not well defined (12, 80, 81). This limited information presents a challenge when attempting to recommend efficacious preventative strategies. Saner et al. (12, 81) implemented a high-intensity interval exercise cycling protocol during a five-night sleep restriction intervention (four hour sleep opportunity each night) which mitigated the adverse effect of reduced sleep on myofibrillar and sarcoplasmic protein synthesis in a group of healthy male adults (Figure 4.4). The results of these studies highlight the possible protective nature of exercise on preserving rates of muscle protein synthesis in the face of disturbed sleep. Further exploration of alternate modalities of exercise warrant investigation as metabolic responses to exercise are mode specific. For example, resistance

exercise has been shown to stimulate myofibrillar protein synthesis to a greater extent than high intensity aerobic-based exercise (216).

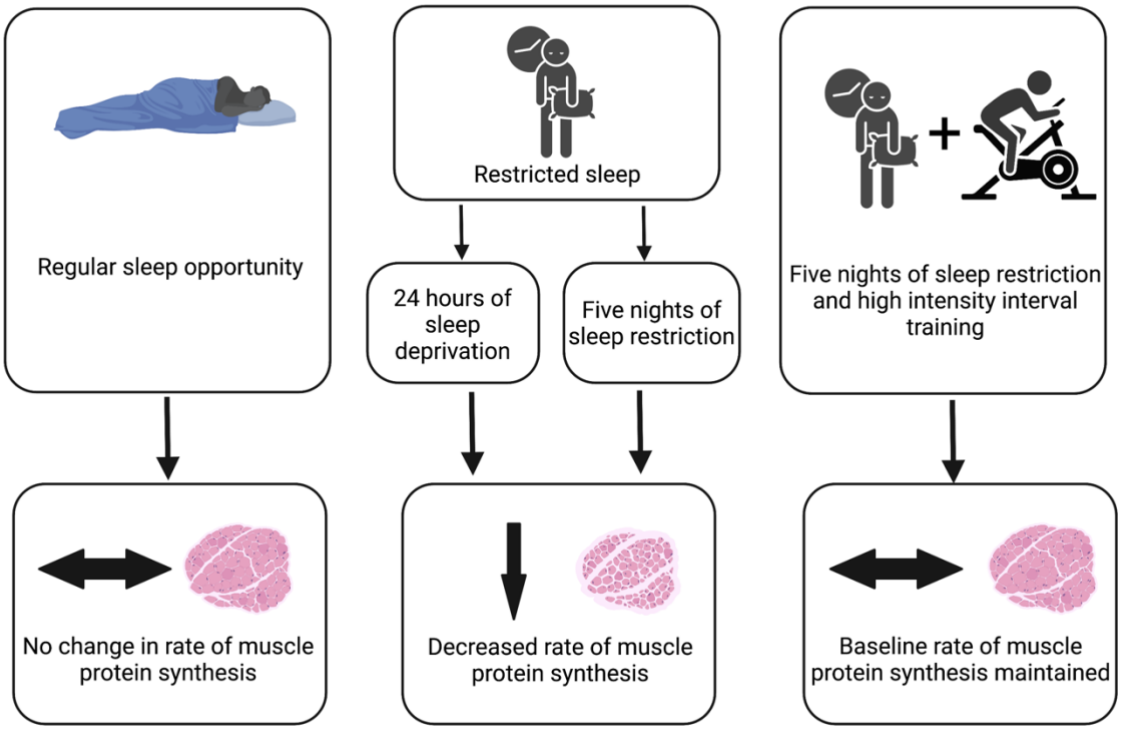

Figure 4.4. Overview of the effects of sleep restriction on rates of skeletal muscle protein synthesis. Reduced sleep durations lower the rate of myofibrillar and sarcoplasmic protein synthesis in the vastus lateralis of healthy adults (12, 81). Implementing high intensity interval training during periods of sleep restriction result in a preservation of the rates of muscle protein synthesis.

The ability to preserve rates of muscle protein synthesis and promote skeletal muscle health has broad appeal to a variety of sleep-restricted populations. Identifying appropriate and effective preventative exercise interventions for sleep-restricted individuals is an important next step. While HIIE appears to promote or maintain rates of muscle protein synthesis during periods of reduced sleep (81), exploring additional modalities of exercise and their efficacy in wider populations is prudent. Establishing the optimal mode, frequency, duration, and intensity of exercise may allow for the development of specific exercise guidelines and recommendations to help mitigate the detrimental effects of restricted sleep on human skeletal muscle. Additionally, developing and promoting efficacious sleep enhancement strategies in synergy with evidence-based exercise guidelines may aid in reducing the host of detrimental effects of insufficient sleep on skeletal muscle and metabolic health.

4.4.9 Exercise as an intervention to improve sleep and realign circadian rhythms

Exercise has a positive effect on sleep-wake behaviours and circadian rhythms (50, 217). Regular exercise increases sleep duration and improves SWS, while also reducing sleep onset latency, REM sleep, and wake after sleep onset (23). However, there are several factors to consider when interpreting these findings, including the exercise prescription (mode, duration, timing, and intensity), and individual characteristics such as age, sex, and fitness level (23). These factors highlight the complex interactive nature between physiological and psychological pathways that influence sleep (17), making it difficult to determine the precise mechanisms that underpin improved sleep after exercise.

There are three independent, but related hypotheses that attempt to explain the beneficial effects of exercise on sleep: the thermoregulatory hypothesis, the energy conservation hypothesis, and the body restoration hypotheses (23). The thermogenic hypothesis proposes that sleep onset coincides with reductions in basal metabolic rate and core body temperature, induced by systemic vasodilation and the accompanying peripheral heat loss (23). The energy conservation and body restoration hypotheses purport that the greater daily exercise-induced energy expenditure may be a catalyst for increased SWS and total sleep time. As exercise is a significant catalyst for increased energy turn-over and overall daily energy expenditure, it is thought that the increased exercise-induced energy deficit may be a driving force underlying improved sleep patterns.

Recently, it has been suggested that exercise-induced peripheral factors may be a potential mechanism for improved sleep after exercise (24). For instance, elevated levels of brain-derived neurotrophic factor (BDNF) have been linked with increased slow wave activity during sleep in rodent models (86). The BDNF-induced improvements in sleep architecture are purportedly underpinned by increases in the synaptic strength of corticortical connections as a response to the elevated brain BDNF (24). In humans, aerobic exercise elevates peripheral BDNF levels in the blood (24). However, increased brain BDNF is likely a result of fibronectin type III domain containing 5 (FNDC5) protein activity, which is cleaved and secreted from skeletal muscle during exercise as the myokine irisin (218). Another mechanism for increased brain BDNF concentrations is through the exercise induced stimulation of the peroxisome proliferator-activated receptor

y coactivator 1α (PGC-1α)/FNDC5 pathway (219). Indeed, studies in rodents demonstrate a PGC-1α/FNDC5/BDNF pathway that is activated in the hippocampus by endurance exercise (218).

Exercise and the molecular clock have an intricate, bi-directional relationship interconnected by numerous signalling pathways. As such, exercise can be used as an effective 'time cue' for realigning circadian rhythms in skeletal muscle (220). This is an important consideration, because when the molecular clock is disrupted in muscle, metabolic dysfunction and muscle atrophy are exacerbated (186). There are several potential mediators linking exercise and circadian rhythms, with the AMP-activated protein kinase (AMPK), PGC-1α, and hypoxia-inducible factor 1α (HIF-1α) all playing regulatory roles in the molecular clock response to exercise (220). Increased metabolic and cellular energy requirements induced by exercise are potent activators of AMPK, which in turn, affect core molecular clock gene expression by reducing the stability of PER and CRY proteins (50). The increased AMPK activity and subsequent CRY1 instability enhance proliferator-activated receptor δ (PPARδ) regulation of lipid metabolism and energy uncoupling in skeletal muscle (50, 221), thus maintaining cellular energy homeostasis.

The transcriptional coactivator PGC1-α regulates several exercise-associated aspects of muscle function including mitochondrial biogenesis and muscle plasticity (i.e., fibre type transitions), mediating many of the beneficial effects of exercise on human health, while also suppressing a broad range of inflammatory responses. During exercise in both humans and rodents, there is a rapid increase in PGC-1α mRNA expression followed by induction of elevated PGC-1α protein expression throughout recovery and for the subsequent 24 h post-exercise (222). The expression of *PGC-1α* exhibits a strong diurnal rhythm in skeletal muscle, with PGC-1α inducing the expression of several core clock genes, particularly *Bmal1*, *Clock*, *Per2* and *Rev-erbα*, in a cell-autonomous manner. The induction of these clock genes is partly mediated through its coactivation of orphan nuclear receptor RORα, with the PGC-1α/ROR and Rev-erbα/HDAC transcriptional complexes playing an antagonistic role in the transcriptional regulation of *Bmal1* expression. The physiological role of PGC-1α in normal clock function is supported by the finding of significant impairments of diurnal rhythms of locomotor activity, body temperature and metabolic rate in *PGC-1α null* mice (223). PGC-1α also plays a role in the exercise-induced increase in

HIF-1α expression, which influences the molecular clock via binding with core clock promoters. Taken collectively, these findings strongly implicate the PGC-1 family of coactivators as a nodal point in integrating energy metabolism and the body clock, and reinforce the notion of exercise as a potent 'time cue' to recalibrate circadian rhythms (50, 220). For recent reviews on the relationship between circadian biology and exercise, the reader is referred to Wolff and Esser (220) and Mansingh and Handschin (50).

4.5 Conclusions

This review highlights the complex relationship between sleep, circadian biology, and skeletal muscle health. At a population level, significant proportions of society experience insufficient sleep and are at increased risk of numerous metabolic and musculoskeletal conditions. Fragmented or insufficient sleep disrupts daily biological rhythms and can cause a cascade of detrimental effects to numerous cells, tissues, and organs, resulting in impaired metabolic and physiological functions. Both acute and chronic sleep restriction have a negative influence on muscle health and function. At the cellular level, inadequate sleep disrupts metabolic function and biological rhythms. Skeletal muscle metabolism is disrupted by poor sleep, with reduced rates of protein synthesis in response to the perturbations in the hormonal environment. The disruption to skeletal muscle metabolism and the hormonal milieu is likely responsible for the atrophic effect on muscle tissue associated with insufficient sleep. However, research in this area is in its infancy, and precisely how circadian clocks interact in with these epigenetic cues is unclear. The ability to mitigate the negative effects of insufficient sleep may have numerous benefits in both regulating and maintaining metabolic health and physical performance.

Investigating the mechanisms that underpin the relationship between sleep, circadian rhythms, and skeletal muscle is warranted and expanding current findings to divergent population groups is important. Determining and quantifying the existence of between-sex responses to sleep deprivation is critical, as embracing such differences will allow for personalised recommendations for improved sleep strategies. Exploring if responses are similar in exercise-trained versus sedentary populations is also needed. Evaluating the utility of various exercise interventions to maintain skeletal muscle metabolism during periods of reduced sleep is prudent. Examining the role of circadian phenotypes ('owls versus larks') and their influence on the interactions between the molecular clock, muscle

metabolism, and sleep is also an area of potential future research and may help to identify the extent to which individual endogenous rhythms need to be accounted for when designing and implementing sleep interventions.

Practice Points

1. Disrupted sleep/wake patterns negatively impact skeletal muscle clocks, with reduced rates of protein synthesis observed following acute periods of sleep restriction.
2. Inadequate sleep durations can misalign peripheral muscle clocks from the central oscillator and perturb daily biological rhythms, with deletrious effects muscle physiology and metabolic health.
3. Exercise may attenuate some of the negative effects of reduced sleep patterns on rates of muscle protein synthesis.

Research Agenda

1. Investigating the effect of chronic sleep disruption on muscle metabolism, as well as population specific responses (e.g., sex, age, shift-workers) would lead to a greater understanding of the relationship between sleep and whole-body metabolic health
2. A greater understanding of the mechanisms associated with sleep, circadian rhythms, and skeletal muscle health is prudent when developing interventions aimed at mitigating the detrimental effects of insufficient sleep.
3. Establishing the efficacy of various exercise strategies (i.e., timing and mode) after periods of disrupted sleep would allow for effective interventions to be designed and implemented to maintain skeletal muscle and whole-body metabolic health.

Chapter 5: Study 2 - Quantifying the effect of afternoon moderate-intensity exercise on sleep quality and quantity in healthy adult males using polysomnography

Publication statement:
This chapter comprised of the following paper submitted to the journal Sleep in February 2024.

5.1 Linking paragraph

As discussed in Chapter four, numerous behaviours have the ability to influence sleep. When individuals consider interventions to enhance their health, incorporating an exercise regime is often considered. However, the specific ways exercise influences sleep is not overtly clear, and the current evidence on the effects of exercise on sleep is equivocal. The varied results observed may be due to the differences in methodologies adapted by researchers or the individual physiology of participants. It appears exercise can affect sleep, but this influence is contingent on the timing and configuration of the exercise bout, yet little is known regarding the influence of afternoon moderate-intensity aerobic exercise. Further, suboptimal sleep is prevalent in Australia, with ~48% of adults estimated to have at least two sleep-related problems (224) and 33% - 45% of adults self-reporting inadequate sleep quality or quantity (6). Additionally, there is a lack of evidence employing gold-standard sleep measurement techniques when examining the influence of exercise on sleep. Given the significance of both sleep and exercise to overall health, it is important to determine how exercise interacts with subsequent sleep. Consequently, Chapter five focuses on determining the effects of moderate-intensity afternoon exercise on subsequent sleep measured using polysomnography in healthy adult males.

Quantifying the effect of afternoon moderate-intensity exercise on sleep quality and quantity in healthy adult males using polysomnography

5.2 Abstract

Introduction: The acute effects of exercise on sleep can be positive or negative, depending on numerous factors including modality, intensity, and timing of exercise. Given the popularity of afternoon exercise, it is important to determine whether it affects sleep. Therefore, the aim of this study was to investigate the effect of afternoon moderate-intensity cycling exercise on objective and subjective sleep in healthy adult males without early or late chronotypes.

Methods: To assess the effect of moderate-intensity afternoon exercise on sleep quality and quantity, 12 healthy adult males who were identified as good sleepers completed a repeated-measures, counter-balanced, crossover study design with two conditions (moderate-intensity aerobic exercise or no exercise). Polysomnography variables assessed were total sleep time; N1, N2, N3 and REM sleep duration; sleep onset latency; wake after sleep onset; sleep efficiency; stage REM onset latency; stage N3 onset latency; arousals; arousals in NREM; arousals in REM; awakenings; and stage shifts. The exercise task involved cycling for 40 minutes at 70%HRmax and was completed at ~15:30h. Polysomnography was used to measure sleep during a 9-hour sleep opportunity (23:00h to 08:00h). Sleep was subjectively assessed using questionnaires 30-minutes after waking. Between-group differences were assessed using Wilcoxon Rank-Sum tests (non-parametric variables) and Welch Two-Sample T-Tests (parametric variables). Magnitude of differences were assessed using Pearson's *r* (non-parametric variables) and Cohen's *d* (parametric variables) effect sizes with 95% confidence intervals.

Results: There were no statistically significant changes in objective or subjective sleep quality or quantity between conditions. The inter-quartile range for total sleep time (exercise: 51.5 mins vs no exercise: 13.4 mins) and sleep efficiency (exercise: 9.5% vs no exercise: 2.5%) suggest that there was more individual variability in subsequent sleep after afternoon exercise compared to no exercise. Exercise appeared to have a moderate effect on reducing total sleep time (mean ± SD; control 493.7 ± 12.6 mins vs exercise: 471.5 ± 55.2 mins; Cohen's *d*: -0.56), sleep efficiency (control 91.4 ± 2.3% vs exercise: 87.3 ± 10.2%; Cohen's *d*: -0.56), and increasing REM onset latency (control: 76.1 ± 45.1 mins vs

exercise: 102.8 ± 46.9 mins; *r*: 0.33), although the results did not reach statistical significance ($p > 0.05$).

Discussion: Healthy adult males can likely complete afternoon moderate-intensity exercise without compromising their subsequent sleep. Individual responses in objective sleep outcomes may vary after exercise.

5.3 Introduction

To maintain health, it is recommended that adults achieve approximately 7-9 hours of sleep per night (5). When sufficient sleep durations are not obtained, various aspects of physiological (157), metabolic (225), and cognitive (226) function may be impaired. For example, during periods of sleep restriction, there are alterations to glucose kinetics, appetite, and net energy expenditure (13). To mitigate the deleterious effects of insufficient sleep, various strategies such as pharmacological interventions (14), nutritional supplements (26), the promotion of sleep health practices (227, 228), and exercise (229) have been suggested to improve the sleep/wake cycle. However, despite these interventions, approximately one third of adults are not currently achieving sufficient sleep durations (7, 34, 39). Therefore, identifying and implementing strategies to improve sleep-wake behaviours is prudent.

Physical activity and exercise are important contributors to both mental and physical health (230). However, modern society promotes a 24-hour lifestyle where extensive work hours are often prioritised. When coupled with unrestricted access to light, food, and entertainment, it becomes evident why the recommended levels of physical activity and sleep are often not met. Furthermore, global data from 1.9 million individuals (via the International Physical Activity Questionnaire (231)) identified that 27.5% of adults were not completing recommended levels of physical activity (232), highlighting the prevalence of sedentary behaviour in society alongside the insufficient sleep. Thus, it appears that the typical demands of modern life and work requirements are often prioritised over essential contributors to good health, such as exercise and sleep, and may compete with one another for time on a daily basis (233).

Exercise is often suggested to have a beneficial effect on sleep, depending on the timing and configuration of the exercise bout (21, 234). Acute exercise appears to improve several indices of sleep, including total sleep time, sleep onset latency, and sleep efficiency across a broad range of demographics (21, 22, 235). However, these improvements are likely influenced by the timing of exercise in proximity to bedtime (229). Laboratory-based studies using polysomnography (PSG) to assess both sleep quality and sleep quantity in healthy adult males have reported equivocal findings (20). For example, increases in sleep efficiency were observed in healthy adult males after evening exercise (236). In contrast,

evening moderate-intensity exercise did not improve sleep quality or quantity in a similar cohort of healthy adult males (129). The differences in sleep observed may contributed to by the aerobic exercise modality, with stationary ergometer cycling (129) vs treadmill running (236), which have differing physiological and metabolic demands (237). Additionally, a quantitative synthesis of findings suggests that exercise completed 3 to 8 hours before bedtime resulted in greater reductions in REM sleep duration than exercise performed > 8 hours or < 3 hours from bedtime (229). These equivocal findings may be attributed to the complex relationship between sleep and exercise, with various factors including light exposure (238), and the composition and timing of exercise interventions (239), having the potential to influence sleep.

Determining the effect of afternoon exercise on sleep is important, as the afternoon is often a popular time of day to undertake exercise. Given the health benefits of exercise and the importance of obtaining sufficient sleep quality and quantity, it is essential to investigate how afternoon exercise affects sleep. As afternoon moderate-intensity exercise may potentially disrupt sleep by reducing REM sleep duration compared to evening or morning exercise (229), exploring this relationship is prudent. Currently, there is a lack of robust, well-controlled investigations examining subsequent objective and subjective sleep quality and quantity following afternoon exercise. When considering the current public health concerns stemming from sedentary behaviours and insufficient sleep, it is important to establish whether the timing of moderate-intensity exercise affects subsequent sleep. If a bout of moderate-intensity exercise in the afternoon can influence subsequent sleep, it would be an important consideration when developing effective strategies for promoting positive health behaviours. Therefore, the aim of this study was to investigate the effect of afternoon moderate-intensity cycling exercise on objective and subjective sleep in healthy adult males.

5.4 Methods

5.4.1 Design and procedures

A repeated-measures, counterbalanced, crossover design was employed to examine the impact of moderate-intensity cycling exercise performed in the afternoon (~15:30h) on subsequent sleep in healthy adult males. Sleep was measured each night using PSG during a 9-hour sleep opportunity (23:00h - 08:00h). On day one, participants were familiarised

with the exercise protocol and commenced a standardised diet. Night one was used to familiarise participants with the sleep measuring equipment and the questionnaires to assess subjective sleep quality. On days two and three, participants completed either a 40-minute moderate-intensity cycling protocol or undertook sedentary activities (i.e., no exercise condition) between 14:30h – 16:30h. After 16:30h, participants undertook sedentary activities for the remainder of the day. Participants rated their subjective sleepiness using a Karolinska Sleepiness Scale (KSS) at 22:30h. At 08:30h the next morning, participants rated their perceptions of sleep quality, sleep quantity, and subjective sleepiness.

5.4.2 Participants

Twelve healthy adult males volunteered to participate in the study (mean ± SD; age: 24.0 ± 4.7 years, height: 177.3 ± 9.4 cm, mass: 76.4 ± 14.9 kg). Participants completed a general health questionnaire, a pre-exercise screening tool developed by Exercise Sports Science Australia (123) (247.5 ± 170.7 weighted minutes of physical activity per week) and The Pittsburgh Sleep Quality Index (PSQI) (131) (PSQI score: 4.0 ± 1.9) to assess eligibility for the study. Participants were excluded if they had been diagnosed with a clinical sleep disorder, suffered from musculoskeletal injuries, experienced a change in medication over the study period known to affect sleep, undertaken transmeridian travel in the previous three months, or if they were smokers or shift-workers. Eligible participants were required to attend the laboratory in the week prior to the study to undertake a submaximal graded exercise cycling test to determine respective workloads for the experimental exercise condition. Participants were informed of the experimental procedures, provided with an opportunity to ask questions, and gave signed written consent prior to commencing the study. Participants were asked to maintain their typical sleep/wake habits in the week prior to the study and to avoid consuming alcohol or caffeine in the 24 hours before the study. The experimental protocol was approved by the CQUniversity's Human Research Ethics Committee (0000022194) and reciprocal approval was obtained from the Australian Catholic University Human Research Committee (2021-181R).

5.4.3 Living conditions

Participants lived and slept in a purpose-built accommodation suite at the CQUniversity's Appleton Institute in Adelaide, Australia. The laboratory does not have any windows and

once inside, participants are not exposed to any natural light. Six participants can be accommodated within the suite concurrently, which is configured similarly to a serviced apartment with each participant having their own private bedroom, lounge room, and bathroom. During the day, when participants were not undertaking exercise testing, they were able to engage in routine sedentary activities such as reading, using laptops and tablets, and watching television. Participants were not permitted to undertake any additional exercise outside the allocated experimental condition and were required to stay awake throughout the day. Researchers monitored participants for compliance using close-circuit television and in-person monitoring.

5.4.4 Meals

Nutritional intake was standardised for each participant for the duration of the study and was consistent with the macronutrient content observed in a typical Western diet (240). All meals provided to participants were calorie-controlled and the number of calories that were made available for consumption at each meal opportunity was consistent for each respective meal (i.e., snacks, breakfast, lunch, and dinner). Participants were provided with breakfast, lunch, and dinner at approximately 9:30h, 12:00h, and 18:30h, respectively. Additional snack opportunities were provided to participants at approximately 10:45h and 15:30h. On average, participants consumed 9044 ± 1480 kJ per day. Water was available ad libitum throughout the day from 08:00h until 23:00h.

Outside of designated meal and snack times, participants were not permitted to consume any food or beverages apart from water. Additionally, participants were not permitted to consume substances known to affect sleep (e.g., alcohol and caffeine (241, 242)) at any time during the protocol.

5.4.5 Sleep

Sleep was recorded using PSG equipment (Grael; Compumedics, Melbourne, VIC) with a standard montage of electrodes. Electrodes were applied in the 60 minutes prior to lights out and included three electroencephalograms (C4-M1, F4-M1, O2-M1), two electrooculograms (left/right outer canthus), and a submental electromyogram. All sleep records were blinded and scored manually in 30-second epochs in accordance with established criteria by the same sleep technician (56). The following dependent variables

were calculated from the sleep recording: total sleep time (min), which identifies the time spent in any stage of sleep (i.e., N1, N2, N3, Rapid Eye Movement (REM)) during time in bed; time spent in stages N1, N2, N3 and REM sleep (min); sleep onset latency (min), which reflects the time between lights-out until the first epoch of any sleep stage (i.e., N1, N2, N3, REM); wake after sleep onset (min), which represents the time spent in bed awake minus sleep onset latency; sleep efficiency (%), which reflects total sleep time divided by time in bed x 100; stage REM onset latency (min); stage N3 onset latency (min); arousals (count); arousals in NREM (count); arousals in REM (count); awakenings (count); and stage shifts (count).

5.4.6 Subjective sleepiness

Subjective sleepiness was assessed using the Karolinska Sleepiness Scale (KSS) (140). The KSS is a 9-point scale where 1 = "extremely alert", and 9 = "very sleepy, great effort to keep awake, fighting sleep". Participants were instructed to indicate their current level of sleepiness by circling the corresponding number on the 9-point scale.

5.4.7 Subjective sleep quality, sleep duration, sleep latency

Subjective sleep quality was assessed using a 7-point scale, where 1 = "extremely poor", 2 = "very poor", 3 = "poor", 4 = "average", 5 = "good", 6 = "very good" and 7 = "extremely good" (129). Subjective sleep quantity and subjective sleep onset latency were assessed verbally, by asking participants "how much sleep do you think you got?" and "how long did it take you to fall asleep?", respectively (129).

5.4.8 Heart rate

Heart rate was continuously monitored using Polar M400 heart rate monitors (M400, Polar Electro; Kempele, Finland) during the submaximal graded exercise test and standardised cycling exercise protocol.

5.4.9 Rating of perceived exertion

Rating of perceived exertion (RPE) was assessed throughout the submaximal graded exercise test and the standardised cycling exercise protocol using the Borg CR10 scale (144). Participants were asked to choose a number on the 1-10 scale that best represented their current level of exertion.

5.4.10 Submaximal graded exercise test

Participants performed a submaximal graded exercise test on a stationary cycle ergometer (Wattbike Trainer, Wattbike Ltd; Nottingham, UK). Prior to the test, participants were instructed to cycle for five minutes at a self-selected pace corresponding to an intensity of approximately five on the Borg CR10 scale of RPE. A 2-minute rest was then provided. The test began with participants cycling at a power output of 55 W, with a cadence of 60 revolutions per minute. Each minute, the required power output increased by 15 W by increasing cadence. Participants rated their RPE each minute during the test and once the participant reached an RPE of seven (i.e., "hard"), the test was concluded. The heart rate and power output for each minute of the test were plotted for each participant and the predicted power output corresponding to 70% of their maximum heart rate (%HRmax) was extrapolated (243).

5.4.11 Standardised cycling exercise protocol

The standardised moderate-intensity cycling protocol began with a 5-minute warm-up with participants cycling at approximately 50%HRmax at a self-selected cadence on a stationary ergometer followed by a 2-minute period. The participant then cycled for 40 minutes at 70%HRmax during which heart rate was monitored continuously. If heart rate deviated from the pre-determined target, the air resistance of the cycle ergometer was adjusted to maintain the relative exercise intensity. Heart rate, RPE, and power output were recorded every five minutes. After the 40 minutes of cycling, participants completed a 2-minute cool down.

5.4.12 Statistical analysis

All statistical analyses were performed in RStudio (Version 2022.12.0+353) using the R programming language (Version 4.2.1). Before statistical analysis, data were examined for normality through visual inspection of density plots using ggplot2 (Version 3.3.6) and objectively via the Shapiro Wilk test. For variables that were significantly different from a normal distribution ($p < 0.05$), non-parametric tests were used. For variables that were normally distributed, parametric tests were used. The Wilcoxon Rank-Sum test and Welch Two Sample T-Test were used to assess differences between conditions. Statistical significance was set with an alpha of $p < 0.05$. For data that were not normally distributed,

the magnitude of differences was assessed by calculating effect sizes (*r*) and 95% confidence intervals as trivial ≤ 0.10 small, ≤ 0.3; medium ≤ 0.5; and large, > 0.5 using the *rcompanion* package (Version 2.4.16) (244). Cohen's *d* effect sizes and corresponding 95% confidence intervals were calculated and interpreted as small > 0.2, medium > 0.5, and large ≥ 0.8 for normally distributed data (147). Separate cutoffs were used for effect sizes due to the differences in underlying statistical approaches. Data are presented as mean ± standard deviation (SD). Interquartile ranges (IQR) were calculated to present the dispersion of the data, allowing for clearer observation of potential variability within each outcome variable.

Individualised objective sleep data points were plotted and visualised using the *raincloudplots* package (Version 0.2.0) (148). Sleep stage distributions during each condition were plotted using histograms. Sleep hypnogram data (recorded in 30-second epochs) were binned into five-minute intervals, and the percentage of each stage (i.e., wake, stage N1, stage N2, stage N3, and REM) was calculated. The percentages were plotted on the y-axis, with each stage stacked on top of each other to represent the overall distribution, while the x-axis shows plots of the five-minute bins.

5.5 Results

5.5.1 Sleep

There were no significant differences in objective or subjective sleep parameters between the exercise and no exercise condition (Table 5.1). However, exercise had a tendency to cause a moderate decrease in total sleep time (mean ± SD; control 493.7 ± 12.6 mins vs exercise: 471.5 ± 55.2 mins; Cohen's *d*: -0.56 (-0.31 to 1.42)), sleep efficiency (control 91.4 ± 2.3 % vs exercise: 87.3 ± 10.2 %; Cohen's *d*: -0.56 (-0.31 to 1.42)), and an increase in REM onset latency (control: 76.1 ± 45.1 mins vs exercise: 102.8 ± 46.9 mins; *r*: 0.33 (-0.64 to 0.10)) compared with no exercise, although these outcomes did not reach statistical significance (all $p > 0.05$). For most sleep variables, the IQR was wider for the exercise condition compared with the no exercise condition (Table 5.1; Figures 5.1-5.2). No changes in subjective sleep or sleepiness were observed after exercise (Table 5.1).

The sleep probability distribution of sleep stages for sleep after exercise vs control are presented in Figure 5.3. After exercise, it appears N3 sleep duration is sustained longer

than compared to no exercise prior to sleep. Additionally, it appears there are greater periods of wake in the final three hours of the sleep episode in the exercise condition compared with the final three hours of the sleep episode in the no exercise condition.

5.5.2 Standardised Cycling Exercise Protocol

Participants maintained an average heart rate of 134 ± 2 beats per minute (bpm), which corresponds with a mean relative heart rate percentage of 70.16% ± 0.55% HRmax during the standardised cycling exercise protocol. Mean power output during the exercise task was 103.6 ± 52.9 W and the mean RPE and final RPE were 4.1 ± 1.2 and 4.0 ± 1.7, respectively.

Table 5.1. Objective and subjective sleep outcomes and subjective sleepiness outcomes

	Conditions				Statistical analysis	
	Control		Exercise			
Variable	Mean ± SD	IQR	Mean ± SD	IQR	*p*-value	Effect size compared to control (95% CI) *interpretation*
Sleep						
Total sleep time (min)	493.7 ± 12.6	13.4	471.5 ± 55.2	51.5	0.198	-0.56 (-0.31 to 1.42)^{d} *medium*
Sleep onset latency (min)	19.7 ± 16.3	20.5	18.9 ± 15.8	16.8	0.839	-0.05 (-0.36 to 0.43)^{r} *small*
Sleep efficiency (%)	91.4 ± 2.3	2.5	87.3 ± 10.2	9.5	0.197	-0.56 (-0.31 to 1.42)^{d} *medium*
Wake after sleep onset (min)	122.7 ± 18.7	25.9	115.8 ± 19.8	19.5	0.391	-0.36 (-0.50 to 1.21)^{d} *small*
Stage N1 duration (min)	33.3 ± 12.9	15.8	31.7 ± 13.9	14.6	0.776	-0.12 (-0.73 to 0.97)^{d} *trivial*
Stage N2 duration (min)	237.0 ± 35.7	33.3	228.1 ± 43.5	44.0	0.583	-0.12 (-0.29 to 0.53)^{r} *small*
Stage N3 duration (min)	100.7 ± 41.4	42.0	95.8 ± 35.2	35.8	0.757	-0.13 (-0.72 to 0.98)^{d} *trivial*
REM duration (min)	122.7 ± 18.7	25.9	115.8 ± 19.8	19.5	0.391	-0.36 (-0.50 to 1.21)^{d} *small*
Stage N3 onset latency (min)	14.3 ± 6.6	5.1	13.0 ± 5.1	5.1	0.595	-0.22 (-0.63 to 1.07)^{d} *small*
REM onset latency (min)	76.1 ± 45.1	32.8	102.8 ± 46.9	55.9	0.112	0.33 (-0.64 to 0.10)^{r} *medium*
Arousals (count)	119.3 ± 33.9	41.8	119.2 ± 47.8	53.8	0.996	-0.002 (-0.85 to 0.85)^{d} *trivial*
Subjective sleep outcomes						
Sleep quantity (h)	7.1 ± 0.8	7.0	6.7 ± 0.8	7	0.377	-0.19 (-0.26 to 0.55)^{r} *small*
Sleep quality (units)	5.3 ± 1.1	6.0	4.9 ± 1.3	5	0.435	-0.17 (-0.25 to 0.55)^{r} *small*
Subjective sleepiness						
KSS bedtime (units)	5.3 ± 1.1	2.0	4.8 ± 1.6	2	0.409	-0.18 (-0.23 to 0.58)^{r} *small*
KSS wake (units)	3.7 ± 1.4	1.3	3.5 ± 0.8	1	0.720	-0.15 (-0.70 to 0.99)^{d} *trivial*

Notes: SD = standard deviation; IQR = Inter-quartile range; 95% CI = 95% Confidence Intervals; ^{d} = Cohen's *d* effect size; ^{r} = *r* effect size; REM = Rapid eye movement; KSS = Karolinska Sleepiness Scale; ^{d} = parametric tests; and ^{r} = non-parametric tests.

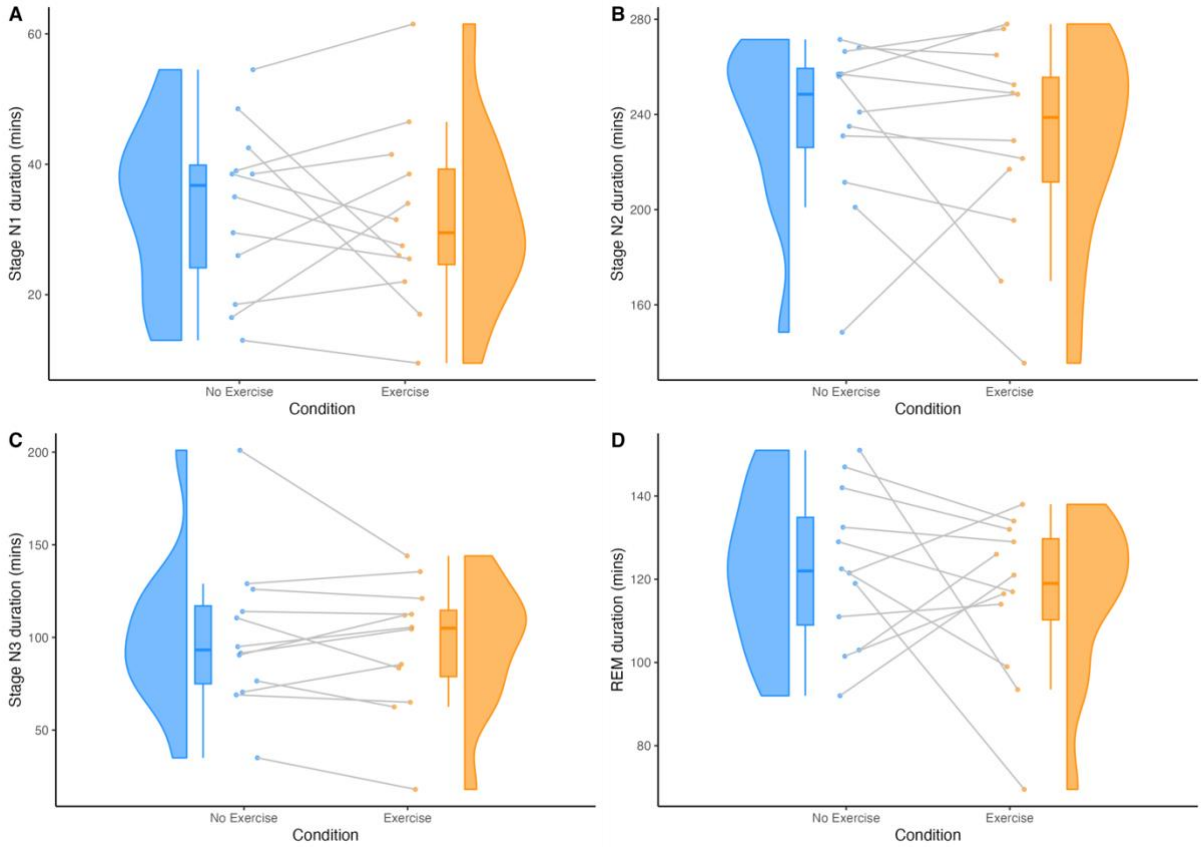

Figure 5.1. Raincloud plots indicating the effect of exercise vs no exercise on stage N1 (plot A), stage N2 (plot B), stage N3 (plot C), and REM (plot D) sleep duration. Adjoining lines connect individual pairs of datapoints between conditions. Vertical box plots represent the median of the data (solid horizontal line), the interquartile range (length of the box plot), and the minimum and maximum values, excluding outliers (whiskers from box plot). The half-violin plots represent the shape and density distribution of data from each condition.

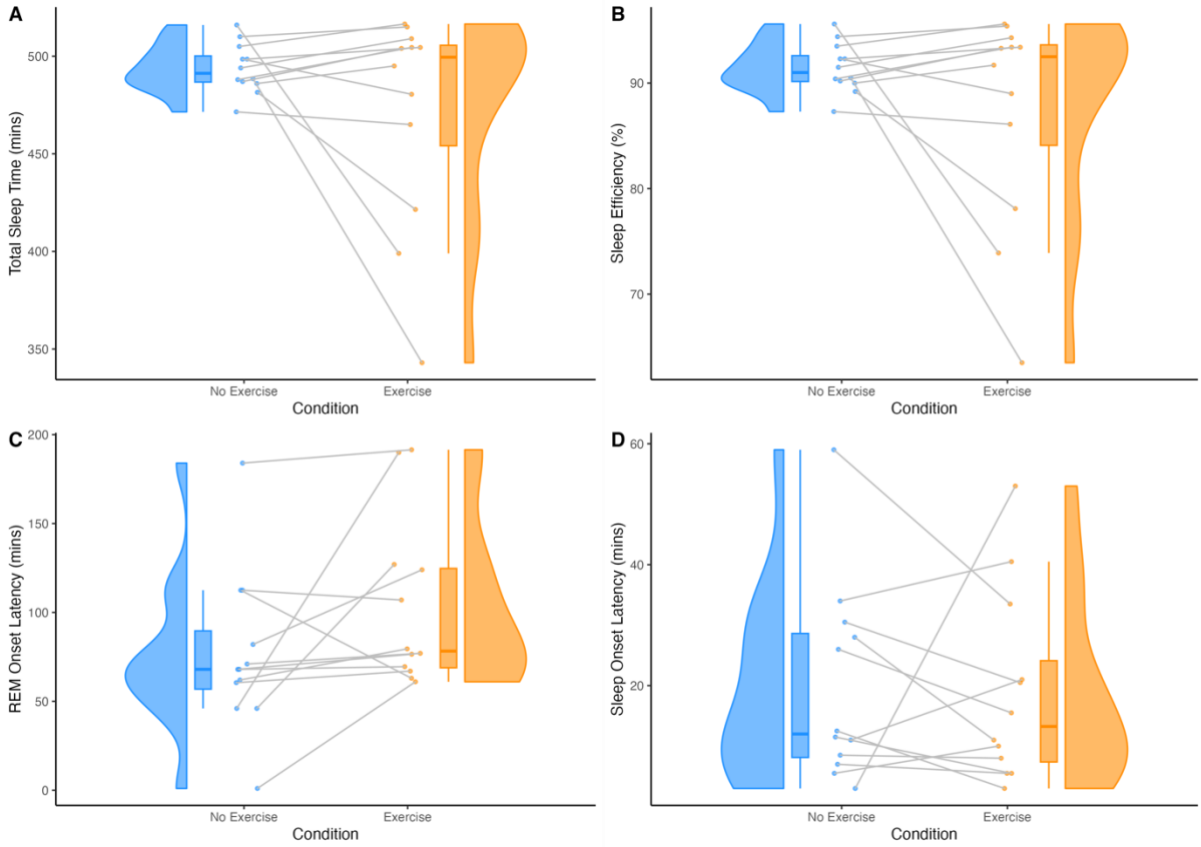

Figure 5.2. Raincloud plots indicating the effect of exercise vs no exercise on total sleep time (plot A), sleep efficiency (plot B), REM onset latency (plot C), and sleep onset latency (plot D). Adjoining lines connect individual pairs of datapoints between conditions. Vertical box plots represent the median of the data (solid horizontal line), the interquartile range (length of the box plot), and the minimum and maximum values, excluding outliers (whiskers from box plot). The half-violin plots represent the shape and density distribution of data from each condition.

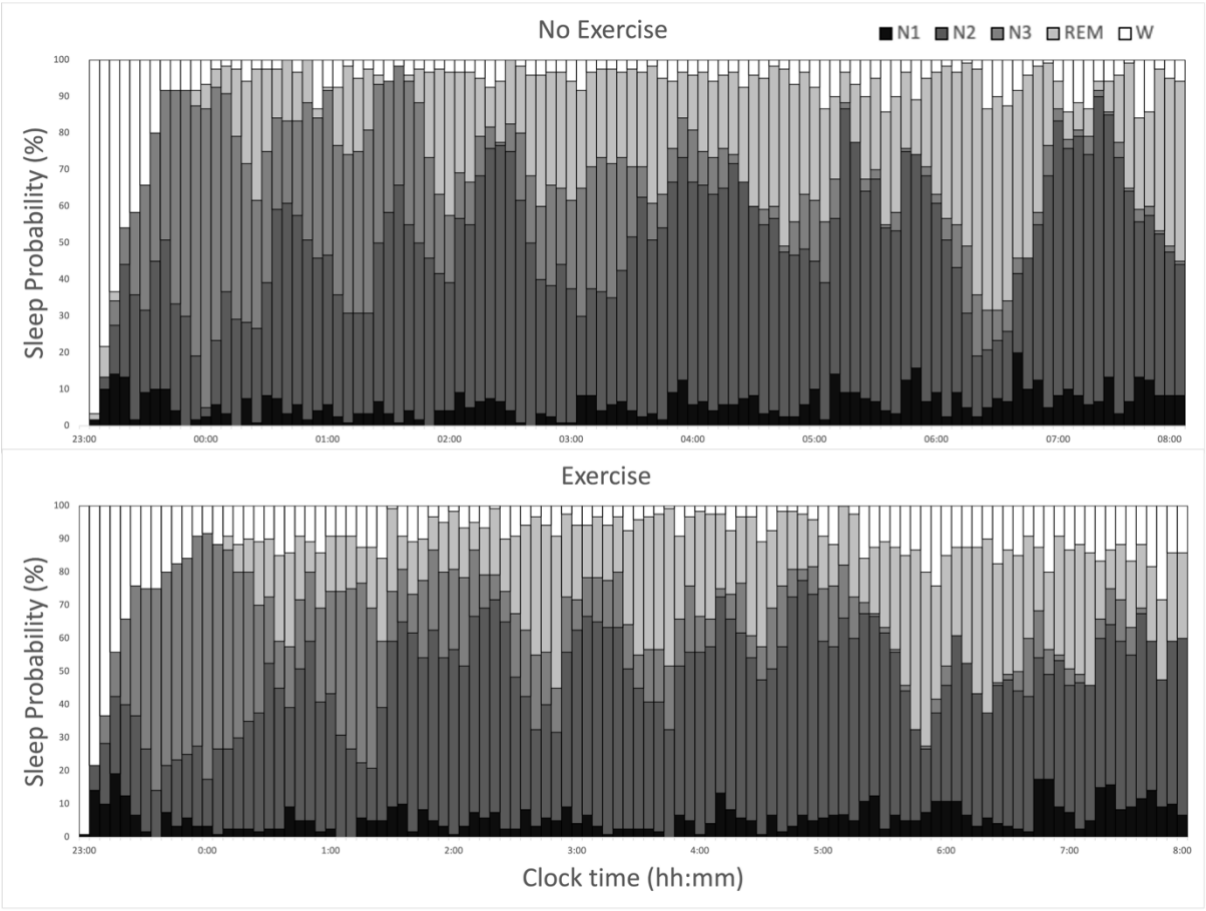

Figure 5.3. Sleep histograms representing the probability distribution of sleep stages between the control (top) and exercise (bottom) condition. Data represent the percentage of epochs scored as Stage N1, Stage N2, Stage N3, REM, and wake (W) in five-minute bins. The concept for this figure was sourced from Sargent et al. (245).

5.6 Discussion

The aim of this study was to investigate the effect of afternoon moderate-intensity cycling exercise on objective and subjective sleep quality and quantity in healthy adult males. There were no statistically significant differences in objective or subjective sleep parameters between the exercise and no exercise condition, which suggests exercise can be performed in the afternoon without negatively influencing sleep. However, some components of sleep appeared to be sensitive to exercise, with a tendency for exercise to cause a moderate impact on reducing total sleep time and sleep efficiency, and a moderate effect on increasing REM onset latency in some participants. Additionally, wide IQRs were observed for total sleep time and sleep efficiency after exercise, suggesting individual sleep responses to exercise are highly variable.

Traditional sleep hygiene guidelines suggest exercise should be performed in the afternoon or early evening, rather than in close proximity to bedtime to avoid impacting sleep. However, contemporary recommendations suggest a 90-minute cut-off for moderate intensity exercise before bed (246). Despite these refined guidelines, equivocal findings explaining the effect of exercise on sleep throughout the day remain and definitive recommendations for the optimal timing for exercising without impairing sleep are lacking. As sleep was not significantly influenced by afternoon exercise in this study, it is likely that the afternoon is an appropriate time to exercise without impairing sleep. Consequently, the findings of this study support the notion of encouraging afternoon exercise promoted by traditional sleep hygiene guidelines. Acknowledging the considerable variability that was observed in this study, it would be prudent to adopt an individualised approach to scheduling exercise in order to avoid influencing sleep. Nonetheless, it is important to note these findings may be limited to healthy adult males conducting moderate-intensity aerobic exercise without early or late chronotypes, and not generalisable to other populations or modalities of exercise, as physical characteristics and exercise prescription may have different effects on sleep.

No improvements in sleep were observed after the moderate-intensity exercise in this study. Exercise showed a tendency to elicit a moderate effect on delaying REM onset latency, suggesting exercise may be able to increase the amount of NREM sleep that occurs prior to the initiation of REM sleep in some participants. This finding aligns with previous research that suggests that exercise may delay REM onset latency (~10 mins) (21, 22). The increase in REM onset latency observed in this study may be due to an increased need for NREM sleep stages to facilitate restorative physiological processes such as nervous system recuperation and assist with energy conservation after exercise (247, 248). However, the current findings also contrast with research that suggests exercise may have a small positive effect on total sleep time (~10 mins) (21, 22). In the current study, there was greater variability in sleep responses observed after exercise compared with no exercise, despite the use of a Latin Square counterbalancing design to avoid potential order effects. This variability may attributed to the individual characteristics of the participants (e,g., age, fitness level). These findings suggest that, in general, moderate-intensity exercise performed in the afternoon can be performed without impairing subsequent sleep.

The effects of acute exercise on sleep may be moderated by variables including the timing, intensity, and duration of exercise (21). Exercise timing has been suggested as one factor that may contribute to the relationship between sleep and exercise (23). Exercise conducted between three and six hours before bedtime has been suggested to aid in sleep, potentially due to thermoregulatory changes in core body temperature, which have been theorised to improve slow wave sleep architecture (249). As exercise was performed ~7 hours prior to bedtime in this study, it is speculated any potential changes in core body temperature that may augment sleep after exercise would have likely resided by bedtime. Additionally, the intensity and duration of exercise are proposed to be important moderating factors for improved sleep architecture (23). For example, longer duration and higher intensity exercise (e.g., 75-80% VO_2max for 1.2h) appears to improve slow wave sleep compared to rest, possibly due to the increased demand in net energy turnover during exercise (23, 250). As sleep did not improve after moderate-intensity and shorter duration exercise, our findings support the notion that higher-intensity and longer duration exercise may be required to augment sleep in healthy adult males. This is possibly due to the lower metabolic and physiological demands required of moderate-intensity exercise compared to high- or vigorous-intensity exercise. However, whether these findings are reproducible with alternate exercise modalities (e.g., resistance training), durations (e.g., > 40 mins) and intensities (e.g., maximal effort) would be important to consider as the physiological response to exercise varies and is individual in nature.

Although this study investigated the effects of moderate-intensity exercise performed in the afternoon on subsequent sleep in healthy adult males, there are limitations that should be noted. In the current study, a potential ceiling effect may have been observed, as participants were provided with an identical sleep opportunity between 23:00h and 08:00h that was free from typical distractions encountered under free-living conditions. Additionally, core body temperature was not measured during the study, which may have provided further insight into the physiological response to exercise and whether core body temperature had returned to baseline prior to bedtime. Finally, the findings in this study should not be generalised to all populations and forms of exercise, as alternate modalities (e.g., resistance training) and intensities (e.g., maximal effort) of exercise may have differing effects on sleep and are not well understood.

The current data suggest that moderate-intensity exercise performed in the afternoon does not have harmful effects on ensuing sleep. The tendency for greater individual variance in some components of sleep after exercise highlights that there may not be a one-size-fits-all approach for scheduling exercise as a tool to improve sleep. Rather, tailored recommendations that consider the needs of the individual may be more appropriate.

Chapter 6: Study 3 – Relationship between sleep quality and quantity and exercise-induced peripheral factors in healthy adult males

Publication statement:

This chapter is comprised of manuscript that is under preparation for future submission.

6.1 Linking paragraph

Chapter five identified that subsequent sleep was not significantly influenced by moderate-intensity exercise performed in the afternoon. However, individual sleep responses observed after exercise were highly-variable. Given these heterogenous findings, it is important to investigate the underlying mechanism to help explain the potential variability observed. Consequently, Chapter six aims to address this by investigating a recently proposed novel hypothesis, that suggests exercise-induced peripheral factors may explain the link between exercise and sleep. It is hypothesised that exercise will increase levels of circulating metabolic factors and these markers may have a relationship with sleep.

Relationship between sleep quality and quantity and exercise-induced peripheral factors in healthy adult males

6.2 Abstract

Introduction: Exercise is often suggested to improve sleep, however the underlying mechanisms explaining exercise-induced improvements in sleep are not well understood. Exercise-induced peripheral factors have been proposed as potential mediators of improvements in sleep after exercise but evidence in humans to support this relationship is lacking. Therefore the aims of this study were: 1) to determine the effect of afternoon, moderate-intensity aerobic exercise on tumor necrosis factor alpha (TNF-α), interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and brain-derived neurotrophic factor (BDNF); and 2) to investigate the relationship between exercise-induced peripheral factors and subsequent sleep in healthy, adult males measured using polysomnography.
Methods: To assess the effect of aerobic exercise on each peripheral factor and subsequent sleep, 12 healthy adult males who were identified as good sleepers completed a repeated-measures, counter-balanced, crossover study design with two conditions (exercise vs no exercise). To measure BDNF, TNF-α, IL-1α, IL-1β, and IL-6, five blood samples were taken across the day, morning fasted, pre-exercise, post-exercise, 1h post-exercise, and 5h post-exercise, with time-matched samples for the control condition. The exercise task consisted of cycling on a stationary cycling ergometer for 40 minutes at 70% of maximal heart rate and was undertaken ~15:50h. Sleep was measured using polysomnography during a 9-hour sleep opportunity (23:00h to 08:00h). Subjective sleep was assessed using questionnaires 30-minutes after waking. Bayesian linear mixed models were used to model the influence of exercise on peripheral factors and the relationship between peripheral factors and objective and subjective sleep.
Results: Exercise had no effect on BDNF, IL-1α, IL-1β. There was a tendency for exercise to have a small effect on increasing IL-6 (posterior probability > 0 = 0.650) and TNF-α (posterior probability > 0 = 0.866), but credible intervals spanned zero. No clear associations were observed between any pre-bed peripheral factor concentrations and objective or subjective sleep outcomes.
Discussion: Moderate-intensity cycling exercise performed in the afternoon does not appear to be a sufficient stimulus to elevate levels of select peripheral metabolic factors.

Sleep quality and quantity do not appear to have an association with low levels of select peripheral metabolic factors that have been proposed to influence the sleep/wake cycle.

6.3 Introduction

Sleep is an essential component of health, with negative effects on health manifesting when sufficient sleep durations are not obtained. For example, during periods of insufficient sleep, metabolism (225), hormonal regulation (157), and circadian rhythms (191) may be disturbed. Furthermore, these disruptions to homeostasis may underpin the relationship between insufficient sleep and health disorders such as hypertension (251), type-2 diabetes (252), and cardiovascular disease (2). Insufficient sleep appears to be a global health concern, with more than a third of American adults not achieving sufficient sleep durations (9). Similar data exist in Australian adults, with inadequate sleep quality or quantity affecting 33-45% of adults (6).

The global prevalence of insufficient sleep has led to growing interest in non-pharmaceutical means of improving sleep. For instance, exercise is commonly believed to have a beneficial effect on the sleep/wake cycle (235, 253). Specifically, improvements in slow wave sleep, sleep onset latency, and reduced wake after sleep onset have been observed after exercise (23, 254). Changes in core body temperature and the cardiovascular response to exercise have been suggested as potential mechanisms for exercise-induced improvements in sleep (17, 249). However, there are equivocal findings pertaining to the effect of exercise on sleep, with disruptions in sleep onset observed after evening vigorous exercise (20, 22, 83), and no effects on sleep observed after evening moderate-intensity exercise (18, 129). The heterogenous sleep responses observed after exercise and the lack of mechanistic understanding of the relationship between sleep and exercise, highlight the need to continue examining potential mediators of the sleep/wake cycle.

There have been no investigations to date examining the role of exercise-induced factors as a potential mechanism to improve sleep following exercise. One possibility is the release of circulating myokines and peripheral factors following exercise; these molecules have roles in inter-organ cross-talk with various organs such as adipose tissue, liver, pancreas, bone, and the brain (255, 256). Several myokines and peripheral factors have been identified as possibly contributing to improved sleep (24). Specifically, brain-derived neurotrophic factor (BDNF), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), irisin, and peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1α) have

been suggested. In rodent models, increased daytime brain BDNF concentrations have been linked with increased slow wave sleep (86). Furthermore, BDNF is suggested to enhance the strength of synaptic transmission in the brain, which is an important component of slow wave sleep (259). In both human (260) and animal (261) models, increased IL-6 appears to have a positive effect on the depth of slow wave sleep in later sleep cycles of a sleep period. An increase in depth and quality of slow wave sleep has been associated with TNF-α in a dose-dependent manner in rodents (262). Therefore, examining the role of exercise-induced myokines and peripheral factors may provide evidence to expand the mechanistic understanding of how exercise impacts sleep.

Recent literature suggests that there is a potential relationship between exercise-induced peripheral factors and sleep in rodent models. However, no study to date has examined the relationship between circulating levels of myokines in response to exercise and subsequent sleep in humans. Therefore, the aims of this study were: 1) to determine the effect of afternoon, moderate-intensity exercise on TNF-α, interleukin-1α (IL-1α), interleukin-1β (IL-1β), IL-6, and BDNF; and 2) to investigate the relationship between exercise-induced peripheral factors and subsequent sleep in healthy, adult males measured using polysomnography.

6.4 Methods

6.4.1 Design and procedures

A repeated-measures, counterbalanced, crossover design was used to examine whether exercise-induced peripheral factors are related to sleep quality and quantity in healthy adult males. The study was conducted at CQUniversity's Appleton Institute for Behavioural Sciences, in Wayville, South Australia. Sleep was measured each night using polysomnography over a 9-hour sleep opportunity (23:00h - 08:00h). On day one, participants were familiarised with the exercise protocol and commenced a standardised diet. Night one was used to familiarise participants with polysomnography and subjective sleep questionnaires. On days two and three, participants either completed sedentary activities (control condition) or undertook a 40-minute moderate-intensity cycling protocol between 14:30h – 16:30h (exercise condition). Blood samples were taken at five timepoints, fasted ~08:30h, pre-exercise ~14:30h, immediately post-exercise ~15:15h, one-hour post-exercise ~16:15h, and five-hours post-exercise ~20:15h by a trained

phlebotomist. Five samples were collected to ascertain a thorough snapshot of the metabolic peripheral factor kinetics. Further, due to the differing kinetics of the five blood markers measured, several samples across the day were taken at timepoints judged most likely to capture pertinent increases. Samples were taken at the same matching timepoints during the control condition without exercise. Following exercise, participants were instructed to undertake sedentary activities for the remainder of the day. Participants rated their subjective sleepiness prior to bedtime using a Karolinska Sleepiness Scale (KSS) at 22:30h, and 30 minutes after waking, at 08:30h. The next morning, at 08:30h, participants completed subjective questionnaires regarding their perception of sleep quality, sleep quantity, and subjective sleepiness.

6.4.2 Participants

Twelve healthy adult male participants completed this study (mean ± SD; age: 24 ± 4.7 years, height: 177.3 ± 9.4cm, mass: 76.4 ± 14.9kg). Participants completed a general health questionnaire, a pre-exercise screening tool by Exercise Sports Science Australia (123) (average physical activity/exercise per week: 247.5 ± 170.7 mins) and The Pittsburgh Sleep Quality Index (PSQI) (131) (PSQI score: 4 ± 1.9) to assess eligibility for the study. Participants were excluded if they had a clinically diagnosed sleep disorder, were extreme chronotypes, were smokers, were consuming or had a change in any medication known to influence sleep, had a musculoskeletal injury, or had undertaken shift-work in the previous three months. In the week prior to the study, participants were required to attend the laboratory and undertake a submaximal graded exercise cycling test to calculate workloads for the experimental exercise condition. Participants were informed of the experimental procedures, given an opportunity to ask questions, and provided signed written consent prior to participation. Participants were instructed to maintain their typical sleep/wake habits in the week prior to the study and to avoid consuming alcohol or caffeine in the 24 hours before the study, which was confirmed by an online questionnaire. The experimental protocol was approved by the CQUniversity's Human Research Ethics Committee (approval number: 0000022194) and reciprocal approval was obtained from the Australian Catholic University Human Research Committee (2021-181R).

6.4.3 Living conditions

Participants lived and slept in a purpose-built accommodation suite at The Sleep Lab at CQUniversity's Appleton Institute. Six participants can be accommodated within the suite concurrently, with each participant having their own private bedroom, lounge room, and bathroom. During the day when participants were not undertaking exercise, they were permitted to engage in routine sedentary activities such as reading, using laptops, and watching television. Throughout the study participants were not exposed to natural light. Participants were not permitted to undertake any additional exercise outside the allocated experimental condition and were required to stay awake throughout the day. Researchers monitored participants for compliance using close-circuit television and in-person checks.

6.4.4 Meals

Nutritional intake was standardised for each participant for the duration of the study. All meals provided to participants were calorie-controlled and the number of calories that were made available for consumption at each meal opportunity were matched across conditions For instance, the same number of calories were available for consumption at breakfast during the control condition as the exercise condition. Participants were provided with breakfast, lunch, and dinner at approximately 9:30h, 12:00h, and 18:30h, respectively. Additionally, snack opportunities were provided to participants at approximately 10:45h and 15:30h. On average, participants consumed 9044 ± 1480 kJ per day. Water was available ad libitum throughout the day from 'lights on' until 'lights out'.

Outside of designated meal and snack times, participants were not permitted to consume any food or beverages apart from water. Additionally, participants were not permitted to consume substances known to affect sleep (e.g., alcohol and caffeine (241, 242)) at any time during the protocol.

6.4.5 Sleep

Sleep was recorded using polysomnography equipment (Grael; Compumedics, Melbourne, VIC) with a standard electrode montage. Electrodes were applied in the 60-minutes prior to bedtime and included three electroencephalograms (C4-M1, F4-M1, O2-M1), two electrooculograms (left/right outer canthus), and a submental electromyogram. All sleep records were blinded and scored manually in 30-second epochs in accordance with

established criteria by the same technician (56). From the sleep recording, the following dependent variables were calculated: total sleep time (min), which identifies the time spent in any stage of sleep (i.e., N1, N2, N3, Rapid Eye Movement (REM)) during time in bed; time spent in stages N1, N2, N3 and REM sleep (min); sleep onset latency (min), which reflects the time between bedtime until the first epoch of any sleep stage (i.e., N1, N2, N3, REM); wake after sleep onset (min), which represents the time spent in bed awake minus sleep onset latency; sleep efficiency (%), which reflects total sleep time divided by time in bed x 100; stage REM onset latency (min); stage N3 onset latency arousals, (count); arousals in NREM (count); arousals in REM (count); awakenings (count); and stage shifts (count).

6.4.6 Subjective sleepiness

Subjective sleepiness was assessed using the Karolinska Sleepiness Scale (KSS) (140). The KSS is a 9-point scale where 1 = "extremely alert", and 9 = "very sleepy, great effort to keep awake, fighting sleep". Participants were instructed to indicate their current level of sleepiness by circling the corresponding number on the 9-point scale.

6.4.7 Subjective sleep quality, sleep duration, sleep latency

Subjective sleep quality was determined using a 7-point scale, where 1 = "extremely poor", 2 = "very poor", 3 = "poor", 4 = "average", 5 = "good", 6 = "very good" and 7 = "extremely good" (129). Assessment of subjective sleep quantity and subjective sleep onset latency were conducted verbally, by asking participants "how much sleep do you think you got?" and "how long did it take you to fall asleep?", respectively (129).

6.4.8 Heart rate

Heart rate was continuously monitored using Polar M400 heart rate monitors (M400, Polar Electro; Kempele, Finland) during the submaximal graded exercise test and standardised cycling exercise protocol.

6.4.9 Rating of perceived exertion

Rating of perceived exertion (RPE) was measured using the Borg CR10 scale of RPE (144). The RPE scale was used to gain a subjective measure of internal load experienced

by the participant. RPE was assessed by asking the participant to select a number from the CR10 scale that best represents their current level of exertion.

6.4.10 Submaximal graded cycling exercise test

Participants performed a submaximal graded exercise test on a stationary cycle ergometer (Wattbike Trainer, Wattbike Ltd; Nottingham, UK). Prior to the test, participants were instructed to cycle for 5-minutes at a self-selected pace with an intensity of approximately five on the RPE Borg CR10 scale. A 2-minute rest was then provided. The test began with participants cycling at a power output of 55 W, with a cadence of 60 revolutions per minute, the required power output increased by 15 W by increasing cadence. Participants rated their RPE each minute during the test and once the participant reached an RPE of seven (i.e., "hard"), the test was concluded. The heart rate and power output for each minute of the test were plotted for each participant and the predicted power output corresponding to 70% of their maximum heart rate (%HRmax) was extrapolated (243).

6.4.11 Standardised cycling exercise protocol

The standardised moderate-intensity cycling protocol began with a 5-minute warm-up with participants cycling at approximately 50%HRmax at a self-selected cadence on a stationary ergometer. Participants were then provided with a 2-minute rest. The participant then cycled for 40-minutes at 70%HRmax, which was monitored continuously. If heart rate deviated from the predetermined target heart rate, the air resistance of the cycle ergometer was adjusted accordingly to maintain the relative intensity. Participant heart rate ((beats per minute (bpm)), RPE, and power output (Watts) were recorded every 5 minutes. After the 40 minutes of cycling, participants were provided with a 2-minute cool down period.

6.4.12 Blood sampling

Blood samples were collected within 30-minutes of waking (fasted and pre-prandial), immediately pre- and post-exercise, one-hour post-exercise, and five-hours post-exercise via either a 21G butterfly needle venepuncture or a 20G intravenous forearm cannula into a 6 mL EDTA vacutainer (Greiner Bio-One, Kremsmünster, Austria). Samples were allowed to clot and then centrifuged at 1500 *g* at 4°C for 10 minutes. The plasma was then separated into five aliquots of approximately 300-400 µL per Eppendorf tube. Samples

were subsequently frozen and stored at -20°C until analysis. Commercially available custom-designed multiplex immunoassay MAGPIX kits were used to analyse IL-1α, IL-1β, IL-6, and TNF-α, while BDNF was measured using ELISA kits (Human Free BDNF Quantikine ELISA kit, USA R&D Systems, Inc; Minneapolis, USA) according to the manufacturer's instructions. After the plasma were analysed, any sample that returned a value below the minimum detectable limit of the kit were populated with the manufacturers recommended minimum detectable limit / sqrt2 as recommended by Croghan et al. (263).

6.4.13 Statistical analysis

A Bayesian framework was employed to model the changes in peripheral metabolic factors following exercise and their relationship to objective and subjective sleep outcomes. Specifically, Bayesian mixed effects models were fit using the *brms* package and the *brm* function in R. These models are more flexible in modelling complex relationships than classic frequentist multi-level approaches. Furthermore, unlike classical frequentist theory, they do not rely on sample size to control type I and type II error rates – small samples will just result in more posterior uncertainty (264).

First, to understand changes in peripheral metabolic factors following exercise, models were fit with a condition (Exercise *vs.* Control) by time interaction. Subject ID was included as a random intercept term. Separate models were fitted for each peripheral metabolic factor; a Gamma log link function was stipulated in the model due to the positively skewed nature of the peripheral metabolic factors. Models were fit with default, non-informative priors due to the exploratory nature of the study. All posterior distributions were estimated by running four separate Markov Chain Monte Carlo (MCMC) chains, with 2000 iterations over each chain. Any convergence issues among the chains were assessed by visually inspecting the plots, ensuring the Gelman-Rubin statistic was close to one, with one indicating perfect convergence, and assessing the effective sample size used after accounting for autocorrelation. Where any divergence issues arose, the adapt delta and maximum tree depth were increased and the model was rerun. Pareto estimates were examined to determine if there were any problematic observations within the data. The influence of exercise on peripheral metabolic factors was interpreted using the *hypothesis* function within *brms*, an evidence ratio less than one suggests that the data supports the null hypothesis and an evidence ratio greater than one shows the data is in

support of the alternative hypothesis. Posterior probabilities were calculated to reflect the updated belief about the effect of exercise on peripheral metabolic factor concentrations, combining priors and likelihood to refine estimates of fixed and random effects.
Second, the relationship between pre-bed peripheral metabolic factors and objective and subjective sleep outcomes were modelled using a multivariate Bayesian mixed effects model, with pre-bed concentrations of TNF-α, interleukin-1α, interleukin-1β, IL-6, and BDNF as fixed effects, subject ID as a random intercept term and objective and subjective sleep outcomes as the outcome variable. A separate model was built for each outcome variable.

6.5 Results

6.5.1 Standardised cycling exercise protocol

During the standardised cycling exercise protocol, participants maintained a mean HR of 134 ± 2 bpm, which corresponds with a mean relative heart rate of 70.2 ± 0.55 %HRmax. Additionally, a mean power output of 103.6 ± 52.9 W, mean RPE of 4.1 ± 1.2, and a final RPE of 4.0 ± 1.7 were recorded during the exercise task.

6.5.2 Exercise and metabolic factors

The credible intervals (CI) spanned zero for all metabolic factors, highlighting the uncertainty in the relationship between exercise and metabolic factors (Table 6.1). There was a tendency for exercise to increase IL-6, with an increase of 0.102-pg/mL [CI: -0.30 to 0.51] (Figure 6.1). Whilst variable, there was a 66% chance that the posterior probability was greater than the control condition. Additionally, exercise tended to increase TNF-α, with an increase of 0.10-pg/mL [CI: -0.10 to 0.49] (Figure 6.2). Whilst there was variability in this response, there was an 86% chance that the posterior probability was greater than the control condition. Consequently, exercise may elicit small increases in each IL-6 and TNF-α in some participants.

Table 6.1. Statistical outcomes for the effect of exercise on metabolic factors

Metabolic factor	Estimate	Estimated standard error	Lower 95% CI	Upper 95% CI	Posterior probability
BDNF	-0.390	0.270	-0.826	0.058	0.075
IL-1α	-0.023	0.100	-0.188	0.146	0.409
IL-1β	-0.066	0.183	-0.359	0.246	0.352
IL-6	0.095	0.236	-0.290	0.486	0.650
TNF-α	0.193	0.178	-0.101	0.485	0.866

Notes: CI = credible intervals; BDNF = brain-derived neurotrophic factor; IL-1α = interleukin-1 alpha; IL-1β = interleukin-1 beta; IL-6 = interleukin-6; TNF-α = tumor necrosis factor alpha

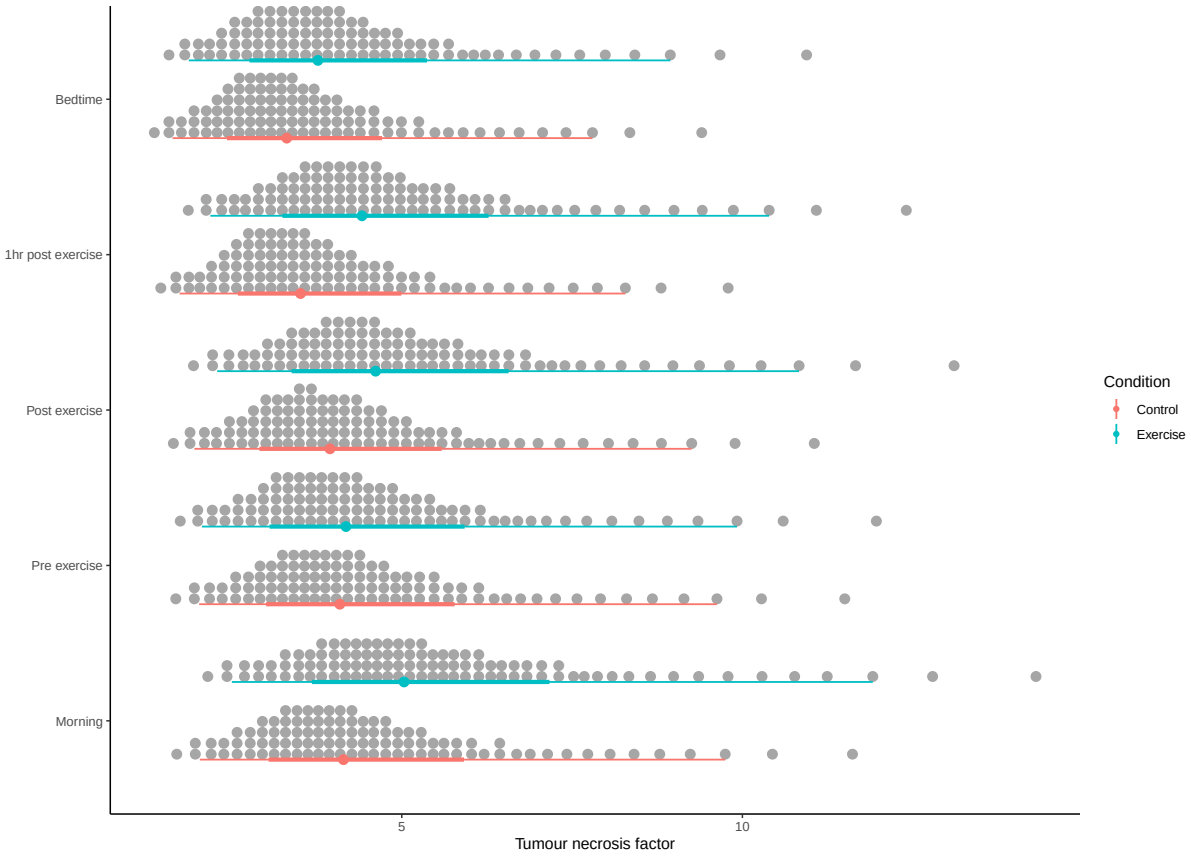

Figure 6.1. Quantile dotplots of the posterior distribution of TNF-α levels across timepoints and between conditions.

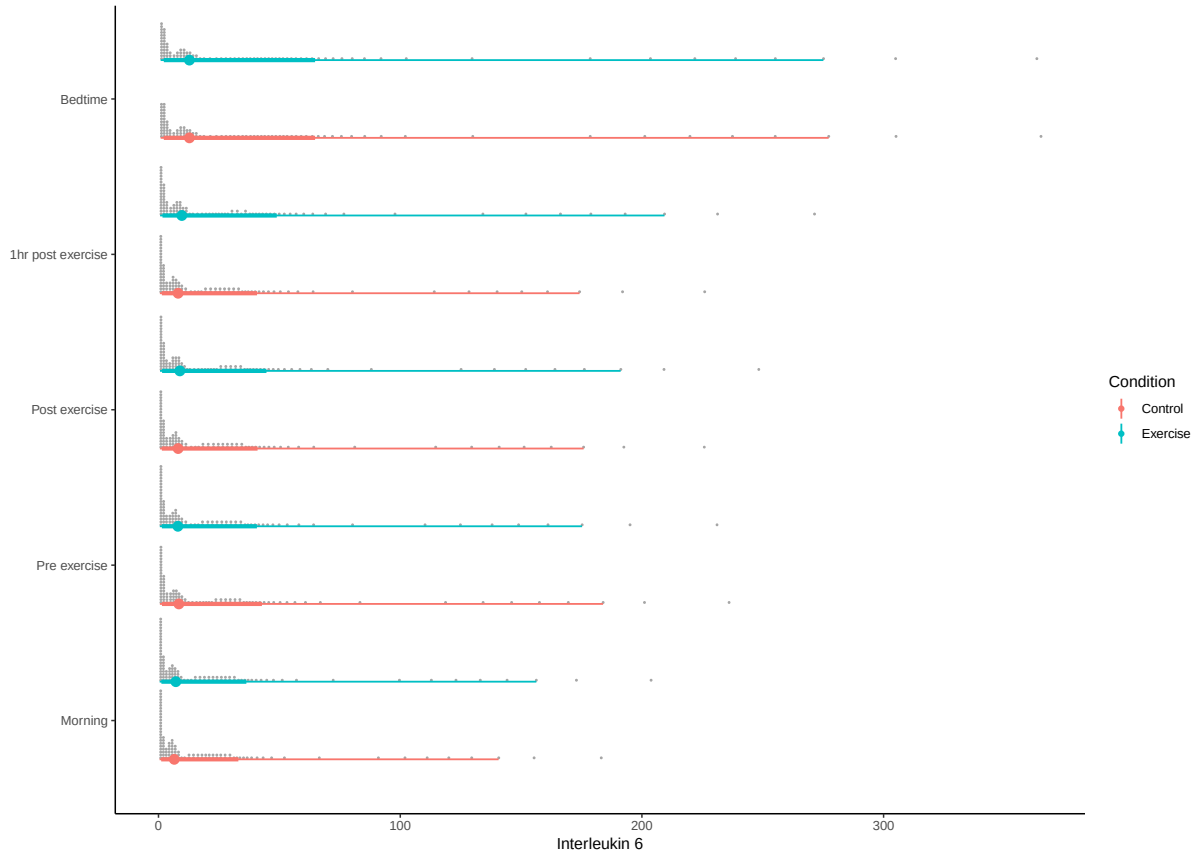

Figure 6.2. Quantile dotplots of the posterior distribution of IL-6 levels across timepoints and between conditions

6.5.3 Sleep and metabolic factors

There were no clear relationships observed between pre-bed metabolic factor concentrations and objective or subjective sleep; CI spanned zero for all metabolic factors except for IL-1β at total sleep time [CI: -58.72 to -4.48], sleep efficiency [CI: -11.6 to -0.50], stage N2 duration [CI: -87.16 to -16.94] highlighting uncertainty in the relationship (Tables 2-3). Good convergence of Markov Chain Monte Carlo (MCMC) sampling process was observed, with rhat values for all variables ranging between 1.000 and 1.006 (Tables 2-3). Raw data for all sleep outcomes and peripheral metabolic factor concentrations are presented in supplementary tables 1 and 2.

There was a tendency for pre-bed IL-6 to increase total sleep time, for every one unit increase in IL-6, there was an estimated 1.96-minute [CI: -0.79 to 4.77] increase in sleep duration. Although this response was variable, there was a 92% chance that the posterior probability was greater than zero. Additionally, pre-bed IL-6 tended to increase sleep efficiency, with an estimated increase of 0.36% [CI: -0.07 to 0.80] for every additional unit

of pre-bed IL-6. Once again, whilst the response was variable, there was a 91% chance that the posterior probability was greater than zero.

There was a tendency for pre-bed IL-1β to delay sleep onset latency, for every one unit increase in pre-bed IL-1β, there was an estimated 11.27-minute [CI: -6.32 to 28.49] increase in sleep onset latency, with an 87% chance that the posterior probability was greater than zero. Similarly, for every unit increase in pre-bed IL-1β, there was an estimated 11.99-minute [CI: -6.32 to 28.49] increase in time spent awake; there was an 85% chance that the posterior probability was greater than zero. Finally, pre-bed IL-1β was associated with REM duration. For every one unit increase in IL-1β, there was an estimated 11.42-minute [CI: -7.48 to 29.84] increase in REM duration with an 85% chance that the posterior probability was greater than zero.

Table 6.2. Relationship between pre-sleep metabolic factors and objective sleep measures

Term	Estimate	Estimated standard error	Lower 95% CI	Upper 95% CI	Rhat	Posterior probability
Total sleep time						
Intercept	491.032	21.017	450.022	532.406	1.000	
BDNF	4.12^{E-05}	0.001	-0.0014	0.002	1.000	0.521
IL-1α	-1.142	1.276	-3.6756	1.337	1.000	0.181
IL-1β	-31.049	16.796	-64.385	2.672	1.001	0.033
IL-6	1.957	1.422	-0.785	4.767	1.001	0.920
TNF-α	2.486	4.093	-5.897	10.397	1.001	0.737
Sleep efficiency						
Intercept	90.892	3.822	83.219	98.409	1.000	
BDNF	8.29^{E-06}	0.000	-0.000	0.000	1.000	0.525
IL-1α	-0.213	0.243	-0.699	0.286	1.001	0.173
IL-1β	-5.735	3.239	-12.176	0.635	1.000	0.037
IL-6	0.364	0.266	-0.153	0.893	1.001	0.913
TNF-α	0.487	0.757	-0.999	2.003	1.000	0.744
Sleep onset latency						
Intercept	15.358	12.185	-7.774	39.678	1.002	
BDNF	0.000	0.000	-0.001	0.001	1.002	0.696
IL-1α	0.050	0.786	-1.507	1.571	1.001	0.539
IL-1β	11.268	10.658	-9.983	31.669	1.002	0.868
IL-6	-0.484	0.856	-2.078	1.275	1.000	0.276
TNF-α	-0.014	2.450	-4.817	4.841	1.001	0.495
REM onset latency						
Intercept	117.142	38.054	41.825	192.694	1.002	
BDNF	-0.002	0.001	-0.005	0.000	1.001	0.052
IL-1α	1.644	2.510	-3.132	6.862	1.003	0.747
IL-1β	31.526	31.061	-30.013	92.809	1.003	0.851

IL-6	-2.772	2.738	-8.285	2.509	1.002	0.149
TNF-α	-1.244	7.172	-15.055	12.909	1.001	0.433
Slow Wave Sleep Onset Latency						
Intercept	12.522	4.609	3.459	21.611	1.003	
BDNF	0.000	0.000	-0.000	0.000	1.001	0.750
IL-1α	0.333	0.288	-0.227	0.877	1.003	0.882
IL-1β	-0.315	3.416	-7.134	6.325	1.003	0.455
IL-6	-0.323	0.302	-0.896	0.255	1.002	0.139
TNF-α	-0.146	0.835	-1.728	1.563	1.002	0.421
Stage N1 duration						
Intercept	256.010	25.752	205.060	308.563	1.000	
BDNF	0.000	0.000	-0.001	0.001	1.001	0.769
IL-1α	0.325	0.737	-1.170	1.758	1.006	0.681
IL-1β	-0.909	9.196	-19.237	16.455	1.002	0.466
IL-6	-0.292	0.795	-1.813	1.297	1.006	0.350
TNF-α	0.040	2.124	-4.260	4.196	1.000	0.512
Stage N2 duration						
Intercept	71.237	26.477	19.632	122.846	1.002	
BDNF	0.001	0.001	-0.001	0.002	1.001	0.713
IL-1α	0.161	1.749	-3.188	3.619	1.003	0.529
IL-1β	-51.351	21.718	-96.357	-11.033	1.002	0.007
IL-6	1.252	1.838	-2.281	4.825	1.001	0.756
TNF-α	-3.442	5.135	-13.388	7.112	1.000	0.243
Stage N3 (SWS) duration						
Intercept	71.237	26.477	19.632	122.846	1.002	
BDNF	0.000	0.001	-0.002	0.002	1.001	0.523
IL-1α	-1.922	1.697	-5.372	1.425	1.002	0.126
IL-1β	22.279	21.759	-23.076	62.881	1.003	0.851
IL-6	1.481	1.741	-1.954	5.0270	1.001	0.817
TNF-α	5.090	5.010	-4.124	15.204	1.003	0.847
REM duration						
Intercept	122.888	14.303	94.029	152.076	1.000	
BDNF	0.000	0.001	-0.001	0.001	1.000	0.637
IL-1α	-0.397	0.886	-2.180	1.357	1.000	0.317
IL-1β	11.418	11.427	-11.152	33.717	1.001	0.847
IL-6	0.033	0.977	-1.834	2.049	1.000	0.506
TNF-α	-1.346	2.946	-7.292	4.268	1.001	0.322
Wake duration						
Intercept	122.725	14.363	93.691	151.221	1.001	
BDNF	0.000	0.001	-0.001	0.001	1.000	0.630
IL-1α	-0.360	0.864	-2.076	1.376	1.000	0.338
IL-1β	11.999	12.011	-11.529	35.936	1.003	0.848
IL-6	-0.019	0.957	-1.874	1.911	1.002	0.483
TNF-α	-1.321	2.952	-7.388	4.387	1.001	0.322
Arousals						
Intercept	147.006	30.312	88.310	205.458	1.003	
BDNF	-0.001	0.001	-0.003	0.001	1.001	0.136
IL-1α	0.522	1.801	-3.147	4.107	1.002	0.613
IL-1β	32.137	20.924	-11.132	74.675	1.001	0.941

IL-6	-1.824	1.917	-5.592	2.084	1.004	0.159
TNF-α	-1.810	5.075	-12.094	8.046	1.001	0.353

Notes: CI = credible intervals; BDNF = brain-derived neurotrophic factor; IL-1α = interleukin-1 alpha; IL-1β = interleukin-1 beta; IL-6 = interleukin-6; TNF-α = tumor necrosis factor alpha.

Table 6.3. Relationship between pre-sleep metabolic factors and subjective sleep and sleepiness measures

Term	Estimate	Estimated standard error	Lower 95% CI	Upper 95% CI	Rhat	Posterior probability
Perceived sleep duration						
Intercept	6.761	0.858	5.045	8.570	1.001	
BDNF	0.000	0.000	-0.000	0.000	1.001	0.400
IL-1α	-0.040	0.054	-0.151	0.065	1.003	0.218
IL-1β	-0.431	0.711	-1.894	0.932	1.001	0.264
IL-6	0.052	0.060	-0.069	0.170	1.003	0.824
TNF-α	0.126	0.168	-0.206	0.465	1.003	0.780
Perceived sleep quality						
Intercept	5.619	1.043	3.669	7.919	1.000	
BDNF	0.000	0.000	-0.000	0.000	1.001	0.400
IL-1α	-0.080	0.059	-0.194	0.038	1.001	0.084
IL-1β	0.595	0.826	-1.013	2.257	1.001	0.784
IL-6	0.064	0.065	-0.065	0.189	1.002	0.842
TNF-α	-0.122	0.208	-0.554	0.273	1.001	0.270
Karolinska Sleepiness Scale bedtime						
Intercept	6.369	1.075	4.121	8.422	1.002	
BDNF	0.000	0.000	-0.000	0.000	1.003	0.129
IL-1α	-0.027	0.069	-0.169	0.105	1.000	0.342
IL-1β	-1.037	0.880	-2.696	0.770	1.001	0.116
IL-6	0.058	0.074	-0.085	0.205	1.000	0.798
TNF-α	-0.094	0.208	-0.528	0.308	1.000	0.318
Karolinska Sleepiness Scale wake						
Intercept	2.882	1.015	0.693	4.775	1.000	
BDNF	0.000	0.000	-0.000	0.000	1.001	0.510
IL-1α	-0.026	0.062	-0.144	0.103	1.003	0.315
IL-1β	-0.617	0.816	-2.250	0.953	1.002	0.215
IL-6	0.046	0.068	-0.098	0.176	1.004	0.767
TNF-α	0.242	0.196	-0.131	0.644	1.002	0.902

Notes: CI = credible intervals; Perceived sleep duration reported in hours; Perceived sleep quality reported using 1-10 Likert scale; BDNF = brain-derived neurotrophic factor; IL-1α = interleukin-1 alpha; IL-1β = interleukin-1 beta; IL-6 = interleukin-6; TNF-α = tumor necrosis factor alpha.

6.6 Discussion

The aims of this study were: 1) to determine the effect of afternoon, moderate-intensity aerobic exercise TNF-α, IL-1α, IL-1β, IL-6, and BDNF; and 2) to investigate the relationship between aerobic exercise-induced peripheral factors and subsequent sleep in healthy, adult males measured using polysomnography. There was no clear evidence that aerobic exercise had a significant effect on BDNF, IL-1α, or IL-1β, but there was some evidence to suggest exercise may have induced a small effect on increasing IL-6 and TNF-α. Additionally, there were no clear associations between any pre-sleep metabolic factors and objective or subjective sleep. When viewed collectively, these results indicate that the duration and intensity of exercise performed in this study was insufficient to cause large changes in select metabolic factors that have been proposed to explain exercise-induced improvements in sleep. The lack of associations observed between metabolic factors measured in this study and sleep, do not support components of a novel hypothesis (24) that suggests exercise-induced peripheral factors are potential mechanisms for exercise-related improvements in sleep.

Moderate-intensity exercise appeared to elicit small increases in IL-6 in this study. This increase in IL-6 aligns with previous findings that show increases in circulating levels of IL-6 after exercise (256, 257, 265). However, the magnitude of plasma IL-6 increases are typically dependent on the exercise intensity, duration, mass of skeletal muscle recruited, and an individual's fitness level (266). For instance, IL-6 has been shown to increase two-fold following six minutes of maximal rowing exercise (during exercise and remaining elevated ~15-minutes post-exercise) (267) and more than 100 times following running a full marathon (~42 km) (sample drawn within 10 minutes of race completion) (268). The small increases observed in this study may be contributed to cycling primarily recruiting musculature from the lower limb, and consequently, recruiting less muscle mass compared to other modalities, such as running and rowing which have shown larger increases in IL-6 (266). The moderate-intensity and relatively short duration of the exercise protocol may explain the small increases in IL-6 observed within this study. Investigating the effects of alternate exercise configurations and modalities on IL-6 levels in relation to sleep may help to further elucidate the relationship between IL-6 and a potential role in sleep regulation.

Within this study there was evidence to suggest moderate-intensity exercise may elicit small increases in TNF-α. This finding contrasts evidence that suggests moderate-intensity exercise does not typically elicit increases in circulating levels of pro-inflammatory cytokines such as TNF-α and IL-1β (269). However, strenuous exercise, such as a marathon, may elicit a two-fold increase in TNF-α and IL-1β but the accompanying increase in anti-inflammatory cytokines often renders the overall response to be primarily anti-inflammatory in nature (270). Pro-inflammatory markers such as TNF-α and IL-1β are typically increased via systemic infection or cellular damage, whereas anti-inflammatory metabolic factors such as IL-6 are often elevated post-exercise. The anti-inflammatory properties of exercise-induced increases of IL-6 are likely to exert inhibitory effects on both TNF-α and IL-1 production (269). IL-6 can increase levels of anti-inflammatory cytokines interleukin-1 receptor agonist (IL-1ra) and IL-10 (271). Consequently, the evidence suggesting acute moderate-intensity exercise may elicit small increases in TNF-α contrasts with the typical response observed after exercise. Collectively, there was evidence to suggest small increases in the anti-inflammatory IL-6 and pro-inflammatory TNF-α in some participants, but whether the magnitude of increase in these markers observed after exercise is likely to be clinically meaningful is unclear.

There was no clear effect of moderate-intensity exercise on BDNF levels. Typically, studies have shown transient increases in peripheral BDNF in healthy adults after acute bouts of exercise, which range from 11.7% to 410% (272). For example, ~20 minutes of acute cycling exercise at ~74%HRmax led to increases in serum BDNF of 14.4% (145). The findings from the current study contrast with a meta-analysis that showed blood concentrations of BDNF increase after acute aerobic exercise (273). The prescription of exercise appears to modulate BDNF concentrations, with modality, intensity, and duration of exercise important factors. For instance, high-intensity exercise and graded exercise tasks likely cause the greatest increases in BDNF. Whilst aerobic exercise has been able to increase BDNF, resistance training has not consistently shown the same effect (273). However, it appears the influence of resistance training on BDNF may be regulated by intensity, with high-intensity resistance training (e.g., five sets of five repetitions at participant's five repetition maximum) able to increase blood concentrations of BDNF (274). Conversely, a meta-regression highlighted exercise duration appears to have a positive association with exercise duration (275), suggesting that a longer duration

exercise protocol may have been required to increase BDNF within this study. Therefore, it appears the moderate-intensity exercise protocol employed within this study was not a sufficient stimulus to influence BDNF concentrations nor yield any improvements in subsequent sleep. Determining whether an alternative configuration or modality of exercise may elicit a greater response in BDNF and interact with sleep would be an important next step in investigating the relationship between exercise-induced peripheral factors and sleep.

Despite the evidence in favour of small possible increases in TNF-α and IL-6 in some participants, no meaningful association was observed between TNF-α or IL-6 and objective or subjective sleep. Additionally, no meaningful associations were observed between IL-1α, IL-1β, BDNF, and objective or subjective sleep. The findings of our study therefore do not support components of a novel hypothesis that exercise-induced peripheral factors are likely mechanisms for improvements in sleep (24). However, it is important to note the relationship between exercise and sleep is modulated by factors such as the configuration and timing of exercise (229). As such, whether an alternate configuration or timing of exercise may have induced larger responses in metabolic factors and consequently influenced sleep quality and quantity would be prudent to explore. Investigating whether other peripheral factors linked with sleep may have been elevated after the moderate-intensity exercise could have further elucidated the relationship between exercise-induced metabolic factors and sleep.

The underlying mechanisms of exercise and sleep interactions are complex and multifaceted. Although the findings of our study do not support components of the hypothesis (24), it is important to acknowledge there are several metabolic factors involved in sleep regulation that were not measured in this study and warrant further investigation. For instance, increases in IL-6 may stimulate elevated levels of interleukin-1 receptor agonist (IL1-ra), which can increase the depth of sleep, particularly during early phases of slow wave sleep (276). Peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) has been implicated in the expression of the core clock gene *Bmal1* in skeletal muscle (193), which may have a downstream influence on myokine secretion and sleep regulation. *Bmal1* is an essential component of circadian rhythmicity and its secretion from skeletal muscle has been linked with sleep regulation in rodents.

Animal models have shown fibronectin type III domain-containing protein (FDNC) increases in response to PGC-1α, which allows for irisin to be cleaved from FDNC and released into circulation due to exercise (277). Increased irisin has been linked to improvements in slow wave sleep via increased BDNF expression in the brain (218). Collectively, the hypothesis that exercise-induced peripheral factors may regulate sleep should be investigated further, with the inclusion of more peripheral factors implicated in sleep regulation, using various exercise configurations and timings, and across different demographics.

Although this is the first study to investigate the relationship between exercise-induced peripheral factors and sleep, it is not without limitations. First, whilst the configuration of exercise used in this study was designed to replicate a typical exercise session, as outlined above the metabolic responses to exercise vary, depending on intensities, durations, and modalities prescribed. Consequently, the findings of this study may not be reproducible after other configurations of exercise and further investigation is therefore warranted. Second, there are exercise-induced peripheral factors that have been suggested to influence sleep beyond those measured in this study that would be important to explore. Examining how chronic vs. acute exercise effects peripheral metabolic factors and subsequent sleep would also be important to investigate.

The current data suggest that a 40-minute bout of leg-cycling in the moderate-intensity domain does not raise levels of several peripheral metabolic factors previously suggested to influence sleep regulation. Although there was some evidence in favour of exercise eliciting small increases in IL-6 and TNF-α, no meaningful associations were observed between any of the peripheral metabolic factors and gold-standard assessments of sleep. The lack of response suggest that alternate forms and configurations of exercise may be required to elucidate the relationship between exercise-induced peripheral factors and sleep. Alternatively, expanding the peripheral metabolic factors examined to include additional myokines that are implicated in sleep regulation may be beneficial.

Supplementary Table 6.1. Sleep and subjective sleepiness outcomes

	Conditions	
	Control	Exercise
Variable	Mean ± SD	Mean ± SD
Sleep		
Total sleep time (min)	493.7 ± 12.6	471.5 ± 55.2
Sleep onset latency (min)	19.7 ± 16.3	18.9 ± 15.8
Sleep efficiency (%)	91.4 ± 2.3	87.3 ± 10.2
Wake after sleep onset (min)	122.7 ± 18.7	115.8 ± 19.8
Stage N1 duration (min)	33.3 ± 12.9	31.7 ± 13.9
Stage N2 duration (min)	237.0 ± 35.7	228.1 ± 43.5
Stage N3 duration (min)	100.7 ± 41.4	95.8 ± 35.2
REM duration (min)	122.7 ± 18.7	115.8 ± 19.8
Stage N3 onset latency (min)	14.3 ± 6.6	13.0 ± 5.1
REM onset latency (min)	76.1 ± 45.1	102.8 ± 46.9
Arousals (count)	119.3 ± 33.9	119.2 ± 47.8
Subjective sleep outcomes		
Sleep quantity (h)	7.1 ± 0.8	6.7 ± 0.8
Sleep quality (units)	5.3 ± 1.1	4.9 ± 1.3
Subjective sleepiness		
KSS bedtime (units)	5.3 ± 1.1	4.8 ± 1.6
KSS wake (units)	3.7 ± 1.4	3.5 ± 0.8

Notes: SD = standard deviation; REM = Rapid eye movement; KSS = Karolinska Sleepiness Scale.

Supplementary Table 6.2. Peripheral metabolic factor concentrations

	Conditions	
	Control	Exercise
Variable	Mean ± SD	Mean ± SD
BDNF (pg/mL)		
Morning fasted	31136.852 ± 28056.496	21990.469 ± 22791.911
Pre-exercise	21981.223 ± 16952.966	23002.096 ± 30626.260
Post-exercise	21508.230 ± 18284.359	22348.973 ± 32158.853
1-h post-exercise	16494.998 ± 6496.301	19862.917 ± 11926.521
Pre-bed	16754.242 ± 10774.729	13859.632 ± 11425.941
IL-1α (pg/mL)		
Morning fasted	32.841 ± 50.345	32.053 ± 46.465
Pre-exercise	33.314 ± 51.927	18.167 ± 17.557
Post-exercise	33.894 ± 58.686	30.459 ± 43.960
1-h post-exercise	32.028 ± 49.364	31.648 ± 46.246
Pre-bed	31.522 ± 52.299	31.435 ± 55.131
IL-1β (pg/mL)		
Morning fasted	1.160 ± 1.440	1.104 ± 1.541
Pre-exercise	0.988 ± 1.242	0.681 ± 0.389
Post-exercise	1.031 ± 1.521	1.053 ± 1.330
1-h post-exercise	1.042 ± 1.267	1.212 ± 1.452
Pre-bed	1.093 ± 1.468	1.061 ± 1.591
IL-6 (pg/mL)		
Morning fasted	28.196 ± 50.796	27.819 ± 44.948
Pre-exercise	28.238 ± 49.129	14.396 ± 16.255
Post-exercise	28.511 ± 52.689	26.238 ± 42.457
1-h post-exercise	28.164 ± 47.715	28.393 ± 45.046
Pre-bed	29.995 ± 49.710	29.304 ± 51.719
TNF-α (pg/mL)		
Morning fasted	4.674 ± 2.899	5.069 ± 2.438
Pre-exercise	4.483 ± 2.238	4.390 ± 2.516
Post-exercise	4.364 ± 2.540	4.915 ± 2.586
1-h post-exercise	3.905 ± 2.184	4.783 ± 2.900
Pre-bed	3.659 ± 2.000	3.591 ± 0.954

BDNF = brain-derived neurotrophic factor; IL-1α = interleukin-1 alpha; IL-1β = interleukin-1 beta; IL-6 = interleukin-6; TNF-α = tumor necrosis factor alpha.

Chapter 7: Study 4 – The Influence of a Formulated Nutritional Intervention on Subsequent Sleep and Next-Morning Physical Performance, Cognitive Function, and Postural Sway in Adult Males

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7.1 Linking paragraph

Chapters 5 and 6 highlight the complex relationship between sleep and exercise. Sleep appears to be relatively stable in response to moderate-intensity exercise, and exercise-induced peripheral factors may not fully explain the relationship between exercise in sleep. However, whether the addition of a formulated nutritional supplement consumed prior to sleep can have a beneficial effect on sleep is unknown. The ability for a nutritional intervention to enhance sleep may be beneficial for people who wish to improve their sleep but do not wish to consume typical sleep medications that may carry unwanted side effects. Given the additive effect that exercise and nutritional behaviours have on health, Chapter 7 aims to determine if the consumption of a pre-sleep formulated nutritional intervention, in conjunction with afternoon exercise, is able to induce a positive influence on sleep. It is hypothesised that the consumption of a pre-sleep formulated nutritional intervention will enhance sleep parameters without inducing any negative next-morning cognitive impairment or declines in physical performance.

The Influence of a Formulated Nutritional Intervention on Subsequent Sleep and Next-Morning Physical Performance, Cognitive Function, and Postural Sway in Adult Males

7.2 Abstract

Background: Athletes often experience poor sleep quality and quantity which may hinder physical performance and cognitive function. Pre-sleep nutritional strategies may be a practical alternative to pharmacological interventions to improve sleep. The aim of this study was to examine the effect of two different doses of a nutritional intervention (both containing high glycaemic index carbohydrate, whey, tryptophan, theanine, and 5'AMP) vs placebo on objective and subjective sleep, next-morning physical performance, cognitive function, and postural sway in trained adult males.

Methods: To assess the effectiveness of each dose of the intervention, 17 healthy, adult, trained males who were identified as good sleepers completed three double-blind trials in a randomised, counter-balanced design. A (i) low-dose, (ii) high-dose, or (iii) placebo drink was provided 90 minutes before sleep each night. Polysomnography was used to measure objective sleep parameters. Cognitive function, postural sway, and subjective sleep quality were assessed 30 minutes after waking. Physical performance was assessed using a 10-minute maximal effort cycling time trial each morning. Participants undertook a standardised high-intensity cycling protocol each afternoon to replicate the typical training demands encountered by athletes. All data were analysed with linear mixed effects models and magnitudes of difference were determined using Cohen's *d* effect sizes.

Results: No improvements were observed in objective or subjective sleep parameters. The low-dose intervention appeared to reduce N3 sleep duration compared to placebo (-13.6 mins) and likely reduced the number of arousals experienced during REM sleep compared to the high-dose (-7.6 count). The high-dose intervention appeared to increase N1 duration compared to placebo (+7.4 mins). Physical performance, cognitive function, postural sway, and gastrointestinal symptoms were not affected by the nutritional interventions.

Conclusion: The nutritional interventions did not improve sleep quality or quantity, nor did they improve next-day physical performance, cognitive function, postural sway, or self-reported sleep quality. Some aspects of sleep architecture were negatively influenced by the high- and low-dose interventions when compared to placebo, but the differences observed were not likely to cause meaningful reductions in sleep quality and quantity.

7.3 Introduction

Sleep is essential for maintaining health and physical performance (278). Without sufficient sleep, a myriad of negative health consequences such as disrupted metabolic and skeletal muscle function, and alterations to the hormonal milieu occur (225). The National Sleep Foundation recommend adults obtain 7-9 hours of sleep per night for maintaining health (279). However, sleep recommendations for athletic populations are less clear, with longer durations likely required, and influenced by the athlete's training load and age (93, 94, 280). Athletes appear to benefit from achieving ~8-hours of sleep per night in order to feel well-rested (280). Nevertheless, most athletes do not achieve sufficient sleep duration or sleep quality (280-282), which is likely underpinned by a combination of various sport-related factors (e.g., timing of training and competition can reduce sleep opportunities (93), long-haul travel can disrupt circadian rhythms (283), and non-sport related factors such as age (284) and sex (285). This is an important issue because athletic performance can be impaired by poor sleep (98).

Pharmacological interventions exist to improve sleep but are often targeted at individuals with clinical sleep disorders such as insomnia (286). As such, the primary aim of the pharmacological agent is to improve overnight sleep, typically without focus on residual next-day effects. Consequently, pharmacological interventions can be associated with impairment of next-day cognitive performance (110). For example, commonly prescribed sleep medications may hinder an individual's ability to perform complex psychomotor tasks such as driving a car (287). Interventions that may impede perceptual performance are likely to be detrimental to athletic performance when considering the need to complete highly demanding cognitive tasks, often under intense time pressure. In addition, pharmacological interventions often carry the risk of addiction and withdrawal upon cessation of use (110). Therefore, it is evident that some pharmacological interventions may not be suitable for use in an athletic population. One potential alternative to enhance sleep without the negative side-effects of pharmacological interventions are nutritional interventions.

Several nutritional substances can improve sleep in healthy adults, including high glycaemic index carbohydrates, tryptophan, theanine, and nucleotides (e.g., 5'AMP) (16, 25, 125, 288, 289). These ingredients have been investigated both in isolation and in

combination in healthy, untrained populations (125). For instance, a nutritional intervention with a combination of ingredients (e.g., high glycaemic index carbohydrates, valerian, tart cherry juicy, and theanine) reduced sleep onset latency (SOL) in healthy adult males without any impairment on next-day alertness, cognitive performance, or postural sway (125). Alertness, cognitive performance, and balance are important contributors to sports performance, but the impact of nutritional interventions targeted towards improving sleep on other aspects of sports performance (e.g., maximal effort) have not been examined. As athletes are often required to train in the morning, determining whether next-morning physical performance is influenced by pre-sleep nutritional interventions is essential. A nutritional intervention that can improve sleep without hindering next-morning physical performance would be beneficial to many athletes.

The aim of the present study was to determine the effect two different doses of a formulated nutritional intervention compared with placebo on objective and subjective sleep and next-morning physical performance, cognitive function, and balance in trained adult males (290) without early or late chronotypes. The two nutritional interventions (i.e., high dose, low dose) contained high glycaemic index carbohydrate, whey, tryptophan, theanine, and 5'AMP and were consumed 90 min before bedtime following a standard bout of daytime exercise.

7.4 Methods

7.4.1 Design and procedures

A double-blind, placebo-controlled, cross-over experimental design was used to examine the effectiveness of two different doses of a nutritional intervention on sleep and next-morning performance. Sleep was measured each night using polysomnography (PSG) during a 10.5-hour sleep opportunity (22:30h – 08:00h). On day one, participants were familiarised with the exercise protocols and were trained on the cognitive and postural sway tasks. Night one was used to familiarise participants with the equipment for monitoring sleep. On nights 2-4, participants received one of the two interventions, (i.e., high-dose or low-dose), or placebo in a randomised, counterbalanced order. The nutritional interventions and placebo were provided to participants in liquid form (250ml in volume). Ingredients for each nutritional intervention and placebo are provided in Table 7.1.

Table 7.1. Ingredients of the nutritional interventions and placebo

Nutritional intervention one	Nutritional intervention two	Placebo
2.7g High glycaemic index carbohydrate	2.7g High glycaemic index carbohydrate	Non-nutritive sweetener
30g Whey	40g Whey	Flavour
0.641g Tryptophan	0.855g Tryptophan	Colour
660mg Theanine	660mg Theanine	
53mcg 5'AMP	53mcg 5'AMP	
Non-nutritive sweetener	Non-nutritive sweetener	
Flavour	Flavour	

Notes: Colour added to match the appearance of the whey protein present in nutritional interventions one and two.

On nights 2-4, participants consumed either a nutritional intervention or placebo at 21:00h. Participants were given five minutes to consume the entire supplement or placebo and were supervised by a member of the research team. After consuming the intervention or placebo, participants were not permitted to consume any water until the morning. Participants rated their subjective sleepiness every 30 minutes from 20:00h to 22:00h. In the thirty minutes after waking in the morning, participants rated their perceptions of sleep (i.e., latency, quality, quantity) and gastrointestinal symptoms. At 09:00h, participants completed a 30-minute test battery to assess subjective alertness, self-perceived capacity, cognitive performance, and postural sway. The tasks in the test battery were completed in the same order each day. At 10:00h, participants completed a 17-minute warm-up followed by a 10-minute maximal effort time trial on a stationary cycling ergometer. Each afternoon, participants completed a high-intensity cycling training session between 15:00h and 17:00h to replicate training demands commonly encountered by athletes.

The nutritional intervention is an extension of a previous supplement designed and formulated by a Box-Behnken predictive model and validated in a similar cohort of adult males (125). However, due to a poor taste profile, due to the inclusion of valerian root, a new formulation of the supplement was required. High glycaemic index carbohydrates, whey protein, theanine, and 5'AMP were included in similar doses to what the Box-Behnken model identified in both the original formulation and the refined version used within this study due to their sleep promoting properties (120, 125, 288). Tryptophan was included within the formulation used in this study due to the affect tryptophan has on improving the sleep/wake cycle, specifically by reducing wake after sleep onset (25, 291).

A low- and high-dose of the supplement were used within this study to determine the optimal dose of the interventions for influencing sleep.

7.4.2 Participants

Seventeen healthy, trained adult males (290) volunteered to participate in the study (mean ± SD; age: 25.4 ± 6.5 years, height: 179.3 ± 7.2 cm, mass: 74.2 ± 10.0 kg). Participants completed a general health questionnaire, a pre-exercise screening tool by Exercise Sports Science Australia (123) (502.6 ± 8.4 minutes of weighted physical activity/exercise per week) and The Pittsburgh Sleep Quality Index (131) (PSQI score: 3.0 ± 1.1) to assess eligibility. Participants were excluded from the study if they had a clinically diagnosed sleep disorder, had a change in medication over the study period known to affect sleep, had any musculoskeletal injuries, were smokers, or were shift-workers. Participants were informed of the experimental procedures, provided with an opportunity to ask questions, and gave signed written consent prior to participation. Participants were instructed to maintain their regular sleep/wake patterns in the week prior to the study and to avoid alcohol in the 24 hours prior to the study. Sample size calculation (n = 18) was based on the number of participants who completed a similar sleep and nutritional intervention protocol which demonstrated an improvement in sleep onset latency (125) and a power calculation using the R package *pwr* (292). The power for the sample size was calculated using a medium effect size of 0.50 (which was the difference between groups in a similar study (125)). The returned power was 0.90, which equates to a 10% chance of Type II error and a 5% chance of Type I error. As such, only 14 participants were required for the study to be powered at the conventional 0.80. The experimental protocol was approved by CQUniversity's Human Research Ethics Committee (0000021915) and reciprocal approval was obtained from the Australian Catholic University Human Research Ethics Committee (2022-2526R).

7.4.3 Living conditions

Participants lived and slept in a purpose-built accommodation suite at CQUniversity's Appleton Institute in Adelaide, Australia. Throughout the study participants were not exposed to natural light. Six participants can be accommodated within the suite concurrently, which is configured similarly to a serviced apartment with each participant having their own private bedroom, lounge room, and bathroom. During the day when

participants were not undertaking testing, they were permitted to engage in routine sedentary activities such as reading, using laptops or tablets, and watching television. Participants were not permitted to undertake any additional exercise outside the two allocated sessions each day and were not permitted to sleep outside of the scheduled time in bed. Researchers monitored participants for compliance using close-circuit television and in-person monitoring.

7.4.4 Meals

Nutritional intake was standardised for each participant for the duration of the study. All meals provided to participants were calorie-controlled and the same approximate number of calories were provided at each respective meal opportunity (e.g., breakfast, lunch, and dinner). Participants were provided with breakfast, lunch, and dinner at 11:30h, 13:30h, and 19:00h, respectively. Additionally, participants were provided with an opportunity to eat a standardised snack (i.e., identical item consumed each day) at 08:45h, and a second snack after the completion of their afternoon exercise training session (~16:30h). Participants were provided with a 250-ml electrolyte sports drink (Gatorade; PepsiCo, Harrison, NY) after the maximal effort time trial and simulated training session. On average, participants consumed 8992 ± 3307 kJ per day. Additionally, water was available ad libitum throughout the day from 08:00h until 21:00h. Participants were not permitted to consume any food or beverages apart from water, outside of the designated meal and snack times. Furthermore, participants were not permitted to consume caffeine or alcohol at any time during the protocol.

7.4.5 Sleep

Sleep was recorded using PSG equipment (Grael V1; Compumedics, Melbourne, VIC) with a standard montage of electrodes. Electrodes were applied in the 60 minutes prior to lights out and included three electroencephalograms (C4-M1, F4-M1, O2-M1), two electrooculograms (left/right outer canthus), and a submental electromyogram. All sleep records were blinded and manually scored in 30-second epochs by the same technician according to established criteria (56). The following dependent variables were calculated from each sleep recording: total sleep time (min), which reflects the time spent in any stage of sleep (i.e., N1, N2, N3, rapid eye movement (REM)) during time in bed; time spent in stages N1, N2, N3 and REM sleep (min); sleep onset latency (min), which

represents the time between lights out to the first epoch of any stage of sleep (i.e., N1, N2, N3, REM); wake after sleep onset (min), which reports the time spent in bed awake minus sleep onset latency; sleep efficiency (%), which represents total sleep time divided by time in bed x 100; arousals (count); arousals in non-rapid eye movement (NREM) (count); arousals in REM (count); awakenings (count); stage shifts (count); stage REM onset latency (min); and stage N3 onset latency. For one participant, objective PSG data were not obtained due to a technical error with the recording.

7.4.6 Subjective sleepiness

Subjective sleepiness was assessed using the Karolinska Sleepiness Scale (KSS) (140). The KSS is a 9-point scale where 1 = "extremely alert", and 9 = "very sleepy, great effort to keep awake, fighting sleep". Participants were instructed to circle the number on the scale that corresponded to their current level of sleepiness.

7.4.7 Subjective sleep quality, subjective sleep duration, subjective sleep latency

Subjective sleep quality was assessed using a 7-point scale, where 1 = "extremely poor", 2 = "very poor", 3 = "poor", 4 = "average", 5 = "good", 6 = "very good" and 7 = "extremely good" (129). Subjective sleep quantity and subjective sleep onset latency were assessed verbally by asking participants "how much sleep do you think you got?" and "how long did it take you to fall asleep?" (129).

7.4.8 Gastrointestinal symptom scale

The presence of gastrointestinal symptoms was assessed using a 16-item questionnaire (125). Participants used a 10-point Likert scale to rate if they had experienced a gastrointestinal symptom since bedtime the previous night. Possible responses ranged from 1, "no problem at all" to 10, "the worst it has ever been".

7.4.9 Subjective alertness and self-perceived capacity

Alertness was assessed using a visual analogue scale (VAS). Participants placed a mark on a 100-mm horizontal line anchored by the statements "struggling to remain awake" and "extremely alert and wide awake". A VAS was also used to measure self-assessed ability to perform the cognitive performance tasks (VAS Performance) (293). Participants responded

to the question “How well do you think you will perform” by placing a mark between the statements “extremely poorly” and “extremely well” on a 100-mm line.

7.4.10 Cognitive performance

Sustained attention was assessed using the psychomotor vigilance task (PVT-192; Ambulatory Monitoring Inc., New York, NY, USA) (294). The PVT is a hand-held device with an upper surface that contains a four-digit LED display and two push-button response keys. Participants attended to the LED display for the duration of the test (10 mins) and pressed the appropriate response key with the thumb of their dominant hand as quickly as possible after the appearance of a visual stimulus (presented at a variable interval of 2-10 seconds). If the correct response key was pressed, the LED display exhibited the participant’s response time (in milliseconds) for 500 ms. If the wrong response key was pressed, an error message was displayed (ERR). If a response was made prior to the stimulus being presented, a false start message was displayed (FS). For all analyses, anticipated responses (i.e., those with response time less than 100 ms) were excluded. Dependent measures were number of lapses, which were defined as a response time greater than 500 ms (count), mean response time (ms), and false starts (count) (295).

7.4.11 Postural sway

Postural sway was assessed using an Accusway computerised force platform (AMTI, Watertown, Massachusetts, USA) in conjunction with Swaywin software (AMTI, Watertown, Massachusetts, USA) (296). The force platform measures both three-dimensional forces (Fx, Fy, Fz) and three-dimensional moments (Mx, My, Mz) involved in balance. These provide centre of pressure (COP) coordinates, which allow postural sway to be calculated. Participants performed two trials each for 30 seconds, one trial with their eyes open and the other trial with their eyes closed. The outcome variable recorded during the postural sway assessment was the area of the 95% confidence ellipse enclosing the COP (Area 95, cm^2).

7.4.12 Heart rate

Heart rate was monitored continuously during both the maximal effort time trial and simulated training session using a Polar M400 heart rate monitor (M400, Polar Electro; Kempele, Finland).

7.4.13 Cycling warm-up protocol

Prior to the maximal effort time trial and simulated training session, participants completed a 17-minute incremental warm-up based on rating of perceived exertion (RPE) using the Borg Scale (143) on a stationary cycling ergometer (Wattbike Trainer, Wattbike Ltd; Nottingham, UK). The warm-up consisted of six minutes of cycling at a self-determined RPE six, progressing to six minutes at RPE 13, followed by three minutes at RPE 16, and then two minutes of rest.

7.4.14 Maximal effort time trial

Exercise performance was measured using a 10-minute maximal effort time trial performed on a stationary cycling ergometer. Participants were instructed to produce the highest average power they could during the time trial. Whilst cycling, participants were blind to heart rate and power output, but were provided with a verbal update of elapsed time every minute. The dependent variables obtained during the time trial were mean RPE (6-20), mean heart rate (beats per minute; bpm), and mean power output (watts; W).

7.4.15 Simulated training session

A standardised exercise session was conducted to replicate a typical training session of an endurance-trained athlete. Participants completed three x five-minute intervals on a stationary cycling ergometer, with the instructions provided to produce the highest average power they could during each interval. A five-minute rest was provided between each interval. Whilst cycling, participants were blind to heart rate and power output, but were provided with a verbal update of time elapsed after every minute.

7.4.16 Statistical analysis

All data were analysed with linear mixed effects models using separate models built for each outcome variable of interest, with condition included as a fixed effect and participant ID included as a random effect using the R package *lme4* (150, 151). A random intercept for participant was included to account for intraindividual dependencies and interindividual heterogeneity arising from the repeated measures on each participant. All models were estimated using Restricted Estimated Maximum Likelihoods from the *lme4* package. All *p*-values were obtained using Type III analysis of variance with

Satterthwaite's tests with Kenward-Roger degrees of freedom as implemented in the R package *CAR* (152). Results were reported as mean estimates with alpha set at $p < 0.05$. The magnitude of differences were assessed using Cohen's d effect size statistic and 95% confidence intervals using the *t_to_d* function in the *effectsize* package, where the t value from the linear mixed model is divided by the square root of the degrees of freedom error from the same model and interpreted as trivial, <0.20; small, 0.20-0.49; moderate, 0.50-0.79, and large ≥0.80 (153). Sleep stage distributions during each condition were plotted using histograms. Sleep hypnogram data (recorded in 30-second epochs) were binned into five-minute intervals, and the percentage of each stage (i.e., wake, stage N1, stage N2, stage N3, and REM) was calculated. The percentages were plotted on the y-axis, with each stage stacked on top of each other to represent the overall distribution, while the five-minute bins were plotted along the x-axis.

7.5 Results

7.5.1 Sleep

For most of the sleep variables, there was no difference between the low-dose intervention, high-dose intervention, and placebo (Tables 7.2-7.3). There were some minor differences between conditions in the duration of stage N1 sleep, stage N3 sleep, and the number of arousals in REM sleep. Specifically, the duration of stage N1 (i.e., 'light' sleep) sleep (Figure 7.1) was likely higher in the high-dose intervention compared to placebo (7.4 mins; Cohen's *d*: 0.4; 95% CI: 0.03 – 0.76; Supplementary Table 7.1); and the duration of stage N3 (i.e., 'deep' sleep) sleep (Figure 7.2) was lower in the low-dose intervention compared to placebo (-13.6 mins; Cohen's *d*: -0.37; 95% CI: -0.72 – 0.004; Supplementary Table 7.1). Additionally, the number of arousals observed during REM sleep (Figure 7.3) was likely lower in the low-dose intervention compared to placebo (-7.6 arousals; Cohen's *d*: -0.46; 95% CI: -0.82 – -0.083; Supplementary Table 7.1). Individual responses for total sleep time, sleep onset latency, wake after sleep onset, and sleep efficiency are presented in Figure 7.4.

The probability distribution of sleep stages for each condition are presented in Figure 7.5. During the first three hours of sleep, the proportion of N3 sleep appears to be sustained in the nutritional intervention groups compared to placebo (Figure 7.5). Additionally, sleep

throughout the final two hours of the high-dose condition appears to contain less periods of wake compared to the low-dose and placebo (Figure 7.5).

Table 7.2. Sleep, subjective sleepiness, and sleep questionnaires outcomes

	Conditions (Mean ± SD)		
Outcome	Placebo	Low dose	High dose
Sleep			
TST (min)	508.3 ± 46.7	494.9 ± 42.8	509 ± 29.7
WASO (min)	49.5 ± 46.1	60.3 ± 47.7	45.1 ± 31.4
SE (%)	89.2 ± 8.2	86.8 ± 7.5	89.3 ± 5.2
SOL (min)	12.2 ± 12.7	13.1 ± 20	15.1 ± 15.4
REM Latency (min)	78.9 ± 22.8	90.5 ± 26	93.8 ± 40.2
Stage 3 Latency (min)	15.4 ± 9.7	16.8 ± 14.1	16.6 ± 7.9
Stage 1 (min)	30.9 ± 10.6	34.9 ± 19.0	37.4 ± 18.4
Stage 2 (min)	240.4 ± 29.9	236.9 ± 34.9	242.1 ± 26.7
Stage 3 (min)	139.6 ± 31.2	125.3 ± 21.9	132.3 ± 22.2
REM (min)	97.4 ± 31.1	97.7 ± 29.8	97.3 ± 29.7
Arousals–total (count)	120.9 ± 41.2	117.6 ± 43.4	126.5 ± 48.3
Arousals–REM (count)	35.2 ± 15.9	31.5 ± 16.9	39.1 ± 19.5
Arousals–NREM (count)	85.8 ± 31.1	86.1 ± 32.1	87.4 ± 34
Awakenings (count)	46.7 ± 46.3	57.2 ± 45.3	39.6 ± 26.5
Stage Shifts (count)	167 ± 30	173.6 ± 34.7	175.6 ± 38.5
Subjective sleepiness			
KSS 2000 h (units)	4.4 ± 1.3	4.7 ± 1.6	4.7 ± 1.6
KSS 2030 h (units)	5 ± 1.7	5 ± 1.4	5.3 ± 1.6
KSS 2100 h (units)	5.3 ± 1.4	5.2 ± 1.3	5.4 ± 1.6
KSS 2130 h (units)	5.5 ± 1.5	5.7 ± 1.4	5.8 ± 1.8
KSS 2200 h (units)	5.9 ± 1.8	6.0 ± 1.4	6.1 ± 1.9
Subjective questionnaires			
Pre-sleep Arousal (units)	4.9 ± 6.5	5.2 ± 6.2	4.6 ± 6.7
Perceived Sleep Quality (units)	4.9 ± 1.1	4.8 ± 1.0	4.8 ± 1.0
Perceived Sleep Quantity (h)	7.6 ± 1.2	7.6 ± 1.4	7.7 ± 0.9
Perceived SOL (min)	24.5 ± 18	22.4 ± 19.9	23 ± 17.7

Notes: TST = total sleep time, WASO = wake after sleep onset, SE = sleep efficiency, SOL = sleep onset latency, REM latency = rapid eye movement latency, REM = rapid eye movement, Arousals NREM = arousals non-rapid eye movement, KSS = Karolinska sleepiness scale.

Table 7.3. Statistical outcomes for sleep, subjective sleepiness, and sleep questionnaires

Outcome	F-statistic	df	*p*-value
Sleep			
TST (min)	1.585	2,31.27	0.221
WASO (min)	1.969	2,31.13	0.157
SE (%)	1.604	2,31.26	0.217
SOL (min)	0.281	2,30.24	0.757
REM Latency (min)	2.194	2,31.37	0.128
Stage 3 Latency (min)	0.112	2,31.43	0.894
Stage 1 (min)	2.518	2,31.05	0.097
Stage 2 (min)	0.214	2,31.47	0.809
Stage 3 (min)	2.140	2,31.24	0.135
REM (min)	0.038	2,31.11	0.963
Arousals–total (count)	1.154	2,31.10	0.329
Arousals–REM (count)	3.254	2,31.14	0.052
Arousals–NREM (count)	0.042	2,31.13	0.959
Awakenings (count)	1.363	2,31.30	0.271
Stage Shifts (count)	1.082	2,31.17	0.351
Subjective sleepiness			
KSS 2000 h (units)	0.348	2,32	0.708
KSS 2030 h (units)	0.723	2,32	0.493
KSS 2100 h (units)	0.320	2,32	0.728
KSS 2130 h (units)	0.254	2,32	0.778
KSS 2200 h (units)	0.208	2,32	0.813
Subjective questionnaires			
Pre-sleep Arousal (units)	0.314	2,32	0.733
Perceived Sleep Quality (units)	0.047	2,32	0.954
Perceived Sleep Quantity (h)	0.130	2,32	0.878
Perceived SOL (min)	0.274	2,32	0.762

Notes: TST = total sleep time, WASO = wake after sleep onset, SE = sleep efficiency, SOL = sleep onset latency, REM latency = rapid eye movement latency, REM = rapid eye movement, Arousals NREM = arousals non-rapid eye movement, KSS = Karolinska sleepiness scale.

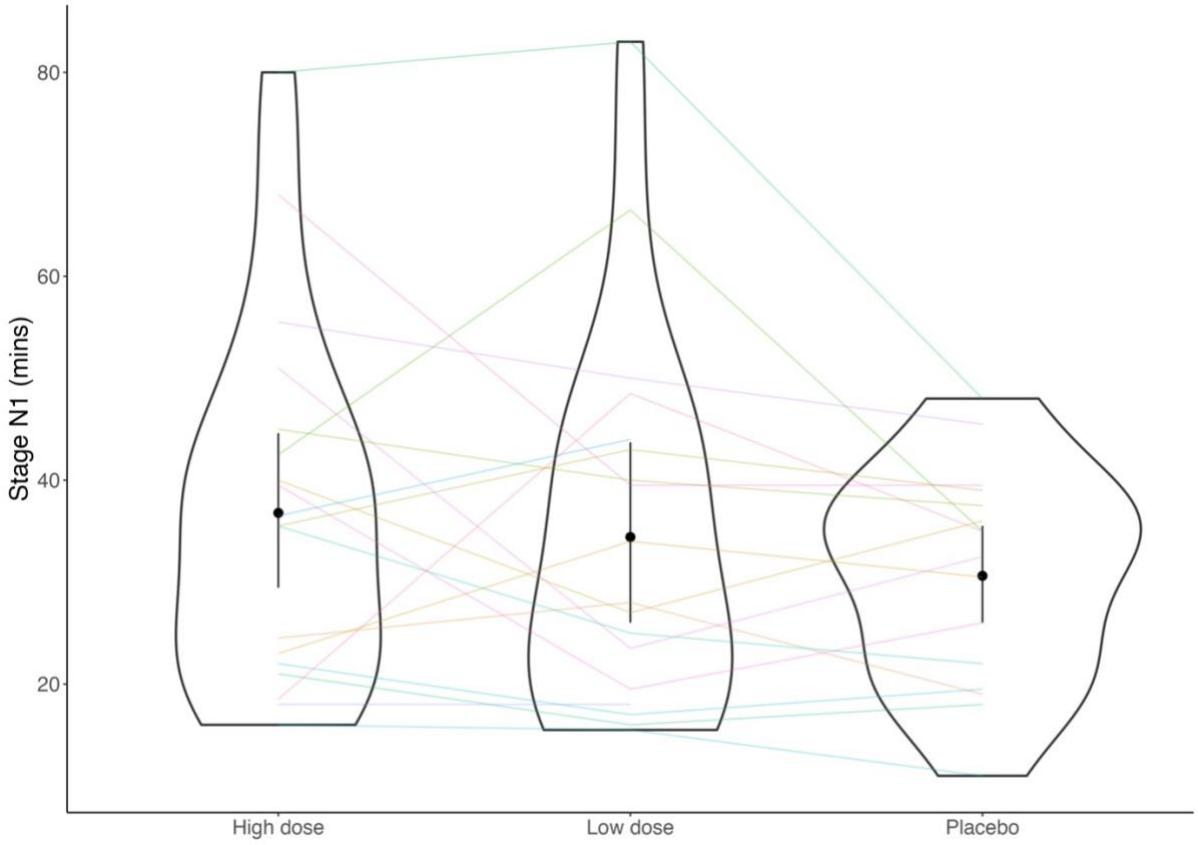

Figure 7.1. Violin plots indicating the effects of two different doses of a nutritional intervention and placebo on stage N1 sleep duration. Coloured lines represent each participant, black dots indicate the mean data point, vertical black lines reflect the standard deviation of the data, and the shape of each plot shows the distribution density of data.

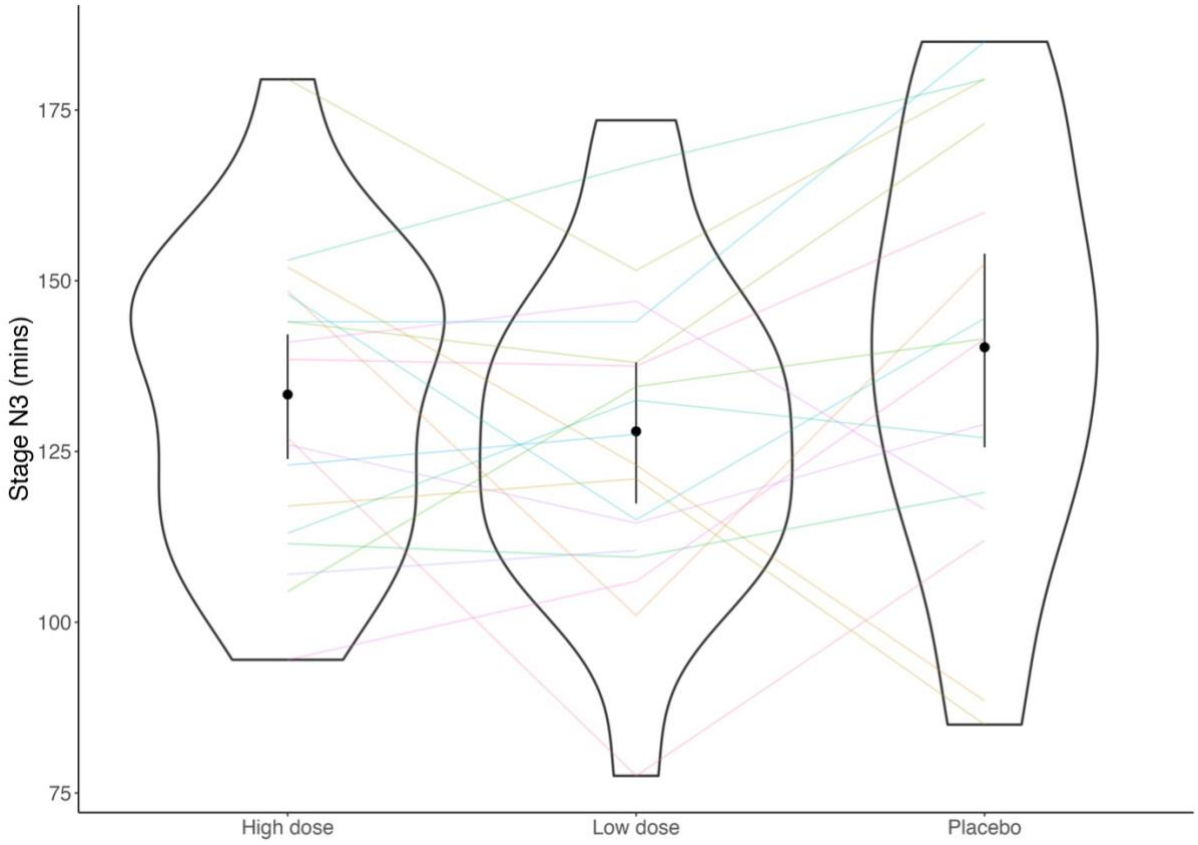

Figure 7.2. Violin plots indicating the effects of two different doses of a nutritional intervention and placebo on stage N3 sleep duration. Coloured lines represent each participant, black dots indicate the mean data point, vertical black lines reflect the standard deviation of the data, and the shape of each plot shows the distribution density of data.

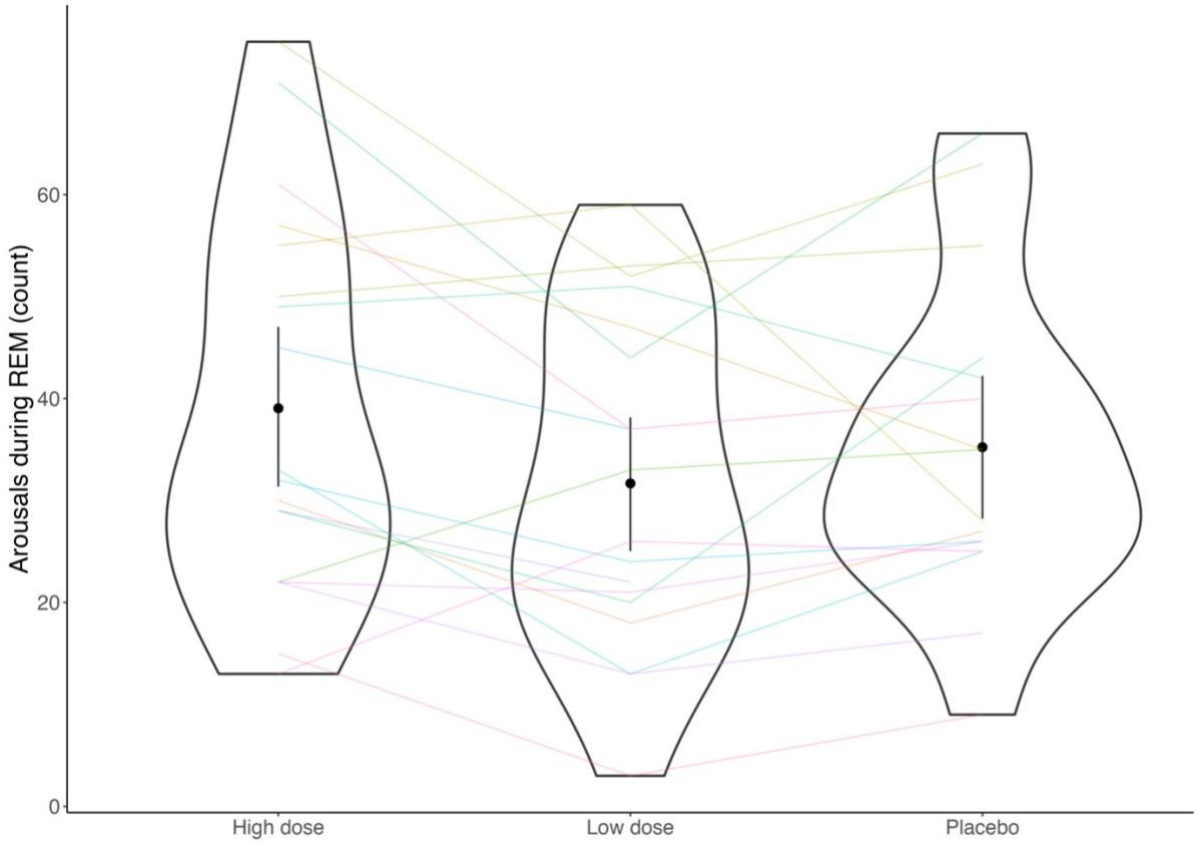

Figure 7.3. Violin plots indicating the effects of two different doses of a nutritional intervention and placebo on arousals during REM. Coloured lines represent each participant, black dots indicate the mean data point, vertical black lines reflect the standard deviation of the data, and the shape of each plot shows the distribution density of data.

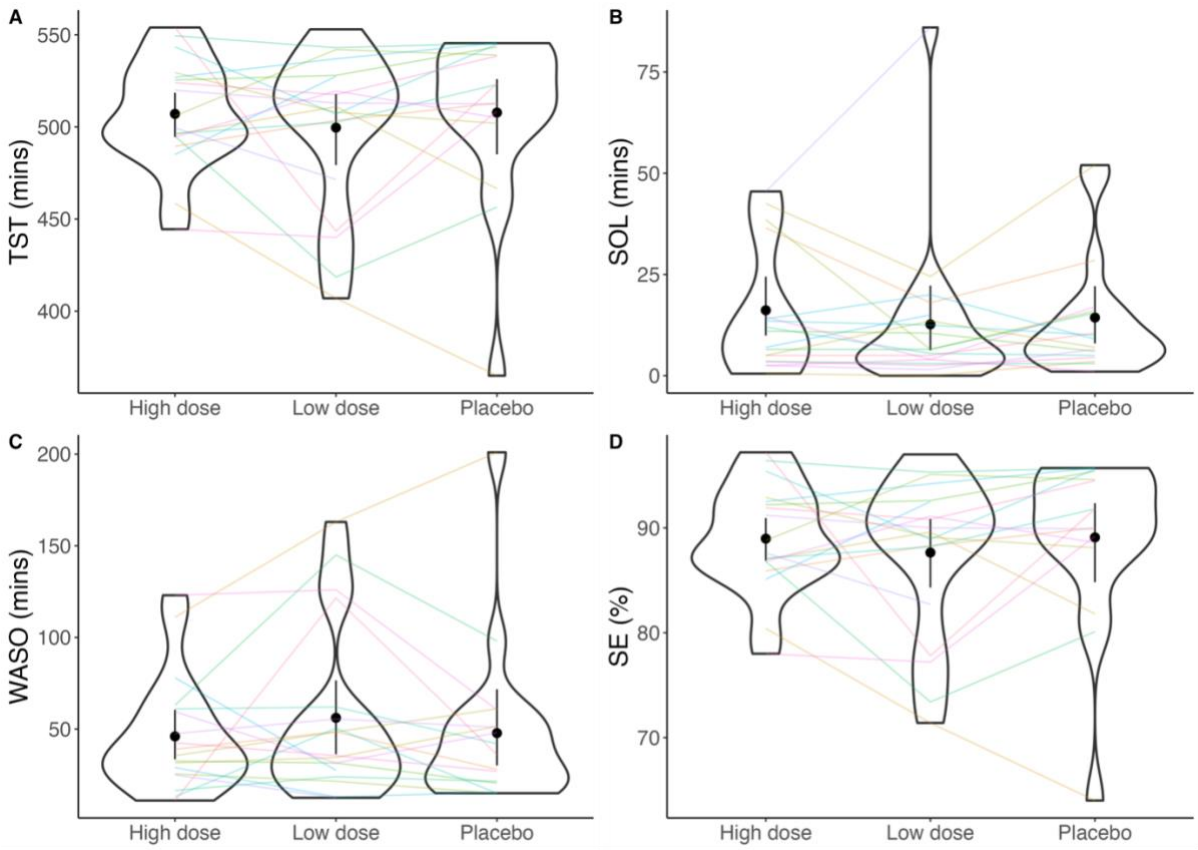

Figure 7.4. Violin plots indicating the effects of two different doses of a nutritional intervention and placebo on (A) total sleep time (TST), (B) sleep onset latency (SOL), (C) wake after sleep onset (WASO), and (D) sleep efficiency (SE). Coloured lines represent each participant, black dots indicate the mean data point, vertical black lines reflect the standard deviation of the data, and the shape of each plot shows the distribution density of data.

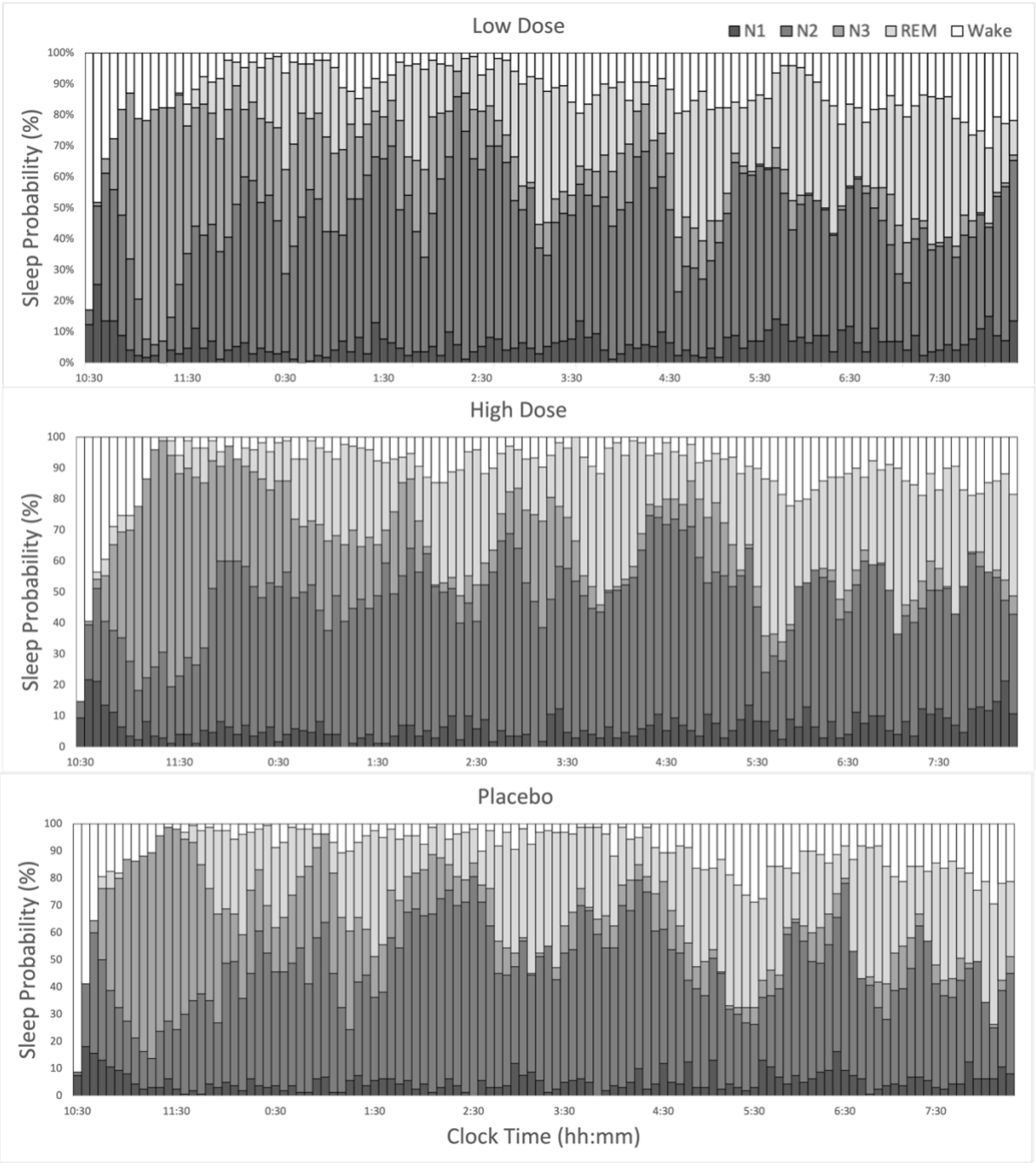

Figure 7.5. Sleep histograms representing the probability distribution of sleep stages across the low-dose (top), and high-dose (middle) nutritional interventions and the placebo (bottom). Data represent the percentage of epochs scored as Stage N1, Stage N2, Stage N3, REM and wake (W) in five-minute bins. The concept for this figure is based on Figure 2 in Sargent et al. (245).

7.5.2 Subjective sleepiness, sleep quality, sleep duration, and sleep latency

No differences were observed in subjective sleepiness between conditions (Tables 7.2-7.3; Supplementary Table 7.2). Additionally, no differences were observed in subjective sleep

quality, subjective sleep duration, or subjective sleep latency between conditions (Tables 7.2-7.3; Supplementary Table 7.2).

7.5.3 Cognitive performance, subjective alertness, self-perceived capacity, and postural sway

There was no difference in mean reaction time, number of lapses, or false starts between conditions during the PVT (Table 7.4-7.5; Supplementary Table 7.2). In addition, there were no differences in subjective alertness, self-perceived capacity, or postural sway between conditions (Table 7.4-7.5; Supplementary Table 7.2).

Table 7.4**.** Cognitive function, postural sway, subjective sleepiness, alertness, and perceived performance testing outcomes

	Conditions (Mean ± SD)		
Outcome	Placebo	Low dose	High dose
PVT – Mean Reaction Time (ms)	244.7 ± 33.2	244.8 ± 28.6	245.7 ± 36.8
PVT – Lapses (count)	1.0 ± 1.5	0.9 ± 1.7	0.9 ± 1.2
PVT – False Starts (count)	1.1 ± 1.6	0.6 ± 1.0	1.3 ± 1.8
KSS (units)	3.8 ± 1.3	3.8 ± 1.1	4.2 ± 1.5
VAS Alertness (units)	64.2 ± 18.1	66.6 ± 17.7	66.3 ± 19.1
VAS Performance (units)	67.7 ± 18.6	68.5 ± 16.6	63.9 ± 17.4
Postural Sway–Area 95 (cm^2)	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.2

Notes: PVT = psychomotor vigilance task, KSS = Karolinska Sleepiness Scale, VAS = visual analogue scale.

Table 7.5. Statistical outcomes for cognitive function, postural sway, subjective sleepiness, alertness, and perceived performance testing

Outcome	F-statistic	df	*p*-value
PVT – Mean Reaction Time (ms)	0.050	2,32	0.951
PVT – Lapses (count)	0.021	2,32	0.979
PVT – False Starts (count)	1.774	2,32	0.186
KSS (units)	1.873	2,32	0.170
VAS Alertness (units)	0.801	2,32	0.458
VAS Performance (units)	1.492	2,32	0.240
Postural Sway–Area 95 (cm^2)	1.297	2,32	0.287

Notes: PVT = psychomotor vigilance task, KSS = Karolinska Sleepiness Scale, VAS = visual analogue scale.

7.5.4 Gastrointestinal symptoms scale

There was no difference in the number of gastrointestinal symptoms between conditions (Mean ± SD; Low dose: 1.8 ± 2.2; high dose: 1.3 ± 1.6; placebo: 1.6 ± 2.2; *p*-value: 0.477) (Supplementary Table 7.2).

7.5.5 Maximal effort time trial and simulated training session

No differences were observed in the maximal effort time trial performance between conditions (Table 7.6). Participants exercised at the same heart rate and power output for each simulated training session (mean ± SD; Low-dose: 144.5 ± 38.5 bpm and 173.3 ± 70 W; High-dose: 153.0 ± 11.4 bpm and 182.4 ± 52.9 W; Placebo: 154.0 ± 11 bpm and 181.4 ± 51.3 W; all *p*-values > 0.05; Supplementary Table 7.2).

Table 7.6. Maximal effort time trial testing and statistical outcomes

	Conditions			Statistical outcomes		
Outcome	Placebo	Low dose	High dose	F-statistic	df	*p*-value
Mean Power Output (watts)	173.3 ± 51.7	175.1 ± 50.6	176.2 ± 52.5	0.294	2,32	0.748
Mean Heart Rate (bpm)	150.3 ± 13.5	152.8 ± 15.3	151 ± 13.3	1.020	2,32	0.372
Mean RPE (units)	16.4 ± 1.1	16.2 ± 1.1	16.4 ± 1.1	1.525	2,32	0.233

Notes: Data are mean ± standard deviation (SD*),* degrees of freedom, RPE = rating of perceived exertion.

7.6 Discussion

The aim of this study was to examine the effect of two different doses of a formulated nutritional intervention compared with placebo on objective and subjective sleep, next-morning physical performance, cognitive function, and postural sway in trained adult males. Objective sleep parameters do not appear to be significantly affected by both doses of the nutritional intervention and no improvements in sleep were observed. However, from a practical standpoint, the respective changes in sleep architecture may lack clinical significance, and are unlikely to cause any deleterious effects on performance (279). No changes in subjective sleep-related outcomes were observed after consumption of either dose of the nutritional intervention (Table 7.2). Additionally, participants were able to maintain next-morning physical performance, cognitive function, and postural sway after the consumption of each intervention. Consequently, the consumption of high- and low-

doses of a proprietary blended supplement that contains tryptophan, high glycaemic index carbohydrate, theanine, 5'AMP, and whey protein does not appear to have a meaningful influence on objective sleep parameters, no effect on subjective sleep outcomes, and does not improve next-day performance.

The two doses of a nutritional intervention examined in the present study did not enhance sleep quality or quantity but likely had an influence on three components of sleep. However, these effects do not represent a meaningful change in sleep quality or quantity. A reduction of 13.5 minutes of 'slow wave sleep' was observed after consuming the lower dose intervention compared to placebo; and a likely increase in 'light sleep' of approximately seven minutes was observed after consuming the higher dose intervention compared to placebo. The findings in this study contrast with previous results where a nutritional intervention with a similar ingredient profile improved sleep onset latency (intervention: 9.9 ± 12.3 min vs placebo: 19.6 ± 32.0 min) in a group of healthy adult males (125). Interestingly, whilst no significant improvement in SOL was observed in the present study, the latencies for all three conditions (low dose: 13.1 ± 20 min, vs high dose: 15.1 ± 15.4 min, vs placebo: 12.2 ± 12.7 min) were very similar to those in the 'improved' SOL in the previous study. This may be attributed to differences in the ingredient profiles of the supplements. In the previously mentioned study with a similar ingredient profile (125), valerian was included in the nutritional intervention, which has been shown to improve sleep outcomes (297). Further, a low-dose of 0.641g and a high-dose of 0.855g of tryptophan were consumed within this study. These doses were below the range of 1g to 12g of L-tryptophan that have been shown to modulate sleep architecture (25, 291, 298). However, it is important to note that tryptophan appears to be more efficacious with daily, regular consumption (25). Therefore, the ingredient profile in the current study may not be as effective at improving sleep compared to interventions with valerian.

Exercise performance was not influenced by either nutritional intervention. This is consistent with evidence reporting the effects of acute pre-sleep alpha-lactalbumin consumption on sleep-quality and time trial performance, in which no improvement in sleep parameters (measured via actigraphy), or changes in 4-km cycling time trial performance were observed (299). In the current study, cycling performance was consistent across trials, highlighting the participants were able to tolerate the exercise demands of the protocol. This is likely due to the participants' previous training experience

and ability to repeat high-intensity efforts across successive days. Furthermore, as there were no decrements in performance observed, the pre-sleep ingestion of the nutritional interventions is likely safe for use by athletes who are required to undertake physical performance tasks in the morning.

Cognitive performance and postural sway were not affected by either dose of the nutritional supplement. This is an important finding as interventions to enhance sleep have the potential to induce a 'hangover effect' and impair cognitive and psychomotor function (14). For example, reduced alertness, slower reaction times, and greater daytime sleepiness may manifest the day after ingestion of prescription sleep medication (300). In an athletic context, a slower reaction time may have negative implications for sporting performance, as numerous components of sport (e.g., agility) rely on fast and accurate decision-making and response times (225, 301). Additionally, no adverse effects that are commonly reported after pharmaceutical sleep medications were observed the morning after either of the nutritional interventions. Therefore, the combination of ingredients in these nutritional interventions appear to not have any detrimental effects on cognitive function or balance in adult male athletes.

Although this study is the first to investigate the effects of two doses of a novel nutritional intervention to enhance sleep in well-trained adult males, there are limitations that should be noted. Athletes are typically reported as poor sleepers due to various sport-related and non-sport-related factors such as early-morning training sessions (302), pre-competition anxiety (303), and travel (94, 304). In the current study, participants were provided a consistent sleep opportunity between 22:30h and 08:00h (i.e., 10.5 hours in bed), in a private distraction-free environment, without the typical demands of early-morning training or evening competition. This may have incurred a ceiling effect, as the sleep environment may be more advantageous for facilitating 'good' sleep than what an athlete would experience in their typical training schedule and living environment. Subsequently, the environment may make it difficult to observe improvements in sleep with the nutritional interventions. Assessing the efficacy of the two doses of the nutritional intervention during an athlete's typical training and competition schedule, under 'free-living' conditions may provide different results. Further, whether the supplements had a different effect on poor sleepers, which athletes often are, would be pertinent to examine. Second, only a single modality of exercise was conducted each morning to assess the

effects of the nutritional interventions on physical performance. Whether performance was affected in a longer duration task or in an alternative modality of exercise (e.g., resistance training) would be valuable to assess, as athletes often undertake various modalities of training in the morning. Third, the study design used a single-day administration of the supplement, rather than a multi-day protocol. It is also important to note the study used a placebo control which were volume, flavour, and colour-matched but not calorie-matched.

In conclusion, this study has determined that the consumption of a high or low dose proprietary blended supplement did not meaningfully influence objective or subjective sleep in trained adult males. The two doses did not appear to improve next-morning physical performance or have any negative effects on cognitive performance or postural sway, suggesting it is safe for use in this context. It is speculated that improvements in sleep-related outcomes were not observed due to the removal of typical stressors associated with an athlete's regular training and competition schedule (e.g., early-morning training and evening competition) that could impair sleep. Alternatively, the combination of ingredients may not be as effective at enhancing sleep-wake behaviours compared to similar nutritional interventions that have used other ingredients for improving sleep. Future research investigating the efficacy of the two nutritional interventions when consumed during periods of typical training and competition in an athlete's 'free-living' conditions may be beneficial.

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Supplementary Table 7.1. Effect sizes and 95% confidence intervals for sleep and sleepiness outcomes

	Low-dose vs placebo	High-dose vs placebo	Low-dose vs High-dose
Sleep			
TST (min)	-0.26 (-0.609 to 0.103)	0.03 (-0.321 to 0.380)	-0.29 (-0.649 to 0.069)
WASO (min)	0.27 (-0.090 to 0.624)	-0.06 (-0.409 to 0.294)	0.34 (-0.029 to 0.694)
SE (%)	-0.26 (-0.611 to 0.102)	0.03 (-0.321 to 0.381)	-0.29 (-0.651 to 0.068)
SOL (min)	-0.11 (-0.462 to 0.243)	0.01 (-0.343 to 0.359)	-0.12 (-0.473 to 0.233)
REM Latency (min)	0.28 (-0.079 to 0.635)	0.36 (-0.006 to 0.716)	-0.08 (-0.432 to 0.273)
Stage 3 Latency (min)	0.08 (-0.274 to 0.425)	0.07 (-0.280 to 0.419)	0.01 (-0.346 to 0.358)
Stage 1 (min)	0.27 (-0.094 to 0.620)	0.40 (0.028 to 0.757)	-0.13 (-0.486 to 0.221)
Stage 2 (min)	-0.07 (-0.423 to 0.278)	0.04 (-0.311 to 0.389)	-0.12 (-0.468 to 0.238)
Stage 3 (min)	-0.37 (-0.728 to -0.004)	-0.18 (-0.530 to 0.176)	-0.20 (-0.549 to 0.161)
REM (min)	-0.03 (-0.384 to 0.319)	-0.05 (-0.400 to 0.303)	0.02 (-0.336 to 0.368)
Arousals–Total (count)	-0.10 (-0.488 to 0.257)	0.17 (-0.188 to 0.520)	-0.27 (-0.626 to 0.091)
Arousals–REM (count)	-0.18 (-0.536 to 0.172)	0.26 (-0.098 to 0.617)	-0.46 (-0.822 to -0.083)
Arousals–NREM (count)	0.00 (-0.356 to 0.347)	0.04 (-0.310 to 0.393)	-0.05 (-0.399 to 0.305)
Awakenings (count)	0.15 (-0.189 to 0.476)	-0.05 (-0.340 to 0.283)	0.21 (-0.132 to 0.544)
Stage Shifts (count)	0.20 (-0.157 to 0.552)	0.25 (-0.108 to 0.605)	0.05 (-0.405 to 0.300)
Subjective sleepiness			
KSS 2000 h (units)	0.13 (-0.221 to 0.475)	0.13 (-0.221 to 0.475)	0 (0 to 0)
KSS 2030 h (units)	0 (0 to 0)	0.18 (-0.167 to 0.532)	-0.18 (-0.532 to 0.167)
KSS 2100 h (units)	-0.07 (-0.417 to 0.277)	0.07 (-0.277 to 0.417)	-0.14 (-0.489 to 0.208)
KSS 2130 h (units)	0.09 (-0.257 to 0.437)	0.12 (-0.228 to 0.468)	-0.03 (-0.377 to 0.317)
KSS 2200 h (units)	0.06 (-0.290 to 0.403)	0.11 (-0.234 to 0.461)	-0.06 (-0.403 to 0.290)

Subjective questionnaires			
Pre-sleep Arousal (units)	0.08 (-0.271 to 0.423)	-0.06 (-0.410 to 0.284)	0.14 (-0.209 to 0.487)
Perceived Sleep Quality (units)	-0.05 (-0.393 to 0.300)	-0.05 (-0.393 to 0.300)	0 (0 to 0)
Perceived Sleep Quantity (h)	0.01 (-0.334 to 0.359)	0.08 (-0.624 to 0.430)	-0.07 (-0.417 to 0.276)
Perceived SOL (min)	-0.05 (-0.393 to 0.300)	-0.05 (-0.393 to 0.300)	0 (0 to 0)

Notes: Data are mean ± SD, Effect sizes presented are Cohen's d, TST = total sleep time, WASO = wake after sleep onset, SE = sleep efficiency, SOL = sleep onset latency, REM latency = rapid eye movement latency, REM = rapid eye movement, Arousals NREM = arousals non-rapid eye movement

Supplementary Table 7.2. Effect sizes and 95% confidence intervals for cognitive testing battery, postural sway, maximal effort time trial, and gastrointestinal symptoms outcomes

	Low-dose vs placebo	High-dose vs placebo	Low-dose vs High-dose
Cognitive testing battery			
PVT–Mean Reaction Time (ms)	0.00 (-0.345 to 0.348)	0.05 (-0.298 to 0.395)	-0.05 (-0.394 to 0.299)
PVT–Lapses (count)	-0.03 (-0.378 to 0.315)	-0.03 (-0.378 to 0.315)	0.00 (0.000 to 0.000)
PVT–False Starts (count)	0.11 (-0.239 to 0.456)	-0.22 (-0.567 to 0.134)	-0.33 (-0.680 to 0.031)
KSS (units)	0.04 (-0.308 to 0.386)	0.31 (-0.043 to 0.666)	-0.27 (-0.626 to 0.080)
VAS Alertness (units)	0.21 (-0.146 to 0.554)	0.18 (-0.171 to 0.528)	0.03 (-0.321 to 0.372)
VAS Performance (units)	0.05 (-0.299 to 0.394)	-0.24 (-0.587 to 0.116)	0.29 (-0.070 to 0.636)
Postural Sway – Area 95 (cm^2)	0.27 (-0.089 to 0.616)	0.04 (-0.304 to 0.389)	-0.22 (-0.572 to 0.130)
Maximal effort time trial			
Power output (watts)	0.08 (-0.266 to 0.428)	0.13 (-0.215 to 0.482)	-0.05 (-0.400 to 0.294)
Heart Rate (bpm)	0.25 (-0.107 to 0.596)	0.07 (-0.273 to 0.420)	0.17 (-0.178 to 0.520)
RPE (units)	-0.21 (-0.557 to 0.143)	0.09 (-0.255 to 0.439)	-0.30 (-0.653 to 0.055)
Gastrointestinal symptoms			
Total symptoms	0.07 (-0.283 to 0.421)	-0.15 (-0.500 to 0.205)	0.22 (-0.140 to 0.569)

Notes: Data are mean ± SD, Effect sizes presented are Cohen's d, KSS = Karolinska sleepiness scale, PVT = psychomotor vigilance task, KSS = Karolinska Sleepiness Scale, VAS = visual analogue scale

Chapter 8: General Discussion, Limitations, Delimitations, Future Directions, and Concluding Remarks

8.1 Summary of findings

The studies conducted in this thesis aimed to examine the relationship between sleep, exercise, and nutrition. The findings help to further elucidate the complex relationships that exist between modifiable health-related behaviours and sleep. The first study in this thesis aimed to review the relationship between sleep, circadian biology, and skeletal muscle to describe the influence of sleep on metabolic health. From this review, it was clear modifiable behaviours such as exercise and nutrition may affect sleep. Therefore, study two investigated the effect of afternoon moderate-intensity aerobic exercise on subsequent sleep quantity and architecture. Afternoon exercise did not disrupt sleep, but variability was observed in individual sleep responses following exercise which indicates the afternoon is likely an appropriate time to exercise without disturbing sleep. Study three investigated a novel hypothesis suggesting that exercise-induced peripheral factors may be potential mechanisms for alterations in sleep following exercise. The moderate-intensity exercise intervention employed in study three appeared to have little effect on circulating plasma cytokine and myokines levels, and there was no association between pre-sleep peripheral factor concentrations and sleep characteristics. These findings led to study four, which explored the combination of both exercise and nutrition on sleep. Specifically, study four examined the influence of a high- and low-dose proprietary nutritional intervention on sleep, next-morning physical performance, cognitive performance, and postural sway. The pre-sleep nutritional interventions did not improve sleep quality or quantity, nor were impairments in next-morning physical or cognitive performance, postural sway, or subjective assessments of sleep observed.

The main findings that emerged from this program of research were: 1) sleep appears to be consistent when measured using gold-standard techniques in highly-controlled environments; 2) individual sleep responses after exercise vary but afternoon aerobic exercise does not appear to interrupt sleep; 3) certain peripheral exercise-induced metabolic factors do not appear to be associated with changes in sleep following exercise; and 4) a proprietary nutritional intervention consumed in conjunction with afternoon exercise does not enhance sleep. While discussions and conclusions have been provided

within each individual study, the aim of this final general discussion is to discuss the emerging findings and themes from this program of research and potential implications for practice. Additionally, limitations, delimitations, future directions for research, and concluding remarks are provided.

8.1.1 Sleep and exercise

The findings of study two add to the existing literature by highlighting that moderate-intensity exercise performed in the afternoon does not have a significant effect on sleep architecture or duration. The individual variability in sleep responses observed in study two may help explain why there are equivocal findings in the literature that show exercise may have a beneficial effect (229), negative effect (20), and at times, no effect (129) on subsequent sleep. In study two, although not statistically significant, exercise appeared to have a moderate effect on total sleep time (mean ± SD; control 493.71 ± 12.59 mins vs exercise: 471.46 ± 55.19 mins; Cohen's *d*: -0.56). However, it is important to note the wide interquartile ranges (IQR) that were observed, which highlight greater variability in sleep responses after exercise compared to no exercise (IQRs: exercise: 51.50 mins vs no exercise: 13.38 mins). Whilst there were no improvements in sleep observed after acute afternoon exercise, the lack of sleep disruption observed suggests that when exercising to improve health, the afternoon would likely be an appropriate time to do so for healthy, physically active adult males without early or late chronotypes. Nonetheless, individualised approaches to afternoon exercise where possible, are recommended.

The configuration of exercise (e.g., modality, intensity, timing, and duration) is an important factor when considering the effect of exercise on sleep. In this thesis, continuous moderate-intensity aerobic exercise and high-intensity interval training were implemented, as these are both commonly undertaken exercise modalities. Whilst moderate-intensity has been suggested to have a positive influence on sleep (234), high-intensity or vigorous exercise has been hypothesised to have a negative effect on sleep (305). The disruption of sleep after higher-intensity exercise is speculated to be due to several factors, including elevations in core body temperature (CBT) (249, 306) and increased psychological and physiological arousal (20). Although CBT was not measured in this thesis, previous research has shown evening moderate-intensity exercise performed between 20:45h and 21:30h elevated CBT before returning to baseline prior to bedtime (129). Thus, exercise-

induced changes in CBT are unlikely to have influenced sleep in this thesis. Additionally, in study four, psychological arousal that often manifests after exercise did not appear to have an influence on sleepiness at bedtime, as there were no differences observed in pre-sleep Karolinska Sleepiness Scales. Nonetheless, while neither of the exercise modalities employed in this thesis appeared to negatively influence sleep, this may be due to the timing of the exercise sessions in relation to the proximity to bedtime.

In this thesis, exercise sessions were typically performed in the afternoon, between 14:00h to 16:00h. Neither exercise intervention significantly influenced sleep. This finding is important, as the effect of exercise on subsequent sleep is not well understood, particularly during the afternoon, which is a common time to exercise. Evening exercise has received more attention in the literature (18, 129, 228) than afternoon exercise, with typical sleep hygiene recommendations suggesting evening exercise may interrupt sleep and should be avoided (227). However, this notion has been challenged (18, 228), with a quantitative synthesis of findings suggesting evening exercise may benefit sleep, provided it ceases 1 h before bedtime (239). By not observing adverse effects of afternoon exercise on sleep, the current findings help to elucidate the relationship between exercise and sleep in healthy adult males and demonstrate that approximately between 14:00h and 16:00h in the afternoon is a suitable time of day to exercise without impairing sleep.

Study one reviewed the complex relationship between sleep, circadian biology, and skeletal muscle. From this review, it became apparent that acute exercise may be a useful tool in preserving metabolic health during periods of insufficient sleep. A host of negative health outcomes can arise from poor sleep quality and quantity, including increased risk of cardiovascular disease, metabolic syndrome, and suppressed immune function (13, 157). Furthermore, during acute periods of sleep restriction, rates of myofibrillar protein synthesis are reduced in skeletal muscle (12, 81). Considering a significant proportion of adults are not meeting recommended sleep guidelines (e.g., sleep durations and regular sleep/wake behaviours) (34), identifying strategies to mitigate some of the deleterious effects of insufficient sleep is prudent. For instance, high-intensity interval exercise performed during periods of acute sleep restriction appears to have a protective effect on rates of skeletal muscle protein synthesis and metabolic health (12, 81). Specifically, the inclusion of high-intensity exercise during periods of acute sleep restriction resulted in the maintenance of baseline rates of myofibrillar protein synthesis in skeletal muscle

compared to sleep restriction without high-intensive exercise (12). However, whether this protective mechanism is present during chronic periods of sleep restriction is unclear and warrants further investigation.

The protective effects of acute exercise interventions during periods of compromised sleep show promise for maintaining metabolic health. However, whether these benefits exist during periods of chronic exercise and/or sleep restriction, is not well understood. Epidemiological evidence has reported some significant positive associations between self-reported exercise habits and subjective measures of sleep (217) but rigorous intervention studies using gold-standard measurement techniques, over extended periods of time are lacking. Considering the health promoting benefits of regular exercise such as strengthening circadian rhythms (220), the prevention of diseases (307), and improving metabolism (307), it is reasonable to propose that chronic exercise would be beneficial for improving both sleep and health. Although not investigated in this thesis, after chronic afternoon exercise, there is likely to be a feedback loop that would invoke positive downstream effects on the sleep/wake cycle, potentially due to exercise acting as a potential circadian time cue to strengthen and promote circadian rhythms.

By further examining the effect of exercise at several timepoints throughout the day on sleep, ongoing refinement of sleep hygiene recommendations is possible. As sleep was unaffected after afternoon exercise in this thesis, and the longstanding recommendation to avoid exercise in the evening is being challenged, sleep hygiene guidelines must continue to adapt in response to emerging evidence. Therefore, continuing to determine the effects of specific timings and configurations of exercise on sleep is essential. Considering the variability in responses observed in the studies within this thesis, it is likely that personalised sleep recommendations based on the needs of the individual would be most appropriate. However, broad generalised sleep guidelines still have utility and should be dynamic in response to the emergence of more evidence elucidating the relationship between sleep and exercise.

8.1.2 Exercise-induced peripheral factors and sleep

Elevated levels of exercise-induced peripheral factors have been proposed to be a potential underlying mechanism for improvements in sleep after exercise (24). Study three was the

first investigation in humans to explore the novel hypothesis proposed by Tan et al. (24) that suggests several exercise-induced peripheral factors including tumour necrosis factor-α (TNF-α), interleukin-1α, interleukin-1β, interleukin-6 (IL-6), and brain-derived neurotrophic factor (BDNF) may influence subsequent sleep. Our findings show that a moderate-intensity aerobic exercise session appears to have little effect on BDNF, interleukin-1α, and interleukin-1β, whilst only inducing small increases in IL-6 and TNF-α. The lack of response observed in the peripheral factors is likely due to the configuration of the exercise stimulus and the individual kinetics of the desired myokines and cytokines. For instance, IL-6 is suggested to have a logarithmic relationship with exercise duration (308), with maximal IL-6 levels peaking almost immediately after a marathon (309), and 60-90 minutes after 1h of single-leg eccentric exercise (310). Furthermore, the magnitude of response also differs between peripheral factors, with a marathon inducing ~100 fold increase in IL-6 but only a two-fold increase in TNF-α (308). However, it was important to investigate whether the novel hypothesis suggested holds true in response to a typical exercise protocol that would likely be undertaken by a physically active healthy adult. Whilst the results observed in study three do not support components of the proposed hypothesis, it is important to acknowledge there were peripheral factors from the hypothesis omitted in this investigation. For instance, due to concerns about the reliability and validity of the assessment of plasma irisin and PGC1-α, they were not measured. Consequently, the hypothesis may still be worth exploring in different populations, different exercise modalities, and with measurements of additional peripheral factors.

The configuration of the exercise component implemented in study three was expected to elicit a pronounced response in exercise-induced peripheral factors. However, given the within-participant repeated-measures study design, it was important to consider the limitations around the intensity and duration of the exercise protocol used. Given the physically active, but not well-trained nature of the participant cohort, implementing highly-fatiguing or muscle damaging exercise protocols may have impaired subsequent performance. As only small increases in IL-6 and TNF-α were observed, it is likely a more strenuous exercise intervention may have elicited greater increases in circulating peripheral factor levels compared to the moderate-intensity exercise bout. It is important to note, moderate-intensity exercise does not typically increase TNF-α (269). Moreover, when IL-6 is elevated after exercise, there may be a release of soluble TNF-α receptors,

which consequently, inhibit the action of TNF-α (269). Further investigation as to whether high-intensity exercise or incurring greater muscle damage increases circulating peripheral factor levels and if there is a subsequent effect on sleep, would be an important next step.

There was no relationship observed between pre-sleep circulating peripheral factors and sleep, regardless of the small increase in IL-6 and TNF-α. This contrasts the proposed hypothesis (24) that suggests increases TNF-α could influence slow wave activity and increases in IL-6 may lead to enhanced slow wave sleep (SWS) via downstream increases in interleukin-1 receptor agonist (IL-1ra), as there were no observed changes in sleep duration or architecture. However, it is important to note that peripheral metabolic factors may display a circadian rhythm of expression. For instance, plasma BDNF typically will peak ~7.5 hours prior to dim-light melatonin onset (DLMO) in women and ~2.5 hours in men (311). In our study, chronotypes were assessed subjectively and used to screen extreme chronotypes from the study, but no objective measures of individual DLMO were collected. Identifying individual DLMO timings and chronotypes may have provided further insight into a potential influence of circadian rhythms on metabolic responses to exercise.

8.1.3 Sleep and nutritional interventions

The findings from studies two and three identified that moderate-intensity exercise did not improve sleep. Study four extends these findings by examining the effects of the consumption of a proprietary nutritional intervention prior to sleep following afternoon exercise. Neither a high- or low-dose of the intervention had beneficial effects on sleep. Rather, some aspects of sleep appeared to be negatively influenced by the ingestion of the supplement although the effects were not clinically meaningful. Consequently, the combination of nutritional interventions and exercise does not appear to have an additive effect on improving sleep. There was considerable inter-individual variability observed in sleep after the consumption of the nutritional supplement and afternoon exercise, which appears to align with variability in sleep after exercise observed in studies two and three. However, it is important to acknowledge intra-individual sleep appears to be quite stable. Nonetheless, when looking at the findings of studies two, three, and four collectively, the observed findings suggest responses in sleep characteristics are highly individual and sleep, in general, remains consistent when measured in a controlled setting.

Study four continues research on a refined version of a previous nutritional intervention that had improved sleep onset latency in healthy adult males (125). In the original formulation of the product, a poor taste profile resulted in the need to reformulate the supplement such that valerian root was removed to augment the taste profile. Anecdotally, the taste profiles of the new version received positive feedback, but the overall efficacy of the new formulation was not as effective as the previous iteration. Therefore, valerian root may have a greater ability to influence sleep/wake behaviours when consumed as part of a nutritional supplement but may not be a viable inclusion due to the poor taste.

The new combination of ingredients did not influence sleep in comparison to the previous blend, likely due to the new ingredient profile without valerian root. The original formula (125) was developed by a Box-Behnken model that was optimised for serum tryptophan levels and validated in a cohort of healthy adult males, similar to the participants recruited in study four. Although the previous iteration was effective, it is important to acknowledge that the original formulation and the new version of the supplement likely act on different mechanisms to influence sleep. For example, valerian root, which was present in the original formula, can reduce sympathetic nervous system activity via acting on the neurotransmitter, gamma-aminobutyric acid (GABA) (297). In contrast, tryptophan, which was present in both versions, is a dietary amino acid that is a pre-cursor to serotonin, which is converted to the hormone melatonin, which is an essential regulator of the sleep/wake cycle (298). As such, the difference in mechanism of action, due to the removal of valerian, may have been the catalyst for the differences observed between the original formulation (125) and the findings within this thesis.

The dose of ingredients and timing of consumption are important considerations when aiming to augment the sleep/wake cycle. In the proprietary formulated supplement used in study four, a low-dose of 0.641g and a high-dose of 0.855g of tryptophan were consumed ~90 minutes before bedtime. These doses were below the range of 1g to 12g of L-tryptophan that have been shown to modulate sleep architecture (25, 291, 298). However, it is important to note that tryptophan appears to be more efficacious with daily, regular consumption (25). Additionally, whether there are additive effects of consuming tryptophan in conjunction with other sleep promoting ingredients is unclear. Nonetheless, determining if the regular consumption of the proprietary formulated supplement improves

sleep over time would be valuable. If sleep was able to be improved with the chronic use of the supplement, there may be downstream implications for performance via increased physiological recovery and improved sleep/wake behaviours. This would be valuable to a wide-range of demographics who are seeking to improve their sleep.

Interventions used to enhance sleep may have increased risk of inducing negative next morning 'hangover' effects. For instance, benzodiazepine medication such as nitrazepam, commonly used to treat insomnia, have been shown to induce a 'hangover' effect upon waking after pre-sleep consumption (312). The nutritional intervention in study four did not induce negative effects on next-morning cognitive performance, postural sway, or subjective sleep which highlights both the low- and high-doses are able to be consumed without any subsequent cognitive impairment. The lack of 'hangover' effects observed supports the trial of the nutritional interventions in a free-living environment, where individuals may be required to undertake complex psychomotor tasks the morning after consumption (e.g., operate a motor vehicle).

Overall, the combination of exercise and the subsequent consumption of a pre-sleep nutritional intervention did not appear to influence sleep in our cohort. However, whether the supplement may have utility in free-living environments which may not be as favourable for sleep may be important to determine. Furthermore, assessing the chronic use of the nutritional intervention may yield promising results as there is potential for a saturation effect to occur with ingredients such as tryptophan, that may help augment sleep and have subsequent implications for physical performance and health.

8.2 Limitations

8.2.1 Participant sample

The cohort of participants recruited for studies two, three, and four were all healthy adult males without early or late chronotypes. The physical activity levels of participants differed between studies two and three, and study four, with physically active participants targeted for the former and well-trained individuals being recruited for the latter. This approach may limit the generalisability of findings of this thesis across different populations. For instance, sleep/wake behaviours differ across the lifespan, with age-related differences in sleep duration and sleep architecture observed (279). Furthermore,

sleep duration and quality (e.g., less SWS and rapid eye movement (REM)) typically decline with age (279). Additionally, circadian biology is also influenced by age and sex, with adolescents and pre-adolescents often reporting later chronotypes (i.e., later bedtimes) and subsequently, having later circadian rhythms (e.g., core body temperature minimum or peak melatonin release) than adults (313). As such, the findings in this thesis may not be replicable in other populations and different outcomes may be observed in different age-groups. Recruiting healthy participants who were identified as good sleepers may also limit the generalisability of the findings of this thesis as a considerable proportion of the population (>30% of Australian adults) are identified as not achieving sufficient sleep quality or quantity (6). How poor sleepers or other populations (e.g., clinical populations, elderly, or youth) respond to the interventions within this thesis would be important to examine. Further, to control for the influence of natural light on altering circadian rhythms, participants were not exposed to natural light during each study in this thesis.

Sleep may also be influenced by the sex of the participant. For instance, the menstrual cycle may have an influence on sleep. During typical ovulatory menstrual cycles, there are cyclical changes in both hormones and core body temperature (314). Specifically, increases in core body temperature during the luteal phase of menstruation have been associated with the proportion of REM sleep obtained (315, 316). Based on these sex-specific differences in physiology and the downstream implications on sleep, the findings from this thesis may not be generalisable to females. Therefore, future research investigating whether the findings of this thesis are replicable in other demographics (e.g., sex and age) may be prudent.

8.2.2 Experimental environment

Conducting robust experiments in a purpose-built sleep laboratory, free of many of the stressors that may contribute to typical sleep interruptions, may have resulted in a ceiling effect. Each participant was housed in their own private room, free from the potential distraction of mobile phones, bed partners, or children. The removal of these potential sleep disrupters may have incurred better sleeping arrangements than the participants' usual sleep environment and thus, introduced a ceiling effect. However, disturbed and insufficient sleep durations are ubiquitous across populations, with athletes also typically not achieving sufficient sleep durations (280). Various sport-related factors such as early

morning training, evening competition times, and pre-competition anxiety may all contribute to poor sleep (94). In study four, when using a physically trained population, the removal of these typical stressors that contribute to poor sleep in athletic populations may have facilitated better sleep opportunities compared to free-living conditions. As such, whether the findings of study four are reproducible in an individual's free-living environment may be prudent to explore.

8.2.3 COVID-19

In 2019, coronavirus disease (COVID-19), caused by the novel coronavirus SARS-CoV-2, triggered a global health pandemic. In response to COVID-19, globally, there were disturbances to the healthcare system, economic crises, travel restrictions, and disruptions to daily life. From a research perspective, strict guidelines were developed, implemented, and adhered to in order to ensure the safety of participants, researchers, and staff when undertaking research projects. One such requirement of the Central Queensland University's Appleton Institute was that participants must be vaccinated against the virus to enter the laboratory. As a result of the mandatory vaccination requirement, potential participants were excluded from participating in the research projects who were unvaccinated. Additionally, in order to undertake any of the studies in this thesis, participants were required to be free from COVID-19 or any related symptoms. The high infection rates observed in the wider community during data collection influenced the number of participants that were able to be recruited for the studies, with several participants who had completed pre-screening and were deemed eligible, unable to participate in the studies due to contracting the virus. Anecdotally, recruitment presented challenges as historical participant pools used for recruitment were not accessible. For instance, face-to-face recruitment of university students from nearby institutions was impacted due to the tendency for students to be learning online and not being present on campus. Additionally, due to the challenges around recruitment, gathering thorough baseline sleep/wake data were challenging, with some participants recruited in close-proximity to the commencement of the respective trial. This was a limitation as greater insight into baseline sleep characteristics may have provided more information into the sleep responses observed of participants.

All data for this thesis were collected between January – August 2022 at the CQU's Appleton Institute, in South Australia amidst the global COVID-19 pandemic. To ensure the safe recruitment, screening, and monitoring of participants during each study, a COVID-safe plan was implemented by CQU's Appleton Institute. Briefly, all staff and researchers were required to undergo daily symptom screening and infrared temperature checks prior to entering the lab. If an individual's temperature exceeded 37.7˚C, COVID-19 protocols to mitigate the risk of exposure to potential COVID-19 positive individuals were activated. Whilst in the lab, participants were subject to the same daily screening. Additionally, all researchers, staff, and participants were required to be vaccinated against COVID-19, and participants were required to produce an official record of their vaccination status prior to entering the lab. Protecting the risk of COVID-19 transmission was of utmost importance to all staff, researchers, and participants. The stringent hygiene and COVID-19 protocols implemented were a strength of the studies in this thesis and once commenced, allowed each trial to occur uninterrupted.

8.3 Delimitations

8.3.1 Experimental environment

The use of a purpose-built sleep laboratory with polysomnography (PSG) was a significant strength of the studies within this thesis. To determine whether sleep was affected by the interventions, it was important to assess sleep using gold-standard techniques in a robust, well-controlled setting. Throughout this thesis, PSG was used to provide a valid measure of sleep onset latency and to determine specific sleep stages, which were both essential outcome variables in studies two, three, and four. Furthermore, the configuration of the laboratory allowed for the standardisation of exposure to variables that are known to influence sleep such as light (317), temperature (318), and nutritional intake (e.g., caffeine (242)). The ability to control the experimental environment to ensure the dependent variable was only influenced by the independent variables (i.e., experimental manipulation) rather than alternate factors is a significant advantage of this thesis.

8.3.2 Study design

All studies in this thesis employed randomised, counterbalanced, repeated-measures, crossover study designs. A significant strength of this thesis was the within-participant comparisons, which minimised the potential effect of inter-individual biological variation

that may present itself if using between-subject comparisons. Additionally, by using a repeated-measures design, less participants were required to be recruited to form adequately powered groups for comparison. Considering the constraints that were present during recruitment (i.e., COVID-19), the within-participant design was a benefit on several levels. The counterbalancing implemented throughout this thesis was informed by a Latin square design, which helped to ensure a similar number of conditional sequences were undertaken in each study. Thus, minimising the possibility of incurring an order-effect. The counterbalancing and crossover designs were strengths of the studies in this thesis, as anecdotally, participants became more comfortable with the laboratory and sleep conditions as the studies progressed. This consequently minimised potential order effects.

8.4 Future directions

This thesis has helped provide insight into the complex relationship between sleep, exercise, and nutrition. From the findings of the studies within this thesis, three primary considerations for future research emerged; 1) examining the effect of alternate exercise configurations and timings on sleep; 2) further elucidating whether different metabolic factors released from skeletal muscle during various modalities and intensities of exercise may be a potential mechanism for exercise-induced improvements in sleep; and 3), determining whether nutritional interventions may be more effective at improving sleep during free-living environments where factors known to influence sleep are present.

Further elucidating the relationship between exercise and sleep is an important next step. Determining how different configurations of exercise, including resistance training and high-intensity training influence sleep would be beneficial, given the vast array of exercise modalities available for individuals to undertake. Additionally, a greater understanding of the influence that the timing of exercise bouts has on sleep would be beneficial, particularly if exercise is being used as a tool to improve sleep. Clarifying the effect of exercise configuration and timing would allow for contemporary sleep hygiene recommendations to be used with greater precision and effectiveness than current guidelines.

The role of exercise-induced peripheral factors in sleep regulation is not fully elucidated. Exploring if a relationship exists between metabolic factors not assessed in this thesis (e.g.,

PGC1-α or irisin) may be a valuable next step. Whilst the components of the proposed hypothesis that were examined do not appear to be confirmed from our findings, determining whether higher intensity exercise elicits a greater metabolic response and subsequently has an influence on sleep may be prudent to investigate. The mechanisms underpinning exercise-induced changes in sleep is clearly complex and likely influenced by multiple factors working in synergy, such as changes in core body temperature, autonomic nervous system activity, and individual chronotype. A greater understanding of the underlying mechanisms will help allow for individualised interventions to be developed to help improve sleep and combat the current global sleep loss epidemic.

Nutritional interventions have shown some promise as a practical alternative to pharmacological substances for improving sleep. However, continuing to refine the optimal combination of ingredients and dosages is important. If nutritional interventions can support the sleep of athletes or users, it may help to offset the myriad of negative health and performance outcomes that arise after insufficient sleep. Thus, leading to improved performance, recovery, and wellbeing without the negative effects associated with typical sleep medications. Additionally, determining whether nutritional interventions may be effective at improving sleep when consumed in free-living situations would be a valuable next step to providing an intervention that can assist in rescuing the poor sleep often encountered by athletes.

Considering the research areas investigated in this thesis still require further exploration, there remains ample scope to continue expanding our knowledge on modifiable behaviours that influence sleep and overall health. Given the wide array of exercise and nutritional interventions available for individuals to adopt throughout their lifelong journey toward health, having a clear understanding on how different combinations of exercise configurations and timings affect sleep would be beneficial. To achieve this, a deeper comprehension of the potential mechanisms responsible for changes in sleep after exercise should continue to be pursued. Additionally, determining if nutritional interventions consumed prior to sleep, as a part of an individual's daily routine, may be an effective tool for improving sleep, holds promise. It is important to keep in mind that when implementing interventions that influence sleep, responses are highly individual and unique to each person. Therefore, when attempting to develop and implement interventions

aimed at improving sleep, it is essential to consider and address the specific needs of each individual.

8.5 Concluding remarks

Sleep is an essential component of health and despite the increased prevalence of sleep-related research, large proportions of the population do not achieve sufficient sleep durations (34). Modifiable behaviours that are known to influence sleep, such as nutritional interventions and exercise are often promoted as tools for improving sleep. However, the relationship between the interplay of sleep, exercise and nutrition is complex, multifaceted, and not extensively examined. The findings from this thesis have provided valuable insights into how exercise and nutritional interventions influence sleep and have highlighted that individual sleep responses to exercise and nutritional interventions are highly variable. Consequently, it is essential to tailor recommendations to the specific needs of the individual. Furthermore, this dissertation has outlined future directions for research, to help elucidate the elaborate relationship between sleep, exercise, and nutrition.

Four main outcomes were identified from this program of research:1) that sleep is consistent when measured using gold-standard techniques in highly controlled environments; 2) individual sleep responses after exercise vary but afternoon exercise does not appear to interrupt sleep; 3) certain peripheral exercise-induced metabolic factors may not be the underlying mechanism for changes in sleep following exercise; and 4) a proprietary nutritional intervention consumed in conjunction with afternoon exercise does not enhance sleep.

Sleep duration and architecture were not meaningfully influenced by exercise or nutritional interventions within this thesis. The use of gold-standard measuring techniques, such as PSG and a highly-controlled sleep laboratory allowed for a valid and reliable assessment of the effects of exercise and nutritional interventions on sleep duration and architecture. When determining if an intervention has an influence on sleep, isolating the effects of the independent variable is essential in determining if the intervention has affected sleep. In studies two, three, and four, no significant differences in sleep were observed between the control condition and the interventions, highlighting the stability of sleep when measured

in a controlled environment, free from typical stressors known to influence sleep. The findings of study two help to inform and support sleep hygiene recommendations by identifying that afternoon exercise does not negatively influence sleep and could be promoted as an appropriate time to plan an exercise bout without disturbing sleep.

Individual sleep responses after exercise and/or the consumption of a pre-sleep nutritional intervention varied in magnitude and direction. However, when assessed collectively, findings did not reach statistical significance in studies two, three, or four. The varied nature of individual responses observed in this thesis add to the equivocal findings present in the literature. Therefore, this program of research highlights the importance of acknowledging the needs and uniqueness of the individual when considering sleep.

The mechanisms explaining the relationship between exercise and sleep are not well understood. Study three was the first study to determine whether exercise-induced peripheral factors may be a potential explanation for changes in sleep after exercise. The findings suggest the peripheral factors examined in study three were not related to sleep. However, there were additional metabolic factors suggested in the novel hypothesis (24) that were not examined and may be prudent to explore in the future. It is likely the mechanisms related to exercise-induced changes in sleep are multifaceted and should continue to be explored. Study three indicates that moderate-intensity exercise performed in the afternoon does not elicit enough of a metabolic response to elevate circulating levels of peripheral factors that have been suggested to be involved in sleep regulation.

The use of nutritional supplements to improve sleep has shown promise as an alternative to pharmacological interventions. Study four examined the use of nutritional interventions to enhance sleep in physically active adults. The findings suggested the doses of the supplement were not effective at improving sleep in the cohort of participants recruited. However, the removal of typical stressors that are present in free-living environments may have mitigated the ability of the supplement to improve sleep in the experimental environment. Consequently, whether the supplements may be more effective when consumed in a more ecologically valid setting would be an important next step. Additionally, the supplement was used in an acute instance, and whether the intervention would be more effective with repeated use remains unknown. Determining whether this

supplement may improve sleep in free-living conditions would be important to several demographics, as an alternative to pharmacological interventions would be valuable.

The interplay between sleep, exercise, and nutrition is vast and complex. Consequently, research investigating the relationships between sleep and modifiable behaviours such as exercise and sleep is still emerging and is currently in its infancy. Therefore, there is scope to continue expanding our knowledge regarding the interaction of these essential contributors to health. The findings of this thesis highlight the variability of sleep responses observed after interventions and reinforce the need to consider how individuals respond to stimuli and the subsequent effects this may have on sleep. Discerning the potential mechanisms by which exercise may influence sleep would also be beneficial in informing strategies aimed at using exercise as a tool to enhance sleep. This program of research helps to elucidate the complex relationship that exists between sleep, exercise, and nutrition. Furthermore, the findings of this thesis can be used to guide future research to develop a framework for individualising interventions to augment sleep and health.

Chapter 9: References

1. Huang W, Ramsey KM, Marcheva B, Bass J. Circadian rhythms, sleep, and metabolism. J Clin Invest. 2011;121(6):2133-41.
2. Mullington JM, Haack M, Toth M, Serrador JM, Meier-Ewert HK. Cardiovascular, inflammatory, and metabolic consequences of sleep deprivation. Prog Cardiovasc Dis. 2009;51(4):294-302.
3. Spiegel K, Leproult R, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. Lancet. 1999;354(9188):1435-9.
4. Spiegel K, Tasali E, Leproult R, Van Cauter E. Effects of poor and short sleep on glucose metabolism and obesity risk. Nat Rev Endocrinol. 2009;5(5):253.
5. Hirshkowitz M, Whiton K, Albert SM, Alessi C, Bruni O, DonCarlos L, et al. National Sleep Foundation's updated sleep duration recommendations. Sleep Health. 2015;1(4):233-43.
6. Adams RJ, Appleton SL, Taylor AW, Gill TK, Lang C, McEvoy RD, et al. Sleep health of Australian adults in 2016: results of the 2016 Sleep Health Foundation national survey. Sleep Health. 2017;3(1):35-42.
7. Ohayon MM. Epidemiological overview of sleep disorders in the general population. Sleep Med Res. 2011;2(1):1-9.
8. Clark I, Landolt HP. Coffee, caffeine, and sleep: A systematic review of epidemiological studies and randomized controlled trials. Sleep Med Rev. 2017;31:70-8.
9. Hafner M, Stepanek M, Taylor J, Troxel WM, Van Stolk C. Why sleep matters—the economic costs of insufficient sleep: a cross-country comparative analysis. Rand Health Q. 2017;6(4).
10. Chattu VK, Manzar MD, Kumary S, Burman D, Spence DW, Pandi-Perumal SR. The Global Problem of Insufficient Sleep and Its Serious Public Health Implications. Healthcare. 2018;7(1).
11. Kecklund G, Axelsson J. Health consequences of shift work and insufficient sleep. BMJ. 2016;355:i5210.
12. Saner NJ, Lee MJ, Pitchford NW, Kuang J, Roach GD, Garnham A, et al. The effect of sleep restriction, with or without high-intensity interval exercise, on myofibrillar protein synthesis in healthy young men. J Physiol. 2020;598(8):1523-36.

13. Knutson KL, Spiegel K, Penev P, Van Cauter E. The metabolic consequences of sleep deprivation. Sleep Med Rev. 2007;11(3):163-78.
14. Brandt J, Leong C. Benzodiazepines and Z-drugs: an updated review of major adverse outcomes reported on in epidemiologic research. Drugs in R&D. 2017;17(4):493-507.
15. Zarcone V. Sleep hygiene Principles and Practice of Sleep Medicine (Vol. 2, pp. 542-546). Philadelphia: WB Saunders Co; 1994.
16. Halson SL. Sleep in elite athletes and nutritional interventions to enhance sleep. Sports Med. 2014;44(1):13-23.
17. Chennaoui M, Arnal PJ, Sauvet F, Léger D. Sleep and exercise: a reciprocal issue? Sleep Med Rev. 2015;20:59-72.
18. Frimpong E, Mograss M, Zvionow T, Dang-Vu TT. The effects of evening high-intensity exercise on sleep in healthy adults: A systematic review and meta-analysis. Sleep Med Rev. 2021;60:101535.
19. Miller D, Sargent C, Roach G, Scanlan A, Vincent G, Lastella M. Moderate-intensity exercise performed in the evening does not impair sleep in healthy males. Eur J Sport Sci. 2020;20(1):80-9.
20. Oda S, Shirakawa K. Sleep onset is disrupted following pre-sleep exercise that causes large physiological excitement at bedtime. Eur J Appl Physiol. 2014;114:1789-99.
21. Youngstedt SD, O'connor PJ, Dishman RK. The effects of acute exercise on sleep: a quantitative synthesis. Sleep. 1997;20(3):203-14.
22. Kubitz KA, Landers DM, Petruzzello SJ, Han M. The effects of acute and chronic exercise on sleep. Sports Med. 1996;21(4):277-91.
23. Driver HS, Taylor SR. Exercise and sleep. Sleep Med Rev. 2000;4(4):387-402.
24. Tan X, van Egmond LT, Cedernaes J, Benedict C. The role of exercise-induced peripheral factors in sleep regulation. Mol Metab. 2020;42:101096.
25. Sutanto CN, Loh WW, Kim JE. The impact of tryptophan supplementation on sleep quality: a systematic review, meta-analysis, and meta-regression. Nutr Rev. 2021.
26. Halson SL. Nutritional interventions to enhance sleep. Sports Sci Exch. 2013;26(116):1-5.
27. Maffei ME. 5-Hydroxytryptophan (5-HTP): Natural Occurrence, Analysis, Biosynthesis, Biotechnology, Physiology and Toxicology. Int J Mol Sci. 2020;22(1).
28. Zisapel N. New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. Br J Pharmacol. 2018;175(16):3190-9.

29. Heslop P, Smith GD, Metcalfe C, Macleod J, Hart C. Sleep duration and mortality: the effect of short or long sleep duration on cardiovascular and all-cause mortality in working men and women. Sleep Med. 2002;3(4):305-14.
30. Borbély AA, Achermann P. Sleep homeostasis and models of sleep regulation. J Biol Rhythms. 1999;14(6):559-70.
31. Lucassen EA, de Mutsert R, le Cessie S, Appelman-Dijkstra NM, Rosendaal FR, van Heemst D, et al. Poor sleep quality and later sleep timing are risk factors for osteopenia and sarcopenia in middle-aged men and women: The NEO study. PLoS One. 2017;12(5):e0176685.
32. Short MA, Louca M. Sleep deprivation leads to mood deficits in healthy adolescents. Sleep Med. 2015;16(8):987-93.
33. Walker MP, Stickgold R. Sleep-dependent learning and memory consolidation. Neuron. 2004;44(1):121-33.
34. Scott H, Naik G, Lechat B, Manners J, Fitton J, Nguyen DP, et al. Are we getting enough sleep? Frequent irregular sleep found in an analysis of over 11 million nights of objective in-home sleep data. Sleep Health. 2023.
35. Schipper SBJ, Van Veen MM, Elders PJM, van Straten A, Van Der Werf YD, Knutson KL, et al. Sleep disorders in people with type 2 diabetes and associated health outcomes: a review of the literature. Diabetologia. 2021;64(11):2367-77.
36. Kochanek K, Murphy S, Xu J, Arias E. Mortality in the United States, 2013. NCHS data brief, no. 178. Natl Health Stat Report. 2014.
37. Gallicchio L, Kalesan B. Sleep duration and mortality: a systematic review and meta-analysis. J Sleep Res. 2009;18(2):148-58.
38. Scott H, Lechat B, Guyett A, Reynolds AC, Lovato N, Naik G, et al. Sleep Irregularity Is Associated With Hypertension: Findings From Over 2 Million Nights With a Large Global Population Sample. Hypertension. 2023;80(5):1117-26.
39. Liu Y, Wheaton AG, Chapman DP, Cunningham TJ, Lu H, Croft JB. Prevalence of healthy sleep duration among adults—United States, 2014. Morb Mortal Wkly. 2016;65(6):137-41.
40. Christensen MA, Bettencourt L, Kaye L, Moturu ST, Nguyen KT, Olgin JE, et al. Direct measurements of smartphone screen-time: relationships with demographics and sleep. PloS one. 2016;11(11):e0165331.
41. Hale L, Guan S. Screen time and sleep among school-aged children and adolescents: a systematic literature review. Sleep Med Rev. 2015;21:50-8.

42. Šušmáková K. Human sleep and sleep EEG. Meas Sci Rev. 2004;4(2):59-74.
43. Hobson JA, Stickgold R, Pace-Schott EF. The neuropsychology of REM sleep dreaming. Neuroreport. 1998;9(3):R1-R14.
44. Tempesta D, Socci V, De Gennaro L, Ferrara M. Sleep and emotional processing. Sleep Med Rev. 2018;40:183-95.
45. Halász P. K-complex, a reactive EEG graphoelement of NREM sleep: an old chap in a new garment. Sleep Med Rev. 2005;9(5):391-412.
46. Fuller PM, Gooley JJ, Saper CB. Neurobiology of the sleep-wake cycle: sleep architecture, circadian regulation, and regulatory feedback. J Biol Rhythms. 2006;21(6):482-93.
47. Aisbett B, Condo D, Zacharewicz E, Lamon S. The Impact of Shiftwork on Skeletal Muscle Health. Nutrients. 2017;9(3).
48. Khalighi S, Sousa T, Pires G, Nunes U. Automatic sleep staging: A computer assisted approach for optimal combination of features and polysomnographic channels. Expert Syst Appl. 2013;40(17):7046-59.
49. Wolff G, Esser KA. Scheduled exercise phase shifts the circadian clock in skeletal muscle. Med Sci Sports Exerc. 2012;44(9):1663-70.
50. Mansingh S, Handschin C. Time to Train: The Involvement of the Molecular Clock in Exercise Adaptation of Skeletal Muscle. Front Physiol. 2022:809.
51. Harfmann BD, Schroder EA, Esser KA. Circadian rhythms, the molecular clock, and skeletal muscle. J Biol Rhythms. 2015;30(2):84-94.
52. Schroder EA, Esser KA. Circadian rhythms, skeletal muscle molecular clocks, and exercise. Exerc Sport Sci Rev. 2013;41(4):224-9.
53. Landolt H-P. Sleep homeostasis: a role for adenosine in humans? Biochem Pharmacol. 2008;75(11):2070-9.
54. Porkka-Heiskanen T, Alanko L, Kalinchuk A, Stenberg D. Adenosine and sleep. Sleep Med Rev. 2002;6(4):321-32.
55. Skocbat T, Haimov I, Lavie P. Melatonin-the key to the gate of sleep. Ann Med. 1998;30(1):109-14.
56. Iber C. The AASM manual for the scoring of sleep and associated events: Rules. Terminology and Technical Specification. 2007.
57. Lee YJ, Lee JY, Cho JH, Choi JH. Interrater reliability of sleep stage scoring: a meta-analysis. J Clin Sleep Med. 2022;18(1):193-202.

58. Van Remoortel H, Giavedoni S, Raste Y, Burtin C, Louvaris Z, Gimeno-Santos E, et al. Validity of activity monitors in health and chronic disease: a systematic review. Int J Behav Nutr Phys Act. 2012;9(1):1-23.
59. Miller DJ, Lastella M, Scanlan AT, Bellenger C, Halson SL, Roach GD, et al. A validation study of the WHOOP strap against polysomnography to assess sleep. J Sports Sci. 2020;38(22):2631-6.
60. Miller DJ, Sargent C, Roach GD. A Validation of Six Wearable Devices for Estimating Sleep, Heart Rate and Heart Rate Variability in Healthy Adults. Sensors. 2022;22(16).
61. Fuller KL, Juliff L, Gore CJ, Peiffer JJ, Halson SL. Software thresholds alter the bias of actigraphy for monitoring sleep in team-sport athletes. J Sci Med Sport. 2017;20(8):756-60.
62. Halson SL. Sleep Monitoring in Athletes: Motivation, Methods, Miscalculations and Why it Matters. Sports Med. 2019;49(10):1487-97.
63. Buysse DJ, Reynolds III CF, Monk TH, Hoch CC, Yeager AL, Kupfer DJ. Quantification of subjective sleep quality in healthy elderly men and women using the Pittsburgh Sleep Quality Index (PSQI). Sleep. 1991;14(4):331-8.
64. Nicassio PM, Mendlowitz DR, Fussell JJ, Petras L. The phenomenology of the pre-sleep state: the development of the pre-sleep arousal scale. Behav Res Ther. 1985;23(3):263-71.
65. Borst SE. Interventions for sarcopenia and muscle weakness in older people. Age Ageing. 2004;33(6):548-55.
66. Piovezan RD, Abucham J, Dos Santos RVT, Mello MT, Tufik S, Poyares D. The impact of sleep on age-related sarcopenia: Possible connections and clinical implications. Age Res Rev. 2015;23:210-20.
67. Børsheim E, Tipton KD, Wolf SE, Wolfe RR. Essential amino acids and muscle protein recovery from resistance exercise. Am J Physiol Endocrinol Metab. 2002.
68. Wolfe RR. Regulation of muscle protein by amino acids. The Journal of nutrition. 2002;132(10):3219S-24S.
69. Marcotte GR, West DW, Baar K. The molecular basis for load-induced skeletal muscle hypertrophy. Calcif Tissue Int. 2015;96:196-210.
70. Wolfe RR. Skeletal muscle protein metabolism and resistance exercise. J Nutr. 2006;136(2):525S-8S.

71. Atherton PJ, Smith K. Muscle protein synthesis in response to nutrition and exercise. J Physiol. 2012;590(5):1049-57.
72. Suchomel TJ, Nimphius S, Stone MH. The Importance of Muscular Strength in Athletic Performance. Sports Med. 2016;46(10):1419-49.
73. Fernandes JFT, Lamb KL, Norris JP, Moran J, Drury B, Borges NR, et al. Aging and Recovery After Resistance-Exercise-Induced Muscle Damage: Current Evidence and Implications for Future Research. J Aging Phys Act. 2020;29(3):544-51.
74. Dáttilo M, Antunes HKM, Galbes NMN, Mônico-Neto M, De SÁ Souza H, Dos Santos Quaresma MVL, et al. Effects of Sleep Deprivation on Acute Skeletal Muscle Recovery after Exercise. Med Sci Sports Exerc. 2020;52(2):507-14.
75. Dáttilo M, Antunes HKM, Medeiros A, Mônico-neto M, Souza HdS, Lee KS, et al. Paradoxical sleep deprivation induces muscle atrophy. Muscle Nerve. 2012;45(3):431-3.
76. de Sá Souza H, Antunes HKM, Dáttilo M, Lee KS, Mônico-Neto M, de Campos Giampa SQ, et al. Leucine supplementation is anti-atrophic during paradoxical sleep deprivation in rats. Amino Acids. 2016;48(4):949-57.
77. Mônico-Neto M, Antunes HKM, Dattilo M, Medeiros A, Souza HSd, Lee KS, et al. Resistance exercise: A non-pharmacological strategy to minimize or reverse sleep deprivation-induced muscle atrophy. Med Hypotheses. 2013;80(6):701-5.
78. Mônico-Neto M, Giampá SQdC, Lee KS, de Melo CM, Souza HdS, Dáttilo M, et al. Negative energy balance induced by paradoxical sleep deprivation causes multicompartmental changes in adipose tissue and skeletal muscle. Int J Endocrinol. 2015;2015.
79. Mônico-Neto M, Antunes HKM, Lee KS, Phillips SM, Giampá SQdC, Souza HdS, et al. Resistance training minimizes catabolic effects induced by sleep deprivation in rats. Appl Physiol Nutr Metab. 2015;40(11):1143-50.
80. Lamon S, Morabito A, Arentson-Lantz E, Knowles O, Vincent GE, Condo D, et al. The effect of acute sleep deprivation on skeletal muscle protein synthesis and the hormonal environment. Physiol Rep. 2021;9(1):e14660.
81. Saner NJ, Lee MJ, Kuang J, Pitchford NW, Roach GD, Garnham A, et al. Exercise mitigates sleep-loss-induced changes in glucose tolerance, mitochondrial function, sarcoplasmic protein synthesis, and diurnal rhythms. Mol Metab. 2021;43:101110.
82. Cedernaes J, Osler ME, Voisin S, Broman JE, Vogel H, Dickson SL, et al. Acute Sleep Loss Induces Tissue-Specific Epigenetic and Transcriptional Alterations to Circadian Clock Genes in Men. J Clin Endocrinol Metab. 2015;100(9):E1255-61.

83. Leota J, Presby D, Czeisler M, Mascaro L, Capodilupo E, Drummond S, et al. O002 High strain evening exercise disrupts sleep: Insights from a real-world examination of~ 7-million nights. Sleep Advances. 2023;4(Supplement_1):A1-A.
84. Kovacevic A, Mavros Y, Heisz JJ, Singh MAF. The effect of resistance exercise on sleep: A systematic review of randomized controlled trials. Sleep Med Rev. 2018;39:52-68.
85. Murphy PJ, Campbell SS. Nighttime drop in body temperature: a physiological trigger for sleep onset? Sleep. 1997;20(7):505-11.
86. Faraguna U, Vyazovskiy VV, Nelson AB, Tononi G, Cirelli C. A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. J Neurosci. 2008;28(15):4088-95.
87. Van Dongen HP, Dinges DF. Circadian rhythms in fatigue, alertness, and performance. Principles and practice of sleep medicine. 2000;20:391-9.
88. Roenneberg T, Kuehnle T, Juda M, Kantermann T, Allebrandt K, Gordijn M, et al. Epidemiology of the human circadian clock. Sleep Med Rev. 2007;11(6):429-38.
89. Thomas JM, Kern PA, Bush HM, McQuerry KJ, Black WS, Clasey JL, et al. Circadian rhythm phase shifts caused by timed exercise vary with chronotype. JCI insight. 2020;5(3).
90. Vitale JA, Weydahl A. Chronotype, physical activity, and sport performance: a systematic review. Sports Med. 2017;47:1859-68.
91. Saidi O, Peyrel P, Del Sordo G, Gabriel B, Maso F, Doré É, et al. Is it wiser to train in the afternoon or the early evening to sleep better? the role of chronotype in young adolescent athletes. Sleep. 2023:zsad099.
92. Lalor BJ, Halson SL, Tran J, Kemp JG, Cormack SJ. A complex relationship: Sleep, external training load, and well-being in elite Australian footballers. Int J Sports Physiol Perform. 2020;15(6):777-87.
93. Lastella M, Roach GD, Vincent GE, Scanlan AT, Halson SL, Sargent C. The impact of training load on sleep during a 14-day training camp in elite, adolescent, female basketball players. Int J Sports Physiol Perform. 2020;15(5):724-30.
94. Walsh NP, Halson SL, Sargent C, Roach GD, Nedelec M, Gupta L, et al. Sleep and the athlete: narrative review and 2021 expert consensus recommendations. Br J Sports Med. 2020.
95. Knowles OE, Drinkwater EJ, Urwin CS, Lamon S, Aisbett B. Inadequate sleep and muscle strength: Implications for resistance training. J Sci Med Sport. 2018;21(9):959-68.

96. Cook C, Beaven CM, Kilduff LP, Drawer S. Acute caffeine ingestion's increase of voluntarily chosen resistance-training load after limited sleep. Int J Sport Nutr Exerc Metab. 2012;22(3):157-64.
97. Thun E, Bjorvatn B, Flo E, Harris A, Pallesen S. Sleep, circadian rhythms, and athletic performance. Sleep Med Rev. 2015;23:1-9.
98. Fullagar HH, Skorski S, Duffield R, Hammes D, Coutts AJ, Meyer T. Sleep and athletic performance: the effects of sleep loss on exercise performance, and physiological and cognitive responses to exercise. Sports Med. 2015;45(2):161-86.
99. Dáttilo M, Antunes HKM, Medeiros A, Mônico-neto M, Souza HSd, Tufik S, et al. Sleep and muscle recovery: endocrinological and molecular basis for a new and promising hypothesis. Med Hypotheses. 2011;77(2):220-2.
100. Mah CD, Mah KE, Kezirian EJ, Dement WC. The effects of sleep extension on the athletic performance of collegiate basketball players. Sleep. 2011;34(7):943-50.
101. Swinbourne R, Miller J, Smart D, Dulson DK, Gill N. The Effects of Sleep Extension on Sleep, Performance, Immunity and Physical Stress in Rugby Players. Sports 2018;6(2).
102. Schwartz J, Simon RD, Jr. Sleep extension improves serving accuracy: A study with college varsity tennis players. Physiol Behav. 2015;151:541-4.
103. Teo W, Newton MJ, McGuigan MR. Circadian rhythms in exercise performance: implications for hormonal and muscular adaptation. J Sports Sci Med. 2011;10(4):600.
104. Starkie RL, Hargreaves M, Lambert DL, Proietto J, Febbraio MA. Effect of temperature on muscle metabolism during submaximal exercise in humans. Exp Physiol. 1999;84(4):775-84.
105. Racinais S, Oksa J. Temperature and neuromuscular function. Scand J Med Sci Sports. 2010;20 Suppl 3:1-18.
106. Martin A, Carpentier A, Guissard N, Van Hoecke J, Duchateau J. Effect of time of day on force variation in a human muscle. Muscle Nerve. 1999;22(10):1380-7.
107. Souissi M, Chtourou H, Zrane A, Cheikh RB, Dogui M, Tabka Z, et al. Effect of time-of-day of aerobic maximal exercise on the sleep quality of trained subjects. Biol Rhythm Res. 2012;43(3):323-30.
108. Chtourou H, Souissi N. The effect of training at a specific time of day: a review. J Strength Cond Res. 2012;26(7):1984-2005.

109. Bruggisser F, Knaier R, Roth R, Wang W, Qian J, Scheer FA. Best Time of Day for Strength and Endurance Training to Improve Health and Performance? A Systematic Review with Meta-analysis. Sports Med Open. 2023;9(1):1-23.
110. Frase L, Nissen C, Riemann D, Spiegelhalder K. Making sleep easier: pharmacological interventions for insomnia. Expert Opin Pharmacother. 2018;19(13):1465-73.
111. Lader M. Benzodiazepine harm: how can it be reduced? Br J Clin Pharmacol. 2014;77(2):295-301.
112. Neutel CI. Risk of traffic accident injury after a prescription for a benzodiazepine. Ann Epidemiol. 1995;5(3):239-44.
113. Gratwicke M, Miles KH, Pyne DB, Pumpa KL, Clark B. Nutritional Interventions to Improve Sleep in Team-Sport Athletes: A Narrative Review. Nutrients. 2021;13(5).
114. Afaghi A, O'Connor H, Chow CM. High-glycemic-index carbohydrate meals shorten sleep onset. Am J Clin Nutr. 2007;85(2):426-30.
115. Halson SL. Nutrition, sleep and recovery. Eur J Sport Sci. 2008;8(2):119-26.
116. Trommelen J, van Lieshout GA, Pabla P, Nyakayiru J, Hendriks FK, Senden JM, et al. Pre-sleep Protein Ingestion Increases Mitochondrial Protein Synthesis Rates During Overnight Recovery from Endurance Exercise: A Randomized Controlled Trial. Sports Med. 2023:1-11.
117. Layman DK, Lönnerdal B, Fernstrom JD. Applications for α-lactalbumin in human nutrition. Nutr Rev. 2018;76(6):444-60.
118. Stretton B, Eranki A, Kovoor J, Bacchi S, Gupta A, Maddern G, et al. Too Sour to be True? Tart Cherries (Prunus cerasus) and Sleep: a Systematic Review and Meta-analysis. Curr Sleep Med Rep. 2023;9(3):225-33.
119. Bell PG, Walshe IH, Davison GW, Stevenson E, Howatson G. Montmorency cherries reduce the oxidative stress and inflammatory responses to repeated days high-intensity stochastic cycling. Nutrients. 2014;6(2):829-43.
120. St-Onge M-P, Mikic A, Pietrolungo CE. Effects of diet on sleep quality. Adv Nutr. 2016;7(5):938-49.
121. Tsaluchidu S, Cocchi M, Tonello L, Puri BK. Fatty acids and oxidative stress in psychiatric disorders. BMC psychiatry. 2008;8:1-3.
122. Lin H-H, Tsai P-S, Fang S-C, Liu J-F. Effect of kiwifruit consumption on sleep quality in adults with sleep problems. Asia Pac J Clin Nutr. 2011;20(2):169-74.

123. Norton K, Norton L. Pre-exercise screening. Guide to the Australian adult pre-exercise screening system exercise and sports science Australia. 2011.
124. Espiritu JRD. Aging-related sleep changes. Clinics in geriatric medicine. 2008;24(1):1-14.
125. Halson SL, Shaw G, Versey N, Miller DJ, Sargent C, Roach GD, et al. Optimisation and Validation of a Nutritional Intervention to Enhance Sleep Quality and Quantity. Nutrients. 2020;12(9).
126. Lakens D. Sample size justification. Collabra: psychology. 2022;8(1):33267.
127. Berry RB, Brooks R, Gamaldo C, Harding SM, Lloyd RM, Quan SF, et al. AASM Scoring Manual Updates for 2017 (Version 2.4). J Clin Sleep Med. 2017;13(5):665-6.
128. Rosenberg RS, Van Hout S. The American Academy of Sleep Medicine inter-scorer reliability program: sleep stage scoring. J Clin Sleep Med. 2013;9(1):81-7.
129. Miller DJ, Sargent C, Roach GD, Scanlan AT, Vincent GE, Lastella M. Moderate-intensity exercise performed in the evening does not impair sleep in healthy males. Eur J Sport Sci. 2020;20(1):80-9.
130. Schade MM, Mathew GM, Roberts DM, Gartenberg D, Buxton OM. Enhancing slow oscillations and increasing N3 sleep proportion with supervised, non-phase-locked pink noise and other non-standard auditory stimulation during NREM sleep. Nat Sci Sleep. 2020:411-29.
131. Buysse DJ, Reynolds III CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193-213.
132. Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: daily temporal patterns of human chronotypes. J Biol Rhythms. 2003;18(1):80-90.
133. Santisteban JA, Brown TG, Gruber R. Association between the munich chronotype questionnaire and wrist actigraphy. Sleep Disorders. 2018;2018.
134. Zavada A, Gordijn MC, Beersma DG, Daan S, Roenneberg T. Comparison of the Munich Chronotype Questionnaire with the Horne-Östberg's morningness-eveningness score. Chronobiol Int. 2005;22(2):267-78.
135. Horne JA, Östberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol. 1976.
136. Thun E, Bjorvatn B, Osland T, Steen VM, Sivertsen B, Johansen T, et al. An actigraphic validation study of seven morningness-eveningness inventories. Eur Psychol. 2012.

137. Taillard J, Philip P, Chastang J-F, Bioulac B. Validation of Horne and Ostberg morningness-eveningness questionnaire in a middle-aged population of French workers. J Biol Rhythms. 2004;19(1):76-86.
138. Akerstedt T, Gillberg M. Karolinska Sleepiness Scale (KSS), a highly sensitive subjective measurement scale for sleepiness. Int J Neurosci. 1990;52(1-2):29-37.
139. Åkerstedt T, Anund A, Axelsson J, Kecklund G. Subjective sleepiness is a sensitive indicator of insufficient sleep and impaired waking function. J Sleep Res. 2014;23(3):242-54.
140. Åkerstedt T, Gillberg M. Subjective and objective sleepiness in the active individual. Int J Neurosci. 1990;52(1-2):29-37.
141. Wainwright B, Cooke CB, O'Hara JP. The validity and reliability of a sample of 10 Wattbike cycle ergometers. J Sports Sci. 2017;35(14):1451-8.
142. Hopker J, Myers S, Jobson S, Bruce W, Passfield L. Validity and reliability of the Wattbike cycle ergometer. Int J Sports Med. 2010;31(10):731-6.
143. Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exerc. 1982.
144. Borg G. Borg's perceived exertion and pain scales: Human kinetics; 1998.
145. Bos I, Jacobs L, Nawrot T, De Geus B, Torfs R, Panis LI, et al. No exercise-induced increase in serum BDNF after cycling near a major traffic road. Neurosci Lett. 2011;500(2):129-32.
146. Chase JD, Roberson PA, Saunders MJ, Hargens TA, Womack CJ, Luden ND. One night of sleep restriction following heavy exercise impairs 3-km cycling time-trial performance in the morning. Appl Physiol Nutr Metab. 2017;42(9):909-15.
147. Cohen J. Statistical power analysis for the behavioral sciences: Academic press; 2013.
148. Allen M, Poggiali D, Whitaker K, Marshall TR, Kievit RA. Raincloud plots: a multi-platform tool for robust data visualization. Wellcome Open Res. 2019;4.
149. Van Ravenzwaaij D, Cassey P, Brown SD. A simple introduction to Markov Chain Monte–Carlo sampling. Psychonomic bulletin & review. 2018;25(1):143-54.
150. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:14065823. 2014.
151. Team RC. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www R-project org/. 2013.

152. Fox J, Weisberg S. Multivariate linear models in R. An R Companion to Applied Regression Los Angeles: Thousand Oaks. 2011.
153. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front Psychol. 2013;4:863.
154. Everson CA, Bergmann BM, Rechtschaffen A. Sleep deprivation in the rat: III. Total sleep deprivation. Sleep. 1989;12(1):13-21.
155. Roenneberg T. The human sleep project. Nature. 2013;498(7455):427-8.
156. Hawley JA, Sassone-Corsi P, Zierath JR. Chrono-nutrition for the prevention and treatment of obesity and type 2 diabetes: from mice to men. Diabetologia. 2020;63(11):2253-9.
157. Banks S, Dinges DF. Behavioral and physiological consequences of sleep restriction. J Clin Sleep Med. 2007;3(5):519-28.
158. Besedovsky L, Lange T, Born J. Sleep and immune function. Pflugers Arch. 2012;463(1):121-37.
159. Kim TW, Jeong J-H, Hong S-C. The impact of sleep and circadian disturbance on hormones and metabolism. Int J Endocrinol. 2015;2015.
160. Minkel J, Moreta M, Muto J, Htaik O, Jones C, Basner M, et al. Sleep deprivation potentiates HPA axis stress reactivity in healthy adults. Health Psychol. 2014;33(11):1430.
161. Chaput J-P, Dutil C. Lack of sleep as a contributor to obesity in adolescents: impacts on eating and activity behaviors. Int J Behav Nutr Phys Act. 2016;13(1):1-9.
162. Knutson KL, Van Cauter E. Associations between sleep loss and increased risk of obesity and diabetes. Ann NY Acad Sci. 2008;1129:287.
163. Leproult R, Copinschi G, Buxton O, Van Cauter E. Sleep loss results in an elevation of cortisol levels the next evening. Sleep. 1997;20(10):865-70.
164. Hobson JA. REM sleep and dreaming: towards a theory of protoconsciousness. Nat Rev Neurosci. 2009;10(11):803-13.
165. Siegel JM. REM sleep: a biological and psychological paradox. Sleep Med Rev. 2011;15(3):139.
166. Peever J, Fuller PM. The Biology of REM Sleep. Curr Biol. 2017;27(22):R1237-R48.
167. Carskadon MA, Dement WC. Normal human sleep: an overview2005. 13-23 p.
168. McCarley RW. Neurobiology of REM and NREM sleep. Sleep Med. 2007;8(4):302-30.

169. Hori T, Sugita Y, Koga E, Shirakawa S, Inoue K, Uchida S, et al. Proposed supplements and amendments to ‘A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects’, the Rechtschaffen & Kales (1968) standard. Psychiatry Clin Neuros. 2001;55(3):305-10.
170. Hastings MH, Maywood ES, Reddy AB. Two decades of circadian time. J Neuroendocrinol. 2008;20(6):812-9.
171. Dyar KA, Lutter D, Artati A, Ceglia NJ, Liu Y, Armenta D, et al. Atlas of Circadian Metabolism Reveals System-wide Coordination and Communication between Clocks. Cell. 2018;174(6):1571-85 e11.
172. Eckel-Mahan K, Sassone-Corsi P. Metabolism and the circadian clock converge. Physiol Rev. 2013;93(1):107-35.
173. Gutierrez-Monreal MA, Harmsen JF, Schrauwen P, Esser KA. Ticking for Metabolic Health: The Skeletal-Muscle Clocks. Obesity 2020;28 Suppl 1:S46-S54.
174. Zierath JR, Hawley JA. Skeletal muscle fiber type: influence on contractile and metabolic properties. PLoS Biol. 2004;2(10):e348.
175. Moholdt T, Parr EB, Devlin BL, Debik J, Giskeodegard G, Hawley JA. The effect of morning vs evening exercise training on glycaemic control and serum metabolites in overweight/obese men: a randomised trial. Diabetologia. 2021;64(9):2061-76.
176. Andrews JL, Zhang X, McCarthy JJ, McDearmon EL, Hornberger TA, Russell B, et al. CLOCK and BMAL1 regulate MyoD and are necessary for maintenance of skeletal muscle phenotype and function. Proc Natl Acad Sci U S A. 2010;107(44):19090-5.
177. Moller-Levet CS, Archer SN, Bucca G, Laing EE, Slak A, Kabiljo R, et al. Effects of insufficient sleep on circadian rhythmicity and expression amplitude of the human blood transcriptome. Proc Natl Acad Sci U S A. 2013;110(12):E1132-41.
178. King AJ, Burke LM, Halson SL, Hawley JA. The Challenge of Maintaining Metabolic Health During a Global Pandemic. Sports Med. 2020;50(7):1233-41.
179. D’Ambrosio C, Redline S. Sleep across the lifespan: Springer; 2014.
180. Parr EB, Coffey VG, Hawley JA. 'Sarcobesity': a metabolic conundrum. Maturitas. 2013;74(2):109-13.
181. Schoenfeld B. Science and development of muscle hypertrophy: Human Kinetics; 2020.
182. Schiaffino S, Dyar KA, Ciciliot S, Blaauw B, Sandri M. Mechanisms regulating skeletal muscle growth and atrophy. FEBS J. 2013;280(17):4294-314.

183. Areta JL, Burke LM, Ross ML, Camera DM, West DW, Broad EM, et al. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. J Physiol. 2013;591(9):2319-31.
184. Cedernaes J, Schönke M, Westholm JO, Mi J, Chibalin A, Voisin S, et al. Acute sleep loss results in tissue-specific alterations in genome-wide DNA methylation state and metabolic fuel utilization in humans. Sci Adv. 2018;4(8):eaar8590.
185. Costamagna D, Costelli P, Sampaolesi M, Penna F. Role of Inflammation in Muscle Homeostasis and Myogenesis. Mediators Inflamm. 2015;2015:805172.
186. Zhang H, Liang J, Chen N. Do not neglect the role of circadian rhythm in muscle atrophy. Age Res Rev. 2020:101155.
187. Zambon AC, McDearmon EL, Salomonis N, Vranizan KM, Johansen KL, Adey D, et al. Time-and exercise-dependent gene regulation in human skeletal muscle. Genome Biol. 2003;4(10):1-12.
188. Dyar KA, Ciciliot S, Tagliazucchi GM, Pallafacchina G, Tothova J, Argentini C, et al. The calcineurin-NFAT pathway controls activity-dependent circadian gene expression in slow skeletal muscle. Mol Metab. 2015;4(11):823-33.
189. Chang SW, Yoshihara T, Machida S, Naito H. Circadian rhythm of intracellular protein synthesis signaling in rat cardiac and skeletal muscles. Biochem Biophys Rep. 2017;9:153-8.
190. Zanchi NE, Lancha AH, Jr. Mechanical stimuli of skeletal muscle: implications on mTOR/p70s6k and protein synthesis. Eur J Appl Physiol. 2008;102(3):253-63.
191. Ackermann K, Plomp R, Lao O, Middleton B, Revell VL, Skene DJ, et al. Effect of sleep deprivation on rhythms of clock gene expression and melatonin in humans. Chronobiol Int. 2013;30(7):901-9.
192. Qiu P, Jiang J, Liu Z, Cai Y, Huang T, Wang Y, et al. BMAL1 knockout macaque monkeys display reduced sleep and psychiatric disorders. Natl Sci Rev. 2019;6(1):87-100.
193. Ehlen JC, Brager AJ, Baggs J, Pinckney L, Gray CL, DeBruyne JP, et al. Bmal1 function in skeletal muscle regulates sleep. Elife. 2017;6.
194. Choi YI, Park DK, Chung J-W, Kim KO, Kwon KA, Kim YJ. Circadian rhythm disruption is associated with an increased risk of sarcopenia: A nationwide population-based study in Korea. Sci Rep. 2019;9(1):1-7.
195. Gabriel BM, Altıntaş A, Smith JA, Sardon-Puig L, Zhang X, Basse AL, et al. Disrupted circadian core-clock oscillations in Type 2 Diabetes are linked to altered rhythmic mitochondrial metabolism. bioRxiv. 2021.

196. Chao C-Y, Wu J-S, Yang Y-C, Shih C-C, Wang R-H, Lu F-H, et al. Sleep duration is a potential risk factor for newly diagnosed type 2 diabetes mellitus. Metabolism. 2011;60(6):799-804.
197. Neufeld-Cohen A, Robles MS, Aviram R, Manella G, Adamovich Y, Ladeuix B, et al. Circadian control of oscillations in mitochondrial rate-limiting enzymes and nutrient utilization by PERIOD proteins. Proc Natl Acad Sci U S A. 2016;113(12):E1673-82.
198. de Goede P, Wust RCI, Schomakers BV, Denis S, Vaz FM, Pras-Raves ML, et al. Time-restricted feeding during the inactive phase abolishes the daily rhythm in mitochondrial respiration in rat skeletal muscle. FASEB J. 2022;36(2):e22133.
199. Tsereteli N, Vallat R, Fernandez-Tajes J, Delahanty LM, Ordovas JM, Drew DA, et al. Impact of insufficient sleep on dysregulated blood glucose control under standardised meal conditions. Diabetologia. 2022;65(2):356-65.
200. Hasselgren P-O. Glucocorticoids and muscle catabolism. Curr Opin Clin Nutr Metab Care. 1999;2(3):201-5.
201. Schakman O, Kalista S, Barbe C, Loumaye A, Thissen JP. Glucocorticoid-induced skeletal muscle atrophy. Int J Biochem Cell Biol. 2013;45(10):2163-72.
202. Areta JL, Burke LM, Camera DM, West DW, Crawshay S, Moore DR, et al. Reduced resting skeletal muscle protein synthesis is rescued by resistance exercise and protein ingestion following short-term energy deficit. Am J Physiol Endocrinol Metab. 2014;306(8):E989-97.
203. Morgan D, Tsai SC. Sleep and the endocrine system. Crit Care Clin. 2015;31(3):403-18.
204. Mihm M, Gangooly S, Muttukrishna S. The normal menstrual cycle in women. Anim Reprod Sci. 2011;124(3-4):229-36.
205. Bremner WJ, Vitiello MV, Prinz PN. Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. J Clin Endocrinol Metab. 1983;56(6):1278-81.
206. Holl RW, Hartman ML, Veldhuis JD, Taylor WM, Thorner MO. Thirty-second sampling of plasma growth hormone in man: correlation with sleep stages. J Clin Endocrinol Metab. 1991;72(4):854-61.
207. Everson CA, Crowley WR. Reductions in circulating anabolic hormones induced by sustained sleep deprivation in rats. Am J Physiol Endocrinol Metab. 2004;286(6):E1060-E70.

208. Kaiser U, Ho KKY. Pituitary Physiology and Diagnostic Evaluation. Williams Textbook of Endocrinology2016. p. 176-231.
209. Breen L, Phillips SM. Skeletal muscle protein metabolism in the elderly: Interventions to counteract the'anabolic resistance'of ageing. Nutr Metab. 2011;8(1):1-11.
210. Allen MJ, Sharma S. Physiology, adrenocorticotropic hormone (ACTH). 2018.
211. Braun TP, Marks DL. The regulation of muscle mass by endogenous glucocorticoids. Front Physiol. 2015;6:12.
212. Rooyackers OE, Nair KS. Hormonal regulation of human muscle protein metabolism. Annu Rev Nutr. 1997;17(1):457-85.
213. Leproult R, Van Cauter E. Effect of 1 week of sleep restriction on testosterone levels in young healthy men. Jama. 2011;305(21):2173-4.
214. Clark RV, Wald JA, Swerdloff RS, Wang C, Wu FCW, Bowers LD, et al. Large divergence in testosterone concentrations between men and women: Frame of reference for elite athletes in sex-specific competition in sports, a narrative review. Clin Endocrinol. 2019;90(1):15-22.
215. Knowles OE. No time to sleep on it - start exercising! J Physiol. 2020;598(11):2059-60.
216. Bell KE, Séguin C, Parise G, Baker SK, Phillips SM. Day-to-day changes in muscle protein synthesis in recovery from resistance, aerobic, and high-intensity interval exercise in older men. J Gerontol A. 2015;70(8):1024-9.
217. Youngstedt SD, Kline CE. Epidemiology of exercise and sleep. Sleep Biol Rhythms. 2006;4(3):215-21.
218. Wrann CD, White JP, Salogiannnis J, Laznik-Bogoslavski D, Wu J, Ma D, et al. Exercise induces hippocampal BDNF through a PGC-1alpha/FNDC5 pathway. Cell Metab. 2013;18(5):649-59.
219. Islam MR, Young MF, Wrann CD. The Role of FNDC5/Irisin in the Nervous System and as a Mediator for Beneficial Effects of Exercise on the Brain. In: Spiegelman B, editor. Hormones, Metabolism and the Benefits of Exercise. Cham (CH)2017. p. 93-102.
220. Wolff CA, Esser KA. Exercise timing and circadian rhythms. Curr Opin Physiol. 2019;10:64-9.
221. Jordan SD, Kriebs A, Vaughan M, Duglan D, Fan W, Henriksson E, et al. CRY1/2 selectively repress PPARδ and limit exercise capacity. Cell Metab. 2017;26(1):243-55. e6.

222. Mathai AS, Bonen A, Benton CR, Robinson DL, Graham TE. Rapid exercise-induced changes in PGC-1α mRNA and protein in human skeletal muscle. J Appl Physiol. 2008;105(4):1098-105.
223. Yin L, Lazar MA. The orphan nuclear receptor Rev-erbα recruits the N-CoR/histone deacetylase 3 corepressor to regulate the circadian Bmal1 gene. Mol Endocrinol. 2005;19(6):1452-9.
224. Adams R, Appleton S, Taylor A, McEvoy D, Antic N. Report to the sleep health foundation 2016 sleep health survey of Australian adults. Adelaide: The Adelaide Institute for Sleep Health & The University of Adelaide. 2016.
225. Morrison M, Halson SL, Weakley J, Hawley JA. Sleep, circadian biology and skeletal muscle interactions: Implications for metabolic health. Sleep Med Rev. 2022;66:101700.
226. Alhola P, Polo-Kantola P. Sleep deprivation: Impact on cognitive performance. Neuropsychiatr Dis and Treat. 2007;3(5):553-67.
227. Irish LA, Kline CE, Gunn HE, Buysse DJ, Hall MH. The role of sleep hygiene in promoting public health: A review of empirical evidence. Sleep Med Rev. 2015;22:23-36.
228. Miller DJ, Roach G, Lastella M, Capodilupo E, Sargent C. Hit the gym or hit the hay: can evening exercise characteristics predict compromised sleep in healthy adults? Front Physiol.14:1231835.
229. Kredlow MA, Capozzoli MC, Hearon BA, Calkins AW, Otto MW. The effects of physical activity on sleep: a meta-analytic review. J Behav Med. 2015;38:427-49.
230. Penedo FJ, Dahn JR. Exercise and well-being: a review of mental and physical health benefits associated with physical activity. Curr Opin Psychiatry. 2005;18(2):189-93.
231. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-95.
232. Ozemek C, Lavie CJ, Rognmo Ø. Global physical activity levels-Need for intervention. Prog Cardiovasc Dis. 2019;62(2):102-7.
233. Yao CJ, Basner M. Healthy behaviors competing for time: associations of sleep and exercise in working Americans. Sleep Health. 2019;5(1):23-30.
234. Wang F, Boros S. The effect of physical activity on sleep quality: a systematic review. Eur J Physiother. 2021;23(1):11-8.

235. Banno M, Harada Y, Taniguchi M, Tobita R, Tsujimoto H, Tsujimoto Y, et al. Exercise can improve sleep quality: a systematic review and meta-analysis. PeerJ. 2018;6:e5172.
236. Flausino NH, Da Silva Prado JM, de Queiroz SS, Tufik S, de Mello MT. Physical exercise performed before bedtime improves the sleep pattern of healthy young good sleepers. Psychophysiology. 2012;49(2):186-92.
237. Millet GP, Vleck VE, Bentley DJ. Physiological differences between cycling and running: lessons from triathletes. Sports Medicine. 2009;39:179-206.
238. Dumont M, Beaulieu C. Light exposure in the natural environment: relevance to mood and sleep disorders. Sleep Med. 2007;8(6):557-65.
239. Stutz J, Eiholzer R, Spengler CM. Effects of evening exercise on sleep in healthy participants: a systematic review and meta-analysis. Sports Med. 2019;49(2):269-87.
240. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, et al. AHA Dietary Guidelines: revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation. 2000;102(18):2284-99.
241. Ebrahim IO, Shapiro CM, Williams AJ, Fenwick PB. Alcohol and sleep I: effects on normal sleep. Alcohol Clin Exp Res. 2013;37(4):539-49.
242. Gardiner C, Weakley J, Burke LM, Roach GD, Sargent C, Maniar N, et al. The effect of caffeine on subsequent sleep: A systematic review and meta-analysis. Sleep Med Rev. 2023:101764.
243. Tanaka H, Monahan KD, Seals DR. Age-predicted maximal heart rate revisited. J Am Coll Cardiol. 2001;37(1):153-6.
244. Gignac GE, Szodorai ET. Effect size guidelines for individual differences researchers. Personality and individual differences. 2016;102:74-8.
245. Sargent C, Kosmadopoulos A, Zhou X, Roach GD. Timing of sleep in the break between two consecutive night-shifts: the effect of different strategies on daytime sleep and night-time neurobehavioural function. Nat Sci Sleep. 2022:231-42.
246. Pacheco D. The best time of day to exercise for sleep. 2021 [
247. Fullagar HH, Duffield R, Skorski S, Coutts AJ, Julian R, Meyer T. Sleep and recovery in team sport: current sleep-related issues facing professional team-sport athletes. Int J Sports Physiol Perform. 2015;10(8):950-7.
248. Vyazovskiy VV, Delogu A. NREM and REM sleep: complementary roles in recovery after wakefulness. Neuroscientist. 2014;20(3):203-19.

249. McGinty D, Szymusiak R. Keeping cool: a hypothesis about the mechanisms and functions of slow-wave sleep. Trends Neurosci. 1990;13(12):480-7.
250. Horne JA, Staff L. Exercise and sleep: body-heating effects. Sleep. 1983;6(1):36-46.
251. Gangwisch JE. A review of evidence for the link between sleep duration and hypertension. Am J Hypertens. 2014;27(10):1235-42.
252. Cappuccio FP, D'Elia L, Strazzullo P, Miller MA. Quantity and quality of sleep and incidence of type 2 diabetes: a systematic review and meta-analysis. Diabetes care. 2010;33(2):414-20.
253. Yang P-Y, Ho K-H, Chen H-C, Chien M-Y. Exercise training improves sleep quality in middle-aged and older adults with sleep problems: a systematic review. J Physiother. 2012;58(3):157-63.
254. Guilleminault C, Clerk A, Black J, Labanowski M, Pelayo R, Claman D. Nondrug treatment trials in psychophysiologic insomnia. Arch Intern Med. 1995;155(8):838-44.
255. Leal LG, Lopes MA, Batista Jr ML. Physical exercise-induced myokines and muscle-adipose tissue crosstalk: a review of current knowledge and the implications for health and metabolic diseases. Frontiers in physiology. 2018;9:1307.
256. Pedersen BK. Exercise-induced myokines and their role in chronic diseases. Brain Behav Immun. 2011;25(5):811-6.
257. Pedersen BK, Åkerström TC, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. J Appl Physiol. 2007.
258. Huh JY. The role of exercise-induced myokines in regulating metabolism. Arch Pharmacal Res. 2018;41(1):14-29.
259. Kang H, Schuman EM. Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science. 1995;267(5204):1658-62.
260. Benedict C, Scheller J, Rose-John S, Born J. 2. Enhancing influence of intranasal interleukin-6 on slow wave activity and memory consolidation during sleep. Brain Behav Immun. 2009(23):S8.
261. Oyanedel CN, Kelemen E, Scheller J, Born J, Rose-John S. Peripheral and central blockade of interleukin-6 trans-signaling differentially affects sleep architecture. Brain Behav Immun. 2015;50:178-85.
262. Shoham S, Davenne D, Cady AB, Dinarello CA, Krueger JM. Recombinant tumor necrosis factor and interleukin 1 enhance slow-wave sleep. Am J Physiol Regul Integr Comp Physiol. 1987;253(1):R142-R9.

263. Croghan C, Egeghy PP. Methods of dealing with values below the limit of detection using SAS. Southern SAS User Group. 2003;22(24):22-4.
264. Bürkner P-C. brms: An R package for Bayesian multilevel models using Stan. J Stat Softw. 2017;80:1-28.
265. Ostrowski K, Rohde T, Asp S, Schjerling P, Klarlund Pedersen B. Chemokines are elevated in plasma after strenuous exercise in humans. Eur J Appl Physiol. 2001;84:244-5.
266. Febbraio MA, Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. FASEB J. 2002;16(11):1335-47.
267. Nielsen HB, Secher NH, Christensen NJ, Pedersen BK. Lymphocytes and NK cell activity during repeated bouts of maximal exercise. Am J Physiol Regul Integr Comp Physiol. 1996;271(1):R222-R7.
268. Suzuki K, Yamada M, Kurakake S, Okamura N, Yamaya K, Liu Q, et al. Circulating cytokines and hormones with immunosuppressive but neutrophil-priming potentials rise after endurance exercise in humans. Eur J Appl Physiol. 2000;81:281-7.
269. Petersen AMW, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol. 2005;98(4):1154-62.
270. Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK. Pro-and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol. 1999;515(1):287-91.
271. Steensberg A, Fischer CP, Keller C, Møller K, Pedersen BK. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am J Physiol Endocrinol Metab. 2003;285(2):E433-E7.
272. Knaepen K, Goekint M, Heyman EM, Meeusen R. Neuroplasticity—exercise-induced response of peripheral brain-derived neurotrophic factor. Sports Med. 2010;40(9):765-801.
273. Dinoff A, Herrmann N, Swardfager W, Liu CS, Sherman C, Chan S, et al. The effect of exercise training on resting concentrations of peripheral brain-derived neurotrophic factor (BDNF): a meta-analysis. PLoS One. 2016;11(9):e0163037.
274. Marston KJ, Newton MJ, Brown BM, Rainey-Smith SR, Bird S, Martins RN, et al. Intense resistance exercise increases peripheral brain-derived neurotrophic factor. J Sci Med Sport. 2017;20(10):899-903.
275. Dinoff A, Herrmann N, Swardfager W, Lanctot KL. The effect of acute exercise on blood concentrations of brain-derived neurotrophic factor in healthy adults: a meta-analysis. Eur J Neurosci. 2017;46(1):1635-46.

276. Schmidt E-M, Linz B, Diekelmann S, Besedovsky L, Lange T, Born J. Effects of an interleukin-1 receptor antagonist on human sleep, sleep-associated memory consolidation, and blood monocytes. Brain Behav Immun. 2015;47:178-85.
277. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 2012;481(7382):463-8.
278. Medic G, Wille M, Hemels ME. Short-and long-term health consequences of sleep disruption. Nat Sci Sleep. 2017:151-61.
279. Ohayon M, Wickwire EM, Hirshkowitz M, Albert SM, Avidan A, Daly FJ, et al. National Sleep Foundation's sleep quality recommendations: first report. Sleep Health. 2017;3(1):6-19.
280. Sargent C, Lastella M, Halson SL, Roach GD. How Much Sleep Does an Elite Athlete Need? Int J Sports Physiol Perform. 2021;1(aop):1-12.
281. Halson SL, Johnston RD, Piromalli L, Lalor BJ, Cormack S, Roach GD, et al. Sleep regularity and predictors of sleep efficiency and sleep duration in elite team sport athletes. Sports Med Open. 2022;8(1):1-10.
282. Fox JL, Scanlan AT, Stanton R, Sargent C. Insufficient sleep in young athletes? Causes, consequences, and potential treatments. Sports Med. 2020;50(3):461-70.
283. Janse van Rensburg DC, Jansen van Rensburg A, Fowler PM, Bender AM, Stevens D, Sullivan KO, et al. Managing travel fatigue and jet lag in athletes: a review and consensus statement. Sports Med. 2021;51(10):2029-50.
284. Swinbourne R, Gill N, Vaile J, Smart D. Prevalence of poor sleep quality, sleepiness and obstructive sleep apnoea risk factors in athletes. Eur J Sport Sci. 2016;16(7):850-8.
285. Schaal K, Tafflet M, Nassif H, Thibault V, Pichard C, Alcotte M, et al. Psychological balance in high level athletes: gender-based differences and sport-specific patterns. PLoS One. 2011;6(5):e19007.
286. Matheson E, Hainer BL. Insomnia: pharmacologic therapy. Am Fam Physician. 2017;96(1):29-35.
287. Gunja N. In the Zzz zone: the effects of Z-drugs on human performance and driving. J Med Toxicol. 2013;9:163-71.
288. Doherty R, Madigan S, Warrington G, Ellis J. Sleep and Nutrition Interactions: Implications for Athletes. Nutrients. 2019;11(4).

289. Vlahoyiannis A, Giannaki CD, Sakkas GK, Aphamis G, Andreou E. A Systematic Review, Meta-Analysis and Meta-Regression on the Effects of Carbohydrates on Sleep. Nutrients. 2021;13(4).
290. McKay AK, Stellingwerff T, Smith ES, Martin DT, Mujika I, Goosey-Tolfrey VL, et al. Defining training and performance caliber: a participant classification framework. Int J Sports Physiol Perform. 2021;17(2):317-31.
291. Spinweber CL, Ursin R, Hilbert RP, Hildebrand RL. l-Tryptophan: effects on daytime sleep latency and the waking EEG. Electroencephalogr Clin Neurophysiol. 1983;55(6):652-61.
292. Champely S, Ekstrom C, Dalgaard P, Gill J, Weibelzahl S, Anandkumar A, et al. Package 'pwr'. R package version. 2018;1(2).
293. Kosmadopoulos A, Zhou X, Roach GD, Darwent D, Sargent C. No first night shift effect observed following a nocturnal main sleep and a prophylactic 1-H afternoon nap. Chronobiol Int. 2016;33(6):716-20.
294. Dinges DF, Powell JW. Microcomputer analyses of performance on a portable, simple visual RT task during sustained operations. Beh Res Meth Instr Comp. 1985;17(6):652-5.
295. Basner M, Dinges DF. Maximizing sensitivity of the psychomotor vigilance test (PVT) to sleep loss. Sleep. 2011;34(5):581-91.
296. Sargent C, Darwent D, Ferguson SA, Roach GD. Can a simple balance task be used to assess fitness for duty? Accid Anal Prev. 2012;45:74-9.
297. Fernández-San-Martín MI, Masa-Font R, Palacios-Soler L, Sancho-Gómez P, Calbó-Caldentey C, Flores-Mateo G. Effectiveness of Valerian on insomnia: a meta-analysis of randomized placebo-controlled trials. Sleep Med. 2010;11(6):505-11.
298. Silber B, Schmitt J. Effects of tryptophan loading on human cognition, mood, and sleep. Neurosci Biobehavioral Rev. 2010;34(3):387-407.
299. MacInnis MJ, Dziedzic CE, Wood E, Oikawa SY, Phillips SM. Presleep α-lactalbumin consumption does not improve sleep quality or time-trial performance in cyclists. Int J Sport Nutr Exerc Metab. 2020;30(3):197-202.
300. Vermeeren A. Residual effects of hypnotics: epidemiology and clinical implications. CNS Drugs. 2004;18:297-328.
301. Sheppard JM, Young WB. Agility literature review: Classifications, training and testing. J Sports Sci. 2006;24(9):919-32.

302. Sargent C, Halson S, Roach GD. Sleep or swim? Early-morning training severely restricts the amount of sleep obtained by elite swimmers. Eur J Sport Sci. 2014;14 Suppl 1:S310-5.
303. Romyn G, Robey E, Dimmock JA, Halson SL, Peeling P. Sleep, anxiety and electronic device use by athletes in the training and competition environments. Eur J Sport Sci. 2016;16(3):301-8.
304. Halson SL, Johnston RD, Appaneal RN, Rogers MA, Toohey LA, Drew MK, et al. Sleep quality in elite athletes: normative values, reliability and understanding contributors to poor sleep. Sports Med. 2021:1-10.
305. Medicine AAoS. International classification of sleep disorders. Diagnostic and coding manual. 2005:148-52.
306. Shapiro CM, Allan M, Driver H, Mitchell D. Thermal load alters sleep. Biol Psychiatry. 1989;26(7):736-40.
307. McGee SL, Hargreaves M. Exercise adaptations: molecular mechanisms and potential targets for therapeutic benefit. Nat Rev Endocrinol. 2020;16(9):495-505.
308. Pedersen BK. Exercise and cytokines. Immunol Cell Biol. 2000;78(5):532-5.
309. Ostrowski K, Rohde T, Zacho M, Asp S, Pedersen B. Evidence that IL-6 is produced in skeletal muscle during intense long-term muscle activity. J Physiol 1998;508:949-53.
310. Rohde T, MacLean DA, Richter EA, Kiens B, Pedersen BK. Prolonged submaximal eccentric exercise is associated with increased levels of plasma IL-6. Am J Physiol Endocrinol Metab. 1997;273(1):E85-E91.
311. Cain SW, Chang A-M, Vlasac I, Tare A, Anderson C, Czeisler CA, et al. Circadian rhythms in plasma brain-derived neurotrophic factor differ in men and women. J Biol Rhythms. 2017;32(1):75-82.
312. Charles R, Kirkham A, Guyatt A, Parker S. Psychomotor, pulmonary and exercise responses to sleep medication. Br J Clin Pharmacol. 1987;24(2):191-7.
313. Fischer D, Lombardi DA, Marucci-Wellman H, Roenneberg T. Chronotypes in the US–influence of age and sex. PLoS One. 2017;12(6):e0178782.
314. Baker FC, Driver HS. Circadian rhythms, sleep, and the menstrual cycle. Sleep Med. 2007;8(6):613-22.
315. Baker FC, Driver HS, Paiker J, Rogers GG, Mitchell D. Acetaminophen does not affect 24-h body temperature or sleep in the luteal phase of the menstrual cycle. J Appl Physiol. 2002;92(4):1684-91.

316. Driver HS, Dijk DJ, Werth E, Biedermann K, Borbély A. Sleep and the sleep electroencephalogram across the menstrual cycle in young healthy women. J Clin Endocrinol Metab. 1996;81(2):728-35.
317. LeGates TA, Fernandez DC, Hattar S. Light as a central modulator of circadian rhythms, sleep and affect. Nat Rev Neurosci. 2014;15(7):443-54.
318. Haskell EH, Palca JW, Walker JM, Berger RJ, Heller HC. The effects of high and low ambient temperatures on human sleep stages. Electroencephalogr Clin Neurophysiol. 1981;51(5):494-501.

Chapter 10: Appendices

Appendix 1: Research portfolio

Study 1: Sleep, circadian biology and skeletal muscle interactions: Implications for metabolic health.

2. **Morrison, M**., Halson, S. L., Weakley, J., & Hawley, J. A. (2022). Sleep, circadian biology and skeletal muscle interactions: Implications for metabolic health. *Sleep Medicine Reviews*, 101700

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Contribution statement: MM was responsible for determining the direction of the review, conducting the literature search, data extraction and synthesis, the writing and submission of the manuscript, responding to reviewer feedback and the approval of the final draft. SH, JW, and JH assisted in refining the aims of the manuscript, writing the manuscript, and responding to reviewer feedback.

Due to copyright limitations, the published version of this journal article is available online at: https://www.sciencedirect.com/science/article/pii/S1087079222001137

Approximate percentage contributions: M. Morrison 70%; S. L. Halson 5%; J. Weakley 5%; J. A. Hawley 20%.

I acknowledge that my contribution to the above manuscript is 70%.

Matthew Morrison Date: 20/12/2023

As principal supervisor, I certify that the above contributions are true and correct:

Jonathon Weakley Date: 20/12/2023

Co-author signatures:

Shona L. Halson Date: 02/01/2024

John A. Hawley Date: 03/01/2024

Study 2: Quantifying the effect of afternoon moderate-intensity exercise on sleep quality and quantity in healthy adult males using polysomnography.

Publication statement and status: Submitted to Sleep in February 2024

Contribution statement: MM was responsible for participant recruitment, data collection, data analysis, statistical analysis, the writing, and preparation of the manuscript for submission. MM assisted SH, JW, GR, and CS with ethical approval, determining the experimental design, and refining the aims of the study. DM and CG assisted with participant recruitment and data collection. All authors assisted with the writing of the manuscript.

Approximate percentage contributions: M. Morrison 60%; J. Weakley 10%; G. D. Roach 7.5%; C. Sargent 7.5%; D. J. Miller 2.5%; C. Gardiner 2.5%; S. L. Halson 10%.

I acknowledge that my contribution to the above manuscript is 60%.

Matthew Morrison Date: 20/12/2023

As principal supervisor, I certify that the above contributions are true and correct:

Jonathon Weakley Date: 20/12/2023

Co-author signatures:

Shona L. Halson Date: 2/01/2024

Gregory D. Roach Date: 25/01/2024

Charli Sargent Date: 25/01/2024

Dean J. Miller Date : 2/01/2024

Carissa Gardiner Date: 31/12/2023

Study 3: Relationship between sleep quality and quantity and exercise-induced peripheral factors in healthy adult males.

Publication statement and status: The manuscript is under preparation for submission. Whilst the final author list for the resulting manuscript is not yet confirmed, I acknowledge I will be responsible for at least a 50% contribution to the manuscript.

Matthew Morrison Date: 20/12/2023

As principal supervisor, I certify the above statement and contribution of Matthew Morrison is true and correct:

Jonathon Weakley Date: 20/12/2023

Study 4: The influence of a formulated nutritional intervention on subsequent sleep and next-morning physical performance, cognitive function, and postural sway in adult males

Publication statement and status: Submitted to Journal of the International Society of Sports Nutrition in January 2024.

Contribution statement: MM was primarily responsible for participant recruitment, data collection, data analysis, statistical analysis, and the writing and preparation of the manuscript for submission. JW assisted with the statistical analysis and interpretation of findings. LN and MP were responsible for the formulation and refinement of the proprietary nutritional supplement. MM, LN, MP, SH, CS, and GR were responsible for the experimental design. MM, SH, JW, CS, and GR were responsible for gaining ethical approval for the study. DM, CG, and GM assisted with participant recruitment and data collection. JW, GR, CS, DM, and SH assisted with writing the manuscript.

Approximate percentage contributions: M. Morrison 60%; J. Weakley 7.5%; G. D. Roach 7.5%; C. Sargent 7.5%; D. J. Miller 2%; L. Nyman 2%; C. Gardiner 2%; M. Pahnke 2%; G. Munteanu 2%; S. L. Halson 7.5%.

I acknowledge that my contribution to the above manuscript is 60%.

Matthew Morrison Date: 20/12/2023

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Matthew Pahnke Date: 2/01/2024

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Appendix 2: Ethics approvals, letters to participants, and consent forms

Study 2 and 3: Letter to participants and consent forms

ACU Human Ethics Committee Register Number: 2021-181R

CQUniversity's Human Research Ethics Committee: 0000021915

The impact of compression garments on sleep after exercise in healthy adults

You have been invited to participate in our research study. Before agreeing to participate, it is important that you read and understand the following explanation of the study and the procedures. This form describes the purpose, procedures, benefits, risks and discomforts associated with the research study. If you choose to participate, you have the right to withdraw from the study at any time and this will not disadvantage you in any way.

The project team
The Principal Investigators are Associate Professor Charli Sargent (CQUniversity), Professor Greg Roach (CQUniversity), Professor Shona Halson (Australian Catholic University) and Mr Dean Miller (CQUniversity). The Associate Investigators are Mr Matthew Morrison (Australian Catholic University), Professor John Hawley (Australian Catholic University), Dr Jonathan Weakley (Australian Catholic University), Dr Jamie Whitfield (Australian Catholic University), and Dr James Broatch (Victoria University). Dr Broatch receives indirect funding from the company 2XU.

Sources of funding
The study is funded by the Australian Catholic University ($20,000) and is supported by in-kind funding from CQUniversity, Victoria University, and 2XU.

Aims and purpose of the research project
Compression tights are elastic garments that are worn over the legs. The tights were originally designed to help people with swelling in the legs, but relatively recently, elite athletes have been using them to improve recovery from training and competition. Some athletes wear the tights to bed to continue the process of recovery. We are interested in measuring what happens to sleep when people wear the compression tights to bed after they have performed a bout of exercise during the day. The study will be carried out at the Appleton Institute's sleep laboratory.

Who CAN participate in this research project?
Healthy, non-smoking, males between 18 to 35 years old, taking no sleep medication and who have a regular sleep pattern are eligible to participate in this study. You must be free from injury and be able to perform exercise on a cycle ergometer in order to participate.

Before commencing the study, you will be asked to complete questionnaires related to your general health, physical activity, work patterns, and sleep habits. You will also be required to complete a 7-night sleep/wake diary and wear a wrist activity monitor (sleep watch). These measures will allow us to assess your regular sleep patterns. After this preliminary period, if you are still interested in continuing, and you meet the inclusion/exclusion criteria, you will be asked to attend the sleep laboratory and to sign an informed consent form.

Who CANNOT participate in this research project?
As we are interested in sleep and performance variables, we must exclude those who are unable to complete all testing. Participants who are injured, ill or have circulatory conditions/problems will not be able to participate in the study. Individuals who have undertaken transmeridian travel in the 3 months prior to testing, those who suffer from, or have been diagnosed with, a sleep disorder such as sleep apnea, insomnia or narcolepsy,

those who undertake shift work, and those who take any form of sleep medication or supplements known to affect sleep (such as melatonin) will also be excluded from the study.

How will my consent be obtained?
All interested participants will be provided with an information sheet, a general health questionnaire, an Adult Pre-Exercise Screening Tool, a Munich chronotype questionnaire, and the Pittsburgh Sleep Quality Index. The general health questionnaire will be used to determine your eligibility for the study. The Adult Pre-Exercise Screening Tool will assess your safety for completing exercise, and the Pittsburgh Sleep Quality Index and the Munich Chronotype Questionnaire will be used to determine your sleep habits. After this, you will be asked to keep a sleep diary and wear a wrist activity monitor to assess your sleep patterns. Those who continue to express interest in participating in the study and who meet the inclusion/exclusion criteria will be asked to attend the sleep laboratory and to sign an informed consent form.

What is required of me to participate in this research project?
At least one week prior to participating in the study, you will complete an incremental cycle ergometer test to exhaustion. This test will be used to determine your individual exercise intensity for the exercise bouts during the study. During this period, you will also complete a 7-night sleep/wake diary and wear a wrist activity monitor (sleep watch).

If you are eligible and agree to participate in the study, you will be required to spend four consecutive days/nights in the sleep laboratory We will be collecting data with participants in groups of six, so you will carry out the study with five other people. Each of you will have your own private bedroom, living room and bathroom.

The first night will be a familiarisation night, so that you can get used to the equipment we will use to monitor your sleep. On the other three nights, you will complete a different condition.
The three conditions are:

1. No exercise and no tights condition – you will not perform any exercise and you will not wear compression tights to bed;
2. Exercise and no tights condition – you will perform a high-intensity exercise session but you will not wear compression tights to bed;
3. Exercise and tights condition – you will perform a high-intensity exercise session and you will wear compression tights to bed.

During the study, we will monitor your sleep, exercise performance, and cognitive function. We will also collect blood samples each morning, before and after each exercise session, and in the evening. We will analyse the blood samples to look at how certain chemicals that are released during exercise (myokines) affect subsequent sleep.

- Sleep. Your sleep will be monitored each night using polysomnography. This process involves the placement of small leads on your head and face using tape and paste. The leads are connected to a small box next to your bed and are long enough such that you are able to sleep in your normal position when in bed. These leads measure brain activity, eye movements, and muscle tone and provide important information about your sleep (i.e., whether you are in light sleep, deep sleep, or dreaming sleep).

- Exercise session. You will be required to perform an exercise screening test when you come for familiarisation prior to the study. This will enable us to quantify your level of fitness. On each day of the study, you will be required to complete one exercise session on a cycle ergometer. The session will be performed in the afternoon and will consist of a 5-minute warm up (~50% of your maximum effort) followed by 40 min at ~70% of your maximum effort. This will be followed by a 2-min warm-down of gentle cycling.

- Heart rate. During all exercise sessions, you will be asked to wear a chest strap and transmitter that will measure your heart rate.

- Blood samples. We will collect samples of your blood in the morning after you wake up, before and after you exercise, and before you go to bed. We will use a small needle and tube (called a catheter) to collect the samples from a vein in your forearm. In total, we will collect ~ 90 ml of blood. The blood that we will collect will be analysed for myokines. Myokines are small proteins that are released during muscular contractions. We are interested in whether myokines affect your sleep after exercise.

- Compression tights. In one of the conditions during the study, you will be asked to wear compression tights while you sleep. The compression tights will be provided to you during the study. The garments are designed to fit firmly and tightly – we will provide you with the correct size according to the manufacturer's recommendations (i.e., based on your body mass and height). You will have an opportunity to try on the compression tights during the familiarisation visit.

- Activity monitor. You will be required to wear an activity monitor at all times during the study. The activity monitor will record your level of movement during the study.

- Subjective assessments. At certain times in the morning, afternoon and evening we will ask you to rate your feelings of sleepiness, fatigue, sleep quality, and muscle soreness using different scales.

In the time when you are not completing exercise sessions or assessments, you may read, watch TV/DVDs, draw, listen to music etc. You will not be able to perform any strenuous activity outside of the planned exercise sessions. You will also be given opportunities at set times each day to use your phone. When you are not using your phone, it will be stored in a locked filing cabinet in the control room of the sleep laboratory.

The room in which you will sleep is similar to a hotel room and it will be dark and quiet during the night. You will not share the room with anyone else and your room has its own bathroom. The room has a video camera so that research staff can monitor your sleep when the lights are out. It also has an audio system, so that research staff can talk to you and hear you from the monitoring area outside the room. Research staff may also come into the room to detach the sleep equipment if you need to get up during the night. You will not be able to sleep at any other time during the study.

All of your meals will be prepared for you during the study and served at the same time each day. You will not be able to bring food with you or eat food outside of the specified times. In addition to meals, snacks will be provided at regular intervals each day. Prior to the start of the study, you will be given a menu to review. You can use this menu to choose meals and food items that you like and to specify any dietary requirements that you have. All of your meals (except snacks) will be served in a dining room that you will share with the other five participants in your group. It is important to remember that throughout the study, and in the 24h prior to the first night, you will be asked to refrain from consuming any alcohol or caffeine (tea, coffee, energy drinks, chocolate).

What do I need to bring?

You will be staying in the sleep laboratory for four consecutive nights, so you will need to bring enough clothes and toiletries to cover this time. You will need comfortable clothes and appropriate shoes to exercise in, and you will need comfortable pyjamas to sleep in. The temperature in the sleep laboratory is maintained between 21 and 23 degrees. We will provide you with all of your linen (sheets, blankets, towels, pillows etc.) but you may bring your own pillow from home if you wish. You will have your own bedroom, living area, and shower and toilet facilities for the duration of the

study. Because there will be periods of spare time throughout the study, please feel free to bring along your own music, books or movies.

What are the risks, inconvenience or discomfort that could reasonably be expected to be experienced during the study?
Each night during the study, we will monitor your sleep using small leads attached to your head and face. You may experience some minor skin irritation from the leads that we will attach to your face. The leads will be taped to your skin next to your eyes, below your chin, in the middle of your forehead and on your collarbone. If you experience any irritation, we will alternate the position of the leads.

There is a low risk of sustaining an injury during the exercise sessions. This is a risk associated with any physical activity. All efforts will be made to reduce the risk of injury by monitoring and maintaining the exercise equipment, and monitoring and maintaining the exercise space to ensure it is safe and free from obstacles. A standard warm up will also be completed prior to each exercise session to reduce the likelihood of injury. You may also experience some discomfort during the exercise sessions – the intensity will be ~70% of your maximum effort.

At times during each day, we will obtain blood from a vein in your forearm. We will use two methods to collect blood samples – for the morning and evening samples, we will use a very small needle. For the samples immediately before and after exercise, we will insert a small tube into a vein in your forearm to avoid using multiple needles. The tube will stay in while you exercise. Veins vary in size from one person to another and from one side of the body to the other. Obtaining a blood sample from some people may be more difficult than from others. Other risks associated with having blood drawn are unlikely but may include fainting or feeling light-headed, bleeding, hematoma (blood accumulating under the skin), or infection (a slight risk any time the skin is broken). To minimise these risks, an experienced phlebotomist will collect all blood samples.

If you decide to participate in the study, you will spend four nights in the laboratory. Because of this, you may experience feelings of isolation, anxiety, mood changes, etc. You will be able to interact frequently with the other participants and researchers and contact your family at allocated times.

What are the benefits to me?
You will not directly benefit from participating in the study. However, upon request, you will receive individual feedback about your sleep and your exercise performance, as well as a summary of the research upon completion of the project. If you complete the study, you will receive $130 ($130 participation payment + $70 completion payment). If for some reason you do not complete the whole study, you will receive a pro rata participation payment based on the amount of time that you spent in the sleep laboratory. You will also be able to keep the pair of compression garments if you wish.

How will my privacy and confidentiality be maintained?
All data from this study will be stored on computers or portable hard drives at the Appleton Institute. These computers will be password-protected and will only be accessed by members of the research team. Data that has been collected in hard copy (e.g., questionnaires) will be stored in lockable filing cabinets at the Appleton Institute. All data collected as part of the study will be stored for 15 years after the publication date of the last publication based upon the data in accordance with CQUniversity policy. All records containing personal information will remain STRICTLY CONFIDENTIAL. You will be asked to keep the identity of your fellow participants confidential. This is in accordance with CQUniversity policy.

What will happen to my information?
Research papers arising from the study will be submitted for publication in scientific journals and presented at conferences. No publications arising from this work will enable any participant to be identified. No information that will lead to the identification of any individual will be released. No case studies will be reported to protect your privacy. Some of the data that we collect with you may be shared with a third party. Any data shared with a third party will be de-identified. The third party will not be able to contact you in any way or identify you in any way.

Right to withdraw
Before deciding whether or not to take part in this study, you may wish to discuss the matter with a relative, friend or your local doctor. You should feel free to do this. It is important that you understand that your participation in this study is voluntary. If you do not wish to take part you are under no obligation to do so. If you decide to take part but later change your mind, you are free to withdraw from the project at any stage without explanation, and without prejudice from any member of the research team. At any time, you can also withdraw any unprocessed data that you have provided.

Any questions regarding this project may be directed to:
Associate Professor Charli Sargent, Research Fellow,
T: 08 8378 4523 E: charli.sargent@cqu.edu.au

Any concerns or complaints may be directed to:
Please contact CQUniversity's Office of Research (Tel: 07 4923 2603; E-mail: ethics@cqu.edu.au; Mailing address: Building 32, CQUniversity, Rockhampton, QLD, 4702) should there be any concerns about the nature and/or conduct of this research project.

This project has been approved by the CQUniversity Human Research Ethics Committee, approval number (22194).

The impact of compression garments on sleep after exercise in healthy adults

I consent to participation in this research project and agree that:

1. I am aged 18 years or older;
2. An Information Sheet has been provided to me that I have read and understood. Any questions I have had about the project have been answered to my satisfaction by the Information Sheet and any further verbal explanation;
3. I understand that I have the right to withdraw from the project at any time without penalty, and to withdraw any unprocessed data;
4. I understand the statement concerning compensation for taking part in the study, which is contained in the Information Sheet;
5. I understand that to preserve anonymity and maintain confidentiality of participants, no personally identifiable information will be used in any publication(s);
6. I understand the research findings will be included in the researchers' publication(s) on the project, and these publications may include articles written for conferences, journals as well as other methods of dissemination;
7. I understand that some of my data may be shared with a third party. I understand that my data will be de-identified and the third party will not be able to contact me or identify me.
8. I acknowledge that the results of the study are unknown and that I may experience some skin irritation and/or minor discomfort due to the equipment used to monitor my sleep. I may experience some discomfort during exercise; and I may experience some discomfit during the procedure to collect blood. This list of symptoms is not exhaustive;
9. I acknowledge that I have been advised that I should avoid driving a car, riding a bike, and/or operating heavy machinery, following the completion of the study, until I have obtained at least one full night of sleep (uninterrupted for 9h or more);
10. I release and indemnify the University, its employees, students and agents against liability in respect of all claims, costs and expenses and for all loss, damage, injury or death to persons or property caused or contributed by me in connection with my failure to follow the after study instructions;
11. I have discussed the risks of the study with, and I have had the opportunity to seek advice from someone independent from the study such as a relative, friend, doctor or lawyer.
12. I have not experienced any COVID-19 symptoms in the last 14 days, nor have I tested positive for COVID-19 in the last 14 days.

CONSENT FORM

The impact of compression garments on sleep after exercise in healthy adults

Participant name (please print): _____

Signature: _____ **Date**: _____

I wish to have a plain English statement of results sent to me	**Yes**	**No**

If yes, please provide E-mail address: _____

I have explained the study to the participant and consider that he/she understands what is involved:

Researcher: _____

Signature: _____ **Date**: _____

Study 4: Letter to participants and consent forms
ACU Human Ethics Committee Register Number: 2022-2526R
CQUniversity's Human Research Ethics Committee: 0000022194

Nutritional Intervention to Enhance Sleep Quality and Sleep Quantity in Athletes (PEP-1911)

You have been invited to participate in our research study. Before agreeing to participate, it is important that you read and understand the following explanation of the study and the procedures. This form describes the purpose, procedures, benefits, risks and discomforts associated with the research study. If you choose to participate, you have the right to withdraw from the study at any time and this will not disadvantage you in any way.

The project team and sources of funding.
The Principal Investigators are Associate Professor Charli Sargent (CQUniversity), Professor Shona Halson (ACU), and Professor Greg Roach (CQUniversity). The Associate Investigators are: Ms Lara Nyman (PepsiCo), Ms Melissa Anderson (PepsiCo), Mr Dean Miller (CQU), Mr Matthew Morrison (ACU), Ms Carissa Gardiner (ACU). The study is funded by PepsiCO and supported in-kind by CQUniversity and Australian Catholic University.

Aims and purpose of the research project
Elite athletes frequently report difficulties initiating sleep on the nights before and after major competition. There is increasing scientific interest in the role that nutrition may play in enhancing sleep. There is some evidence to suggest that nutrition intake before sleep may influence the quantity and quality of sleep. The aim of this project is to examine whether a nutritional intervention, in the form of a drink, may influence sleep and/or affect next day exercise and cognitive performance.

Who CAN participate in this research project?
Healthy, non-smoking, well-trained males between 18 to 40 years old, taking no sleep medication and who have a regular sleep pattern are eligible to participate in this study. To be considered well-trained, you must undertake 2 hours of training at least 3 days per week for a minimum of 3 years. Before commencing the study, you will be asked to complete questionnaires related to your general health, physical activity and sleep habits.

Who CANNOT participate in this research project?
As we are interested in sleep and performance variables, we must exclude those who are unable to complete all testing. Participants who are injured or ill will not be able to participate in the study. Individuals who suffer from, or have been diagnosed with, a sleep disorder such as sleep apnea, insomnia or narcolepsy, those who undertake shift work, those who take any form of sleep medication or supplements known to affect sleep (such as melatonin) and those who have participated in another clinical trial within the past 30 days or another PepsiCo study within the past 6 months.

How will my consent be obtained?
All interested participants will be provided with an information sheet, a General Health Questionnaire, an Adult Pre-Exercise Screening Tool and the Pittsburgh Sleep Quality Index. The General Health Questionnaire will assess your medical history; the Adult Pre-Exercise Screening Tool will assess your safety for completing exercise; and the Pittsburgh Sleep Quality will be used to determine your sleep habits. Those who continue to express interest in participating in the study and who meet the inclusion/exclusion criteria will be asked to attend the sleep laboratory and to sign an informed consent form.

What is required of me to participate in this research project?
The study will be carried out at the Appleton Institute's sleep laboratory in Adelaide. We will be collecting data with participants in groups of up to six, so you will carry out the study with up to five other people. Each of you will have your own private bedroom, living room and bathroom.

In the week prior to the study, you will be asked to wear a wrist activity monitor and to complete a sleep diary. This will provide us with a record of how well you slept in the week before taking part in the study.

If you are eligible and agree to participate in the study, you will be required to attend the sleep laboratory for 4 consecutive nights and the 5 corresponding days. The first night will be a familiarisation night, so that you can get used to the equipment we will use to monitor your sleep. On the following three nights, you will be asked to consume one of three drinks prior to sleep and perform exercise tasks before and after each night of sleep.

During the study, we will monitor your sleep, physical performance, and cognitive function.

- Sleep. Your sleep will be monitored each night using polysomnography. This process involves the placement of small leads on your head and face using tape and paste. The leads are connected to a small box next to your bed and are long enough such that you are able to sleep in your normal position when in bed. These leads measure brain activity, eye movements, and muscle tone and provide important information about your sleep (i.e., whether you are in light sleep, deep sleep, or dreaming sleep). A member of the research team will remove the leads each morning. The residue from the tape and paste washes off with warm water and soap. You will also be asked questions regarding how sleepy you are, your perceived sleep quality and quantity how long it took you to fall asleep.

- Sleep drink. On three of the nights in the laboratory, you will be required to consume one of three sleep drinks (250ml). One of the drinks will be a placebo, and will contain no active ingredients, and the other two drinks will contain the active ingredients. The active drink does not contain any drugs – it contains nutritional ingredients such as proteins and carbohydrates. You may experience some minor gastrointestinal discomfort from the sleep drinks.

- Exercise cycling task and exercise performance test. You will be required to perform two cycling familiarisation tasks, one which replicates a typical cycling interval training session (cycling task) and the other a test of maximal performance (performance test). During the study, you will be required to complete three cycling tasks and three performance tests. The cycling task will be completed in the afternoon and will consist of a 17-minute warm up, followed by 25 minutes of high intensity cycling intervals (70-85% of maximal heart rate). This will be followed by a warm-down. The performance task will be completed in the morning and consists of a 17-minute warm-up followed by a 10-minute time trial on a cycling ergometer.

- Heart rate. During all exercise sessions, you will be asked to wear a chest strap and transmitter that will measure your heart rate.

- Cognitive function. On the days when you are in the laboratory, we will assess your cognitive function using different tasks. These tasks will include a reaction time task and a postural balance task. Each task takes between 2 and 20 min to complete, resulting in a total of approx. 30min. You will also be asked questions relating to your alertness, speed and accuracy.

- Activity monitor. You will also be required to wear an activity monitor at all times during the study. The activity monitor will record your level of movement during the study.

In the time when you are not completing performance tasks or exercise sessions, you may read, watch TV/DVDs, draw, listen to music etc. You will not be able to perform any strenuous activity outside of the planned exercise sessions. You will be able to use your phone during the day (except when we are assessing your cognitive function in the morning) but you will not be able to use your phone in the 2 hours prior to bed or during the night when you are in bed. When you are not using your phone, it will be stored in a locked filing cabinet in the control room of the sleep laboratory.

The room in which you will sleep is similar to a hotel room and it will be dark and quiet during the night. You will not share the room with anyone else and your room has its own bathroom. The room has a video camera so that research staff can monitor your sleep when the lights are out. It also has an audio system, so that research staff can talk to you and hear you from the monitoring area outside the room. Research staff may also come into the room to detach the sleep equipment if you need to get up during the night. You will not be able to sleep at any other time outside of 22:30 to 08:00 during the study.

All of your meals will be prepared for you during the study and served at the same time each day. You will not be able to bring food with you or eat food outside of the specified times. In addition to meals, snacks will be provided at regular intervals each day. Prior to the start of the study, you will be given a menu to review. You can use this menu to choose meals and food items that you like and to specify any dietary requirements that you have. All of your meals (except snacks) will be served in a dining room that you will share with the other five participants in your group. It is important to remember that throughout the study, and in the 24h prior to the first night of the study, you will be prohibited from consuming any alcohol or caffeine (e.g., tea, coffee, energy drinks, chocolate).

What do I need to bring?
You will be staying in the sleep laboratory for three consecutive nights, so you will need to bring enough clothes and toiletries to cover this time. You will need comfortable clothes and appropriate shoes to exercise in, and you will need comfortable pyjamas to sleep in. The temperature in the sleep laboratory is maintained between 21 and 23 degrees. We will provide you with all of your linen (sheets, blankets, towels, pillows etc.) but you may bring your own pillow from home if you wish. You will have your own bedroom, living area, and shower and toilet facilities for the duration of the study. There will be periods of spare time throughout each day – you will have access to music and movies using a television in your room. There is also a small collection of books, magazines and newspapers that you can access, but please feel free to bring along your own reading materials.

What are the risks, inconvenience or discomfort that could reasonably be expected to be experienced during the study?
Each night during the study, we will monitor your sleep using small leads attached to your head and face. You may experience some minor skin irritation from the leads that we will attach to your face. The leads will be taped to your skin next to your eyes, below your chin, in the middle of your forehead and on your collarbone. If you experience any irritation, we will alternate the position of the leads.

There is a low risk of sustaining an injury during the exercise sessions. This is a risk associated with any physical activity. All efforts will be made to reduce the risk of injury by monitoring and maintaining the exercise equipment, and monitoring and maintaining the exercise space to ensure it is safe and free from obstacles. A standard warm up will also be completed prior to each exercise session to reduce the likelihood of injury. You may also experience some discomfort during the exercise sessions when you are performing at high intensities. The blocks of high intensity exercise will be 5 min in duration and you will have 5 min of recovery between each 5-min bout.

You may experience some minor gastrointestinal discomfort from the sleep drinks. It is important to tell a member of the research staff if you experience any symptoms after consuming the drinks.

If you decide to participate in the study, you will spend four nights in the laboratory. Because of this, you may experience feelings of isolation, anxiety, mood changes, etc. You will be able to interact frequently with the other participants and researchers and contact your family at allocated times.

What are the benefits to me?
You will not directly benefit from participating in the study. However, upon request, you will receive individual feedback about your sleep and your exercise performance, as well as a summary of the

research upon completion of the project. If you complete the study, you will receive $280 ($186 participation payment + $94 completion payment). If for some reason you do not complete the whole study, you will receive a pro rata participation payment based on the amount of time that you spent in the sleep laboratory.

How will my privacy and confidentiality be maintained?

All data from this study will be stored on computers or portable hard drives at the Appleton Institute. These computers will be password-protected and will only be accessed by members of the research team. Data that has been collected in hard copy (e.g., questionnaires) will be stored in lockable filing cabinets at the Appleton Institute. All data collected as part of the study will be stored for 15 years after the publication date of the last publication based upon the data in accordance with CQUniversity policy. All records containing personal information will remain STRICTLY CONFIDENTIAL. You will be asked to keep the identity of your fellow participants confidential. This is in accordance with CQUniversity policy.

What will happen to my information?

Research papers arising from the study will be submitted for publication in scientific journals and presented at conferences. No publications arising from this work will enable any participant to be identified. No information that will lead to the identification of any individual will be released. No case studies will be reported to protect your privacy. The deidentified data that we collect from you, as well as a final report describing the results of the study, will be provided to the sponsor of the study (PepsiCo). PepsiCo will also have direct access to your de-identified data for the purpose of audits, ethical review and regulatory inspections. No information that will lead to the identification of any individual will be released to PepsiCo.

Right to withdraw

Before deciding whether or not to take part in this study, you may wish to discuss the matter with a relative, friend or your local doctor. You should feel free to do this. It is important that you understand that your participation in this study is voluntary. If you do not wish to take part you are under no obligation to do so. If you decide to take part but later change your mind, you are free to withdraw from the project at any stage without explanation, and without prejudice from any member of the research team. At any time, you can also withdraw any unprocessed data that you have provided. The principal researcher reserves the right to terminate an individual's participation for non-compliance of the study protocol.

…..

Any questions regarding this project may be directed to:

Associate Professor Charli Sargent, Research Fellow,
T: 08 8378 4523 E: charli.sargent@cqu.edu.au

Professor Shona Halson,
T: 0422 224491 E: shona.halson@acu.edu.au

Any concerns or complaints may be directed to:

Please contact CQUniversity's Office of Research (Tel: 07 4923 2603; E-mail: ethics@cqu.edu.au; Mailing address: Building 32, CQUniversity, Rockhampton, QLD, 4702) should there be any concerns about the nature and/or conduct of this research project.

This project has been approved by the CQUniversity Human Research Ethics Committee, approval number (21915).

Nutritional intervention to enhance sleep quality and quantity in athletes (PEP-1911)

I consent to participation in this research project and agree that:

13. I am aged 18 years or older;
14. An Information Sheet has been provided to me that I have read and understood. Any questions I have had about the project have been answered to my satisfaction by the Information Sheet and any further verbal explanation;
15. I understand that I have the right to withdraw from the project at any time without penalty;
16. I understand the statement concerning compensation for taking part in the study, which is contained in the Information Sheet;
17. I understand that to preserve anonymity and maintain confidentiality of participants, no personally identifiable information will be used when transferring data to the sponsor or in any publication(s);
18. I understand the research findings will be included in the researchers' publication(s) on the project, and these publications may include articles written for conferences, journals as well as other methods of dissemination;
19. I understand that some of my data may be shared with a third party. I understand that my data will be de-identified and the third party will not be able to contact me or identify me.
20. I acknowledge that the results of the study are unknown and that I may experience some skin irritation and/or minor discomfort due exercise and the equipment used to monitor my sleep. I may experience some gastrointestinal discomfort following consumption of the sleep drink. This list of symptoms is not exhaustive;
21. I acknowledge that I have been advised that I should avoid the following activities: driving a car, riding a bike, and/or operating heavy machinery, following the completion of the study, until I have obtained at least one full night of sleep (uninterrupted for 9 hours or more);
22. I have not experienced any COVID-19 symptoms in the last 14 days, nor have I tested positive for COVID-19 in the last 14 days. I acknowledge that I have been provided information on the COVID-safe plan and agree to abide by these guidelines;
23. I release and indemnify the University, its employees, students and agents against liability in respect of all claims, costs and expenses and for all loss, damage, injury or death to persons or property caused or contributed by me in connection with my failure to follow the after study instructions;
24. I have discussed the risks of the study with, and I have had the opportunity to seek advice from someone independent from the study such as a relative, friend, doctor or lawyer.

Project Title:

Nutritional intervention to enhance sleep quality and quantity in athletes

Signature of participant: _____ Date: _____

Name (please print): _____

Signature of researcher:_____ Date: _____

Name (please print): _____

	YES	NO
I wish to have a Plain English statement of results sent to me.		

Appendix 3: Pre-screening questionnaires

Pittsburgh Sleep Quality Index

PITTSBURGH SLEEP QUALITY INDEX (PSQI)

INSTRUCTIONS: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the past month, when have you usually gone to bed at night?

   USUAL BED TIME _____

2. During the past month, how long (in minutes) has it usually take you to fall asleep each night?

   NUMBER OF MINUTES _____

3. During the past month, when have you usually gotten up in the morning?

   USUAL GETTING UP TIME _____

4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spend in bed.)

   HOURS OF SLEEP PER NIGHT _____

INSTRUCTIONS: For each of the remaining questions, check the one best response. Please answer all questions.

5. During the past month, how often have you had trouble sleeping because you...

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
(a) ...cannot get to sleep within 30 minutes	☐	☐	☐	☐
(b) ...wake up in the middle of the night or early morning	☐	☐	☐	☐
(c) ...have to get up to use the bathroom	☐	☐	☐	☐
(d ...cannot breathe comfortably	☐	☐	☐	☐
(e) ...cough or snore loudly	☐	☐	☐	☐
(f) ...feel too cold	☐	☐	☐	☐
(g) ...feel too hot	☐	☐	☐	☐
(h) ...had bad dreams	☐	☐	☐	☐
(i) ...have pain	☐	☐	☐	☐
(j) Other reason(s), please describe _____ _____				
How often during the past month have you had trouble sleeping because of this?	☐	☐	☐	☐

PSQI Page 1

	Very good	Fairly good	Fairly bad	very bad
6. During the past month, how would you rate your sleep quality overall?	☐	☐	☐	☐

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
7. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?	☐	☐	☐	☐
8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?	☐	☐	☐	☐

	No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?	☐	☐	☐	☐

	No bed partner or roommate	Partner/ roommate in other room	Partner in same room, but not same bed	Partner in same bed
10. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?	☐	☐	☐	☐

If you have a roommate or bed partner, ask him/her how often in the past month you have had...

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
(a) ...loud snoring	☐	☐	☐	☐
(b) ...long pauses between breaths while asleep	☐	☐	☐	☐
(c) ...legs twitching or jerking while you sleep	☐	☐	☐	☐
(d) ...episodes of disorientation or confusion during sleep	☐	☐	☐	☐
(e) Other restlessness while you sleep; please describe	☐	☐	☐	☐

_____

_____

SCORING INSTRUCTIONS FOR THE PITTSBURGH SLEEP QUALITY INDEX:

The Pittsburgh Sleep Quality Index (PSQI) contains 19 self-rated questions and 5 questions rated by the bed partner or roommate (if one is available). Only self-rated questions are included in the scoring. The 19 self-rated items are combined to form seven "component" scores, each of which has a range of 0-3 points. In all cases, a score of "0" indicates no difficulty, while a score of "3" indicates severe difficulty. The seven component scores are then added to yield one "global" score, with a range of 0-21 points, "0" indicating no difficulty and "21 " indicating severe difficulties in all areas.

Scoring proceeds as follows:

Component 1: Subjective sleep quality

Examine question #6, and assign scores as follows:

Response	Component 1 score
"Very good"	0
"Fairly good"	1
"Fairly bad"	2
"Very bad"	3

*Component 1 score:*_____

Component 2: Sleep latency

1. Examine question #2, and assign scores as follows:

Respo\nse	Score
≤15 minutes	0
16-30 minutes	1
31-60 minutes	2
> 60 minutes	3

Question #2 score: _____

2. Examine question #5a, and assign scores as follows:

Response	Score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Question #5a score: _____

3. Add #2 score and #5a score

Sum of #2 and #5a: _____

4. Assign component 2 score as follows:

Sum of #2 and #5a	Component 2 score
0	0
1-2	1
3-4	2
5-6	3

*Component 2 score:*_____

Component 3: Sleep duration

Examine question #4, and assign scores as follows:

Response	Component 3 score
> 7 hours	0
6-7 hours	1
5-6 hours	2
< 5 hours	3

Component 3 score: _____

Component 4: Habitual sleep efficiency

1. Write the number of hours slept (question #4) here: _____
2. Calculate the number of hours spent in bed:

   Getting up time (question #3): _____

   Bedtime (question #1): _____

   _____

   Number of hours spent in bed: _____

3. Calculate habitual sleep efficiency as follows:

   (Number of hours slept/Number of hours spent in bed) X 100 = Habitual sleep efficiency (%)

   (_____/_____) X 100 = %

4. Assign component 4 score as follows:

Habitual sleep efficiency %	Component 4 score
> 85%	0
75-84%	1
65-74%	2
< 65%	3

Component 4 score: _____

Component 5: Step disturbances

1. Examine questions #5b-5j, and assign scores for each question as follows:

Response	Score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

5b score: _____
5c score: _____
5d score: _____
5e score: _____
5f score: _____
5g score: _____
5h score: _____
5i score: _____
5j score: _____

2. Add the scores for questions #5b-5j:

Sum of #5b-5j: _____

3. Assign component 5 score as follows:

Sum of #5b-5j	Component 5 score
0	0
1-9	1
10-18-4	2
19-27	3

Component 5 score: _____

Component 6: Use of sleeping medication

Examine question #7 and assign scores as follows:

Response	Component 6 score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Component 6 score: _____

Component 7: Daytime dysfunction

1. Examine question #8, and assign scores as follows:

Response	Score
Never	0
Once or twice	1
Once or twice each week	2
Three or more times each week	3

Question#8 score: _____

2. Examine question #9, and assign scores as follows:

Response	Score
No problem at all	0
Only a very slight problem	1
Somewhat of a problem	2
A very big problem	3

Question #9 score: _____

3. Add the scores for question #8 and #9:

Sum of #8 and #9: _____

4. Assign component 7 score as follows:

Sum of #8 and #9	Component 7 score
0	0
1-2	1
3-4	2
5-6	3

Component 7 score: _____

Global PSQI Score

Add the seven component scores together:

Global PSQI Score: _____

Munich ChronoType Questionnaire (MCTQ)

Instructions:
In this questionnaire, you report on your typical sleep behaviour over the past 4 weeks. We ask about work days and work-free days separately. Please respond to the questions according to your perception of a standard week that includes your usual work days and work-free days.

Personal Data

Date: _____

Name: _____

eMail: _____

Age: _____ years

Sex: female ☐ male ☐

Height: _____ cm

Weight: _____ kg

Country: _____

City: _____

Postal Code: _____

Participant ID:

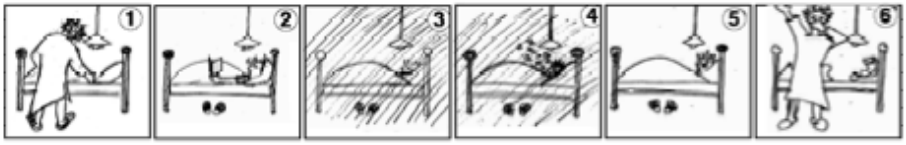
MCTQ

I have a regular work schedule (this includes being, for example, a housewife or househusband):

Yes ☐ I work on 1☐ 2☐ 3☐ 4☐ 5☐ 6☐ 7☐ day(s) per week.

No ☐

Is your answer "*Yes, on 7 days*" or "*No*", please consider if your sleep times may nonetheless differ between regular 'workdays' and 'weekend days' and fill out the MCTQ in this respect.



Please use 24-hour time scale (e.g. 23:00 instead of 11:00 pm)!

Workdays

Image 1: I go to bed at _____ o'clock.

Image 2: Note that some people stay awake for some time when in bed!

Image 3: I actually get ready to fall asleep at _____ o'clock.

Image 4: I need _____ minutes to fall asleep.

Image 5: I wake up at _____ o'clock.

Image 6: After _____ minutes I get up.

I use an alarm clock on workdays: Yes ☐ No ☐

If "Yes": I regularly wake up BEFORE the alarm rings: Yes ☐ No ☐

Free Days

Image 1: I go to bed at _____ o'clock.

Image 2: Note that some people stay awake for some time when in bed!

Image 3: I actually get ready to fall asleep at _____ o'clock.

Image 4: I need _____ minutes to fall asleep.

Image 5: I wake up at _____ o'clock.

Image 6: After _____ minutes I get up.

My wake-up time (Image 5) is due to the use of an alarm clock: Yes ☐ No ☐

There are particular reasons why I cannot freely choose my sleep times on free days:

Yes ☐ If "Yes": Child(ren)/pet(s) ☐ Hobbies ☐ Others ☐, for example:_____

No ☐

Participant ID:

MCTQ, English, Version 2015-01

Work Details

In the last 3 months, I worked as a shift worker.

No ☐ Yes ☐ (please continue with "*My work schedules are ...*").

My usual work schedule …

… starts at _____ o´clock.

… ends at _____ o´clock.

My work schedules are …

… very flexible ☐ … a little flexible ☐ … rather inflexible ☐ … very inflexible ☐

I travel to work …

… within an enclosed vehicle (e.g. car, bus, underground). ☐

… not within an enclosed vehicle (e.g. on foot, by bike). ☐

I work at home. ☐

For the commute to work, I need ___ hours and ___ minutes.

For the commute from work, I need ___ hours and ___ minutes.

Time Spent Outdoors

On average, I spend the following amount of time outdoors in daylight (without a roof above my head):

on workdays: _____ hours _____ minutes

on free days: _____ hours _____ minutes

Participant ID:

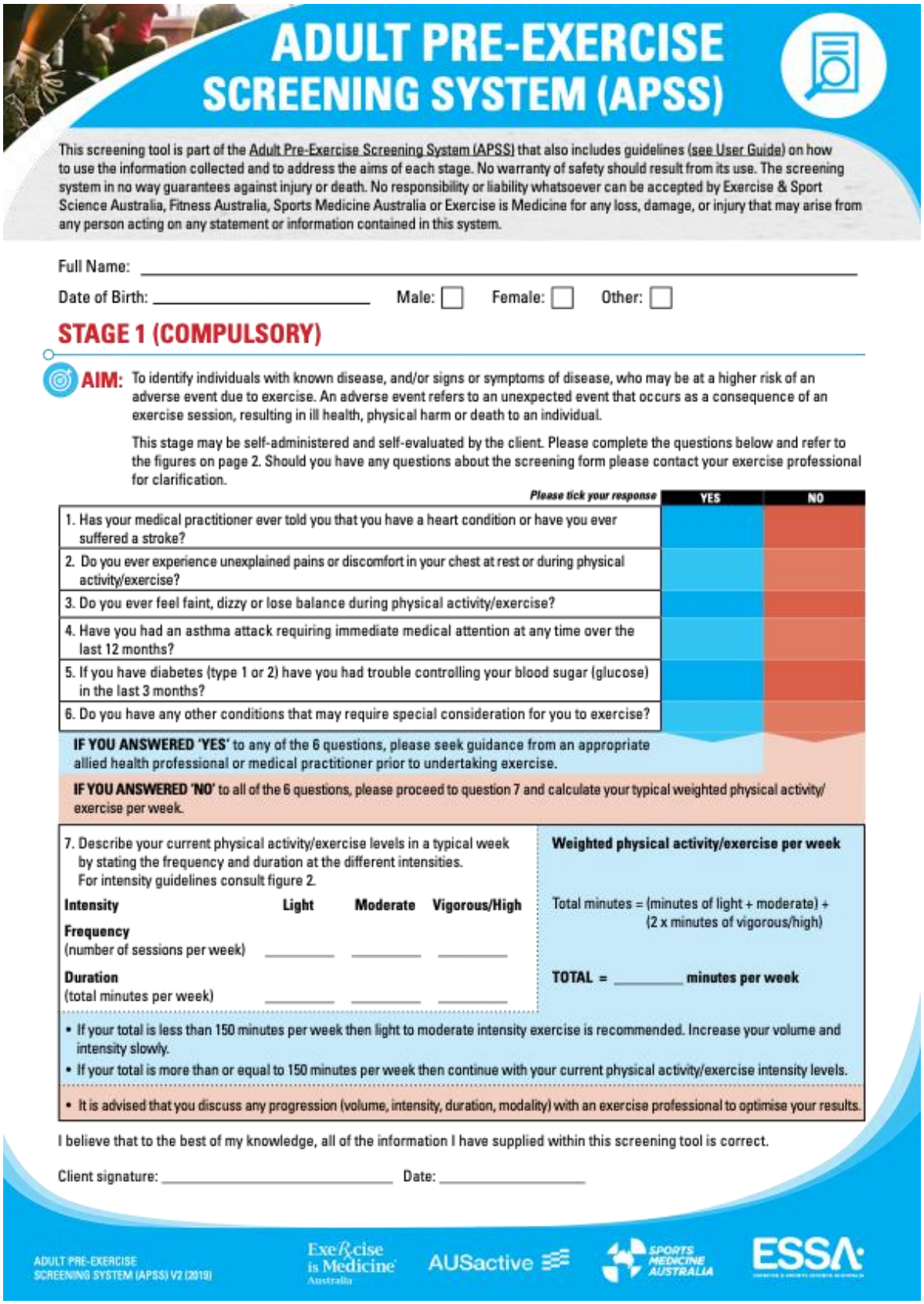
Stimulants

Please give approximate/average amounts!

		per →	day	/ week	/ month
I smoke	_____ cigarettes ...		☐	☐	☐
I drink	_____ glasses of beer ...		☐	☐	☐
I drink	_____ glasses of wine ...		☐	☐	☐
I drink	_____ glasses of liquor/whiskey/gin etc. ...		☐	☐	☐
I drink	_____ cups of coffee …		☐	☐	☐
I drink	_____ cups of black tea ...		☐	☐	☐
I drink	_____ cans of caffeinated drinks (soft-drinks) …		☐	☐	☐
I take sleep medication _____ times ...			☐	☐	☐

Participant ID:

Exercise and Sport Science Australia's Adult Pre-Exercise Screening System



ADULT PRE-EXERCISE SCREENING SYSTEM (APSS)

This screening tool is part of the Adult Pre-Exercise Screening System (APSS) that also includes guidelines (see User Guide) on how to use the information collected and to address the aims of each stage. No warranty of safety should result from its use. The screening system in no way guarantees against injury or death. No responsibility or liability whatsoever can be accepted by Exercise & Sport Science Australia, Fitness Australia, Sports Medicine Australia or Exercise is Medicine for any loss, damage, or injury that may arise from any person acting on any statement or information contained in this system.

Full Name: _____

Date of Birth: _____ Male: ☐ Female: ☐ Other: ☐

STAGE 1 (COMPULSORY)

AIM: To identify individuals with known disease, and/or signs or symptoms of disease, who may be at a higher risk of an adverse event due to exercise. An adverse event refers to an unexpected event that occurs as a consequence of an exercise session, resulting in ill health, physical harm or death to an individual.

This stage may be self-administered and self-evaluated by the client. Please complete the questions below and refer to the figures on page 2. Should you have any questions about the screening form please contact your exercise professional for clarification.

Please tick your response	YES	NO
1. Has your medical practitioner ever told you that you have a heart condition or have you ever suffered a stroke?		
2. Do you ever experience unexplained pains or discomfort in your chest at rest or during physical activity/exercise?		
3. Do you ever feel faint, dizzy or lose balance during physical activity/exercise?		
4. Have you had an asthma attack requiring immediate medical attention at any time over the last 12 months?		
5. If you have diabetes (type 1 or 2) have you had trouble controlling your blood sugar (glucose) in the last 3 months?		
6. Do you have any other conditions that may require special consideration for you to exercise?		

IF YOU ANSWERED 'YES' to any of the 6 questions, please seek guidance from an appropriate allied health professional or medical practitioner prior to undertaking exercise.

IF YOU ANSWERED 'NO' to all of the 6 questions, please proceed to question 7 and calculate your typical weighted physical activity/exercise per week.

7. Describe your current physical activity/exercise levels in a typical week by stating the frequency and duration at the different intensities. For intensity guidelines consult figure 2.

Intensity	Light	Moderate	Vigorous/High
Frequency (number of sessions per week)	_____	_____	_____
Duration (total minutes per week)	_____	_____	_____

Weighted physical activity/exercise per week

Total minutes = (minutes of light + moderate) + (2 x minutes of vigorous/high)

TOTAL = _____ minutes per week

- If your total is less than 150 minutes per week then light to moderate intensity exercise is recommended. Increase your volume and intensity slowly.
- If your total is more than or equal to 150 minutes per week then continue with your current physical activity/exercise intensity levels.
- It is advised that you discuss any progression (volume, intensity, duration, modality) with an exercise professional to optimise your results.

I believe that to the best of my knowledge, all of the information I have supplied within this screening tool is correct.

Client signature: _____ Date: _____

ADULT PRE-EXERCISE SCREENING SYSTEM (APSS) V2 (2019)

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FIGURE 1: Stage 1 Screening Steps

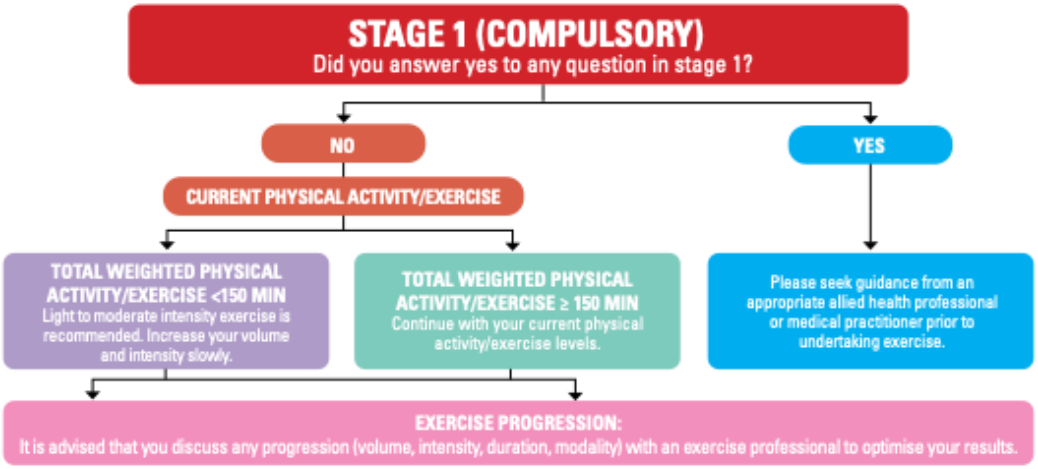

FIGURE 2: Exercise Intensity Guidelines

INTENSITY CATEGORY	HEART RATE MEASURES	PERCEIVED EXERTION MEASURES	DESCRIPTIVE MEASURES
LIGHT	40 to <55% HRmax*	VERY LIGHT TO LIGHT RPE# 1-2	• An aerobic activity that does not cause a noticeable change in breathing rate • An intensity that can be sustained for at least 60 minutes
MODERATE	55 to <70% HRmax*	MODERATE TO SOMEWHAT HARD RPE# 3-4	• An aerobic activity that is able to be conducted whilst maintaining a conversation uninterrupted • An intensity that may last between 30 and 60 minutes
VIGOROUS	70 to <90% HRmax*	HARD RPE# 5-6	• An aerobic activity in which a conversation generally cannot be maintained uninterrupted • An intensity that may last up to 30 minutes
HIGH	≥ 90% HRmax*	VERY HARD RPE# 7	• An aerobic activity in which it is difficult to talk at all • An intensity that generally cannot be sustained for longer than about 10 minutes

* HRmax = estimated heart rate maximum. Calculated by subtracting age in years from 220 (e.g. for a 50 year old person = 220 - 50 = 170 beats per minute).

= Borg's Rating of Perceived Exertion (RPE) scale, category scale 0-10.

Modified from Norton K, L. Norton & D. Sadgrove. (2010). Position statement on physical activity and exercise intensity terminology. J Sci Med Sport 13, 496-502.

ADULT PRE-EXERCISE SCREENING SYSTEM (APSS) V2 (2019)

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STAGE 2 (RECOMMENDED)

AIM: This stage is to be completed with an exercise professional to determine appropriate exercise prescription based on established risk factors.

CLIENT DETAILS	GUIDELINES FOR ASSESSING RISK
8. Demographics Age: _____ Male ☐ Female ☐ Other ☐	Risk of an adverse event increases with age, particularly males ≥ 45 yr and females ≥ 55 yr.
9. Family history of heart disease (e.g. stroke, heart attack)? Relationship (e.g. father) Age at heart disease event _____ _____ _____ _____ _____ _____	A family history of heart disease refers to an event that occurs in relatives including parents, grandparents, uncles and/or aunts before the age of 55 years.
10. Do you smoke cigarettes on a daily or weekly basis or have you quit smoking in the last 6 months? Yes ☐ No ☐ If currently smoking, how many per day or week? _____	Smoking, even on a weekly basis, substantially increases risk for premature death and disability. The negative effects are still present up to at least 6 months post quitting.
11. Body composition Weight (kg) _____ Height (cm) _____ Body Mass Index (kg/m²) _____ Waist circumference (cm) _____	Any of the below increases the risk of chronic diseases: BMI ≥ 30 kg/m² Waist > 94 cm male or > 80 cm female
12. Have you been told that you have high blood pressure? Yes ☐ No ☐ If known, systolic/diastolic (mmHg) _____ Are you taking any medication for this condition? Yes ☐ No ☐ If yes, provide details _____	Either of the below increases the risk of heart disease: Systolic blood pressure ≥ 140 mmHg Diastolic blood pressure ≥ 90 mmHg
13. Have you been told that you have high cholesterol/ blood lipids? Yes ☐ No ☐ If known: Total cholesterol (mmol/L) _____ HDL (mmol/L) _____ LDL (mmol/L) _____ Triglycerides (mmol/L) _____ Are you taking any medication for this condition? Yes ☐ No ☐ If yes, provide details _____	Any of the below increases the risk of heart disease: Total cholesterol ≥ 5.2 mmol/L HDL < 1.0 mmol/L LDL ≥ 3.4 mmol/L Triglycerides ≥ 1.7 mmol/L

CLIENT DETAILS	GUIDELINES FOR ASSESSING RISK
14. Have you been told that you have high blood sugar (glucose)? Yes ☐ No ☐ If known: Fasting blood glucose (mmol/L) _____ Are you taking any medication for this condition? Yes ☐ No ☐ If yes, provide details _____	Fasting blood sugar (glucose) ≥ 5.5 mmol/L increases the risk of diabetes.
15. Are you currently taking prescribed medication(s) for any condition(s)? These are additional to those already provided. Yes ☐ No ☐ If yes, what are the medical conditions? _____	Taking medication indicates a medically diagnosed problem. Judgment is required when taking medication information into account for determining appropriate exercise prescription because it is common for clients to list 'medications' that include contraceptive pills, vitamin supplements and other non-pharmaceutical tablets. Exercise professionals are not expected to have an exhaustive understanding of medications. Therefore, it may be important to use common language to describe what medical conditions the drugs are prescribed for.
16. Have you spent time in hospital (including day admission) for any condition/illness/injury during the last 12 months? Yes ☐ No ☐ If yes, provide details _____	There are positive relationships between illness rates and death versus the number and length of hospital admissions in the previous 12 months. This includes admissions for heart disease, lung disease (e.g., Chronic Obstructive Pulmonary Disease (COPD) and asthma), dementia, hip fractures, infectious episodes and inflammatory bowel disease. Admissions are also correlated to 'poor health' status and negative health behaviours such as smoking, alcohol consumption and poor diet patterns.
17. Are you pregnant or have you given birth within the last 12 months? Yes ☐ No ☐ If yes, provide details _____ _____ _____	During pregnancy and after recent childbirth are times to be more cautious with exercise. Appropriate exercise prescription results in improved health to mother and baby. However, joints gradually loosen to prepare for birth and may lead to an increased risk of injury especially in the pelvic joints. Activities involving jumping, frequent changes of direction and excessive stretching should be avoided, as should jerky ballistic movements. Guidelines/fact sheets can be found here: 1) www.exerciseismedicine.com.au 2) www.fitness.org.au/Pre-and-Post-Natal-Exercise-Guidelines
18. Do you have any diagnosed muscle, bone, tendon, ligament or joint problems that you have been told could be made worse by participating in exercise? Yes ☐ No ☐ If yes, provide details _____ _____	Almost everyone has experienced some level of soreness following unaccustomed exercise or activity but this is not really what this question is designed to identify. Soreness due to unaccustomed activity is not the same as pain in the joint, muscle or bone. Pain is more extreme and may represent an injury, serious inflammatory episode or infection. If it is an acute injury then it is possible that further medical guidance may be required.

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