The effect of creatine monohydrate loading on cognitive performance, mood, sleepiness, and perceived workload following sleep restriction

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science in Nutrition and Dietetics

Massey University, Albany

New Zealand

Juliette Victoire Constance Janvresse

2023

Abstract

Background: Sleep restriction (SR; i.e. not getting enough sleep per 24 hours for one or multiple nights) impairs cognitive performance and mood, which can compromise capability and safety. SR-related performance deficits may be underpinned by disruptions to brain energy metabolism. Sleep loss appears to reduce brain phosphorylcreatine (PCr; also called creatine phosphate and phosphocreatine), which supports the regeneration of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). Since creatine supplementation increases tissue PCr, including in the brain, it may mitigate possible SR-related cognitive impairments.

Aim: To assess the effect of a 7-day creatine monohydrate (CrM) loading protocol on cognitive performance, mood, subjective sleepiness, and perceived workload following one night of SR (3 hours time-in-bed; TIB).

Methods: A double-blinded, randomised, placebo-controlled cross-over trial was conducted with 7 healthy participants (3 men and 4 women); age, 28.0 ± 4.6 years (range, 22-35 years). Participants ingested 20 $g \cdot day^{-1}$ of CrM or placebo (i.e. tapioca) divided into 4 equal 5 g doses for 7 days followed by a single SR night (3 hours TIB). Cognitive performance (10-minute Psychomotor Vigilance Task; PVT), mood (fatigue and vigour), subjective sleepiness, and perceived workload were measured following ~8 hours of sleep prior to creatine supplementation and following the creatine loading protocol and SR. A minimum of a 5-week wash-out period separated the two trial arms. Linear mixed effects models were employed to analyse data.

Results: SR slowed mean reciprocal reaction time (p = 0.01) and mean 10% fastest reaction time (p = 0.02), increased fatigue (p = 0.004), subjective sleepiness (p = 0.009), workload (p = 0.02), and reduced vigour (p <0.001); however, it did not alter the number of lapses (p = 0.60) and mean 10% slowest reaction time (p = 0.60). CrM loading had no effect on cognitive performance, mood, subjective sleepiness, or workload.

Conclusion: A single night of SR (3 hours TIB) negatively affected aspects of PVT performance, mood, subjective sleepiness, and perceived workload; however, CrM loading (20 g·day^{-1} for 7 days) did not appear to mitigate the effects of SR.

Key words: sleep loss; psychomotor vigilance task; randomised cross-over trial

Acknowledgement

This thesis has been a very challenging journey, and I want to express my gratitude toward all who were directly and indirectly involved in this study.

First, I would like to thank my supervisors Dr David Shaw and Dr Margo van den Berg. Although the research has not always gone to plan, your expertise, invaluable feedback and support throughout this process will always be appreciated. Thank you for the time and the effort you put into this research.

I would like to thank all the participants who generously offered their time and accepted to take part of this challenging protocol. Thank you for making this study possible.

To my mum, dad, sister, Manon and my best friend, Mathilde, who despite living on the other side of the planet, reassured me and gave me strength when I needed it. Thank you for your continued support and encouragement.

Last but not least, I want to thank my wonderful partner, Sumit. Without you, I doubt I would have managed to finish this thesis, and I can't thank you enough for all the financial, emotional, and technical support you provided me over the intense months of work. Thank you for always believing in me.

Table of Contents

List of Figures

List of Tables

1 Introduction

1.1 Background

Obtaining an adequate amount of sleep (7 to 9 hours for most adults) is essential for optimal well-being and performance (Hirshkowitz et al., 2015; Watson et al., 2015). However, due to work schedules, long duty hours, personal or family commitments, social and sporting engagements, as well as individuals' life choices or medical conditions (Banks et al., 2017; McLellan et al., 2016; Rupp et al., 2012), a significant proportion (37%) of the population regularly fail to achieve sleep recommendations (Lee & Sibley, 2019). Sleep restriction (SR; i.e. not getting enough sleep per 24 hours for one or multiple nights) increases fatigue, sleepiness and impairs cognitive performance and mood (Banks et al., 2017; Lowe et al., 2017). Unintentional human errors are estimated to contribute between 30% to 90% of all serious incidents across industries (Reason, 1990), with fatigue and sleepiness being primary causes (Dinges, 1995). This is a concern in work areas affected by shift work, such as military, healthcare and transport, as it can compromise capability and safety (Caldwell et al., 2019; McLellan et al., 2016). Strategies to mitigate the detrimental effects of inadequate sleep on cognition are required.

The brain is a highly metabolic organ, consuming ~20-25% of the resting metabolic rate in humans (Bailey, 2019), and therefore requires a constant energy supply. Following sleep loss, brain energy metabolism is disrupted, which likely underpins some cognitive impairments (Engle-Friedman, 2014; Thomas et al., 2000). Glucose is the primary energy source for the brain (Dienel, 2019), but it can also utilise alternative non-oxidisable substrates, such as phosphorylcreatine (PCr) (Greenhaff, 2001; Walker, 1979), to generate adenosine triphosphate (ATP), the energy currency of the cell. ATP is hydrolysised to adenosine diphosphate (ADP) and inorganic phosphate to produce energy (Greenhaff, 2001). The re-synthesis of ATP is modulated by PCr due to its ability to donate a phosphate group to adenosine di-phosphate (ADP) via the enzyme creatine kinase (Greenhaff, 2001; Walker, 1979). PCr is synthesised by phosphorylating creatine using the same enzyme, creatine kinase, in a reverse reaction (Greenhaff, 2001; Walker, 1979). The availability of PCr, however, can decline when energy demand is increased, such as during exhausting mental tasks, or following sleep loss (Dworak et al., 2010; Lyoo et al., 2003; Plante et al., 2014). Increasing brain

creatine, and therefore PCr stores, is a possible strategy to mitigate cognitive impairments following sleep loss, which can be achieved by supplementing oral intake of creatine (Dworak et al., 2017; McMorris et al., 2007; McMorris et al., 2006).

Creatine is a naturally occurring guanidine compound involved in storage and transport of energy (Walker, 1979). Creatine is mostly found in muscles due to their large mass, but it is also stored in the brain (Rae & Bröer, 2015). Creatine comes from dietary sources (mostly meat and fish) and endogenous synthesis (Andres et al., 2008; East, 2002; Wallimann et al., 2007). Creatine can also be ingested as a supplement, usually in the form of creatine monohydrate (CrM), to augment tissue concentrations (Kreider et al., 2017). CrM supplementation is safe (European Food Safety Acency, 2004; Kim et al., 2011) and typically ingested over several days-to-weeks (i.e. CrM loading). Whereas dietary creatine does not seem to influence brain creatine stores (Kaviani et al., 2020; Solis et al., 2014), CrM loading protocols have demonstrated to increase brain creatine and PCr stores by 4.7 to 11.5% (depending on the brain areas), and 9.2%, respectively (Dechent et al., 1999; Turner et al., 2015). The benefits of creatine supplementation on cognitive performance and mood seem most pronounced under physiological stress, such as demanding cognitive tasks (van Cutsem et al., 2020; Watanabe et al., 2002), sleep deprivation (SD; i.e. no sleep) (McMorris et al., 2007; McMorris et al., 2006) and hypoxia (Turner et al., 2015). Mitigating cognitive fatigue with creatine supplementation may also reduce perceived workload associated with demanding cognitive tasks, yet such effects are still to be elucidated (van Cutsem et al., 2020).

Whether creatine supplementation can support cognition following SR remains uncertain, but as SR is more common within most communities than total SD, findings would be highly relevant. A single night of SR (i.e. ~5 hours time-in-bed; TIB) impairs performance across multiple cognitive domains (Lowe et al., 2017), particularly simple and monotonous tasks, such as those assessing vigilance (Durmer & Dinges, 2005). Vigilance can be measured using the Psychomotor Vigilance Task (PVT) (Dinges & Powell, 1985), which is a simple, valid, and reliable measure of the effects of sleep loss, and circadian rhythm in cognitive performance (Lim & Dinges, 2008, 2010). Supporting measures of subjective sleepiness, mood (fatigue and vigour), and perceived workload would complement observations on cognitive performance. To the author's knowledge, however, the effects of creatine supplementation on cognition following SR have not yet been investigated.

1.2 Research aims

The aim of this research thesis is to assess the effect of a 7-day CrM loading protocol on psychomotor vigilance performance, mood (vigour and fatigue), subjective sleepiness, and perceived workload following one night of SR (3 hours TIB) in healthy men and women.

1.3 Objectives

The objectives of this research thesis are to examine whether 7 days of ingesting 20 $g \cdot day^{-1}$ of CrM affects aspects of cognition following a single night of SR (3 hours TIB), including:

- Simple cognitive performance (vigilance and sustained attention), using a 10-minute version of the PVT (PVT-192).
- Mood (fatigue and vigour), using a the validated computerised ANAM® mood scale.
- Subjective sleepiness, using the Stanford Sleepiness Scale.
- Perceived workload, using the NASA Task Load Index (NASA-TLX)

1.4 Hypothesis

1. A single sleep restriction night (3 hours TIB) will negatively affect cognitive performance, mood, subjective sleepiness, and perceived workload.
2. CrM loading will mitigate the adverse effects of a single night of sleep restriction (3 hours TIB) on cognitive performance, mood, subjective sleepiness, and perceived workload.

1.5 Structure of the thesis

This thesis consists of four chapters. The first chapter is an introduction identifying the purpose of the study. Chapter two is a narrative literature review on sleep, the effect of SR on cognition and brain energy metabolism, creatine supplementation, and the effects of creatine supplementation on cognition and possible implications for SR. Chapter three is the research manuscript, providing a complete presentation of the study, followed by the discussion of the findings and the conclusion. The fourth and final chapter is a conclusion, including the strengths and limitations of the study, potential application of observations and suggestions for future research. Appendices, including participant information sheet, participant calendar, and caffeine handout are added at the end of the thesis.

1.6 Contributors to the research

Table 1.1: Researchers' Contributions.

Researchers	Contributions
Dr David Shaw, PhD Academic main supervisor	Main contributor to study design, ethics application, participant recruitment, study implementation and data collection; creation of the R script for data analyses; academic support of the thesis write up.
Dr Margo van den Berg, PhD Academic co-supervisor	Provision of actigraphs and PVT devices; scoring of participants' actigraphy data; interpretation of results; academic support of the thesis write up.
Juliette Janvresse, Student Dietitian	Review of literature; creation of participant resource (i.e. calendar); study implementation and data collection; data processing and interpretation; author of the thesis.
Associate Professor Nick Gant, PhD	Guidance on the use of creatine supplementation and use of the Exercise Neurometabolism Laboratory, University of Auckland, to create the creatine and placebo supplements.
Peter Bloomfield, PhD Candidate	Data collection.

2 Literature Review

2.1 Sleep

Sleep is essential for optimal health and performance (Hirshkowitz et al., 2015; Watson et al., 2015). Sleep is "a reversible behavioural state of perceptual disengagement from and unresponsiveness to the environment" (Carskadon & Dement, 2017, p. 1). Although the function of sleep is not completely understood, sleep seems to be required for brain processing, memory consolidation and restoring brain energy stores (Inoué et al., 1995; Stickgold, 2005; Xie et al., 2013). There are large individual differences in sleep need (Dennis et al., 2017; Van Dongen et al., 2004) with some individuals requiring up to 10 hours to function optimally, whereas others need as few as 6 hours (Hirshkowitz et al., 2015). However, the recommended optimal sleep duration for adults (18 to 64 years old) is between 7 to 9 hours per night on a regular basis (Hirshkowitz et al., 2015; Watson et al., 2015). Unfortunately, lack of sleep is commonly experienced in our society due to social drivers and work demands (Chatzitheochari & Arber, 2009; Lee & Sibley, 2019). Indeed, in a recent survey, 37% of adults in New Zealand reported achieving less than the minimum recommended sleep duration (Lee & Sibley, 2019). People working in occupations and industries such as the military, medical care, transport, search and rescue, are also at risk of inadequate sleep due to work schedules and long duty hours overlapping with the person's normal sleep periods (McLellan et al., 2016). Inadequate sleep increases fatigue and sleepiness, which impairs cognitive performance and vigilance, thus increasing the risk of errors and accidents (Banks et al., 2017; Caldwell et al., 2019; van Dongen et al., 2017). Strategies mitigating the adverse effects of sleep loss are therefore warranted.

2.1.1 Structure of sleep

There are two distinct states of sleep; rapid-eye movement (REM) and non-rapid eye movement (NREM) sleep (Patel et al., 2021). These alternate cyclically, with each sleep cycle lasting about 90 minutes, although cycle duration varies across the sleep period and between individuals (Březinová, 1974). NREM sleep is further divided into 3 stages called N1, N2 and N3, based on brainwave characteristics (i.e. the electrical activity occurring in the brain), eye movements and muscle tone. Stage N1 is the lightest, and stage N3 is the deepest stage of sleep. Stage N3, also referred to as slow wave sleep (SWS) or deep sleep, is characterised by delta waves, or slow-wave activity. REM sleep is characterised by complete loss of muscle

tone, bursts of rapid eye movements, and dreaming (Carskadon & Dement, 2017). During REM sleep, brainwave activity looks similar to waking brainwave activity (Carskadon & Dement, 2017).

2.1.2 Measuring sleep

Polysomnography (PSG) is considered to be the gold standard for measuring sleep (de Souza et al., 2003). PSG records cortical brain wave activity (electro-encephalogram), eye movements (electro-oculogram), and surface muscle tone (electromyogram), which are used to determine sleep onset latency, sleep efficiency, total sleep duration and sleep stages (McCall & McCall, 2012). PSG is often performed in a laboratory setting and is not practical for recording sleep across multiple days in field settings as it is expensive, time consuming and setting up the recording requires prior training (Arnal et al., 2020; Chinoy et al., 2021). Alternatively, actigraphy can be used to measure sleep, but unlike PSG, it cannot measure sleep states (McCall & McCall, 2012). Actigraphy is inexpensive and can easily be used for recording sleep-wake periods across several days-to-weeks (Ancoli-Israel et al., 2003; Ancoli-Israel et al., 2015). An actigraph is placed on the non-dominant wrist and utilises an accelerometer to record activity. One minute is an epoch length commonly used, and when the device is set to record data in 1-minute epochs, movement is recorded as the number of activity counts per minute (i.e. wrist movement per epoch) (Ancoli-Israel et al., 2015). Sleep-wake cycles are then identified using a sleep-scoring algorithm which has been validated against PSG (Ancoli-Israel et al., 2015). The sensitivity of actigraphy (i.e. the ability of actigraphy to detect sleep when PSG did the same) is reported to be high and satisfactory (Marino et al., 2013). However, the specificity of actigraphy (i.e. the ability of actigraphy to correctly identify wakefulness) is reported to be low (de Souza et al., 2003; Sadeh, 2011). Actigraphy can overestimate total sleep time and efficiency, and underestimate sleep onset latency (Paquet et al., 2007). This overestimation is due to the limited capacity of actigraphy to detect wakefulness when an individual is immobile in bed, but not asleep (de Souza et al., 2003). Despite this, actigraphy has acceptable validity and reliability in healthy individuals with relatively good sleep patterns (Sadeh, 2011).

2.1.3 Sleep regulation

Two main processes have been proposed to regulate sleep: 1) the homeostatic drive for sleep (process S) and 2) the circadian drive (process C) (Borbély et al., 2016), as illustrated in Figure

2.1. The longer an individual is awake, the more slow-wave activity will be present in the next sleep period, suggesting that slow wave activity reflects the brain's need for sleep (Carskadon & Dement, 2017). Brain energy stores also predominantly replenish during SWS (Krueger et al., 2016). The homeostatic drive for sleep is thought to be triggered by the increased concentration of adenosine in the brain (Dworak et al., 2017; Reichert et al., 2022). Adenosine is a neurotransmitter produced by the increased breakdown of adenosine triphosphate (ATP) (Dworak et al., 2010). Adenosine inhibits the release of excitatory neurotransmitters, and, when adenosine accumulates during wakefulness, it decreases neuronal activity (Porkka-Heiskanen & Kalinchuk, 2011). Adenosine may also influence the circadian clock functioning, but it still needs to be further investigated (Reichert et al., 2022).

Circadian rhythms are daily biological cycles (the word 'circadian' comes from the Latin 'circa diem' = about a day), lasting approximately 24 hours (Duhart et al., 2021; Sharma & Kavuru, 2010). Circadian rhythms are generated by the body's internal master clock located within the suprachiasmatic nucleus (SCN) of the hypothalamus, and peripheral clocks located elsewhere in the brain and body (e.g. liver, lung, and skeletal muscle) (Yamanaka, 2020). They control the daily rhythm of sleep and wakefulness, and most biological processes (e.g. hormone production, metabolism, body temperature and cell regeneration) (Garbarino, 2020). In the absence of external time cues, the SCN produces rhythms with a period that, on average, is slightly longer than 24 hours. Due to a process of active synchronisation or entrainment, the SCN's internal rhythm is able to keep pace with the external 24-hour light-dark cycle (Garbarino, 2020). Zeitgebers (meaning "time givers" in German) are time cues that regulate the SCN's internal rhythm, with light being the most important one (Borbély et al., 2016). Sleep/wake, resting/active and feeding/fasting cycles also contribute to synchronising the SCN and circadian rhythms (Beersma & Gordijn, 2007; Duhart et al., 2021; Garbarino, 2020). Shift work, insufficient sunlight exposure, nocturnal exposure to artificial light and travel across multiple time zones, disrupt circadian rhythms and impair sleep (Garbarino et al., 2020).

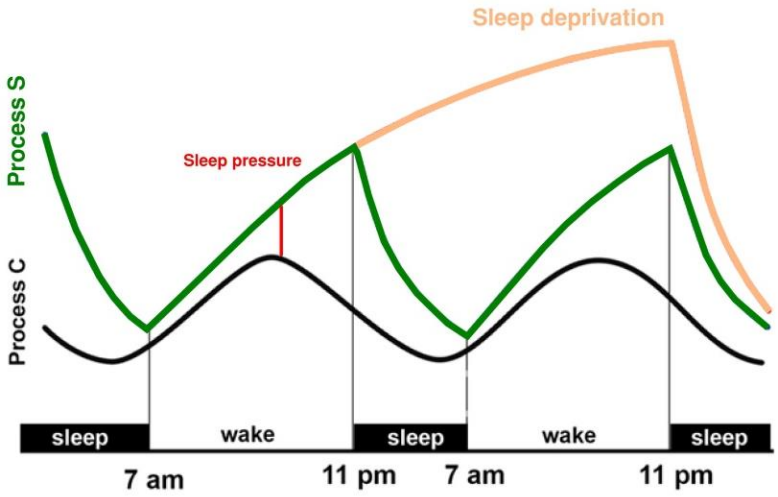


Figure 2.1: The Two-Process Model of Sleep Regulation (adapted from Patanaik, 2015).

This figure illustrates how process S (i.e. sleep homeostat) and process C (i.e. circadian process) interact. Whereas process C follows a pattern of an approximate 24-hour cycle, process S varies with sleep: it increases during the wake period and decreases during sleep. The difference between the two processes quantifies the sleep pressure. Sleep pressure increases with wakefulness and reduces with sleep (Borbély et al., 2016).

2.1.4 Sleep restriction

Sleep loss results from sleep restriction (SR) or total sleep deprivation (SD). To date, the majority of research has investigated SD as it generally induces large and consistent effects on cognition (Lim & Dinges, 2010). However, SR is experienced more frequently and by a greater proportion of the population (Banks et al., 2017; Rupp et al., 2012). Total SD is defined as being awake for a period beyond 16 to 18 hours (Banks et al., 2017), whereas SR is defined as not getting enough sleep per 24 hours for one or multiple nights (Banks et al., 2017). Insufficient sleep can be due to irregular work schedules, and long duty hours, personal or family commitments, social and sporting engagements, as well as individuals' life choices or medical conditions (Banks et al., 2017; McLellan et al., 2016).

Some individuals are more sensitive to sleep loss (Lowe et al., 2017), with resilience to sleep loss appearing to be a trait-like characteristic (Van Dongen et al., 2004), and cognitive domains affected by sleep loss may not be the same between individuals (Lowe et al., 2017). This

highlights the importance of controlling sleep duration prior to SR, or adjusting the magnitude of SR based on individuals prior sleep.

2.1.4.1 Sleep restriction, cognition, and mood

SR impairs cognitive functions and mood. In a recent meta-analysis by Lowe et al. (2017), SR was shown to compromise cognitive performance across multiple domains, particularly vigilance, reaction time, working memory and executive functions. The decline in cognitive performance following SR appears to have a dose-response effect (van Dongen et al., 2003). For example, Belenky et al. (2003) demonstrated that 7 days of either 3, 5, 7 hours TIB impaired 10-minute PVT performance (mean reciprocal response time; RRT, mean fastest 10% response time; RT, number of lapses) and increased subjective sleepiness compared with 9 hours TIB. In the 5 and 7 hours TIB conditions, PVT performance decreased for the initial 5 days before plateauing, whereas in the 3 hours TIB condition, PVT performance and subjective sleepiness continued to deteriorate (i.e. no stabilisation); however, sleepiness in the 5 and 7 hours TIB conditions did not increase (Belenky et al., 2003). Similarly, van Dongen et al. (2003) demonstrated that 4 and 6 hours TIB per night for 14 consecutive days cumulatively impaired 10-minute PVT performance (i.e. mean RRT, mean fastest 10% RT, number of lapses) and working memory measured using a computerised Digit Symbol Substitution Task relative to 8 hours TIB. Similarly to Belenky et al. (2003), subjective sleepiness increased markedly during the initial 2 days, but only exhibited minor increases across subsequent days. This suggests that increases in subjective sleepiness does not always reciprocate reductions in cognitive performance following sleep restriction.

It appears that a single night of SR is enough to affect cognition. Miyata et al. (2010) demonstrated that 4 hours TIB impaired reaction time and vigilance (assessed using a continuous performance test). Banks et al. (2010) also reported impaired performance on a 10-minute PVT (i.e. number of lapses and mean fastest 10% RT) and 3-minute Digit Symbol Substitution Task (i.e. number of correct responses), and increased sleepiness following the first of five nights of SR (4 hours TIB). These performance effects typically coincide with impaired mood. For example, Baum et al (2014) showed that mood and vigour (assessed using the Profile of Mood States questionnaire) deteriorated following a few days of sleep restriction (6.5 hours TIB for 5 nights). Additionally, Kahn et al. (2014) reported higher fatigue and reduced vigour (assessed using the Profile of Mood States questionnaire), and increased

omission and commission errors in an online continuous performance test following a single night of SR (4 hours TIB). Overall, a single night of SR is likely to impair multiple aspects of cognition and mood.

2.1.4.2 Vigilance, reaction time and Psychomotor Vigilance Task

Vigilance, or sustained attention, is the "ability of an individual to maintain concentrated attention on specific target stimuli over a prolonged period" (Kim et al., 2017, p. 343). Vigilance is sensitive to the effects of SR (Lim & Dinges, 2010), and is commonly measured using the PVT (Dinges & Powell, 1985). The PVT is a popular, valid and reliable measure of cognitive performance during SR and total SD, and is sensitive to both circadian and homeostatic influences (Lim & Dinges, 2008, 2010). The PVT is simple to use and has minimal practice effects as performance does not improve by repeating the test, making it a preferred method for measuring vigilance in many laboratory and field-based studies (Basner et al., 2018). The PVT requires a response to a visual stimulus that appears at random intervals, between 2 to 10 seconds, for a 10-minute test (Dinges & Powell, 1985). Shorter test durations often have a smaller inter-stimulus interval (e.g. 2-5 seconds) (Basner et al., 2018). The PVT can be easily administered via a computerised, handheld device and the most frequent measurements include reaction speed (reciprocal response time), mean 10% fastest and slowest reaction times and number of lapses (e.g. reaction times >500 ms).

2.1.4.3 Sleep loss, brain energy metabolism and high-energy phosphates

The brain is a highly metabolic organ, consuming ~20-25% of the resting metabolic rate in humans (Bailey, 2019). It is able to utilise various substrates for adenosine triphosphate (ATP) production (i.e. the energy currency of the cell), in particular glucose and, to a lesser extent, lactate, ketones and phosphorylcreatine (PCr). However, sleep loss disrupts brain energy metabolism, which may underpin impairments to cognitive functions. For example, during SD, the cerebral metabolic rate of glucose is reduced (Thomas et al., 2000) and glycogen (i.e. glucose polymer) may also decline (Kong et al., 2002). Recent interest, however, has focussed on the decline of PCr following sleep loss (Plante et al., 2014; Dowark et al., 2010), which is important as PCr acts by donating a phosphate group to adenosine diphosphate (ADP) to regenerate ATP following the hydrolysis of ATP to generate energy, ADP, and an inorganic phosphate (Pi). For example, Plante et al. (2014) sleep deprived 8 healthy adults (mean age 35

years old) for 40 hours and demonstrated with use of ^{31}P Magnetic Resonance Spectroscopy that PCr declined (albeit non-significantly) by 3.9%, then increased by 7.3% after the first night of recovery sleep, and increased by a further 10.1% after the second night of recovery sleep. These findings were supported by Gordji-Nejadet al. (2018), who demonstrated, also with use of ^{31}P Magnetic Resonance Spectroscopy, that cerebral PCr decreased during the day, but was restored after a 20-minute nap in 30 healthy adults (mean age 36 years old). Interestingly, in the same study, there was no significant change in cerebral ATP levels (Gordji-Nejad et al., 2018). In rodents, Dworak et al. (2010) demonstrated that PCr decreased by 57% in the frontal cortex after 3 hours of SD while creatine levels tended to increase compared to undisturbed time matched controls. Together, these findings indicate that PCr is depleted to maintain ATP levels during extended wakefulness.

2.1.5 Summary

SR is common within the population and its detrimental effects accumulate with increased sleep loss. SR increases the risk of mishaps and accidents, and in catastrophic situations can lead to loss of life. Cognitive deficits related to SR are potentially influenced by disruptions to brain energy metabolism. Sleep loss appears to reduce PCr levels, which may limit the resynthesis of ATP, particularly during fatiguing cognitive tasks. Therefore, manipulating brain PCr stores prior to SR may alter subsequent energy metabolism and cognitive function. This can be achieved via orally ingesting supplemental creatine.

2.2 What is creatine?

Creatine is a naturally occurring guanidine compound involved in storage and transport of energy (Figure 2.2) (Walker, 1979). It supports high-energy phosphate metabolism, which is important for maintaining homeostasis of ATP and appears to act as a cellular energy buffer (Walker, 1979). During periods of accelerated ATP degradation, such as when performing a cognitive-demanding task, creatine facilitates rapid energy provision by transferring a phosphate from PCr to ADP to regenerate ATP (Greenhaff, 2001; Walker, 1979). This reaction is reversibly catalysed by the enzyme, creatine kinase (CK) (Greenhaff, 2001; Walker, 1979). This energy pathway is typically referred to as the PCr/CK/ATP system and has been named the Lohman reaction (Figure 2.3). At energy producing sites in the cell, creatine is phosphorylated by CK to PCr, which joins the cell's PCr pool and is used to phosphyorylate adenine compounds, primarily ADP. PCr serves as a rapidly available reserve of high-energy

phosphates in the brain to recycle ATP. The generated ATP contributes to energy-required within the cell.

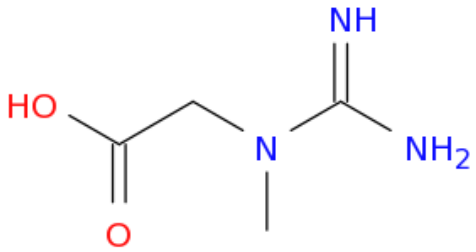


Figure 2.2: Creatine molecule.

Creatine is found mostly in muscle tissue, hence its name which derives from *kreas,* the Greek word for meat (Rae & Bröer, 2015). Creatine is also stored in other tissues, such as the heart and the brain (Jacobus & Diffley, 1986; Monge et al., 2008). Creatine supplements have been largely used by athletes and weightlifters to increase muscle creatine stores and enhance physical performance, particularly for explosive and high-intensity exercise (Kreider, 2003). Relative to muscle, the brain contains less than 5% of the total body creatine (i.e. total of ~120 g in a 70 kg human), but similarly, it is highly metabolically active and requires creatine for energy production (Gualano et al., 2010; Turner et al., 2015). This has led to research assessing how increasing brain creatine affects its function, particularly when under stress and in diseased states (Gualano et al., 2010; Turner et al., 2015). For example, creatine supplementation has been shown to mitigate symptoms (e.g. decline in vigilance) in situations where neural energy provision is impaired such as hypoxia (Turner et al., 2015). It is also possible that increasing brain creatine could be beneficial during other stressors that disrupt metabolic energy production, such as sleep loss.

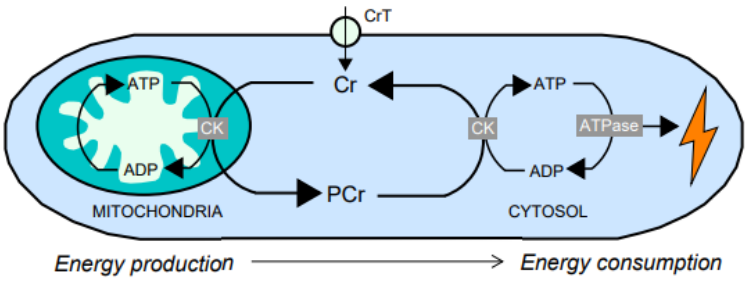


Figure 2.3: Lohman reaction (adapted from Turner, 2015).

2.2.1 Sources of creatine

Humans require ~2 g·day^{-1} of creatine to maintain creatine stores (Wyss & Kaddurah-Daouk, 2000). Approximately 1 g can be obtained via an omnivorous diet including meat and fish (Andres et al., 2008; Wallimann et al., 2007), and the other half is via endogenous biosynthesis (East, 2002), which is an inter-organ process involving the kidneys, pancreas, and liver (Walker, 1979). The brain can also synthesise creatine and does not necessarily rely on dietary or endogenous production from other organs (Braissant et al., 2007).

Endogenous synthesis of creatine is a two-step reaction and requires three amino acids: glycine, arginine, and methionine (Brosnan et al., 2011). Initially, the amino acids arginine and glycine are combined within the kidneys by an enzyme (L-arginine-glycine amidino transferase) to form guanidinoacetate, and the amino acid ornithine is released as a secondary product. Guadinoacetate travels to the liver and is methylated by S-adenosyl-L-methionine in a reaction catalysed by the enzyme guanodinoacetate- methyltransferase. This produces creatine and S-adenosylhomocysteine (Figure 2.4). Creatine is then predominantly transported to the skeletal muscle and the brain. Once in the cells, creatine can be phosphorylated to PCr in a Lohman reaction (Figure 2.3). The phosphate group comes from ATP (Wyss & Kaddurah-Daouk, 2000).

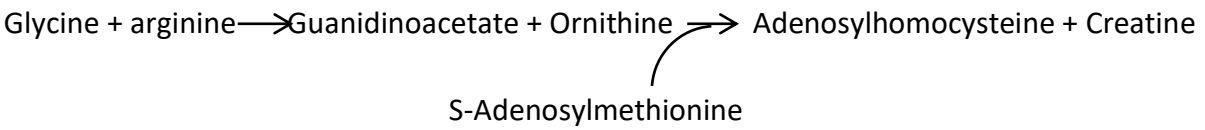


Figure 2.4: Creatine synthesis.

Creatine biosynthesis and absorption are regulated by dietary creatine sources (Lukaszuk et al., 2002). Individuals frequently ingesting creatine via meat or fish have lower rates of endogenous creatine synthesis than individuals who ingest little or no dietary creatine (i.e. vegetarians and vegans) (Venderley & Campbell, 2006). In addition to dietary and endogenous sources, creatine can also be ingested in supplemental form. Creatine supplements are generally safe, cheap, and easily accessible, and are often used to increase tissue creatine content (Kim et al., 2011; Persky & Rawson, 2007; Yoshizumi & Tsourounis, 2004).

2.2.2 Optimal protocol for creatine supplementation

Creatine supplements are available in various forms; however, creatine monohydrate (CrM) is the most commonly used and will be the focus of this review. Similar to the muscle, brain creatine stores can be increased via creatine supplementation (Dechent et al., 1999; Lyoo et al., 2003; Turner, 2015; Turner et al., 2015). Unlike the muscle, however, optimal creatine supplementation protocols to increase brain creatine levels are less clear (Roschel et al., 2021). Whilst short term creatine supplementation (e.g. 20 $g \cdot day^{-1}$ for 5-7 days) can increase intramuscular creatine stores by ~10% (Becque et al., 2000) to 40% (Kreider, 2003), it appears creatine supplementation can only increase brain creatine and PCr by ~5-10% (Dechent et al., 1999; Turner et al., 2015). For example, in 15 individuals (mean age 31 years old) ingesting 20 $g \cdot day^{-1}$ of CrM split evenly into 4 daily 5 g doses for 7 days, brain creatine increased by 9.2% on average (Turner et al., 2015). A similar dose given for 4 weeks in 6 healthy participants (mean age 26.8 years old) has also shown to increase total brain creatine by 8.7% (Dechent et al., 1999). As such, the brain appears less sensitive to exogenous creatine sources than muscles, which may be due to mechanisms preventing orally ingested creatine from being transported into brain cells (Dolan et al., 2019; Roschel et al., 2021). Therefore, high doses (i.e. ~20 g) of creatine across several days are likely needed to increase brain creatine and PCr (Roschel et al., 2021).

2.2.3 Safety of creatine supplementation

CrM is considered safe to ingest within healthy individuals (European Food Safety Acency, 2004). Some possible side effects include a slight weight gain caused by increased storage of water, muscle and gastrointestinal cramps (Juhn, 1999; Vandenberghe et al., 1997); however, side effects often appear anecdotal and are likely to be due to inappropriate creatine dosing protocols (e.g. >10 g for a single dose for 28 days) (Ostojic & Ahmetovic, 2008), or may not be specifically related to creatine supplementation (e.g. muscle cramps can result from dehydration) (Dalbo et al., 2008; Kim et al., 2011). Additionally, there is no evidence that creatine supplementation adversely affects the kidneys in healthy individuals (Hall & Trojian, 2013). Altogether, CrM may be used without concern by healthy individuals when ingested in recommended quantities.

2.2.4 Creatine supplementation and cognition

The effect of creatine supplementation on cognition at rest (i.e. under no physiological stress) appears equivocal. Several studies have demonstrated no effect (Benton & Donohoe, 2011; Rae et al., 2003; Rawson et al., 2008; van Cutsem et al., 2020; Watanabe et al., 2002), but a selected few have demonstrated beneficial effects (Rae et al., 2003; van Cutsem et al., 2020; Watanabe et al., 2002). These favourable effects may have been due to the population being vegetarians (Rae & Bröer, 2015), who typically have lower creatine stores and are more sensitive to creatine supplementation (Solis et al., 2017), or because the cognitive tasks were prolonged and fatiguing (van Cutsem et al., 2020; Watanabe et al., 2002), thus stressing brain energy metabolism. For example, Watanabe et al. (2002) demonstrated that 8 $g \cdot day^{-1}$ CrM for 5 days increased the number of correct responses on a 15-minute serial calculation task in 24 healthy individuals (mean age 24.3 years old). Moreover, van Cutsem et al. (2020) demonstrated that 20 $g \cdot day^{-1}$ CrM for 7 days improved accuracy during a 90-minute Stroop task; however, it did not alter short cognitive performance (Flanker task) and vigour. Furthermore, when concurrently experiencing a physiological stressor, the benefits of creatine supplementation may be more pronounced. For example, 20 $g \cdot day^{-1}$ CrM for 7 days improved multiple aspects of cognition during exposure to hypoxia (10% oxygen) in 15 healthy individuals (mean age 31 years old) (Turner et al., 2015). Altogether, it appears that creatine supplementation may be beneficial for improving performance on sufficiently fatiguing cognitive tasks, and that these benefits may more likely manifest when cerebral energy metabolism is stressed, such as following SR.

2.2.5 Creatine supplementation, sleep loss and cognition

Creatine supplementation may influence cognition following sleep loss. To date, the effect of creatine supplementation on cognition in humans following sleep loss has been investigated in three studies (Cook et al., 2011; McMorris et al., 2007; McMorris et al., 2006) (Table 2.1). McMorris et al. (2006) supplemented 10 healthy adults with 20 $g \cdot day^{-1}$ of CrM for 7 days and, when compared to the placebo group (n = 9), found no cognitive performance or mood effects after 6 and 12 hours of SD; however, after 24 hours of SD, changes in working memory (measured via random movement generation test), choice reaction time, and mood were smaller in the creatine supplemented group than in the placebo group. In another study, McMorris et al. (2007) also supplemented 10 healthy adults with 20 $g \cdot day^{-1}$ of CrM for 7 days

(placebo, *n* = 9) and demonstrated that after 36 hours of SD, but not 18 or 24 hours, performance on a central executive task was less degraded compared to the placebo group. In that same study, there were no differences between groups for mood, choice reaction time or working memory (McMorris et al., 2007). Collectively, these findings suggest that creatine loading can mitigate degradation in aspects of cognition following sufficient sleep loss, but these effects seem equivocal.

Creatine supplementation may also be able to alter cognition following SR. In a study by Cook et al. (2011), 10 elite rugby players were supplemented with an acute dose of CrM of 50 to 100 $mg \cdot kg^{-1}$ 1.5 hours prior to repeated performance on a passing task. Interestingly, pass accuracy was less impaired following a single night of 3-5 hours of sleep (Cook et al., 2011), suggesting CrM supplementation had a positive effect on psychomotor skills. However, effects on cognitive performance and mood were not assessed. The mechanism of how CrM could have positively effect skill performance however is uncertain, as there is no evidence regarding the effect of a single acute dose of creatine on cerebral energy metabolism. In rats, SD induced-increase in extracellular adenosine was attenuated after CrM supplementation (a rodent diet supplemented with 2% of CrM for 4 weeks). This finding suggests that CrM supplementation may also be able to reduce sleep need and homeostatic sleep pressure following SD (Dworak et al., 2017), although these effects have not been replicated in humans. Further research is required to assess if creatine supplementation can alter cognitive performance following SR and whether it also influences other aspects of cognition, such as sleepiness and mood.

Table 2.1: The effect of creatine supplementation on cognition following sleep loss.

Author	Population	Study design	SR/SD protocol	Creatine supplementation protocol	Cognitive and/or performance tests	Primary outcomes
McMorris et al. (2006)	*n* = 19, mean age = 21.1, young athletic male and female	Double-blinded, placebo-controlled, parallel design	Series of tests at baseline and after 6, 12 and 24 h of SD w/ moderate intermittent exercise after a 7-day CrM loading or placebo intake	5 g 4 times per day for 7 days	Random movement generation and adjacency, verbal and spatial recall, choice reaction time, static balance, and mood state	Performance reduction was attenuated in the CrM group for random movement generation, choice reaction time, balance, and mood following 24 h of SD
McMorris et al. (2007)	*n* = 19, mean age = 21.1, young athletic male	Double blinded, placebo-controlled, parallel design	Series of tests at baseline and after 18, 24 and 36 h of SD w/ moderate intermittent exercise after a 7-day CrM loading or placebo intake	5 g 4 times per day for 7 days	Random number generation test, verbal short memory test, choice reaction time, dynamic balance test, shortened version of the Profile of Mood States questionnaire, NASA-TLX	CrM supplementation only affects the random number generation test performance following 36 h of SD. CrM had no effect on mood state
Cook et al. (2011)	*n* = 10, mean age = 20, elite male rugby players	Blinded, placebo-controlled crossover trial	Complete 5 trials on a simple rugby passing skill test after 7-9 h of sleep and 5 trials after 3-5 h of sleep	1 or 5 $mg \cdot kg^{-1}$ 1 of caffeine, 50 to 100 $mg \cdot kg^{-1}$ CrM 1.5 h before each trial	Simple rugby passing skill test (20 repeats per trial)	SD reduced passing accuracy and CrM supplementation reversed this effect (trend for greater effect in larger dose)

2.3 Conclusion

The brain is a highly metabolically active organ and relies on a constant energy supply. During times of accelerated brain ATP turnover, such as fatiguing cognitive tasks, creatine supplementation over several days can facilitate a more constant regeneration of ATP in the brain. Therefore, prophylactic creatine supplementation may protect brain function in the event of acute stressors, such as SR. Despite promising research demonstrating some benefit of creatine supplementation on cognition following total SD, there is currently no evidence in the context of SR, except for a single study assessing psychomotor performance. Further research is clearly required.

2.3.1 Comment from the author

Chapter 3 contains a subset of data from the main study as time restrictions, and the scope of this thesis, did not permit the inclusion of all data collected. The additional variables measured, but not reported on, are listed in the 'Project Procedures' section of the Participant Information Sheet, which can be found in Appendix A.

3 Research Manuscript

Title

The effect of creatine monohydrate loading on cognitive performance, mood, sleepiness, and perceived workload following sleep restriction.

Authors

Janvresse, Juliette[1]

van den Berg, Margo[2]

Bloomfield, Peter[3]

Gant, Nick[3]

Shaw, David M[1,4]

Institutions

[1]School of Sport, Exercise and Nutrition, Massey University, New Zealand

[2]Sleep/Wake Research Centre, School of Health Sciences, Massey University, New Zealand

[3]Department of Exercise Sciences, University of Auckland, New Zealand

[4]Aviation Medicine Unit, Royal New Zealand Air Force Base Auckland, Whenuapai, Auckland, New Zealand

3.1 Abstract

Background: Sleep restriction (SR; i.e. not getting enough sleep per 24 hours for one or multiple nights) impairs cognitive performance and mood, which can compromise capability and safety. SR-related performance deficits may be underpinned by disruptions to brain energy metabolism. Sleep loss appears to reduce brain phosphorylcreatine (PCr; also called creatine phosphate or phosphocreatine), which supports the regeneration of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). Since creatine supplementation increases tissue PCr, including in the brain, it may mitigate possible SR-related cognitive impairments.

Aim: To assess the effect of a 7-day creatine monohydrate (CrM) loading protocol on cognitive performance, mood, subjective sleepiness, and perceived workload following one night of SR (3 hours time-in-bed; TIB).

Methods: A double-blinded, randomised, placebo-controlled cross-over trial was conducted with 7 healthy participants (3 men and 4 women); age, 28.0 ± 4.6 years (range, 22-35 years). Participants ingested 20 $g \cdot day^{-1}$ of CrM or placebo (i.e. tapioca) divided into 4 equal doses for 7 days followed by a single SR night (3 hours TIB). Cognitive performance (10-minute Psychomotor Vigilance Task; PVT), mood (fatigue and vigour), subjective sleepiness, and perceived workload were measured following ~8 hours of sleep prior to creatine supplementation and following the CrM loading protocol and SR. A minimum of a 5-week wash-out period separated the two trial arms. Linear mixed effects models were employed to analyse data.

Results: SR impaired mean reciprocal reaction time (p = 0.01) and mean 10% fastest reaction time (p = 0.02), increased fatigue (p = 0.004), subjective sleepiness (p = 0.009), and workload (p = 0.02), and reduced vigour (p <0.001); however, it did not alter the number of lapses (p = 0.60) or mean 10% slowest reaction time (p = 0.60). CrM loading had no effect on cognitive performance, mood, subjective sleepiness, or workload.

Conclusion: CrM loading may not alter the detrimental effects of a single night of SR (3 hours TIB) on 10-minute PVT performance, mood, subjective sleepiness, or perceived workload.

Key words: sleep loss; psychomotor vigilance task; randomised cross-over trial

3.2 Introduction

Sleep is essential for optimal health and performance (Hirshkowitz et al., 2015; Watson et al., 2015). Whilst the recommended sleep duration for adult is 7 to 9 hours per night (Hirshkowitz et al., 2015; Watson et al., 2015), SR (i.e. not getting enough sleep per 24 hours for one or multiple nights) is common (Banks et al., 2017; McLellan et al., 2016; Rupp et al., 2012). SR can result from work schedules overlapping with the person's normal sleep time, long duty hours, personal or family commitments, or medical conditions (Banks et al., 2017; McLellan et al., 2016; Rupp et al., 2012). SR increases daytime fatigue and impairs cognitive performance and mood (Banks et al., 2017; Lowe et al., 2017), resulting in increased risk of incidents and accidents (Caldwell et al., 2019). Caffeine consumption remains the main dietary strategy to mitigate these effects (Irwin et al., 2020); however, high caffeine intakes can exacerbate SR by negatively affecting sleep quality and sleep duration (Temple et al., 2018). Alternative strategies should therefore be considered, such as creatine supplementation.

Creatine is derived from dietary sources (~50%), mostly meat and fish, and via endogenous synthesis in the kidneys and liver (Andres et al., 2008; East, 2002; Wallimann et al., 2007). In order to increase tissue creatine content, supplementation is typically required. For example, 20 $g \cdot day^{-1}$ of CrM for 7 days increased brain creatine by 9.2% (Turner et al., 2015). CrM supplementation is safe (European Food Safety Acency, 2004; Kim et al., 2011) and is typically ingested over several days-to-weeks (Lyoo et al., 2012). Several studies have assessed the effect of creatine supplementation on cognition (Avgerinos et al., 2018), but few have demonstrated beneficial effects (Rae et al., 2003; van Cutsem et al., 2020; Watanabe et al., 2002). Creatine supplementation also appears more beneficial for cognitive performance during physiological stress, such as hypoxia (Turner, 2015). Considering sleep loss appears to reduce brain PCr concentration (Plante et al., 2014), which is important for the resynthesis of the brain's primary energy source (i.e. ATP), creatine supplementation may be able to support cognition following SR.

Indeed, creatine supplementation has demonstrated beneficial effects on cognition following total sleep deprivation (SD) (McMorris et al., 2007; McMorris et al., 2006). Considering SR is more common than SD and creatine supplementation effects may differ when sleep is attained but in insufficient amounts, further research is required specifically in the context of SR. Therefore, the aim of this research was to assess the effect of a 7-day creatine loading

protocol on simple cognitive task performance, mood, subjective sleepiness, and perceived workload following one night of SR (3 hours TIB). Findings will contribute to determining whether creatine can be ingested as a countermeasure to sleep loss related performance impairment.

3.3 Material and Methods

3.3.1 Study overview

A double-blinded, randomised, placebo-controlled, cross-over design trial was conducted in healthy men and women to compare the effect of CrM loading and a placebo (PLA) on cognitive performance, mood, subjective sleepiness, and perceived workload following SR (3 hours TIB). Ethical approval was provided by the Massey University Ethics Committee (SOA 21/47). Participants were fully informed of the study's rationale and possible risks of the experimental procedures before providing their written consent.

3.3.2 Eligibility criteria

Participants were recruited via emails, posters dispatched at Massey University and word-of-mouth. Participants were required to be: 1) healthy; 2) aged 18-50 years; 3) body mass index <30 kg·m^2; 4) consuming a mixed diet; 5) habitually going to bed between 2100 and 0000 and waking between 0600 and 0900. In addition, women were eligible to participate ensuring both trial arms were during the same periods of their menstrual cycle. Participants were excluded if they: 1) supplemented with creatine in the previous 3 months; 2) smoked; 3) consumed >3 cups caffeinated beverages per day; 4) habitually slept <7 hours or >9 hours per night; 5) regularly consumed medications acting on the central nervous system; 6) had a history of drug or alcohol abuse; 7) engaged in shift work or trans-meridian travel within the 28 days prior to the study. Participants were also required to have: Epworth Sleepiness Scale score <10 (Johns, 1991); global Pittsburg Sleep Quality Index score ≤5 (Buysse, Reynolds, Monk, Berman, &Kupfer, 1989); normal scores on the 21-item Depression Anxiety Stress Scale (i.e. 0-7) (Lovibond & Lovibond, 1995); and scoring as either moderately evening or intermediate chronotype on the Horne-Östberg Morningness/Eveningness Questionnaire (Horne & Östberg, 1976). Participants were fully informed of the rationale of the study and possible risks of the experimental procedures (Appendix A) before providing their written consent;

however, they were not informed of the potential cognitive effects of a CrM loading. Each participant received a calendar summarizing their daily task (Appendix B).

3.3.3 Experimental procedures

Figure 3.1. provides an overview of the experimental design. Prior to the study, participants were familiarised with the cognitive test battery by performing it 5 times to minimise practice effects. In the morning following two nights of at least 8 hours TIB (approximately 2200-2230 to 0600-0630), participants presented to the laboratory between 0730 and 0930 in a fasted and rested state to complete the testing protocol (day 1; baseline). Immediately afterwards, participants commenced their dietary supplement allocation (i.e. CrM or PLA) for 7 days as per prior randomisation (www.randomizer.org). Diet records were reported on 2 non-consecutive days and sleep was monitored daily via wrist actigraphy. Participants were requested to maintain habitual bed and rise times (approximately 8 hours TIB) between days 1 to 6. On day 7, participants were requested to restrict sleep to 3 hours TIB (0300 to 0600). Regular text messages on WhatsApp and actigraphy data confirmed participants respected the protocol. During the study, participants were requested to abstain from over-the-counter medications, alcohol, napping, and remain hydrated. Caffeine intake was limited to <100 $mg \cdot day^{-1}$ ingested before 1200 and prohibited for the 24 hours prior to testing (Appendix C). Ingestion of meat and fish was prohibited during the 24 hours prior to testing. Physical activity was requested to be maintained at moderate levels. All other lifestyle choices were allowed to vary naturally during the study period. Following SR (day 8; post-intervention), participants presented to the laboratory between 0730 and 0930 in a fasted state to repeat the testing protocol. In order to avoid participants driving themselves following SR and risk a fatigue-related accident, participants' transport to and from the laboratory was arranged and paid for. Following completion, participants were asked to state what supplement protocol they were randomised to. The washout period between treatments was at least 5 weeks as this appears to reduce tissue creatine concentrations to approximately baseline (pre-supplement) levels (Hultman et al., 1996; McKenna et al., 1999; Preen et al., 2003). After the washout period, participants repeated the experimental protocol with the alternative supplement treatment.

3.3.4 Dietary supplementation

Participants ingested 20 g·day^{-1} of CrM (NZ Muscle, New Zealand) or tapioca (Pams, New Zealand). Supplements were made in a research grade kitchen and provided in powdered form mixed with 3 g of a flavoured drink powder (Vitafresh, New Zealand) to be mixed with water and taste matched for blinding. Participants were requested to ingest 4 CrM doses of 5 g each daily at equally spaced intervals on days 1 to 7. A CrM loading strategy of 20 g·day^{-1} for 7 days was chosen as this has been shown to augment creatine stores within the brain of men and women (Turner et al., 2015). Participants were requested to send a text message to the researchers to confirm ingestion at the end of each day.

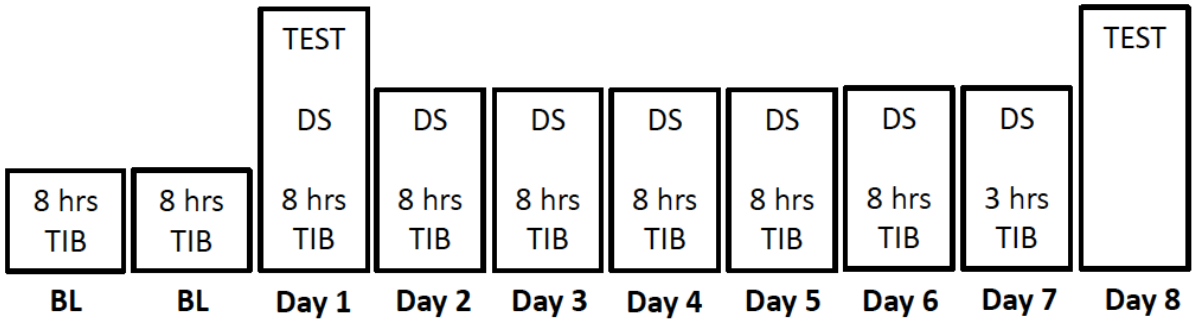


Figure 3.1: An overview of the experimental design. Abbreviations: TIB, time-in-bed; DS, dietary supplementation.

3.3.5 Testing protocol

Following arrival at Massey University (Albany Campus), participants rested in a quiet room for 10 min, then performed a 10-minute PVT. Immediately afterwards, mood and sleepiness were measured using the Automated Neuropsychological Assessment Metrics test battery (ANAM®) and subjective workload on a written handout. All testing was performed at the same time of day, in a room under fluorescent lighting and constant temperature (~20 °C).

3.3.5.1 PVT-192 task

Vigilant attention was measured using a 10-minute version of the PVT (Dinges & Powell, 1985). Participants respond to the presentation of a visual stimulus as quickly as possible by pressing a button with the thumb of their dominant hand (Dinges & Powell, 1985). After a random time interval (between 2 to 10 seconds), the stimulus reappears. The PVT is included in this study

because of its sensitivity to the effects of fatigue and short administration time (Lim & Dinges, 2008, 2010). The PVT is also very reliable with little evidence of practice effects (Balkin et al., 2004). Variables for analysis included number of lapses (i.e. reaction times exceeding 500 ms), mean reciprocal response time (RRT; 1/mean response time (RT)] × 1000), mean fastest 10% RT and mean slowest 10% RT.

3.3.5.2 Mood

Fatigue and vigour were measured using the computerised ANAM® mood scale. Validated against the Profile of Mood Scores (POMS) (Johnson, Vincent, Johnson, Gilliland, & Schlegel, 2008), each variable consists of six adjectives displayed on a 7-point Likert scale anchored with 0 (not at all) to 6 (very much). Fatigue adjectives included lazy, inactive, tired, weary, sluggish, and drowsy, whilst vigour adjectives included energetic, lively, alert, spirited, active, and vigorous. Higher scores indicated more fatigue or vigour.

3.3.5.3 Subjective sleepiness

Subjective sleepiness was measured in the ANAM test battery using the Stanford Sleepiness Scale (Hoddes, Zarcone, Smythe, Phillips, & Dement, 1973). Participants were asked to rate their sleepiness on a 7-point Likert scale which was anchored from 1 (feeling active, vital, alert, or wide awake) to 7 (cannot stay awake, sleep onset appears imminent). Higher scores indicated greater perceived sleepiness.

3.3.5.4 Subjective workload

Subjective workload was measured using the modified National Aeronautics and Space Administration Task Load Index (NASA-TLX) (G.Hart & E.Staveland, 1988), whereby all subscales have an equal weight (i.e. Raw-TLX) (Hart, 2006). Each bipolar sub-scale (mental demand, physical demand, temporal demand, performance, effort, and frustration) is anchored with the verbal descriptions ‘Low’ to ‘High’ (except for the performance sub-scale, which is anchored with the verbal descriptions ‘Good’ to ‘Poor’) and divided into twenty equal intervals. Participants were instructed to place an ‘X’ in the position that best describes their experience from 0 to 100. Overall workload scores were calculated through averaging the sum of the subscales, with a higher mean score indicating higher perceived workload.

3.3.6 Dietary monitoring

Participants were asked to complete two diet records, one prior intervention and one during the dietary supplementation. Diet records were ascertained using image-assisted (alongside a fiducial marker), weighed diet records reported in real-time to a New Zealand registered dietitian via a mobile phone application (WhatsApp, Facebook, San Francisco, CA). Diet records were assessed via visual inspection.

3.3.7 Sleep monitoring

Sleep was monitored using a wrist actigraph (Micro Motionlogger Watch; Ambulatory Monitoring Inc., Ardsley, New York, USA), worn on the participant's non-dominant wrist. Participants were instructed to press the event marker on the actigraph to indicate when they began trying to sleep and when they finished trying to sleep. A standardised sleep diary was also completed to support actigraph data. Actigraphy objectively assesses sleep-wake patterns and has been validated against the gold standard polysomnography (Ancoli-Israel et al., 2015). Data was recorded in 1-minute epochs and analysed by an experienced sleep researcher using ActionW2.7 software with the Cole-Kripke sleep-scoring algorithm applied, in conjunction with information from the sleep log. The variable chosen for analysis was sleep duration, defined as the total number of minutes scored as sleep within the sleep interval (excluding any minutes scored as wake) (Cole et al., 1992). Participants were asked to aim for 7 to 9 hours of sleep per night and restrict sleep on the 7th night of the intervention (3 hours TIB; 0300 to 0600). A text message requesting a response was sent to participants on the morning following the SR night to ensure participants were awake at 0600.

3.3.8 Statistical Analysis

All statistical analyses were conducted in R version 3.6.0 with RStudio version 1.1463 (R Core Team, 2020). Sleep duration was analysed using paired t-tests or Wilcoxon signed rank test for normally and non-normally distributed data, respectively; however, as a data point was missing for sleep duration in the supplementation period, a Welch test was employed. Normality was assess using a Shapiro-Wilk test. All other data were analysed using a series of linear mixed models with restricted maximum likelihood using the R package "lme4". Fixed effects included condition (2 levels; creatine and placebo) and test (2 levels; baseline and sleep restricted). A random intercept for participant was included to adjust for inter-individual

variability and a random slope for sleep restriction was included to adjust for differences in the effect of sleep between participants. Models were controlled for supplement order. Normality of distribution and homoscedasticity of the model's residuals were determined by visual inspection of Q-Q plots; if violated, data were either log, square-root or inverse transformed and assessed for best fit prior to extracting *p*-values. *P*-values for fixed-effects factors were obtained using Type II Wald F tests with Kenward-Roger degrees of freedom in the R package "car". *P*-values for pairwise comparisons were obtained using Holm adjustment for multiplicity in the R package "emmeans". Significance was inferred when $p \leq 0.05$. Graphs were produced in GraphPad Prism Version 9.1.1 (GraphPad Software, San Diego, California USA).

3.3.9 Comment from the author

R scripts for data analyses were written by David Shaw; however, I executed each step and interpreted the outputs.

3.4 Results

3.4.1 Participants

15 individuals volunteered for this study and were assessed for eligibility; however, 5 were either ineligible or decided not to participate. 10 participants commenced the study but 3 were removed due to not adhering to the study's requirements. A total of 7 participants (3 men and 4 women); age, 28.0 ± 4.6 years (range, 22-35 years); body mass, 68.1 ± 14.0 kg; height, 1.70 m ± 0.12 m; body mass index, 23.1 ± 2.0 kg.m^2 were included in the data analyses (Figure 3.2). Two of the participants were part of the research team, but the double-blinding study setting ensured their responses were not biased. Due to the resulting low sample size; the study was treated as a pilot study.

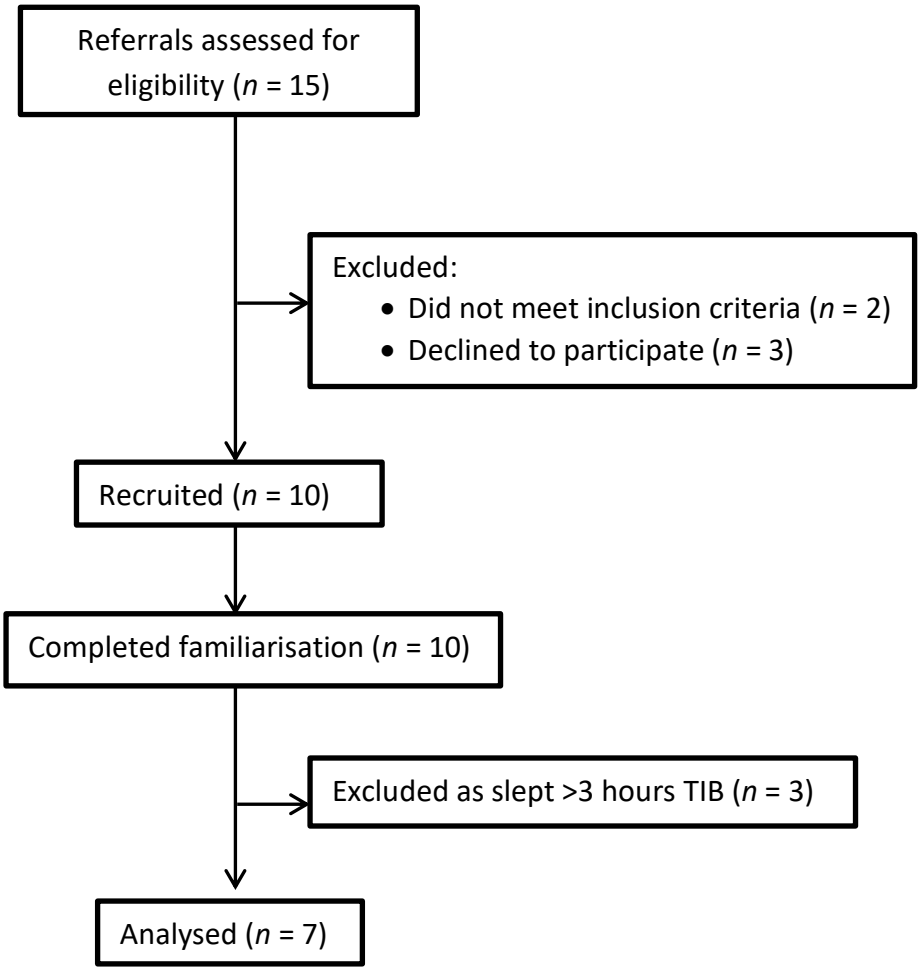


Figure 3.2: Participant selection.

3.4.2 Supplement and dietary compliance

Participants reported consistent dietary habits during each supplement intervention, which was confirmed by a visual assessment of their 2-day weighed image-assisted dietary records. Participants also confirmed not consuming meat or fish on the day prior to performing the cognitive tests. Compliance to the supplementation protocols appeared to be excellent as confirmed by participants' daily messages of ingestion to the researchers.

3.4.3 Sleep duration

3.4.3.1 2-day baseline

Average sleep duration on baseline days did not significantly differ between the placebo and CrM interventions (p = 0.973) (Table 3.1). All participants went to bed between 2100 and 0000 at baseline for both placebo and CrM, except for participant 3 who went to sleep at 0036 on the first baseline night in the CrM intervention. Most participants obtained at least 7 hours sleep each night; however, participant 3 slept <7 hours on all baseline nights (6.15 - 6.83 hours) and participant 11 obtained 5.31 hours sleep on the first baseline night in the placebo condition. Since sleep on baseline days was not expected to affect test results following SR (day 8), data from participants 3 and 11 remained included.

3.4.3.2 7-day supplement intervention

Average sleep duration on days 1 to 6 did not differ between placebo and CrM interventions (p = 0.393) (Table 3.1). All participants went to bed between 2100 and 0000 and slept at least 7 hours each night during the 7-day CrM and placebo interventions. Participant 7 had a short nap on day 5 during the placebo intervention, but only obtained 1 minute sleep; since this was not expected to affect results, participant's 7 data remained included. For the placebo intervention, participants 5, 7 and 11 slept >9 hours (9.17 - 10.48 hours) for three to four nights. For the CrM intervention, participants 1, 6 and 7 slept >9 hours for one night (9.18 - 9.62 hours) and participant 5 slept >9 hours for four nights (9.32 - 10.02 hours). As participant 6 did not wear his watch on day 7 during the placebo intervention, the sleep duration for this day was not available. During the CrM intervention, participants 11 and 6 had one split sleep (i.e. night-time sleep was divided in two parts) on days 2 and 8, and participant 3 had two split sleeps on days 3 and 7.

3.4.3.3 Sleep restriction night

Sleep duration on the SR night did not significantly differ between conditions (p = 0.435) (Table 3.1). For the placebo intervention, participant 11 slept slightly more than 3 hours (i.e. 3.18 hours). For the CrM intervention, participants 6 and 11 spent >3 hours TIB (i.e. 3.48 and 3.62 hours), with participant 11 obtaining 3.03 hours sleep, while participant 6 obtained 1.98 hours of sleep.

Table 3.1: Sleep duration for baseline, 7-day intervention and SR for CrM and placebo (in hours).

	Placebo				CrM			
Study period	Mean ± SD	Median	Range	*n*	Mean ± SD	Median	Range	*n*
Baseline Days	7.75±1.17	7.87	5.32-9.83	14	7.76±0.76	7.83	6.15-9.23	14
7-day Intervention	7.86±0.93	7.93	6.10-9.92	48[a]	7.72± 0.70	7.82	5.90-9.53	49
SR Night	2.78±0.22	2.78	2.52-3.18	7	2.70±0.36	2.75	1.98-3.03	7

[a] Includes one outlier.

3.4.3.4 Duration of wakefulness prior to the cognitive tests

There were no differences between interventions for time awake prior cognitive tests at baseline ($p = 0.501$) or following SR ($p = 0.578$). Within each supplement condition, there were no differences between baseline between baseline and SR for time awake prior cognitive testing (placebo: $p = 0.318$; CrM: $p = 0.219$).

Table 3.2: Duration of wakefulness prior to each testing time (in hours).

	Placebo				CrM			
Study period	Mean ± SD	Median	Range	*n*	Mean ± SD	Median	Range	*n*
Baseline	1.54 ± 0.44	1.48	1.02-2.08	7	1.41 ± 0.61	1.22	0.60-2.55	7
SR	1.72 ± 0.59	1.67	0.98-2.48	7	1.63[a] ± 0.33	1.53	1.05-2.02	7

[a] Data not normally distributed.

3.4.4 PVT performance

SR significantly increased mean fastest 10% RT ($p = 0.017$; Figure 3.3B) and decreased mean RRT ($p = 0.013$; Figure 3.3D) but did not affect mean slowest 10% RT ($p = 0.602$, Figure 3.3A) or number of lapses ($p = 0.599$; Figure 3.3C). CrM supplementation did not affect mean slowest 10% RT ($p = 0.694$, Figure 3.3A), mean fastest 10% RT ($p = 0.693$; Figure 3.3B), number of lapses ($p = 0.533$; Figure 3.3C) or the mean RRT ($p = 0.390$; Figure 3.3D) following SR.

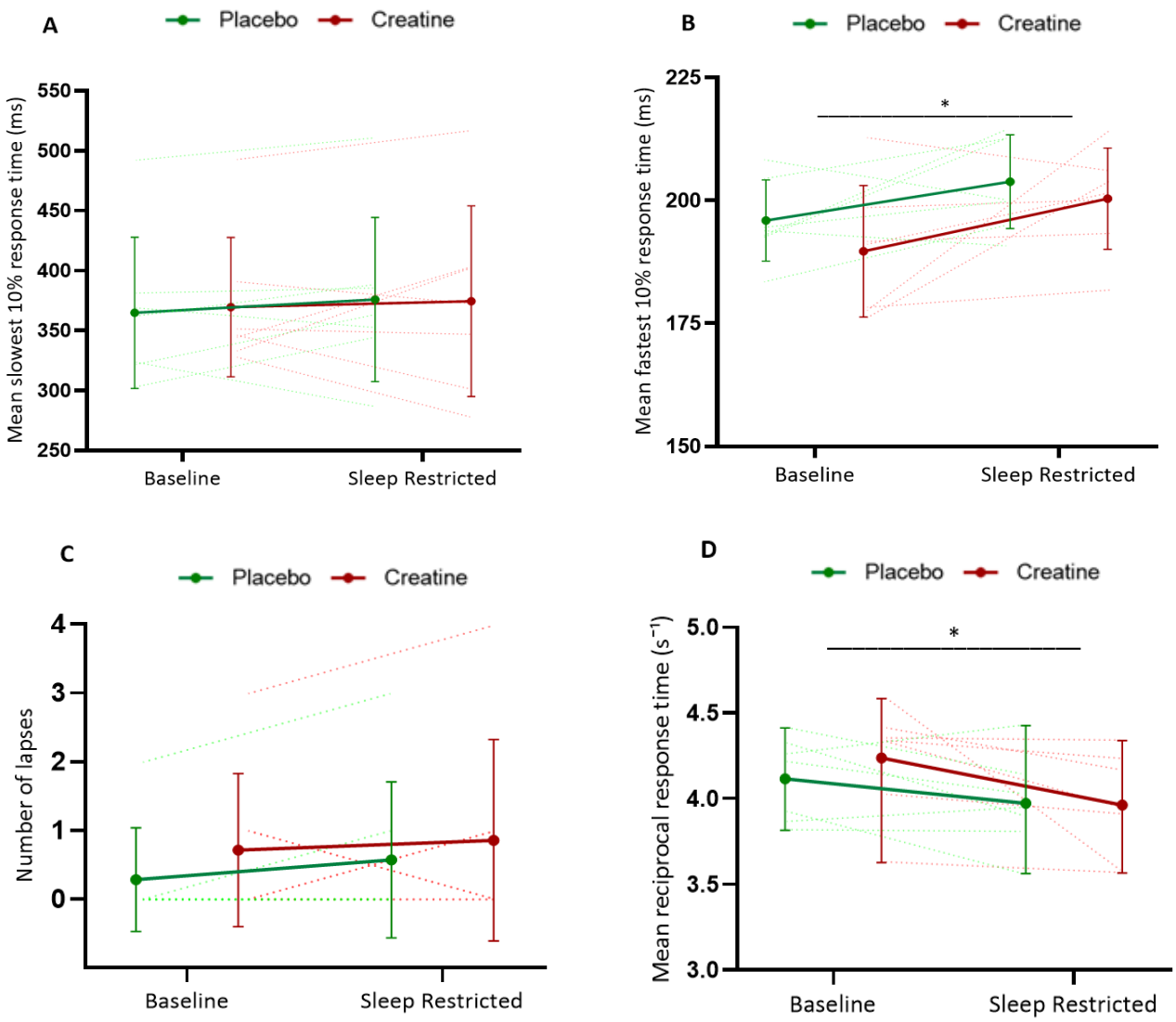


Figure 3.3: Psychomotor vigilance task performance at baseline and after SR (3 hours TIB) following 7 days of CrM supplementation for: (A) mean slowest 10% response time; (B) mean fastest 10% response time; (C) number of lapses; and (D) mean reciprocal response time. Values are presented as mean ± standard deviation. Dotted lines represent each participant.

Table 3.3: *P*-values of main effects and interactions for PVT variables.

	Lapses	Mean RRT	Mean 10% fastest RT	Mean 10% slowest RT
Test	0.599	**0.013**	**0.017**	0.602
Condition	0.061	0.461	0.181	0.942
Test x condition	0.533	0.390	0.693	0.694

3.4.5 Mood, subjective sleepiness, and workload

SR significantly reduced vigour ($p < 0.001$; Figure 3.4A), and increased fatigue ($p = 0.004$; Figure 3.4B), subjective sleepiness ($p = 0.009$; Figure 3.4C) and subjective workload ($p = 0.017$; Figure

3.4D). However, CrM supplementation did not affect vigour (p = 0.818; Figure 3.4A), fatigue (p = 0.591; Figure 3.4B), subjective sleepiness (p = 0.858; Figure 3.4C) or workload following SR (p = 0.229; Figure 3.4D).

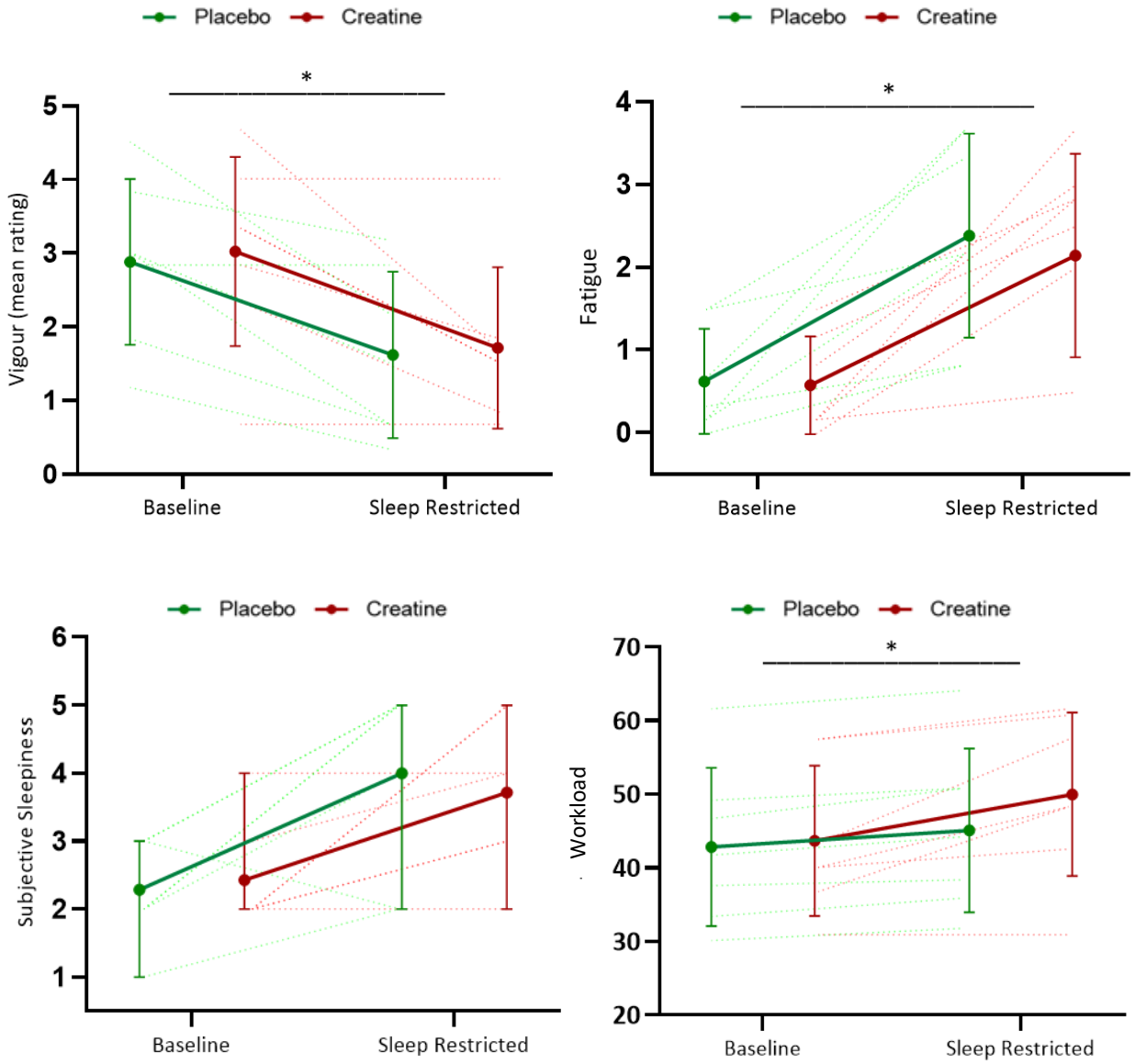


Figure 3.4: Mood, subjective sleepiness, and workload at baseline and after SR (3 hours TIB) following 7 days of CrM supplementation for: (A) vigour; (B) fatigue; (C) subjective sleepiness; and (D) workload. Values are presented as mean ± standard deviation. Dotted lines represent each participant.

Table 3.4: *P*-values of main effects and interactions for mood, subjective sleepiness, and workload.

	Vigour	Fatigue	Subjective sleepiness	Workload
Test	**<0.001**	**0.004**	**0.009**	**0.017**
Condition	0.849	0.300	0.931	0.153
Test x condition	0.818	0.591	0.858	0.229

3.5 Discussion

This study investigated the effects of a 7-day CrM supplementation protocol compared with placebo on cognitive performance, mood, subjective sleepiness, and perceived workload following a single night of SR (3 hours TIB). SR impaired some cognitive performance variables (mean RRT, mean 10% fastest RT), mood (vigour and fatigue), subjective sleepiness and workload; whereas other cognitive performance variables (number of lapses, mean slowest 10% RT) remained unaltered. In contrast to our hypothesis, CrM supplementation did not mitigate the effects of SR on cognitive performance, mood, sleepiness, and workload following SR compared with the placebo condition. These observations suggest that increasing brain PCr, by ingesting 20 g·day^{-1} of CrM for 7 days, is insufficient to ameliorate potential metabolic derangements and cognitive deficits associated a single night of SR.

3.7.1 Effect of sleep restriction

The single night of SR in the present study, which limited sleep from 0300 to 0600, impaired multiple aspects of PVT performance. Mean RRT decreased and mean fastest 10% RT increased, indicating mean speed and fastest responses were slower. In the present study, SR was not sufficient to increase the number of lapses, which have been shown to increase following multiple nights of SR (Belenky et al., 2003) or during total SD (van Dongen et al., 2003). Consistent with the effects on cognitive performance were increases in subjective fatigue, sleepiness, and perceived workload, and a reduction in vigour. These observations suggest that our protocol impaired multiple aspects of cognition. Cognitive performance impairments could have been related to disruptions in energy metabolism, for which the efficacy of creatine loading was being assessed.

3.5.1 Effect of creatine supplementation following sleep restriction

Creatine supplementation did not appear to affect any aspects of cognition following SR. Considering SR is likely to reduce brain PCr stores (Plante et al., 2014), CrM supplementation was expected to elicit a favourable effect due to its ability to increase brain creatine (Turner et al., 2015). As brain creatine stores were not measured in the present study, it is impossible to confirm whether the SR protocol was sufficient to elicit a metabolic effect which creatine loading could mitigate. For example, the benefits of creatine on cognitive performance and mood were not observed *until* 18 to 24 hours of SD (McMorris et al., 2006), suggesting 3 hours TIB may have been insufficient to disrupt energy metabolism for which creatine supplementation could ameliorate. Future studies should therefore include measures of brain energy metabolism to explain cognitive outcomes.

The cognitive task load may also not have elicited sufficient metabolic stress in addition to SR for which the benefits of creatine could manifest. In the present study, the 10-minute PVT is known to enhance time-on-task effects (Lim & Dinges, 2008). However, while participants reported increased workload following SR, the PVT may not have been adequately demanding. For example, in studies demonstrating the beneficial effects of creatine supplementation at rest, Watanabe et al. (2002) used a 2 x 15-minute mathematical calculation task, and van Cutsem et al. (2020) used a 90-minute Stroop test. Moreover, in resting (van Cutsem et al., 2020; Watanabe et al., 2002) and SD (McMorris et al., 2007; McMorris et al., 2006) studies assessing the effect of creatine supplementation on cognitive performance, tasks favourably affected by creatine supplementation were typically more complex than the PVT (e.g. random number generation task). As such, the effort required during shorter and simpler tasks was likely insufficient for observing a favourable effect of creatine supplementation on cognition following sleep loss.

3.5.2 Brain creatine stores

Since brain creatine concentration was not measured in the present study, the efficacy of the creatine dosing protocol (i.e. 20 $g \cdot day^{-1}$) increasing brain creatine and PCr stores cannot be confirmed. As such, it is possible that brain creatine and PCr stores were not sufficiently increased to alter cognition, despite using a validated loading protocol. For example, Wilkinson et al. (2006) used the same dosing protocol and found no increase in brain creatine in 18 young sportsmen (mean age 22.7 years old). Additionally, the effect of creatine

supplementation on total brain creatine appears to vary between individuals (Dechent et al., 1999) and according to age, gender and body weight (Dechent et al., 1999; Solis et al., 2017). Increases in brain creatine, compared to muscle, appear less consistent, which may be due to the brain being less sensitive to dietary and supplemental creatine sources (Dolan et al., 2019; Roschel et al., 2021). It is therefore possible that the creatine supplementation has failed to increase brain creatine in the present study. Future studies should aim to measure brain energy dynamics either via measurement of substrate or alternative measures of activation (e.g. neurophysiological or haemodynamic markers).

3.5.3 Study limitations

In addition to not measuring brain energy metabolism and creatine stores, the present study may have failed to identify effects of creatine supplementation due to the following methodological reasons. Firstly, the small sample size (i.e. n = 7) due to challenging recruitment amidst COVID restriction, meant the study was probably underpowered to detect the subtle effects on cognition; however, the study was a within-participant design (i.e. crossover), meaning each participant received both supplements (i.e. CrM and PLA), increasing the possibility of detecting an effect. Other creatine loading and sleep loss studies (McMorris et al., 2007; McMorris et al., 2006) were typically parallel design which compare two different groups, thus highlighting a strength of the present investigation. Secondly, as participants slept at home during the entire protocol, sleep periods could not be controlled as in laboratory-based studies and therefore there were some minor deviations with the protocols. Although participants sleeping significantly longer than 3 hours during the SR night were excluded from data analyses, sleeping in uncontrolled conditions could have increased variability in sleep duration and quality. Nevertheless, all participants were closely monitored via wrist actigraphy and sleep duration and waking time prior to testing did not differ between supplemental conditions.

3.5.4 Conclusion

In conclusion, a single night of SR (3 hours TIB) impaired aspects of PVT performance, increased fatigue, subjective sleepiness and perceived workload, and reduced vigour. Whilst CrM supplementation has previously been associated with favourable effects on cognition in stressful situations, such as sleep loss, the present study indicated that a 7-day CrM supplementation protocol does not mitigate the cognitive effects of a single night of SR (3

hours TIB). However, the current observations are preliminary and further research using larger sample sizes and multiple nights of SR, which is common in various occupational environments, is required.

4 Conclusion and recommendations

4.1 Study summary

This double-blinded controlled, cross-over trial was designed to examine the effect of a 7-day CrM loading protocol (20 g·day^{-1} of CrM or placebo divided into 4 equal 5 g doses) on cognitive performance, mood, subjective sleepiness, and perceived workload following a single night of SR (3 hours TIB). To the author's knowledge, this is the first study to investigate the effect of CrM supplementation on cognitive responses following SR.

This study was conducted under a hybrid setting of home and laboratory environments. Whilst all testing were performed in the laboratory, participants slept at home, including on the SR night. To ensure participants adhered to the study's requirements at home, rigorous monitoring strategies were employed, which included text messages from participants reporting CrM ingestion, wrist-worn actigraphs and sleep diaries to monitor sleep duration and timing, and image-assisted diet records to monitor dietary intake. Caffeine was also prohibited, and physical activity limited in the 24 h prior to testing. Participants' sleep duration, reported dietary intake and exercise load did not differ between the placebo and CrM conditions and were, therefore, not expected to have confounding effects on cognitive outcomes. As such, the study design was reasonably well controlled to elucidate if there was an effect of CrM.

Several challenges were encountered during the study. This study started whilst the country was under COVID-related restrictions, making participant recruitment and data collection difficult. Unfortunately, two participants were infected with COVID during the intervention, meaning they were not able to test after the SR period and consequently their data could not be used. Additionally, two participants were excluded because they exceeded the sleep duration requirements on the SR night (i.e 3.71 and 5.53 hours). Due to a lack of time and availability, and unwillingness to repeat the study protocol, participants did not undertake the intervention and SR again; therefore, their data were also unable to be used. Due to the resulting low sample size (i.e. n = 7), the study was treated as a pilot study and these data considered preliminary.

The objectives of this research thesis were to examine whether 7 days of ingesting 20 g·day^{-1} of CrM affects aspects of cognition following a single night of SR (3 hours TIB), including:

- Simple cognitive performance (vigilance and sustained attention), using a 10-minute version of the PVT (PVT-192).
- Mood (fatigue and vigour), using a the validated computerised ANAM® mood scale.
- Subjective sleepiness, using the Stanford Sleepiness Scale.
- Perceived workload, using the NASA Task Load Index (NASA-TLX)

4.2 Main findings

4.2.1 Hypothesis 1: A single sleep restriction night (3 hours TIB) will negatively affect cognitive performance, mood, subjective sleepiness, and perceived workload.

Cognitive performance, as measured by a 10-minute PVT, demonstrated some variables were degraded following SR (mean RRT and mean fastest 10% RT), whereas others were stable (i.e. number of lapses and mean slowest 10% RT). Further, SR impaired mood (i.e. increased fatigue and reduced vigour), and increased subjective sleepiness and perceived workload. These findings support the hypothesis by indicating that a single night of SR (3 hours TIB) can be sufficient to impair some aspect of cognition.

4.2.2 Hypothesis 2: CrM loading will mitigate the adverse effect of a single night of sleep restriction night (3 hours TIB) on cognitive performance, mood, subjective sleepiness, and perceived workload.

The absence of a test (i.e. baseline and SR) and supplementation (i.e. creatine vs placebo) interaction indicates that CrM loading did not moderate SR-related deficits in cognitive performance variables (i.e. lapses, mean RRT, mean slowest 10% RT and mean fastest 10% RT), mood (fatigue and vigour), subjective sleepiness, and perceived workload following SR (3 hours TIB). However, further investigation is warranted as a small sample size and methodological limitations prevent making definitive conclusions.

4.3 Study strengths

1. This double-blinded study was conducted under a hybrid setting of home and laboratory environments. Whilst participants slept at home, participants were monitored for key variables that may influence the study's primary outcomes, such as

sleep duration and diet, and requirements standardising physical activity were implemented. The testing environment was strictly controlled, and participants were provided with a quiet room with no distraction and constant temperature. Participants' testing time between baseline and following SR for both interventions was kept the same, thus eliminating potential time of day effects.

2. A 10-minute PVT was used, which is a valid and reliable task to measure reaction time and vigilance following sleep loss. The PVT is widely used for measuring these variables in sleep studies as it is sensitive to the effects of sleep loss. The PVT has been used in controlled laboratory and fields setting. Also, the PVT reduces intra-subject variability as it has minimal practice effects (Lim & Dinges, 2008, 2010). Participants were familiarised in the laboratory with the 10-minute PVT test prior to both interventions to minimise any possible practice effects (Balkin et al., 2004).
3. To reduce the likelihood of abnormal sleep habits influencing outcome measures, participants were selected based on their habitual sleep pattern (i.e. typically sleeping from 2100 and 0000 to 0700 and 0900), sleep quality measured using the Pittsburg Sleep Quality Index score, and chronotype measured using the Horne-Östberg Morningness/Eveningness Questionnaire. If a participant had a Pittsburg Sleep Quality Index score >5 or scored to be definitely a morning or an evening chronotype, they were not eligible for the study. Participants' sleep duration at baseline (2 days), during the 7-day intervention and during the SR night were monitored by wrist actigraphy and sleep diary to ensure these sleeping patterns were maintained and participants adhered to the SR requirements. Average sleep duration did not differ between placebo and CrM interventions, thereby any confounding effects on cognitive performance and subjective variables were not expected following SR.
4. The participant inclusion and exclusion criteria were established to minimise potential confounding effects on cognitive responses. Participants were recruited with strict inclusion and exclusion criteria to ensure participants were healthy, within an age range of 18-50 years, had a BMI within the healthy weight range (i.e. <30 $kg \cdot m^2$), were free from chronic illness and sleep disorders, consumed <3 cups caffeinated beverages per day, and were not involved in shift work or trans-meridian travel within the 28 days prior to the study to reduce possible confounding effects on cognition.

5. Participants and researchers communicated daily during each intervention to ensure participants complied with sleep, supplement, and dietary requirements.
6. Linear mixed effect models were used to analyse data, which included a random intercept for participant and a random slope for test. These are more appropriate than basic linear models due to: 1) the repeated-measures crossover design, which means data are non-independent; 2) the potential for individual variability; and 3) the effect of SR could vary within each individual between supplement conditions. Whereas basic linear models assume each data point is discrete and does not account for variability in individual responses and the effect of sleep restriction.

4.4 Study limitations

1. The initial aim was to recruit 15 to 20 participants; however, due to the difficulty of recruiting participants (e.g. COVID-related restrictions, and strict eligibility/exclusion criteria) and adhering to the challenging protocol, and the limited time frame to collect data, only a small sample size of $n = 7$ could be attained. This reduced the power of the study to detect a statistically significant effect of CrM on cognition following SR and, therefore, the data in considered preliminary and the study was reduced to a pilot study.
2. Participant characteristics in this study do not reflect all demographics within the New Zealand adult population. Therefore, the results cannot be directly extrapolated to other populations, such as shift workers, older adults, overweight-obese individuals, anxious, depressed, and stressed individuals, smokers, high caffeine consumers and individuals with abnormal sleep habits.
3. The efficacy of the CrM loading protocol on increasing brain creatine content could not be confirmed as pre- and post- brain creatine content was not measured. It is possible that the dosing protocol failed to increase brain creatine stores, which could explain why no effect was found after CrM loading. Honest reporting from participants to take the supplements was expected; however, it is not ascertained that participants took the supplements as instructed (i.e. 5 g 4 times per day for 7 days).
4. Sleep and SR took place at home, meaning participants were not as closely monitored as what occurs in laboratory-based studies. However, participants were monitored with actigraph watches and those not compliant with the restrictions were detected.

Further, as participants were sleeping in a familiar environment, it is more likely they adhered to their normal sleep patterns and ascertain higher quality sleep. Whereas, sleeping in uncontrolled conditions would have increased the potential for poor sleep.

5. The PVT is a valid and reliable measure of cognitive performance during SR (Lim & Dinges, 2008, 2010); yet, the 10-minute version of the PVT may have not been demanding enough to elicit sufficient metabolic stress in addition to SR for which the benefits of creatine could manifest.
6. Physical activity was limited, and caffeine consumption forbidden before testing to limit the known effects to mask sleep loss related deficits (Irwin et al., 2020; McLellan et al., 2016; Temple et al., 2018; Tomporowski, 2003). However, adherence was not monitored, and we relied on participants' honesty.

4.5 Final recommendations

4.5.1 Impact

The study findings highlight that a single night of SR can negatively affect cognition, mood, sleepiness, and perceived workload. Sleep loss increases unintentional human error and can lead to dramatic outcomes. Given the high prevalence of SR in our society, including in the military, medical care, transport, and search and rescue, strategies to mitigate the effects of sleep loss are critical. Previous research has demonstrated the beneficial effects of CrM supplementation on cognitive performance when brain metabolism is impaired, such as following sleep loss; however, little is known about the effect of CrM on cognition, mood, sleepiness, and perceived workload following a single night of SR. Although this study was unable to demonstrate any effect of CrM supplementation on cognitive responses following SR, it can provide methodological considerations and recommendations for further research.

4.5.2 Recommendations

The following recommendations should be considered for future research examining the effect of CrM loading on cognition, mood, sleepiness, and perceived workload following SR:

1. The present study's findings do not support for or against the use of CrM supplementation for individuals who experience periods of SR. However, if an individual chooses to pursue CrM supplementation for their own interest, it seems that this will not exacerbate the effects of SR on cognitive functions and will be safe for the

consumer if used correctly (under the guidance of a registered dietitian or qualified professional).

2. Due to the resulting low sample size (*n* = 7), findings from the present study need to be confirmed with larger population samples. A power calculation indicated a sample size of 20 participants was required to detect a large effect size (Cohen's f = 0.4-0.5) in the outcome measures within a repeated-measured design. Thus, to be able to detect an effect of creatine supplementation on cognition, future studies should include a sample of at least *n* = 20. To facilitate the recruitment process, strategies to encourage participants to enrol should be proposed, for example, offering renumeration for their time or organising an incentive prize draw.
3. To assess the effect of CrM loading and SR on brain creatine and PCr content, future studies should consider measuring brain stores of PCr and creatine, or potentially use indirect measures that could infer differences to cerebral metabolic activation, such as electro-encephalography or functional near-infrared spectroscopy.
4. In the present study, the use of the PVT was chosen as it has been shown to be sensitive to sleep loss; yet, the 10-minute PVT may not be as demanding as other cognitive tests in studies elucidating an effect of creatine supplementation. More complex cognitive tasks (e.g. random number generation task) or longer mentally fatiguing task (e.g. 90-minute Stroop accuracy) could therefore be considered to assess the effects of creatine on cognitive performance following sleep loss.

5 References

Ancoli-Israel, S., Cole, R., Alessi, C., Chambers, M., Moorcroft, W., & Pollak, C. P. (2003). The role of actigraphy in the study of sleep and circadian rhythms. *Sleep*, *26*(3), 342-392.

Ancoli-Israel, S., Martin, J. L., Blackwell, T., Buenaver, L., Liu, L., Meltzer, L. J., Sadeh, A., Spira, A. P., & Taylor, D. J. (2015). The SBSM guide to actigraphy monitoring: Clinical and research applications. *Behavioral Sleep Medicine*, *13*(sup1), S4-S38.

Andres, R. H., Ducray, A. D., Schlattner, U., Wallimann, T., & Widmer, H. R. (2008). Functions and effects of creatine in the central nervous system. *Brain Research Bulletin*, *76*(4), 329-343.

Arnal, P. J., Thorey, V., Debellemaniere, E., Ballard, M. E., Bou Hernandez, A., Guillot, A., Jourde, H., Harris, M., Guillard, M., & Van Beers, P. (2020). The Dreem Headband compared to polysomnography for electroencephalographic signal acquisition and sleep staging. *Sleep*, *43*(11), zsaa097.

Avgerinos, K. I., Spyrou, N., Bougioukas, K. I., & Kapogiannis, D. (2018). Effects of creatine supplementation on cognitive function of healthy individuals: A systematic review of randomized controlled trials. *Experimental Gerontology*, *108*, 166-173.

Bailey, D. M. (2019). Oxygen, evolution and redox signalling in the human brain; quantum in the quotidian. *The Journal of Physiology*, *597*(1), 15-28.

Balkin, T. J., Bliese, P. D., Belenky, G., Sing, H., Thorne, D. R., Thomas, M., Redmond, D. P., Russo, M., & Wesensten, N. J. (2004). Comparative utility of instruments for monitoring sleepiness-related performance decrements in the operational environment. *Journal of Sleep Research*, *13*(3), 219-227.

Banks, S., Jill Dorrian, Lathias Basner, & David Dinges. (2017). Sleep deprivation. In Elesvier (Ed.), *Principles and Practice of Sleep Medicine* (6th ed.).

Banks, S., Van Dongen, H. P., Maislin, G., & Dinges, D. F. (2010). Neurobehavioral dynamics following chronic sleep restriction: dose-response effects of one night for recovery. *Sleep*, *33*(8), 1013-1026.

Basner, M., Hermosillo, E., Nasrini, J., McGuire, S., Saxena, S., Moore, T. M., Gur, R. C., & Dinges, D. F. (2018). Repeated administration effects on psychomotor vigilance test performance. *Sleep*, *41*(1), zsx187.

Baum, K. T., Desai, A., Field, J., Miller, L. E., Rausch, J., & Beebe, D. W. (2014). Sleep restriction worsens mood and emotion regulation in adolescents. *Journal of Child Psychology and Psychiatry*, *55*(2), 180-190.

Becque, M. D., Lochmann, J. D., & Melrose, D. R. (2000). Effects of oral creatine supplementation on muscular strength and body compositioin. *Medicine and Science in Sports and Exercise*, *32*(3), 654-658.

Beersma, D. G., & Gordijn, M. C. (2007). Circadian control of the sleep–wake cycle. *Physiology & Behavior*, *90*(2-3), 190-195.

Belenky, G., Wesensten, N. J., Thorne, D. R., Thomas, M. L., Sing, H. C., Redmond, D. P., Russo, M. B., & Balkin, T. J. (2003). Patterns of performance degradation and restoration during sleep restriction and subsequent recovery: A sleep dose-response study. *Journal of Sleep Research*, *12*(1), 1-12.

Benton, D., & Donohoe, R. (2011). The influence of creatine supplementation on the cognitive functioning of vegetarians and omnivores. *British Journal of Nutrition*, *105*(7), 1100-1105.

Borbély, A. A., Daan, S., Wirz-Justice, A., & Deboer, T. (2016). The two-process model of sleep regulation: A reappraisal. *Journal of Sleep Research*, *25*(2), 131-143.

Braissant, O., Bachmann, C., & Henry, H. (2007). Expression and function of AGAT, GAMT and CT1 in the mammalian brain. *Creatine and Creatine Kinase in Health and Disease*, 67-81.

Březinová, V. (1974). Sleep cycle content and sleep cycle duration. *Electroencephalography and Clinical Neurophysiology*, *36*, 275-282.

Brosnan, J. T., Da Silva, R. P., & Brosnan, M. E. (2011). The metabolic burden of creatine synthesis. *Amino Acids*, *40*(5), 1325-1331.

Caldwell, J. A., Caldwell, J. L., Thompson, L. A., & Lieberman, H. R. (2019). Fatigue and its management in the workplace. *Neuroscience & Biobehavioral Reviews*, *96*, 272-289.

Carskadon, M. A., & Dement, W. C. (2017). Normal Human Sleep: An Overview. *Principles and Practice of Sleep Medicine*.

Chatzitheochari, S., & Arber, S. (2009). Lack of sleep, work and the long hours culture: evidence from the UK Time Use Survey. *Work, Employment and Society*, *23*(1), 30-48.

Chinoy, E. D., Cuellar, J. A., Huwa, K. E., Jameson, J. T., Watson, C. H., Bessman, S. C., Hirsch, D. A., Cooper, A. D., Drummond, S. P., & Markwald, R. R. (2021). Performance of seven consumer sleep-tracking devices compared with polysomnography. *Sleep*, *44*(5), zsaa291.

Cole, R. J., Kripke, D. F., Gruen, W., Mullaney, D. J., & Gillin, J. C. (1992). Automatic sleep/wake identification from wrist activity. *Sleep*, *15*(5), 461-469.

Cook, C. J., Crewther, B. T., Kilduff, L. P., Drawer, S., & Gaviglio, C. M. (2011). Skill execution and sleep deprivation: effects of acute caffeine or creatine supplementation-a randomized placebo-controlled trial. *Journal of the International Society of Sports Nutrition*, *8*(1), 1-8.

Dalbo, V. J., Roberts, M. D., Stout, J. R., & Kerksick, C. M. (2008). Putting to rest the myth of creatine supplementation leading to muscle cramps and dehydration. *British Journal of Sports Medicine*, *42*(7), 567-573.

de Souza, L., Benedito-Silva, A. A., Pires, M. L. N., Poyares, D., Tufik, S., & Calil, H. M. (2003). Further validation of actigraphy for sleep studies. *Sleep*, *26*(1), 81-85.

Dechent, P., Pouwels, P., Wilken, B., Hanefeld, F., & Frahm, J. (1999). Increase of total creatine in human brain after oral supplementation of creatine-monohydrate. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *277*(3), R698-R704.

Dennis, L. E., Wohl, R. J., Selame, L. A., & Goel, N. (2017). Healthy adults display long-term trait-like neurobehavioral resilience and vulnerability to sleep loss. *Scientific Reports*, *7*(1), 1-10.

Dienel, G. A. (2019). Brain glucose metabolism: Integration of energetics with function. *Physiological Reviews*, *99*(1), 949-1045.

Dinges, D. F. (1995). An overview of sleepiness and accidents. *Journal of Sleep Research*, *4*, 4-14.

Dinges, D. F., & Powell, J. W. (1985). Microcomputer analyses of performance on a portable, simple visual RT task during sustained operations. *Behavior Research Methods, Instruments, & Computers*, *17*(6), 652-655.

Dolan, E., Gualano, B., & Rawson, E. S. (2019). Beyond muscle: the effects of creatine supplementation on brain creatine, cognitive processing, and traumatic brain injury. *European Journal of Sport Science*, *19*(1), 1-14.

Duhart, J. M., Inami, S., & Koh, K. (2021). Many faces of sleep regulation: beyond the time of day and prior wake time. *The FEBS Journal*.

Durmer, J., & Dinges, D. (2005). Neurocognitive Consequences of Sleep Deprivation [Internet]. Seminars in Neurology, Vol 25. In: Thieme Medical Publishers Inc.

Dworak, M., Kim, T., Mccarley, R. W., & Basheer, R. (2017). Creatine supplementation reduces sleep need and homeostatic sleep pressure in rats. *Journal of Sleep Research*, *26*(3), 377-385.

Dworak, M., McCarley, R. W., Kim, T., Kalinchuk, A. V., & Basheer, R. (2010). Sleep and brain energy levels: ATP changes during sleep. *Journal of Neuroscience*, *30*(26), 9007-9016.

East, D. B. (2002). Biochemical Pathways of Creatine and Creatine Phosphate.

Engle-Friedman, M. (2014). The effects of sleep loss on capacity and effort. *Sleep Science*, *7*(4), 213-224.

European Food Safety Acency. (2004). Creatine monohydrate for use in foods for particular nutrional uses (Question number EFSA-Q-2003-125). *EFSA J*, *36*, 1-6.

Garbarino, S. (2020). Sleep Disorders across the Lifespan: A Different Perspective. In (Vol. 17, pp. 9025): Multidisciplinary Digital Publishing Institute.

Gianfredi, V., Nucci, D., Tonzani, A., Amodeo, R., Benvenuti, A. L., Villarini, M., & Moretti, M. (2018). Sleep disorder, Mediterranean Diet and learning performance among nursing students: inSOMNIA, a cross-sectional study. *Ann Ig*, *30*(6), 470-481.

Gordji-Nejad, A., Matusch, A., Li, S., Kroll, T., Beer, S., Elmenhorst, D., & Bauer, A. (2018). Phosphocreatine levels in the left thalamus decline during wakefulness and increase after a nap. *Journal of Neuroscience*, *38*(49), 10552-10565.

Greenhaff, P. L. (2001). The creatine-phosphocreatine system: there's more than one song in its repertoire. *The Journal of physiology*, *537*(Pt 3), 657.

Gualano, B., Artioli, G. G., Poortmans, J. R., & Lancha Junior, A. H. (2010). Exploring the therapeutic role of creatine supplementation. *Amino Acids*, *38*(1), 31-44.

Hall, M., & Trojian, T. H. (2013). Creatine supplementation. *Current Sports Medicine Reports*, *12*(4), 240-244.

Hirshkowitz, M., Whiton, K., Albert, S. M., Alessi, C., Bruni, O., DonCarlos, L., Hazen, N., Herman, J., Hillard, P. J. A., & Katz, E. S. (2015). National Sleep Foundation's updated sleep duration recommendations. *Sleep Health*, *1*(4), 233-243.

Inoué, S., Honda, K., & Komoda, Y. (1995). Sleep as neuronal detoxification and restitution. *Behavioural Brain Research*, *69*(1-2), 91-96.

Irwin, C., Khalesi, S., Desbrow, B., & McCartney, D. (2020). Effects of acute caffeine consumption following sleep loss on cognitive, physical, occupational and driving performance: A systematic review and meta-analysis. *Neuroscience & Biobehavioral Reviews*, *108*, 877-888.

Jacobus, W., & Diffley, D. (1986). Creatine kinase of heart mitochondria. Control of oxidative phosphorylation by the extramitochondrial concentrations of creatine and phosphocreatine. *Journal of Biological Chemistry*, *261*(35), 16579-16583.

Juhn, M. S. (1999). Oral creatine supplementation in male collegiate athletes: a survey of dosing habits and side effects. *Journal of the Academy of Nutrition and Dietetics*, *99*(5), 593.

Kahn, M., Fridenson, S., Lerer, R., Bar-Haim, Y., & Sadeh, A. (2014). Effects of one night of induced night-wakings versus sleep restriction on sustained attention and mood: a pilot study. *Sleep Medicine*, *15*(7), 825-832.

Kaviani, M., Shaw, K., & Chilibeck, P. D. (2020). Benefits of creatine supplementation for vegetarians compared to omnivorous athletes: a systematic review. *International Journal of Environmental Research and Public Health*, *17*(9), 3041.

Kim, H. J., Kim, C. K., Carpentier, A., & Poortmans, J. R. (2011). Studies on the safety of creatine supplementation. *Amino Acids*, *40*(5), 1409-1418.

Kim, J.-H., Kim, D.-W., & Im, C.-H. (2017). Brain areas responsible for vigilance: an EEG source imaging study. *Brain Topography*, *30*(3), 343-351.

Kong, J., Shepel, P. N., Holden, C. P., Mackiewicz, M., Pack, A. I., & Geiger, J. D. (2002). Brain glycogen decreases with increased periods of wakefulness: implications for homeostatic drive to sleep. *Journal of Neuroscience*, *22*(13), 5581-5587.

Kreider, R. B. (2003). Effects of creatine supplementation on performance and training adaptations. *Molecular and Cellular Biochemistry*, *244*(1), 89-94.

Kreider, R. B., Kalman, D. S., Antonio, J., Ziegenfuss, T. N., Wildman, R., Collins, R., Candow, D. G., Kleiner, S. M., Almada, A. L., & Lopez, H. L. (2017). International Society of Sports Nutrition position stand: safety and efficacy of creatine supplementation in exercise, sport, and medicine. *Journal of the International Society of Sports Nutrition*, *14*(1), 1-18.

Krueger, J. M., Frank, M. G., Wisor, J. P., & Roy, S. (2016). Sleep function: Toward elucidating an enigma. *Sleep medicine reviews*, *28*, 46-54.

Lee, C. H., & Sibley, C. G. (2019). Sleep duration and psychological well-being among New Zealanders. *Sleep Health*, *5*(6), 606-614.

Lim, J., & Dinges, D. F. (2008). Sleep deprivation and vigilant attention. *Annals of the New York Academy of Sciences*, *1129*(1), 305-322.

Lim, J., & Dinges, D. F. (2010). A meta-analysis of the impact of short-term sleep deprivation on cognitive variables. *Psychological Bulletin*, *136*(3), 375.

Lowe, C. J., Safati, A., & Hall, P. A. (2017). The neurocognitive consequences of sleep restriction: a meta-analytic review. *Neuroscience & Biobehavioral Reviews*, *80*, 586-604.

Lukaszuk, J. M., Robertson, R. J., Arch, J. E., Moore, G. E., Yaw, K. M., Kelley, D. E., Rubin, J. T., & Moyna, N. M. (2002). Effect of creatine supplementation and a lacto-ovo-vegetarian diet on muscle creatine concentration. *International Journal of Sport Nutrition and Exercise Metabolism*, *12*(3), 336-348.

Lyoo, I. K., Kong, S. W., Sung, S. M., Hirashima, F., Parow, A., Hennen, J., Cohen, B. M., & Renshaw, P. F. (2003). Multinuclear magnetic resonance spectroscopy of high-energy phosphate metabolites in human brain following oral supplementation of creatine-monohydrate. *Psychiatry Research: Neuroimaging*, *123*(2), 87-100.

Lyoo, I. K., Yoon, S., Kim, T.-S., Hwang, J., Kim, J. E., Won, W., Bae, S., & Renshaw, P. F. (2012). A randomized, double-blind placebo-controlled trial of oral creatine monohydrate augmentation for enhanced response to a selective serotonin reuptake inhibitor in women with major depressive disorder. *American Journal of Psychiatry*, *169*(9), 937-945.

Marino, M., Li, Y., Rueschman, M. N., Winkelman, J. W., Ellenbogen, J., Solet, J. M., Dulin, H., Berkman, L. F., & Buxton, O. M. (2013). Measuring sleep: accuracy, sensitivity, and specificity of wrist actigraphy compared to polysomnography. *Sleep*, *36*(11), 1747-1755.

McCall, C., & McCall, W. V. (2012). Comparison of actigraphy with polysomnography and sleep logs in depressed insomniacs. *Journal of Sleep Research*, *21*(1), 122-127.

McLellan, T. M., Caldwell, J. A., & Lieberman, H. R. (2016). A review of caffeine's effects on cognitive, physical and occupational performance. *Neuroscience & Biobehavioral Reviews*, *71*, 294-312.

McMorris, T., Harris, R. C., Howard, A. N., Langridge, G., Hall, B., Corbett, J., Dicks, M., & Hodgson, C. (2007). Creatine supplementation, sleep deprivation, cortisol, melatonin and behavior. *Physiology & Behavior*, *90*(1), 21-28.

McMorris, T., Harris, R. C., Swain, J., Corbett, J., Collard, K., Dyson, R. J., Dye, L., Hodgson, C., & Draper, N. (2006). Effect of creatine supplementation and sleep deprivation, with mild exercise, on cognitive and psychomotor performance, mood state, and plasma concentrations of catecholamines and cortisol. *Psychopharmacology*, *185*(1), 93-103.

Miyata, S., Noda, A., Ozaki, N., Hara, Y., Minoshima, M., Iwamoto, K., Takahashi, M., Iidaka, T., & Koike, Y. (2010). Insufficient sleep impairs driving performance and cognitive function. *Neuroscience Letters*, *469*(2), 229-233.

Monge, C., Beraud, N., Kuznetsov, A. V., Rostovtseva, T., Sackett, D., Schlattner, U., Vendelin, M., & Saks, V. A. (2008). Regulation of respiration in brain mitochondria and synaptosomes: restrictions of ADP diffusion in situ, roles of tubulin, and mitochondrial creatine kinase. *Molecular and Cellular Biochemistry*, *318*(1), 147-165.

Ostojic, S. M., & Ahmetovic, Z. (2008). Gastrointestinal distress after creatine supplementation in athletes: are side effects dose dependent? *Research in Sports Medicine*, *16*(1), 15-22.

Paquet, J., Kawinska, A., & Carrier, J. (2007). Wake detection capacity of actigraphy during sleep. *Sleep*, *30*(10), 1362-1369.

Patel, A. K., Reddy, V., & Araujo, J. F. (2021). Physiology, sleep stages. *StatPearls [Internet]*.

Plante, D. T., Trksak, G. H., Jensen, J. E., Penetar, D. M., Ravichandran, C., Riedner, B. A., Tartarini, W. L., Dorsey, C. M., Renshaw, P. F., & Lukas, S. E. (2014). Gray matter-specific changes in brain bioenergetics after acute sleep deprivation: a 31P magnetic resonance spectroscopy study at 4 Tesla. *Sleep*, *37*(12), 1919-1927.

Porkka-Heiskanen, T., & Kalinchuk, A. V. (2011). Adenosine, energy metabolism and sleep homeostasis. *Sleep Medicine Reviews*, *15*(2), 123-135.

Porkka-Heiskanen, T., Strecker, R. E., Thakkar, M., Bjørkum, A. A., Greene, R. W., & McCarley, R. W. (1997). Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. *Science*, *276*(5316), 1265-1268.

Rae, C., Digney, A. L., McEwan, S. R., & Bates, T. C. (2003). Oral creatine monohydrate supplementation improves brain performance: a double–blind, placebo–controlled, cross–over trial. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1529), 2147-2150.

Rae, C. D., & Bröer, S. (2015). Creatine as a booster for human brain function. How might it work? *Neurochemistry International*, *89*, 249-259.

Rawson, E. S., Lieberman, H. R., Walsh, T. M., Zuber, S. M., Harhart, J. M., & Matthews, T. C. (2008). Creatine supplementation does not improve cognitive function in young adults. *Physiology & Behavior*, *95*(1-2), 130-134.

Reason, J. (1990). *Human error*. Cambridge university press.

Reichert, C. F., Deboer, T., & Landolt, H. P. (2022). Adenosine, caffeine, and sleep–wake regulation: state of the science and perspectives. *Journal of Sleep Research*.

Roschel, H., Gualano, B., M Ostojic, S., & S Rawson, E. (2021). Creatine Supplementation and Brain Health. *Nutrients*, *13*(2), 586.

Rupp, T. L., Wesensten, N. J., & Balkin, T. J. (2012). Trait-like vulnerability to total and partial sleep loss. *Sleep*, *35*(8), 1163-1172.

Sadeh, A. (2011). The role and validity of actigraphy in sleep medicine: an update. *Sleep Medicine Reviews*, *15*(4), 259-267.

Sharma, S., & Kavuru, M. (2010). Sleep and metabolism: an overview. *International Journal of Endocrinology*, *2010*.

Solis, M. Y., Artioli, G. G., Otaduy, M. C. G., Leite, C. d. C., Arruda, W., Veiga, R. R., & Gualano, B. (2017). Effect of age, diet, and tissue type on PCr response to creatine supplementation. *Journal of Applied Physiology*, *123*(2), 407-414.

Solis, M. Y., de Salles Painelli, V., Artioli, G. G., Roschel, H., Otaduy, M. C., & Gualano, B. (2014). Brain creatine depletion in vegetarians? A cross-sectional 1H-magnetic resonance spectroscopy (1H-MRS) study. *British Journal of Nutrition*, *111*(7), 1272-1274.

Stickgold, R. (2005). Sleep-dependent memory consolidation. *Nature*, *437*(7063), 1272-1278.

Temple, J. L., Hostler, D., Martin-Gill, C., Moore, C. G., Weiss, P. M., Sequeira, D. J., Condle, J. P., Lang, E. S., Higgins, J. S., & Patterson, P. D. (2018). Systematic review and meta-analysis of the effects of caffeine in fatigued shift workers: implications for emergency medical services personnel. *Prehospital emergency care*, *22*(sup1), 37-46.

Thomas, M., Sing, H., Belenky, G., Holcomb, H., Mayberg, H., Dannals, R., Wagner Jr, H., Thorne, D., Popp, K., & Rowland, L. (2000). Neural basis of alertness and cognitive performance impairments during sleepiness. I. Effects of 24 h of sleep deprivation on waking human regional brain activity. *Journal of Sleep Research*, *9*(4), 335-352.

Tomporowski, P. D. (2003). Effects of acute bouts of exercise on cognition. *Acta Psychologica*, *112*(3), 297-324.

Turner, C. E. (2015). *Effect of Dietary Creatine Supplementation and Hypoxia on Motor and Cognitive Function* [Doctoral dissertation, University of Auckland].

Turner, C. E., Byblow, W. D., & Gant, N. (2015). Creatine supplementation enhances corticomotor excitability and cognitive performance during oxygen deprivation. *Journal of Neuroscience*, *35*(4), 1773-1780.

van Cutsem, J., Roelands, B., Pluym, B., Tassignon, B., Verschueren, J., De Pauw, K., & Meeusen, R. (2020). Can creatine combat the mental fatigue-associated decrease in visuomotor skills. *Med. Sci. Sports Exerc*, *52*, 120-130.

van Dongen, H., Maislin, G., Mullington, J. M., & Dinges, D. F. (2003). The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep*, *26*(2), 117-126.

van Dongen, H., Thomas Balkin, & Hursch, S. (2017). Performance Deficits during sleep loss and their operational consequences. In *Principles and Practice of Sleep Medicine* (6th ed.). Elsevier.

Van Dongen, P., Baynard, M. D., Maislin, G., & Dinges, D. F. (2004). Systematic interindividual differences in neurobehavioral impairment from sleep loss: evidence of trait-like differential vulnerability. *Sleep*, *27*(3), 423-433.

Vandenberghe, K., Goris, M., Van Hecke, P., Van Leemputte, M., Vangerven, L., & Hespel, P. (1997). Long-term creatine intake is beneficial to muscle performance during resistance training. *Journal of Applied Physiology*, *83*(6), 2055-2063.

Venderley, A. M., & Campbell, W. W. (2006). Vegetarian diets. *Sports Medicine*, *36*(4), 293-305.

Walker, J. B. (1979). Creatine: biosynthesis, regulation, and function. *Adv Enzymol Relat Areas Mol Biol*, *50*(177), 2.

Wallimann, T., Tokarska-Schlattner, M., Neumann, D., Epand, R. M., Epand, R. F., Andres, R. H., Widmer, H. R., Hornemann, T., Saks, V., & Agarkova, I. (2007). The phosphocreatine circuit: molecular and cellular physiology of creatine kinases, sensitivity to free radicals, and enhancement by creatine supplementation. *Molecular system bioenergetics: Energy for life*, 195-264.

Watanabe, A., Kato, N., & Kato, T. (2002). Effects of creatine on mental fatigue and cerebral hemoglobin oxygenation. *Neuroscience Research*, *42*(4), 279-285.

Watson, N. F., Badr, M. S., Belenky, G., Bliwise, D. L., Buxton, O. M., Buysse, D., Dinges, D. F., Gangwisch, J., & Grandner, M. A. (2015). Recommended amount of sleep for a healthy adult: a joint consensus statement of the American Academy of Sleep Medicine and Sleep Research Society. *Journal of Clinical Sleep Medicine*, *11*(6), 591-592.

Wyss, M., & Kaddurah-Daouk, R. (2000). Creatine and creatinine metabolism. *Physiological reviews*.

Xie, L., Kang, H., Xu, Q., Chen, M. J., Liao, Y., Thiyagarajan, M., O'Donnell, J., Christensen, D. J., Nicholson, C., & Iliff, J. J. (2013). Sleep drives metabolite clearance from the adult brain. *Science*, *342*(6156), 373-377.

Appendices

Appendix A: Participant information sheet

The effect of creatine monohydrate loading on cognition, mood and sleepiness following sleep restriction

Researchers Introduction

We are investigating 1) the effects of creatine loading on cognitive function, mood and sleepiness following sleep restriction and 2) cerebral oxygenation during task performance. The findings of this research will contribute towards dietary recommendations to manage fatigue, a Master of Sciences, Nutrition and Dietetics research thesis and publication in academic journals and conferences.

The research will be conducted at Massey University (Albany Campus), by Dr. David Shaw, Dr. Margo van den Berg, Associate Professor Nick Gant, Juliette Janvresse and Peter Bloomfield. Please ensure you read and understand all pages. If you have any queries, please contact Dr. David Shaw (lead researcher; see details below).

Email: david.shaw2@nzdf.mil.nz

Phone: (09) 399 8939

Project Description and Invitation

Sleep restriction (i.e. sleep duration less than 7 hours) is common in the military. Insufficient sleep impairs cognitive performance, particularly vigilance, sustained attention, and memory. This increases the risk of incident, accident and, potentially, loss of life. Caffeine is often used to temporarily reduce the detrimental effects of sleep restriction; however, excessive caffeine intake or when ingested close to bedtime will compromise sleep – worsening sleep restriction! Also, not everyone consumes caffeine in amounts that can enhance cognitive performance. Another option could be creatine supplementation, which has shown positive effects on cognitive performance in individuals deprived of sleep (i.e. no sleep at all). Whether this extends to sleep restriction is uncertain.

Creatine is naturally found in the diet, particularly in meat and chicken. When supplementing with creatine, it is typically ingested in the form of creatine monohydrate, a white powder often mixed into a drink. Creatine is ingested over several days to "load" body tissues and is often used to improve muscular performance. Since creatine loading also increases brain creatine stores, it can augment energy production in the brain and have beneficial effects when brain energy systems are compromised, such as sleep restriction. This can be monitored by measuring brain oxygenation, which has shown to be sensitive to both creatine loading and sleep restriction. Based on this, it is important to examine whether creatine loading could have a benefit following sleep restriction.

We would, therefore, like to invite you to participate in this study. Whether or not you take part is your choice. If you do not want to take part, you do not have to give a reason. If you do want to take part now, but change your mind later, you can pull out of the study at any time. This participant information sheet will help you decide if you would like to take part. It explains what your participation will involve, what the benefits and risks to you might be, and what happens after the study ends.

Participant Identification and Recruitment

Recruitment for this study is via word-of-mouth and poster advertising (e.g. the Albany campus, local gyms, supermarkets). Participation for this study is on a voluntary basis. Your name and contact details will only be obtained following your expression of interest.

To be deemed eligible, you will need to meet all of the following inclusion criteria:

1) Healthy man or woman.
2) aged 18-50 years.
3) body mass index less than 30 $kg.m^2$.
4) consuming a mixed diet.
5) habitually going to bed between 2100 and 0000 and waking between 0600 and 0900.

However, you will be deemed ineligible if you meet one of the following exclusion criteria:

1) supplemented with creatine in the previous 3 months.
2) smoke.
3) consume>3 cups caffeinated beverages per day.

4) habitually sleep less than 7 hours or more than 9 hours per night.

5) consuming medications acting on the central nervous system.

6) have a history of drug or alcohol abuse.

7) engaged in shift work or trans-meridian travel within the 28 days prior to the study.

You will also be required to meet additional specifications for sleepiness, sleep quality, mood, and circadian preference, which will be assessed using standardised questionnaires.

If you are identified as a poor sleeper and are deemed ineligible for the study, you will be provided with information on good sleep practices, and if you have any concerns about your sleep, we will recommend that you follow up with your GP.

- *Number of participants involved in the study.*

The aim is to recruit 20 participants in total. Selection for participating will occur on a first in, first-served policy.

- *Compensation/reimbursement of expenses/payments.*

As you must not drive on the day following sleep restriction as sleep restriction increases the risk of incidents and accidents. Transport will be arranged for you to and from Massey University (Albany Campus).

- *Discomforts and risks.*

The only discomfort you may experience during the study is tiredness following sleep restriction. It is unlikely that creatine supplementation will cause an issue. All of the other study procedures will be undertaken as part of your normal daily routine. You will also be rigorously screened prior to your participation to minimise any potential risk of an adverse event.

How do I agree to participate in this research?

By signing the consent form, you are agreeing to take part in this study. However, your participation in this research is voluntary and whether or not you choose to participate will neither advantage nor disadvantage you. You are able to withdraw from the study at any time. If you choose to withdraw from the study, you will be offered the choice between having any

data that is identifiable as belonging to you removed or allowing it to continue to be used. However, once the findings have been produced, removal of your data may not be possible.

Project Procedures

Figure 1 provides an overview of the experimental design. You will be familiarised with the cognitive test battery (each test completed 10 times separated by at least 5 min) to mitigate practice effects; this will be completed before commencing the study during a separate visit to Massey University (Albany Campus).

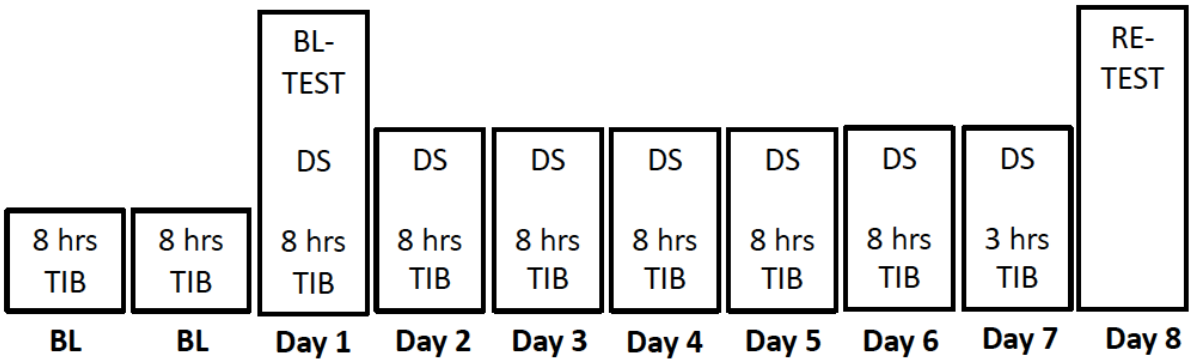


Figure 1: An overview of the experimental design. Abbreviations: BL, baseline; TIB, time-in-bed; DS, dietary supplementation.

You will also be given an actigraph and sleep diary, for monitoring your sleep/wake patterns.

The actigraph is the same size as a wrist watch and is worn on your non-dominant wrist. It should be worn all the time, except during showering, swimming. It measures movement, and this information is used to estimate when, how long, and how well you have slept. The actigraph collects information about activity, light intensity and the temperature of the actigraph case (to detect times when it is off-wrist). The actigraph cannot collect any biological or other information. The sleep/duty diary provides additional information on when you try to sleep and has spaces for you to write any comments about your sleep.

In the morning following two nights of at least 8-hour time-in-bed (2230-0630 h), you will present to Massey University (Albany Campus) between 0730-0930 h in a fasted state to complete the testing protocol (i.e. day 1; baseline). Immediately afterwards, you will commence your dietary supplement allocation for 7 days. You will report your dietary intake on 2 non-consecutive days of your choice and your sleep daily. You will be requested to maintain your habitual bed and rise times (at least 8-hour time-in-bed) between days 1 to 6. On day 7, you will restrict your sleep to 3-hour time-in-bed (0330 to 0630 h).

During the study, you will be requested to:

- abstain from over-the-counter medications, alcohol, and napping.
- remain hydrated.
- restrict caffeine intake to less than 100 $mg \cdot day^{-1}$, which is to be ingested before 1200 daily and abstain from caffeine in the 24 hours prior to testing.
- abstain from meat and fish during the 24 hours prior to testing.
- maintain physical activity at moderate levels, which will be monitored with an actigraph.
- all other lifestyle choices will be allowed to vary naturally during the study period.

Following sleep restriction, you will present to Massey University (Albany Campus) between 0730-0930 h in a fasted stated to repeat the testing protocol (i.e. day 8; post-intervention).

Following completion, you will be asked to state what dosing protocol you think you were randomised to.

The washout period between treatments will be at least 5 weeks as this reduces tissue creatine concentrations to approximately baseline (pre-supplement) levels. During this time, you will not receive any interventions or be monitored. After the washout period, you will repeat the experimental protocol with the alternative supplement treatment.

Dietary supplementation

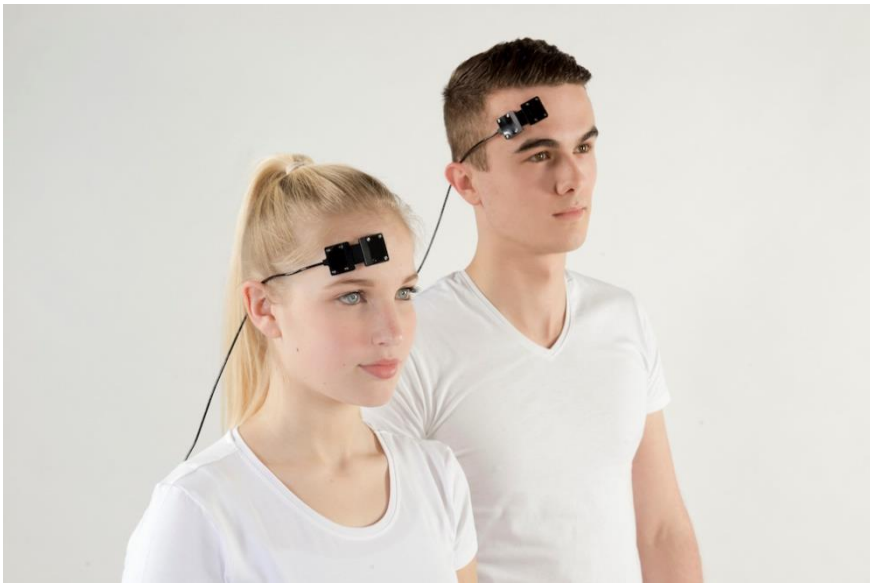
You will ingest 20 g·day^{-1}of creatine monohydrate or 20 g·day^{-1}of maltodextrin; you won't know which. Supplements will be made in a research grade kitchen and provided in powdered form to be mixed with water. Both will taste the same. You will ingest 4 doses per day, at equally spaced intervals on days 1 to 7 and will be asked to send a text to the researchers to confirm ingestion at the end of each day.

Testing protocol

Following arrival to Massey University (Albany Campus), you will rest in a quiet room. The cognitive test battery takes about 20 minutes to complete and will be divided into: 1) psychomotor vigilance task and measurement of cerebral oxygenation; and 2) working memory, logical relations and executive function using the computerised Automated Neuropsychological Assessment Metrics (ANAM®). Immediately afterwards, mood and sleepiness will be measured using the ANAM® and subjective workload will be recorded on a written handout.

Cerebral oxygenation measurement

Cerebral oxygenation will be measured using single channel near-infrared spectroscopy by placing a cap over your head.



Sleep/wake and physical activity monitoring

You will wear a wrist actigraph on your non-dominant wrist to monitor sleep and activity. You will also report subjective sleep quality rating upon wakening each morning using a visual analogue scale.

Dietary monitoring

You will be trained on how to report your dietary intake using an image-assisted, weighed diet record reported in real-time via a mobile phone application. You will be provided with written and verbal education on how to do this by a registered dietitian.

The time involved.

For each supplement treatment (remember, there are two treatments), the time involved includes:

- 2 hours for testing familiarisation
- Two 30 min periods for testing (i.e. baseline and post-intervention)
- 30 min for diet reporting
- 10 min for entering sleep diary information
- Additional for travel and will depend on your location

Total amount of time for each supplement treatment: **3 hours and 40 minutes**

Total amount of time for the study: **7 hours and 20 minutes**

Any conflict of financial interest and/or role.

The researchers have no immediate or foreseeable conflicts of interest.

Any support processes in place to deal with adverse physical or psychological risk.

You will be provided with the contact details of the research team in case you have any queries or concerns. If you are identified as a poor sleeper, you will be provided with information on good sleep practices, and if you have any concerns about your sleep, we will recommend that you follow up with your GP.

Data Management

What will happen to the data when it is obtained.

All data will be stored electronically on the Massey University for a minimum of 10 years after the study has been completed and will then be archived. Paper based data will be stored for 5 years. Data will not be shared or reported beyond the research team in any way that may identify participants.

Storage and disposal of data.

All data will be de-identified. Hard copy data will be stored in a locked cabinet at the School of Sport, Exercise and Nutrition, Massey University. All electronic data will be stored on the Massey University password protected networks. Hard copy data will be destroyed after 5 years.

Method for accessing a summary of the project findings.

Following your participation in the study, you will be provided a 1-2 page summary of your individual responses. On your request, these will be explained to you by one of the researchers.

Method for preserving confidentiality of identity.

All data will be stored, as previously described, to protect your privacy. Only de-identified / aggregated data will be published.

Participant's Rights

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- decline to answer any particular question;
- withdraw from the study (at any time before or during your participation);
- ask any questions about the study at any time during participation;
- provide information on the understanding that your name will not be used unless you give permission to the researcher;
- be given access to a summary of the project findings when it is concluded.

Your participation in this study is voluntary and you are free to remove yourself from the study at any stage, without any costs, repercussions or disadvantages. You have the right to access all of your personal information at any stage during the study and are able to ask for the results to be explained if you are uncertain of their meaning. You may decline to answer any question and also ask any question about the study. However, you will not own any intellectual property arising from this study or analysis of your samples.

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted, you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Project Contacts

Dr. David Shaw | PhD

Aerospace Physiologist, Aviation Medicine Unit, Royal New Zealand Air Force Base Auckland

School of Sport, Exercise and Nutrition, Massey University, Auckland
David.shaw2@nzdf.mil.nz
Dr. Margo van den Berg | PhD
Lecturer, Sleep/Wake Research Centre,
School of Health Sciences, Massey University, Wellington
m.j.vandenberg@massey.ac.nz

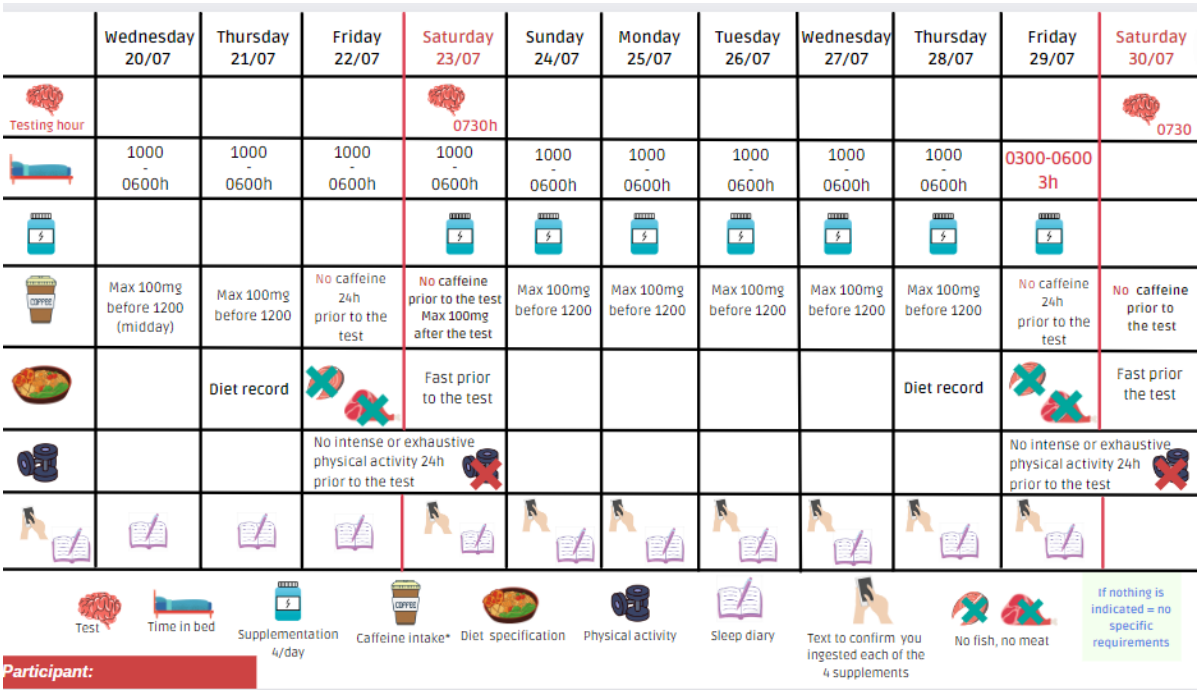
Associate Professor Nick Gant | PhD
Department of Exercise Sciences, University of Auckland, Auckland
n.gant@auckland.ac.nz

Juliette Janvresse | Dietetic Student
School of Sport, Exercise and Nutrition, Massey University, Auckland
J.Janvresse@massey.ac.nz

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 21/47. If you have any concerns about the conduct of this research, please contact Dr Negar Partow, Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63363, email humanethicsoutha@massey.ac.nz.

Note from the author: cerebral oxygenation measurement was not part of the present thesis.

Appendix B: Participant calendar



	Wednesday 20/07	Thursday 21/07	Friday 22/07	Saturday 23/07	Sunday 24/07	Monday 25/07	Tuesday 26/07	Wednesday 27/07	Thursday 28/07	Friday 29/07	Saturday 30/07
Testing hour				0730h							0730
	1000 - 0600h	1000 - 0600h	1000 - 0600h	1000 - 0600h	1000 - 0600h	1000 - 0600h	1000 - 0600h	1000 - 0600h	1000 - 0600h	0300-0600 3h	
	Max 100mg before 1200 (midday)	Max 100mg before 1200	No caffeine 24h prior to the test	No caffeine prior to the test Max 100mg after the test	Max 100mg before 1200	Max 100mg before 1200	Max 100mg before 1200	Max 100mg before 1200	Max 100mg before 1200	No caffeine 24h prior to the test	No caffeine prior to the test
		Diet record		Fast prior to the test					Diet record		Fast prior the test
			No intense or exhaustive physical activity 24h prior to the test							No intense or exhaustive physical activity 24h prior to the test	

Test · Time in bed · Supplementation 4/day · Caffeine intake* · Diet specification · Physical activity · Sleep diary · Text to confirm you ingested each of the 4 supplements · No fish, no meat · If nothing is indicated = no specific requirements

Participant:

Appendix C: Caffeine handout

During this study, please limit your caffeine intake to **100 mg per day** prior to 1200 (midday) and avoid caffeine after 1200.

Natural sources of caffeine include coffee beans, cocoa beans, kola nuts, tea leaves, yerba mate and guarana. Below is a list of common caffeine containing beverages and foods.

Food/drink	Average caffeine content (mg)
Coffee/coffee based products	
Brewed coffee black (250mls)	100-170
Espresso (single shot) (30mls)	70-90
Instant coffee (1 cup)	60-80
Cappuccino, Latte or Flatwhite (250mls)	70-90
Decaffeinated instant	3-5
Teas	
Black tea (1 cup)	50
Tea (green, oolong, white)	25-48
Herbal teas	0
Decaffeinated teas (250mls)	0-5
Soft drinks and energy drinks	
Energy drinks (250mls)	80-120
Coca cola (250ml)	25
Diet Coke (250ml)	32
Coke Zero (250ml)	24
Energy shots	60
Cocoa products	
Dark chocolate (40g)	27
Milk chocolate (40g)	8-12
Chocolate milk (250ml)	3-5
Chocolate ice-cream (1/2 cup)	2

Please use this as a guide and ask Dave or Juliette if you have any questions.