The effect of a two-week ketogenic diet, versus a carbohydrate-based diet, on cognitive performance, mood and subjective sleepiness during 36 hours of extended wakefulness in military personnel

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

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2022

Abstract

Background: Sleep deprivation (SD) compromises cognitive performance of military personnel, jeopardising operational performance and safety. Since SD-related performance deficits coincide with decreased glucose metabolism in associated brain regions, the ketogenic diet (KD) may mitigate cognitive impairments by providing an alternative fuel source (i.e. ketone bodies [KB]).

Aim: To investigate the effect of a 2-week KD compared with a carbohydrate (CHO)-based diet on cognitive function, mood and sleepiness during 36 hours of extended wakefulness.

Methods: A randomised, cross-over trial was conducted with 7 military personnel (range, 26-45 years). Participants ingested a KD (~25 g·day^{-1} CHO) or CHO-based diet (~285 g·day^{-1} CHO) for 14 days, immediately followed by 36 hours of wakefulness and separated by a 12-day washout period. Cognitive performance (5-minute Psychomotor Vigilance Task; PVT), mood (fatigue and vigour), subjective sleepiness, and capillary blood glucose and D-β-hydroxybutyrate (D-βHB) concentrations were measured every 2 hours (1, 3 and 5 hours after each meal). Linear mixed models tested the effect of diet, period (6 x 6-hourly bins), test time (1-3) within periods, and their interactions.

Results: D-βHB was higher (+0.75 to +1.45 mM; $p < 0.001$) and glucose was lower (-0.26 to -1.16 mM; $p < 0.01$) in the KD compared with the CHO-based diet. The KD improved performance for all PVT variables (number of lapses, mean reciprocal reaction time [RRT], slowest 10% RT and fastest 10% RT) ($p < 0.05$), mood ($p = 0.001$), and sleepiness ($p < 0.001$) compared with the CHO-based diet; however, there were no interactions with period or test. Number of lapses and subjective sleepiness increased, and mood, mean RRT and slowest 10% RT deteriorated during the 36 hours of extended wakefulness independent of diet (all $p <0.01$). Circadian effects were also observed for fastest 10% RT, mood and sleepiness independent of diet (all $p < 0.01$).

Conclusion: The KD appeared to improve cognitive performance, mood and sleepiness during 36 hours of extended wakefulness compared with the CHO-based diet. This suggests the KD could be considered for military operations when sleep deprivation is anticipated.

Keywords: Sleep deprivation; Psychomotor Vigilance Task; Keto-adaptation; Randomised cross-over trial.

Acknowledgements

This thesis has been a challenging, but immensely rewarding journey. I feel very lucky to look back on this process as something I have enjoyed being a part of. This is largely due to the incredible amount of support I have received along the way.

First and foremost, I would like to thank my supervisors Dr David Shaw and Dr Margo van den Berg. Your expertise, mentorship and generous support throughout this process has been invaluable. Dave, you went over and above the role of a supervisor, ensuring I was gaining experience in as many areas of the research process as possible. Thank you for the time and effort you put into this project. Margo, thank you for taking the time to explain sleep science concepts and for providing helpful resources. Your knowledge and eye for detail is greatly appreciated.

I would like to thank the staff at the AMU for welcoming me and providing their assistance throughout the year. A special thanks to Colin and Louise who helped to supervise the participants during the 36 hours of extended wakefulness. I would also like to thank Molly Illingworth who volunteered her time to help supervise the participants overnight.

To the participants, who pushed through to the end of a very challenging study with good humour and great energy. Thank you for offering your time to take part. I really enjoyed getting to know you all and have fond memories of playing Cube at 3am in the morning. Thank you for making this study possible.

Finally, to mum, dad, Thea and Dan. I appreciate the time you gave, listening to me read aloud my writing, discuss my results and panic about time lines. My drive to finish this thesis, and look ahead to a very exciting career, is undoubtedly due to you all. Thank you for your continued support and encouragement.

This thesis is dedicated to my grandmother. She was one of the greatest influences for my drive to further my education and pursue a Master's degree. Although she is unable to see this thesis in its completion, I hope it would have made her proud.

Table of Contents

List of Tables

List of Figures

'Fatigue is the invisible enemy of safe practices in any industry, especially Military Aviation. It is endemic and often unavoidable due to long duty hours, inadequate sleep, poor nutrition ... Whilst humans are resilient, the effects of fatigue are insurmountable and can be catastrophic. There is a need to understand how we can improve the cognitive performance of those affected.'

SQNLDR Gus Cabre
Officer Commanding Aviation Medicine Unit
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1 Introduction

1.1 Background

Fatigue-related impairments are associated with catastrophic real-world consequences. For example, nuclear disasters such as Chernobyl and Three Mile Island have been attributed, in part, to human error caused by fatigue (Mitler et al., 1988). There are many different definitions of fatigue (Balkin & Wesensten, 2011), but for the purpose of this thesis, fatigue is defined as "a physiological state of reduced mental or physical performance capability resulting from sleep loss, extended wakefulness, circadian phase, and/or workload (mental and/or physical activity) that can impair a person's alertness and ability to perform safety-related operational duties" (International Civil Aviation Organization, 2016). Early start times, late finishes, night work and extended working hours contribute to the development of fatigue and are common in occupations such as transport, medical and emergency services, law enforcement and the military (Williamson et al., 2011). The examination of potential fatigue management strategies is, therefore, critical for occupations where the development of fatigue is inevitable.

In the military, fatigue persists due to training and operational demands, as well as the collective 'can do' mindset (Johnson, 2007; National Commission on Military Aviation Safety, 2020). This is of particular concern as it compromises the safety and success of operations (Caldwell & Caldwell, 2016; Caldwell, Caldwell, Thompson, & Lieberman, 2019). Management strategies are important to prevent fatigue-related incidents, accidents and loss of limb or life, particularly ensuring personnel consistently achieve adequate sleep (i.e. optimising sleep-wake schedules) (Caldwell & Caldwell, 2016). Most adults require between 7-9 hours of sleep a night to maintain cognitive performance and prevent fatigue (Caldwell et al., 2019; Hirshkowitz et al., 2015). Nevertheless, sleep deprivation (SD) in the military occurs and personnel may be required to operate under fatigue to complete missions (Caldwell et al., 2019; Miller, Shattuck, & Matsangas, 2011).

Sleep deprivation, or extended wakefulness, increases the homeostatic drive for sleep to impair performance across multiple cognitive domains (Lim & Dinges, 2010). Deficits appear greatest for simpler, monotonous tasks, such as those assessing alertness and vigilance (Durmer & Dinges, 2005), whilst more complex tasks requiring critical thinking and logical reasoning seem less affected (Lim & Dinges, 2010; Pilcher, Band, Odle-Dusseau, & Muth, 2007).

Vigilant attention is required to complete various tasks in military operations (Lieberman et al., 2006) and can be measured using the Psychomotor Vigilance Task, which is sensitive to the effects of SD (Lim & Dinges, 2008). Mood also declines with SD (Durmer & Dinges, 2005) and this may also impact the success of military operations. Cognitive deficits are typically observed within the first 24 hours of SD and further increase after 2-3 nights without sleep (Thomas et al., 2000). However, it is rare that military operations would continue across multiple nights without sleep; therefore, assessing fatigue management strategies for up to ~36 hours SD is more reflective of real-world operations.

The circadian process interacts with the homeostatic drive for sleep to regulate levels of alertness and cognition (Borbély, Daan, Wirz-Justice, & Deboer, 2016; Valdez, 2019). This process is controlled by an endogenous biological clock, located in the suprachiasmatic nucleus (SCN) in the brain (Yamanaka, 2020). Although the SCN produces a rhythm that is on average slightly longer than 24 hours, it is entrained to the external 24 hour day/light cycle by time cues, such as light (Czeisler & Buxton, 2017). It not only modulates the sleep-wake cycle, but also cognitive function; typically, cognitive performance is low in the morning (0700-1000 h), increases towards noon (1000-1400 h), dips mid-afternoon (1400-1600 h), and improves into the evening (1600-2200 h) before dropping to its lowest point in the early hours of the morning (Valdez, 2019). The circadian process modulates the effects of SD on alertness and cognitive performance, leading to improved performance after a period of SD when the circadian drive for wakefulness is high. However, after ~24 hours of SD, alertness and cognitive performance is reduced when compared to same time of day 24 hours earlier (Gabehart & Van Dongen, 2017).

Sleep deprivation dysregulates brain function and metabolism, which could underpin some cognitive impairments. Typically, the brain relies almost exclusively on glucose for energy production (Mergenthaler, Lindauer, Dienel, & Meisel, 2013), accounting for ~98% of whole brain energy metabolism (Courchesne-Loyer et al., 2017); however, the cerebral metabolic rate of glucose declines during SD, which has been associated with impaired cognition (Thomas et al., 2000; Wu, Gillin, Buchsbaum, & Hershey, 1991). Brain regions particularly vulnerable to SD include the thalamus and prefrontal cortex, which mediate alertness, attention and higher order cognitive processes (Thomas et al., 2000; Wu et al., 1991). The provision of an alternative energetic substrate for cerebral oxidation during SD could mitigate reductions in glucose metabolism, and thus cerebral activity, to attenuate declines in cognitive performance.

The ketone bodies (KBs), acetoacetate (AcAc) and beta-hydroxybutyrate (βHB), can provide an alternative energetic substrate for the brain (Owens, Parker, & Benton, 1997). Ketone bodies are 4-carbon molecules that are able to cross the blood-brain-barrier via monocarboxylate transporters (Halestrap & Wilson, 2012). A very low-carbohydrate (CHO), ketogenic diet (KD) stimulates hepatic ketogenesis to increase the concentration of circulating KBs. A typical KD comprises <5% energy intake (EI) from CHO, 15-20% EI from protein and 75% EI from fat (Phinney, Bistrian, Wolfe, & Blackburn, 1983; Shaw, Maunder, Dulson, Merien, & Braakhuis, 2019). The predominant circulating KB is the D- enantiomer of beta-hydroxybutyrate (D-βHB), which increases from ~0.1-0.2 mM to >0.4 mM (i.e. ketosis) within days of induction to a KD (Robinson & Williamson, 1980; Shaw et al., 2019). After several days of ingesting a KD, KBs can provide up to 33% of the brains total energy requirements to reduce the reliance on glucose (Courchesne-Loyer et al., 2017).

Research into the effect of a KD on cognition in healthy individuals is limited. Short-term (1 week) KDs (Edwards et al., 2011), or non-ketogenic, high-fat diets (Holloway et al., 2011), appear to have unfavourable effects on cognition; however, these effects appear to dissipate with longer (e.g. 3 weeks) KD interventions (Iacovides, Goble, Paterson, & Meiring, 2019). Only one study has investigated the effect of a low(er) CHO, high-fat diet on cognitive performance during SD. A 1-week, non-ketogenic (40% EI from CHO), high-fat diet compared with a high-CHO diet (65% EI from CHO), examined changes in cognitive performance and sleepiness across 24 hours of SD (6 x 4-hour periods, with each commencing with a meal) (Lowden et al., 2004). Simple reaction time deteriorated across the post-prandial period (i.e. after a meal) for the high-CHO diet, compared with a more level response observed for the high-fat diet. Higher levels of subjective sleepiness were also observed for the high-CHO diet compared with the high-fat diet (Lowden et al., 2004). Since participants were likely to not be in a state of nutritional ketosis as CHO intake was not sufficiently low to increase ketogenesis, it is possible a KD may further alter cognitive responses during SD. In addition, the 1-week intervention period may have been inadequate to optimise metabolic adaptations necessary to overcome the initial abrupt changes in CHO availability.

To the author's knowledge, the KD is yet to be investigated for its effects on cognitive performance, sleepiness and mood during SD. It is plausible that the KD may provide a further

benefit than what was observed with non-ketogenic, high-fat diets due to the provision of KBs when cerebral glucose metabolism is impaired.

1.2 Purpose of the Research Study

Mitigating SD-related cognitive impairments within the military is a priority (Johnson, 2007). The impact of dietary interventions are unclear due to limited research, with the exception of caffeine (Chaudhary, Taylor, Grandner, Troxel, & Chakravorty, 2021), although interest in optimising military personnel's cognitive performance via diet is increasing (Teo et al., 2017). The KD has also been implicated within some military groups, primarily for its role in weight management and physical performance (LaFountain et al., 2019; Zinn et al., 2017), with little understanding of its impact on cognition. This study will contribute to future dietary and fatigue-management guidelines within the military.

1.3 Aim

To investigate the effect of a 2-week KD, compared with a CHO-based diet, on cognitive performance, mood and sleepiness during 36 hours of extended wakefulness.

1.4 Objectives

1. To assess the effect of a 2-week KD, compared with a CHO-based diet, on cognitive performance every 2 hours during a 36-hour period of extended wakefulness.
2. To assess the effect of a 2-week KD, compared with a CHO-based diet, on mood (fatigue and vigour) every 2 hours during a 36-hour period of extended wakefulness.
3. To assess the effect of a 2-week KD, compared with a CHO-based diet, on subjective sleepiness every 2 hours during a 36-hour period of extended wakefulness.

1.5 Hypothesis

1. The KD will mitigate SD-related impairments in cognitive performance, mood and sleepiness compared with the CHO-based diet.
2. Differences in cognitive performance, mood and sleepiness between dietary conditions will increase towards the end of the post-prandial period, particularly in the latter stages of SD.

1.6 Structure of Thesis

This thesis is divided into 4 chapters. **Chapter one** provides an introduction to the background, purpose, aims, objectives and hypotheses of this study. **Chapter two** is a review of relevant literature relating to key concepts and background of the study. **Chapter three** presents the manuscript for publication including a full description of study methods, results and discussion. The final chapter of this thesis, **Chapter 4,** presents the study conclusions, contribution to the literature and recommendations for future research. The **appendices** include supplementary results, participant information, questionnaires and guides.

1.7 Researcher's Contributions

Table 1.1 Researcher's Contributions

Author	Contribution
Lydia Henderson Master of Science Nutrition and Dietetics Student	Review of ethics application; review of literature; creation of participant resources including the caffeine guide, KD guidebook, example meal plans and participant schedules; study implementation and data collection; development of participant meal plans; supervising participants during SD; data processing and interpretation; preparation of final manuscript; and primary author of thesis.
Dr David Shaw Main academic supervisor	Main contributor to study design, ethics application and trial registration; participant recruitment; study implementation and data collection; creation of the R script for data analyses; data processing; and supervision of thesis write up.
Dr Margo van den Berg Academic co-supervisor	Assisted with study design, ethics application and registration; provision of actigraphs and PVT devices; scoring of participants' actigraphy data; interpretation of results; co-supervision of thesis write up.

2 Literature review

2.1 Fatigue

Fatigue has become an unavoidable consequence of modern day society (Caldwell et al., 2019). Early starts, late finish times, extended work periods and shift work contribute to inadequate sleep and circadian disruption (Caldwell et al., 2019), which degrades cognitive performance (Durmer & Dinges, 2005). Fatigue-related cognitive impairments are so substantial that they are the leading causes of incidents in road transport (Williamson & Feyer, 2000). In fact, 17 hours of wakefulness (from 0800) has been shown to be as detrimental to cognition as a blood alcohol concentration of 0.05% (equivalent to the NZ legal driving limit) (Dawson & Reid, 1997). Some occupations are at a greater risk of experiencing fatigue to levels that compromise performance and safety, including transportation, medical/emergency services, law enforcement and the military (Caldwell et al., 2019). In these occupations, fatigue-related risk must be managed effectively to avoid incidents and accidents (Van Dongen, Maislin, Mullington, & Dinges, 2003).

2.1.1 Fatigue in the military

Extended duty periods (Johnson, 2007), more deployments and increasingly complex missions contribute to fatigue within the military (Frone & Blais, 2019). Advances in technology, such as night-vision devices and extended-range fuel systems, have also made around the clock missions achievable, increasing expectation on human productivity and efficiency (Johnson, 2007). Whilst machinery can operate continuously, the human body cannot and requires sufficient periods of recovery to maintain performance (Caldwell et al., 2019). Many operations provide little opportunity for recovery, which limit the amount of quality sleep attained by personnel. This can lead to fatigue-related cognitive impairments, such as errors of omission (lapses in attention), errors of commission (false responding) and poor decision making (Johnson, 2007). Despite implementation of fatigue management strategies, such as optimising sleep-wake schedules, napping and/or the use of caffeine (Caldwell & Caldwell, 2016; Chaudhary et al., 2021), military personnel may be required to operate while fatigued as some missions must be completed (Johnson, 2007).

2.1.2 Defining fatigue

Fatigue is defined as "a physiological state of reduced mental or physical performance capability resulting from sleep loss, extended wakefulness, circadian phase, and/or workload (mental and/or physical activity) that can impair a person's alertness and ability to perform safety-related operational duties" (International Civil Aviation Organization, 2016). Fatigue causes a broad range of symptoms, outlined in Table 2.1, which can lead to injuries and accidents.

Table 2.1. Signs, symptoms and effects of fatigue from sleep loss and/or circadian disruption. Adapted from Caldwell et al. (2019).

Signs and symptoms of fatigue	Effects of fatigue
• Rubbing the eyes • Head nodding • Forgetting instructions • Long eye blinks • Yawning • Fidgeting • Uncommunicative • Inability to solve routine work problems • Lack of motivation • Depression • Giddiness	• Reduced decision making ability • Reduced ability to do complex planning • Reduced communication skills • Reduced productivity or performance • Reduced attention and vigilance • Reduced ability to handle stress • Increased reaction time in speed and thought • Loss of memory or the ability to recall details • Failure to respond to changes in surroundings or information provided • Inability to stay awake (e.g. falling asleep while operating machinery or driving vehicles) • Increased tendency for risk-taking • Increased forgetfulness • Increased errors in judgement • Increased medical costs • Increased rates of adverse incidents

2.1.3 Sleep

Sleep is defined as "a reversible state in which conscious control of the brain is absent and processing of sensory information from the environment is minimal" (International Civil Aviation Organization, 2016) and is imperative for the maintenance of cognition whilst awake (Caldwell et al., 2019). Most adults require between 7-9 hours of sleep per night (Hirshkowitz et al., 2015) to prevent deficits in cognitive domains such as alertness, vigilance, sustained attention, consolidation of memories and emotional regulation (mood) (Diekelmann & Born, 2010; Minkel et al., 2012). Two distinct types of sleep have been identified, non-rapid eye movement sleep and rapid eye movement sleep, which alternate cyclically (Carskadon & Dement, 1989). Non-rapid eye movement sleep is divided further into 4 stages identified by electroencephalography (EEG) (Carskadon & Dement, 1989; Kales & Rechtschaffen, 1968), with stages 3 and 4 regarded as slow wave sleep. Slow wave sleep, characterised by the presence of slow wave activity, is thought to play an integral role in cerebral restoration and recovery (Benington & Heller, 1995; Kales & Rechtschaffen, 1968; Roth, 2009). .

The two-process model of sleep regulation, first proposed by Borbély (Borbély et al., 2016), describes how sleep is regulated by two main processes (Figure 2.1); 1) the homeostatic drive for sleep and 2) the circadian process (Borbély et al., 2016). As the hours spent awake increase, the homeostatic drive for sleep increases. Slow wave activity has been shown to have a dose response relationship with prior sleep/wakefulness and is a physiological marker for sleep pressure (Borbély et al., 2016). The circadian process interacts by setting boundaries to the homeostatic process, with threshold levels that oscillate depending on time of day (Borbély et al., 2016; Durmer & Dinges, 2005). Once the upper threshold is reached, falling asleep becomes involuntary and can be observed by micro-sleeps (Bougard et al., 2018; Goel, Rao, Durmer, & Dinges, 2009). Slow wave activity dissipates during sleep and once the drive for sleep is low and the lower threshold is reached, sustained wakefulness is invoked (Borbély et al., 2016). These two processes interact to regulate alertness and cognition throughout the day and night.

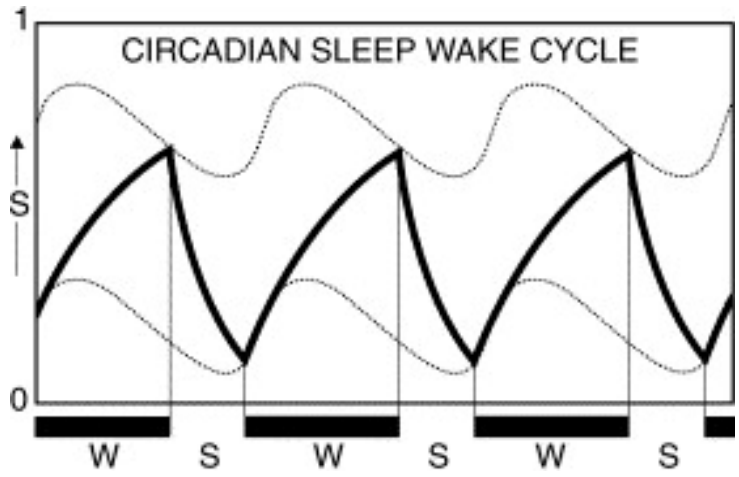

Figure 2.1. The two process model of sleep regulation. Process S represents the homeostatic drive for sleep. It increases during waking (W) and decreases during sleep (S). Thresholds for sleep and wake are determined by the circadian process (dotted lines), which vary with time of day. Reproduced with permission from rights holder (Beersma & Gordijn, 2007) .

2.1.3.1 Measuring sleep

The gold standard objective measure of sleep is polysomnography (PSG). Polysomnography measures sleep using electroencephalography, electrooculography, and electromyography (Jaworski, Roshan, Tae, & Park, 2019). However, PSG is typically not practical for recording sleep across multiple days outside of a laboratory setting as it is expensive, time consuming and requires trained staff (Chinoy et al., 2020). Alternatively, sleep can be measured objectively using actigraphy (Ancoli-Israel et al., 2015). Actigraphy utilises an accelerometer to record wrist movement per epoch (e.g. 60 seconds) to identify sleep-wake states using a sleep-scoring algorithm validated against PSG (Ancoli-Israel et al., 2015). An event marker is utilised to indicate when the individual starts trying to sleep and when they wake, which is supported by sleep diary entries (Ancoli-Israel et al., 2015). Actigraphy is effective for measuring sleep-wake periods across multiple days as it is less expensive and intrusive than PSG, and more user friendly (Ancoli-Israel et al., 2015; Chinoy et al., 2020; Jaworski et al., 2019). Nevertheless, actigraphy can overestimate total sleep time and efficiency and underestimate sleep onset latency (Paquet, Kawinska, & Carrier, 2007). This is due to the difficulty detecting wakefulness when an individual is immobile in bed, but not asleep (de Souza et al., 2003).

2.1.4 Circadian rhythms

Circadian rhythms are biological cycles that respond to daily fluctuations in the internal and external environment (Garbarino et al., 2020). These rhythms are generated by a central biological clock within the suprachiasmatic nucleus (SCN) of the hypothalamus and peripheral "clocks" located elsewhere in the brain and body (Yamanaka, 2020). These rhythms are, on average, slightly longer than 24 hours but are entrained to the external 24-hour day/light cycle by time cues (Zeitgebers) such as light (Garbarino et al., 2020; Tharumalay, Din, & Ahmad, 2020). In turn, the SCN relays its 24-hour rhythm to other processes including behavioural (e.g. sleep-wake activity), autonomic (e.g. core body temperature [CBT]) and hormonal rhythms (Garbarino et al., 2020). Humans are a diurnal species intrinsically programmed to be active and awake during the day, but inactive and asleep during the night (Tharumalay et al., 2020). Shift work, insufficient sunlight exposure, nocturnal exposure to artificial light and travel across multiple time zones can disrupt circadian rhythms (Garbarino et al., 2020). Circadian rhythms have also been identified for glucose and lipid metabolism, and diet-induced thermogenesis (Johnston, 2014; Yamanaka, 2020).

2.1.4.1 Circadian variation in alertness and cognitive performance

Daily fluctuations in alertness and cognitive performance reflect circadian rhythms that control our waking behaviours (Gabehart & Van Dongen, 2017). Throughout history, many disasters have occurred at night, with the risk of traffic accidents up to 10-fold higher, coinciding with when we are inherently programmed to be asleep (Williamson & Feyer, 2000). Typically, cognitive performance (attention, working memory and executive function) is low in the morning (0700-1000) due to the circadian low, increases towards noon (1000-1400), dips mid-afternoon (1400-1600), and improves again in the evening (1600-2200) before dropping to the lowest point in the early hours of the morning (Valdez, 2019). As it is not possible to measure the SCN's activity directly, biological markers strongly controlled by the SCN, such as CBT, are used to track an individual's circadian phase (Kleitman & Jackson, 1950). In a healthy individual who is entrained with the 24-hour light/dark cycle, the rhythm in sleep propensity (i.e. sleepiness) is inversely related to the rhythm in CBT (Kleitman & Jackson, 1950). During extended wakefulness, cognitive performance deficits can have a 1-4 hour discrepancy with the rhythm in CBT, likely due to the increased homeostatic pressure for sleep, which accumulates with time awake (Gabehart & Van Dongen, 2017).

2.1.5 Sleep deprivation

Acute total sleep deprivation (SD) is typically defined as a period of wakefulness extending beyond 16-18 hours (Banks, Dorrian, Basner, & Dinges, 2017). Whereas, sleep restriction is when insufficient sleep is attained within each 24-hour cycle for one or more nights (Banks & Dinges, 2007). Whilst SD is not as common as sleep restriction (Lim & Dinges, 2010), it still occurs within the military, particularly when operations are extended. Research to date has assessed physiological and cognitive responses to SD up to 90 hours (Banks et al., 2017); however in the military, SD beyond 36 hours is unlikely to occur. When SD occurs, the homeostatic drive for sleep increases to the extent that it impairs waking behaviours/functions, even when the circadian drive for wakefulness is high (Durmer & Dinges, 2005; Goel, Basner, Rao, & Dinges, 2013).

2.1.5.1 Cognitive function and subjective states during sleep deprivation

Sleep deprivation impairs performance across multiple cognitive domains (Durmer & Dinges, 2005; Lim & Dinges, 2010). Deficits in cognitive domains accumulate as SD prolongs (Cirelli & Tononi, 2017), diminishing productivity and increasing the risk of accidents (Lowe, Safati, & Hall, 2017). Occupations which operate around the clock, such as the military, are more at risk. Deficits appear greatest for simpler, monotonous tasks such as those assessing alertness and vigilance (Durmer & Dinges, 2005), whilst more complex tasks requiring critical thinking and logical reasoning appear less affected (Lim & Dinges, 2010; Pilcher et al., 2007). For example, SD increases reaction time and lapses of attention, as well as exacerbates the effect of time on task (Durmer & Dinges, 2005; Lim & Dinges, 2008). Lapses of attention also indicate increased moment-to-moment variability caused by sleep-initiating mechanisms that begin to interfere with wakefulness and progressively destabilise cognition (Doran, Van Dongen, & Dinges, 2001). The psychomotor vigilance task is used to measure vigilant attention as it has shown to be valid and reliable during SD and is sensitive to both circadian and homeostatic influences (Lim & Dinges, 2008). In addition, all forms of SD result in negative mood states, especially feelings of fatigue and loss of vigour (Durmer & Dinges, 2005). Some individuals display minimal impairment from SD, whilst others are more vulnerable to sleep loss (Van Dongen, Maislin, & Dinges, 2004). Repeated exposure to SD indicates that these individual differences in response to SD are trait-like and may reflect underlying genetic differences (Van Dongen, Baynard, Maislin, & Dinges, 2004). This variability must be considered when assessing the effects of SD.

2.1.5.2 Changes in brain function and metabolism during sleep deprivation

Sleep deprivation alters brain function and cerebral glucose metabolism. Typically, the brain relies almost exclusively on glucose for energy production (Mergenthaler et al., 2013), accounting for ~98% of whole brain energy production (Courchesne-Loyer et al., 2017). However, after 24 hours of SD, the cerebral metabolic rate of glucose declines in areas such as the thalamus and prefrontal cortex, which mediate alertness, attention and higher order cognitive processes (Thomas et al., 2000). As a result, the reduction in cerebral glucose metabolism appears to coincide with impaired performance in these cognitive domains (Thomas et al., 2000; Wu et al., 1991). Preventing sleep, particularly slow wave sleep (Gip, Hagiwara, Ruby, & Heller, 2002), may also prevent the restoration of cerebral glycogen stores (glucose polysaccharide) that progressively deplete while being awake (Benington & Heller, 1995).

Moreover, six hours of SD in mice significantly increased blood concentrations of the ketone bodies (KBs), acetoacetate (AcAc) and β-hydroxybutyrate (βHB), which returned to baseline concentrations after 6 hours of sleep (Chikahisa, Shimizu, Shiuchi, & Séi, 2014). Gene expression of ketogenic enzymes also increased, although there was a concomitant reduction in gene expression of ketolytic enzymes in the brain (Chikahisa et al., 2014); however, it is uncertain whether these responses altered the brain's KB oxidation rates. This suggests KB metabolism has a role in sleep-wake regulation and could alter brain function during prolonged SD. Therefore, the provision KBs for cerebral oxidation during SD could mitigate reductions in cerebral glucose metabolism to attenuate declines in cognitive performance.

2.2 Ketogenic diet

The ketogenic diet (KD) is a very low-CHO, high-fat diet typically comprising <5% energy intake (EI) from CHO, 15-20% EI from protein and >75% EI from fat (Phinney et al., 1983; Shaw, Merien, Braakhuis, Maunder, & Dulson, 2020). Initially introduced as a treatment for epilepsy in the 1920's (Wheless, 2008), the KD has since been investigated for its effects on weight management (Paoli, 2014), neurological disease (Pavón et al., 2021) and exercise performance (Burke, 2021; Shaw et al., 2019). The term, ketogenic, refers to the generation of KB's which can bypass the blood brain barrier via monocarboxylate transporters (Halestrap & Wilson, 2012) to support the energy demands of the brain when glucose availability is low

(Robinson & Williamson, 1980). Typical post-prandial concentrations of blood KBs are ~0.1-0.2 mM (Robinson & Williamson, 1980) and elevate to >0.4 mM within the initial days of induction to a KD (Shaw et al., 2019; Volek, Noakes, & Phinney, 2015). In starvation, blood KB concentrations can increase to 5-7 mM (Cahill, 2006); however, in an energy-balanced, KD concentrations are often 0.5-2 mM (Shaw et al., 2020). Nutritional ketosis is the sustained elevation of blood KBs above typical post-prandial concentrations and is often used to confirm compliance to a KD (Robinson & Williamson, 1980). The KD is generally deemed safe, with some populations choosing to adhere to it for several months (Volek et al., 2016). Ketogenic diets have also been assessed within military populations, primarily for weight management and physical performance, with preliminary research having been conducted within the New Zealand Defense Force (Zinn et al., 2017) and American military (LaFountain et al., 2019). There is continued interest by the military, including the New Zealand Defense Force, into the effects of a KD on various stressors applicable to operational demands, such as sleep deprivation.

2.2.1 Ketone bodies

The KBs (AcAc, acetone and βHB) are water soluble molecules involved in energy production, cell signalling and gene expression (Robinson & Williamson, 1980). They are predominantly produced in the liver (e.g. hepatic ketogenesis) via beta-oxidation from fatty acids when CHO availability is depleted (Dhillon & Gupta, 2021; Robinson & Williamson, 1980). Insulin levels decline and glucagon increases to upregulate KB production (Robinson & Williamson, 1980). β-hydroxybutyrate is the predominant circulating KB, which reversibly reforms AcAc for oxidation in extrahepatic tissues, such as the brain, heart and skeletal muscle. Compared to glucose, βHB is a more efficient substrate, as it produces more ATP per 2-carbon unit (Veech, 2004). β-hydroxybutyrate exists in two isoforms; D-βHB, which is the isomer produced during hepatic ketogenesis and contributes to energy metabolism, and L-βHB, which exists in much lower concentrations and is a by-product of fat metabolism that are predominantly used for free fatty acid and sterol production (Lincoln, Rosiers, & Brunengraber, 1987; Webber & Edmond, 1977). Acetone is largely produced via the spontaneous decarboxylation of AcAc; it is not involved in energy production and is mostly excreted in the urine and breath (Robinson & Williamson, 1980). Peripheral tissues differ in their capacity to adapt and metabolise KBs (Robinson & Williamson, 1980). For example, skeletal muscle has a lower capacity to oxidise

KBs than the brain and the heart, which is likely due to prioritising the preservation of cognition and circulation (Lauritsen, Søndergaard, Luong, Møller, & Gormsen, 2020).

2.2.2 Cerebral adaptation to the ketogenic diet

The brain is a metabolically flexible organ capable of oxidising substrate other than glucose, such as lactate, pyruvate and KBs (Achanta & Rae, 2017). Brain KB oxidation increases when: (1) plasma KB concentrations increases; (2) cerebral KB uptake increases; and (3) ketolytic enzyme activity increases (Owen et al., 1967). Typically, the metabolism of βHB contributes to <3% of total cerebral metabolism (Courchesne-Loyer et al., 2017); however, within days of induction to a KD, plasma concentrations of KBs increase and cerebral KB uptake through monocarboxylate transporters upregulates (Achanta & Rae, 2017). In response to increased cerebral βHB concentrations, mitochondrial enzymes involved in KB utilisation and oxidation are upregulated and βHB can be metabolised by all brain cells (Achanta & Rae, 2017). For example, a study with 10 healthy adults demonstrated an 8-fold increase in plasma KB concentration and a 24% reduction in plasma glucose concentrations after 4 days of nutritional ketosis (Courchesne-Loyer et al., 2017). After 4 days, the cerebral metabolic rate of glucose reduced by 20% and the cerebral metabolic rate of AcAc increased 6-fold, which was approximately 17% of the brain's energy production (Courchesne-Loyer et al., 2017). When combined with βHB, total KBs contributed to approximately 33% of the brain's energy production (Courchesne-Loyer et al., 2017). The optimal period for adaptation to the KD, however, remains uncertain. Although longer periods may be required to overcome initial abrupt changes to substrate availability, it is likely to be shorter than suggestions for muscular adaptation due to the brain's superior ability to metabolise KBs.

2.2.3 The ketogenic diet and cognitive performance

The effect of the KD on cognitive performance in healthy individuals is unclear. A short term 7-day KD (1.5% EI from CHO, 74.4% EI from fat and 24.1% EI from protein) and a 5-day high-fat diet (4% EI from CHO, 70% EI from fat and 26% EI from protein; blood βHB was not measured) were associated with an increase in simple reaction time, impaired attention, alertness and reduced speed of processing (Edwards et al., 2011; Holloway et al., 2011). In contrast, a ~ 29-day KD (15% EI from CHO, 60% EI from fat and 25% EI from protein; average capillary blood βHB 1.0 ± 0.5 mM) had no effect on cognitive outcome measures (vigilance, visual learning and memory, working memory and executive function) compared to a high-

CHO diet (Iacovides et al., 2019). Short-term CHO restricted protocols (<1 week) are potentially inadequate for sufficient cerebral adaptation to overcome deficits in cognitive performance resulting from changes in substrate metabolism. Therefore, adequate adaptation to a KD (i.e. >1 week) and strict dietary adherence appears imperative to distinguish the effects of a KD compared with other high-fat, non-KDs on cognitive function. In alignment with recommendations to examine whole dietary patterns on cognitive function (Teo et al., 2017), further research needs to evaluate the effects of a KD.

2.2.4 The ketogenic diet and mood

The effect of a KD, and low-CHO diets, on mood in healthy individuals also is unclear. A 7-day KD demonstrated a negative effect on mood by reducing calmness and awareness in sedentary men (Edwards et al., 2011), whilst a 5-day high-fat, non-KD appeared to decrease contentedness (Holloway et al., 2011). In contrast, a ~29-day KD did not appear to effect mood, compared to a high-CHO diet (Iacovides et al., 2019). Therefore, similar to cognitive performance, adequate dietary duration and adherence appear important to maintain mood.

2.2.5 The ketogenic diet and sleep deprivation

Only one study has examined the effect of reduced CHO intake on cognitive performance and sleepiness during SD (Lowden et al., 2004). In crossover design, seven male participants consumed a 1-week high-CHO diet (15% EI from protein, 65% EI from CHO, 20% EI from fat) and high-fat, non-KD (15% EI from protein, 40% EI from CHO, 45% EI from fat) with a 1-month wash-out between conditions. On the morning of day 7, participants commenced a period of 24 hours of SD, split into 6 x 4-hour periods, each beginning with a meal. Cognitive performance (simple reaction time and an arithmetic task), subjective sleepiness and mood were assessed hourly (Lowden et al., 2004). Simple reaction time deteriorated across the 4-hour post-prandial period for the high-CHO diet, compared with a more level response observed for the high-fat diet. There was an overall decline in simple reaction time for the final two periods of the study (2400-0800 h) independent of diet. In addition, subjective sleepiness was higher for the high-CHO diet compared with the high-fat, non-KD diet towards the end of the post prandial period (2-4 hours after eating) and during blocks 2 (1200-1600 h) and 5 (2400-0400 h). Considering CHO was ~40% of EI and circulating D-βHB was not measured, it is unlikely participants were in nutritional ketosis and glucose remained the primary substrate for cerebral energy metabolism. Therefore, a KD could exert different, potentially favourable,

effects on cognition during SD as impairments related to reduced cerebral glucose metabolism could be mitigated. Furthermore, by extending the SD period beyond 24 hours, which is associated with suppression of cerebral glucose metabolism (Thomas et al., 2000; Wu et al., 1991) and decreased glycogen stores (Kong et al., 2002), the potential interaction of the KD and SD could be more profound.

2.3 Summary

Fatigue-related impairments resulting from sleep loss pose a threat to the safety and success of military operations. Despite efforts to implement fatigue management strategies, it remains a prevalent issue. Cognitive deficits during SD are associated with decreases in cerebral glucose metabolism and glycogen synthesis, suggesting that the provision of alternative energetic substrates, such as KBs, may mitigate impairments to brain function. As KBs have been implicated in sleep-wake regulation and are an area of interest for militaries to improve resilience of their personnel, further research into dietary strategies inducing ketosis is required before it's widespread implementation. To date, no studies have been published investigating the effect of a KD on cognition, mood and sleepiness during SD; therefore, this research thesis will aim to address this gap in the literature.

3 Research Manuscript

Title

The effect of a 2-week ketogenic diet, versus a carbohydrate-based diet, on cognitive performance, mood and subjective sleepiness during 36 hours of extended wakefulness in military personnel

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3.1 Abstract

Sleep deprivation (SD) compromises cognitive performance of military personnel, jeopardising operational performance and safety. Sleep deprivation-related performance deficits coincide with decreased glucose metabolism in associated brain regions, suggesting the potential utility of a ketogenic diet (KD) to provide an alternative fuel source during SD. A randomised, cross-over trial was conducted with 7 military personnel. Participants ingested an iso-energetic KD (~25 g carbohydrate·day^{-1}) or carbohydrate-based diet (~285 g carbohydrate·day^{-1}) for 14 days, immediately followed by 36 hours of extended wakefulness and separated by a 12-day washout. Cognitive performance, mood (fatigue and vigour), subjective sleepiness, capillary blood glucose and D-β-hydroxybutyrate concentrations were measured every 2 hours (1, 3 and 5 hours after each meal). Linear mixed models tested the effect of diet, period (6 x 6-hourly bins), test time (1-3) within periods, and their interactions. D-βHB was higher (+0.75 to +1.45 mM; $p < 0.001$) and glucose was lower (-0.26 to -1.16 mM; $p < 0.01$) in the KD compared with the CHO-based diet. The KD improved performance for all cognitive performance variables (Mean reciprocal response time, number of lapses, mean slowest 10% response time and mean fastest 10% response time) ($p < 0.05$), mood ($p = 0.001$) and sleepiness ($p < 0.001$) compared with the carbohydrate-based diet. Sleep deprivation-related deficits were found for number of lapses, mean reciprocal response time, mean slowest 10% response time, mood and subjective sleepiness, independent of diet (all p <0.01). Circadian effects were also observed independent of diet; fastest 10% response time was slower in periods 4 and 5 (0130-1330) compared with periods 1, 2 and 3 (0730-0130), but was faster in period 6 (1330-1930) compared with period 4 (all $p < 0.01$); and mood declined and sleepiness increased from period 1 (0730-1330) to period 4 (0130-0730) ($p < 0.001$), but stabilised across periods 4, 5 and 6 (1330-1830). In conclusion, a KD demonstrated beneficial effects on cognitive performance, mood and sleepiness during 36 hours of extended wakefulness compared with the carbohydrate-based diet. This suggests the KD could be considered for military operations when sleep deprivation is anticipated.

Key words

Sleep deprivation, psychomotor vigilance task, randomised controlled trial

3.2 Introduction

Sleep deprivation (SD) in the military occurs when operational duties are extended, often unexpectedly. Fatigue manifests and cognitive performance is impaired, particularly alertness, vigilance, and sustained attention (Durmer & Dinges, 2005). Consequently, there is an increased risk of incidents, accidents and, in some circumstances, loss of life (Caldwell et al., 2019). It is, therefore, a priority for the military to reduce the risk of fatigue-related impairments on personnel, as safety is paramount. Despite implementation of fatigue-mitigation strategies, such as optimising sleep-wake schedules, napping, and/or use of caffeine (Caldwell & Caldwell, 2016), SD may be unpreventable as some operations must be completed. Except for caffeine (Chaudhary et al., 2021), dietary strategies rarely feature when mitigating SD-related cognitive deficits largely due to limited research. As the impact of diet on cognition is of interest to the military (Teo et al., 2017), research investigating the interaction of diet and SD on cognitive performance is warranted.

Sleep deprivation impairs cerebral carbohydrate metabolism (Thomas et al., 2000). The human brain depends on glucose as its primary energy source (Mergenthaler et al., 2013), yet impaired cognition experienced during SD appears to coincide with a decrease in the cerebral metabolic rate of glucose (Thomas et al., 2000; Wu et al., 1991). It is also hypothesised that extended wakefulness depletes cerebral glycogen stores that are typically restored during slow wave sleep (Benington & Heller, 1995). These metabolic impairments suggest the need to consider an alternative energetic substrate for the brain during SD, such as ketone bodies (KBs; acetoacetate [AcAc] and beta-hydroxybutyrate [βHB]). The predominant circulating KB is D-beta-hydroxybutyrate (D-βHB), which has a typical post-prandial concentration of ~0.1-0.2 mM (Robinson & Williamson, 1980) and increases to >0.4mM within days following induction to a ketogenic diet (KD) (Shaw et al., 2019; Volek et al., 2015). The KD comprises <5% energy intake (EI) from carbohydrate (CHO), 15-20% EI from protein and >75% EI from fat (Burke et al., 2017; Phinney et al., 1983; Shaw et al., 2019). Once adapted to a KD, KBs can provide up to 33% of the brains total energy requirements (Courchesne-Loyer et al., 2017).

Research on the effect of a KD on cognitive performance and mood in healthy individuals is limited. In non-sleep deprived conditions, short-term (<1 week) KD (Edwards et al., 2011) and non-ketogenic, high-fat diets (Holloway et al., 2011) appear to elicit unfavourable effects on cognition; however, impairments appear to dissipate with longer adaptation periods (Iacovides

et al., 2019). The metabolic stresses imposed by SD may help to delineate the effect of a KD on cognition. However, only one study has investigated the effect of a low-CHO, high-fat on cognition during SD (Lowden et al., 2004). A 1-week, non-ketogenic (40% EI from CHO), high-fat diet, compared with a high-CHO diet (65% EI from CHO), examined cognitive performance and sleepiness across 24 hours of SD (6 x 4-hour periods, with each commencing with a meal) (Lowden et al., 2004). Simple reaction time declined in the final two periods (2400-0800 h) for both diets, indicating the detrimental effect of SD. For the high-CHO diet, simple reaction time declined across the post-prandial period (after each meal) compared with a more level response for the high-fat, non-KD, and subjective sleepiness was higher towards the end of the post-prandial period (2-4 hours after eating) and during period 2 (1200-1600 h) and 5 (2400-0400 h) compared with the high-fat, non-KD diet. Considering CHO intake in the high-fat diet was ~40% EI, which is typically insufficient to increase ketogenesis, and circulating KBs were not measured, it is unlikely that participants were in nutritional ketosis and glucose remained the primary substrate for cerebral energy metabolism. It is, therefore, possible that a KD could exert different, potentially favourable, effects on cognition during SD, as impairments related to reduced cerebral glucose metabolism could be mitigated.

The aim of this study was to examine the effect of a 2-week KD, versus a CHO-based diet, on cognitive performance, mood and subjective sleepiness during 36 hours of extended wakefulness. It is hypothesised that SD-related impairments in cognitive performance, mood and sleepiness will be mitigated by the KD compared with the CHO-based diet. Furthermore, differences in cognitive performance, mood and sleepiness between dietary conditions will increase towards the end of the post-prandial period, particularly in the latter stages of SD.

3.3 Materials and methods

3.3.1 Study overview

A randomised, controlled, cross-over trial was conducted in military personnel from the Royal New Zealand Air Force. No *a priori* sample size calculation was performed for this pilot study and the aim was to recruit 15 participants based on a similar study (Gupta et al., 2019). Participants completed seven days of baseline testing and then were randomised (www.randomizer.org) to either a 14-day CHO-based diet or a KD (i.e. dietary adaptation). Immediately following dietary adaptation, participants completed a 36-hour period of extended wakefulness at the Aviation Medicine Unit, Royal New Zealand Air Force Base Auckland. A 12-day washout period separated the two trial arms. Ethical approval was provided by the New Zealand Defence Force and Massey University Ethics Committees (SOA 20/47). The study was retrospectively registered with the Australian New Zealand Clinical Trials Registry (ACTRN12621000105842). The study was funded by Massey University School of Sport Exercise and Nutrition Post-Graduate Research Supporting Funding and the Aviation Medicine Unit, Royal New Zealand Air Force.

3.3.2 Participants

Participants were recruited via a base-wide email and all interested personnel volunteered for the study. Participants were required to be: 1) healthy; 2) aged 18-50 years; 3) body mass index <28 kg/m^2; 4) consuming a mixed diet; 5) habitually going to bed between 2100-0000 and waking between 0600-0900. In addition, only men were recruited. Participants were excluded if they: 1) habitually consumed a KD or exogenous ketone supplements in the 2 years prior to the study; 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) had food allergies or restrictive dietary patterns; 8) 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 (Lovibond & Lovibond, 1995); and scoring as either moderately evening or intermediate chronotype on the Horne-Östberg Morningness/Eveningness Questionnaire (Horne & Ostberg, 1976). Participants were fully informed of the rationale of the study and possible risks of the

experimental procedures before providing their written consent; however, they were not informed of the potential effects of a KD and were requested to refrain from personal investigation to prevent biasing their results.

3.3.3 Dietary intervention

Participants commenced ingesting their dietary allocation immediately following baseline testing. Dietary interventions were matched to each participant's habitual energy intake ascertained during the baseline period using a 3-day, nonconsecutive diet record. Participants were provided with education from a registered dietitian (RD) and student dietitian and meal plans specific to their dietary allocation within 500 kJ of their habitual dietary intake. Additional education was provided to participants prior to commencing the KD in the form of a handout (Appendix C-E). The prescribed KD comprised <5% energy intake (EI) (<40 g·day^{-1}) from CHO, 15-20% EI from protein and >75% EI from fat. Participants were provided with electrolyte capsules (Pure Electrolyte Replacement Capsule; Pure Sports Nutrition, New Zealand) and were requested to ingest one capsule twice daily during the KD to mitigate potential reductions in blood electrolyte concentration. Whereas, the prescribed CHO-based diet comprised >45% EI from CHO, 15-20% EI from protein and <40% EI from fat. During both dietary interventions, participants were requested to abstain from over the counter medications, alcohol, napping, and to maintain their normal sleep and physical activity routines. Caffeine intake was limited to <100 mg per day ingested prior to 1200; participants were provided with a handout detailing caffeine content in common dietary items (Appendix D). All other lifestyle choices were allowed to vary naturally; however, participants were requested to replicate these during both dietary interventions.

3.3.4 Dietary monitoring

Dietary compliance was monitored via an image-assisted, weighed diet record reported remotely in real-time via a mobile phone application (WhatsApp, Facebook, San Francisco, CA). Participants were trained in how to provide accurate dietary reports, which were provided on three non-consecutive days between days 1-7 and 8-14. Each record was coded (FoodWorks Professional Edition, Version 10, Xyris Software, Queensland, Australia) using images for validation by a student dietitian, which were checked for accuracy by a RD. To verify compliance to the KD, participants measured their waking (i.e. fasted) capillary whole-blood D-βHB concentration (Freestyle Optimum Neo; Abbott Diabetes Care, Australia) using

standardised techniques. Nutritional ketosis was classified as having D-βHB concentration ≥0.4 mM. To help maintain energy balance throughout the study, participants were requested to prevent a >2% fluctuation in body mass. Participants had daily access to a RD for support to promote compliance; however, if under-reporting or noncompliance was suspected, the RD immediately intervened and the diet record was repeated the subsequent day.

3.3.5 Sleep monitoring

Sleep was monitored during dietary adaptation using a wrist actigraph (Micro Motionlogger Watch; Ambulatory Monitoring Inc., Ardsley, New York, USA), worn on the participants 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 log 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 Sadeh sleep-scoring algorithm applied (Haghayegh, Khoshnevis, Smolensky, Diller, & Castriotta, 2019) 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) (Signal, Gale, & Gander, 2005). On the night of day 14 during dietary adaptation, participants were asked to aim for 8 hours of sleep in preparation for the period of extended wakefulness. Whilst our screening criteria required participants to habitually sleep for >7 hours per night for study inclusion, participants not meeting this threshold were not excluded due to the already limited sample size and shorter sleep durations being reflective of the wider working population.

3.3.6 Extended wakefulness

On the morning of day 15, participants were woken by phone call at 0630 and were not allowed to fall back to sleep or consume caffeine after waking. Participants presented to the Aviation Medicine Unit at approximately 0700 h to commence a 36-hour period of extended wakefulness from approximately 0730 h. This period comprised of 6 x 6-hour blocks, each commencing with a meal. Cognitive performance (5-min psychomotor vigilance task [PVT]), subjective sleepiness, mood, capillary blood glucose and D-β-hydroxybutyrate were measured 1, 3 and 5-h following each meal (i.e. 2-h between tests) (Figure 3.1). Participants remained

awake in the laboratory and were continuously monitored; a New Zealand Defence Force Medic was on call during this time. The environment was strictly controlled in terms of activities and environmental conditions. Participants had free time to read, watch movies, play board games, and interact with other participants and research staff; however, no vigorous physical activity, interaction with people external to the study or use of technology for work purposes (i.e. non-leisure) was allowed. Typical office light exposure was maintained at a constant level and ambient temperature was maintained at 20-22 °C. Following completion, participants were provided with transport home and requested to refrain from duty for a 24-hour period to allow for sufficient recovery.

3.3.6.1 Diet during extended wakefulness

During the 36-hour period of extended wakefulness, participants were provided meals by the research team in accordance with their dietary allocation. Meals were provided at approximately 0730 h (breakfast), 1330 h (lunch), 1930 h (dinner) and 0130 h (night meal), with each comprising 25% of the participant's daily energy requirements (based on habitual energy intake minus 10% for sedentariness). Each meal contributed approximately the same percentage of protein, fat and carbohydrate in alignment with the prescribed dietary allocation (i.e. CHO or KD). Participants on the KD ingested an electrolyte capsule three times daily (i.e. approximately 0800, 1600 and 0000). Participants were instructed not to talk about food and the researchers refrained from discussing food throughout the period of extended wakefulness. All meals were formulated by a student dietitian, overseen by a registered dietitian. For each group, no caffeine or alcohol was allowed and only non-caffeinated beverages were made available, which could be consumed as required. During the 36-hour period, participants were only able to consume food provided by the research team. Participants measured capillary blood D-βHB concentrations every two hours to verify dietary compliance using standardised techniques.

3.3.7 Psychomotor Vigilance Task

The 5-minute version of the PVT was employed to measure vigilant attention. Participants responded to a visual stimulus, appearing at random intervals (between 2-10 seconds), by pressing a button on the hand held PVT-192 device (Dinges & Powell, 1985) with the finger or thumb of their dominant hand. The PVT is highly sensitive to the effects of fatigue, making it a suitable tool for use in SD studies (Lim & Dinges, 2008). The PVT also shows negligible

practice effects (Balkin et al., 2004). Variables chosen for analysis included number of lapses, mean reciprocal response time (RRT; 1/mean response time (RT)] × 1000), mean fastest 10% RT and mean slowest 10% RT.

3.3.8 Subjective sleepiness

The Stanford Sleepiness Scale (Hoddes, Zarcone, Smythe, Phillips, & Dement, 1973) was used to quantify changes in subjective sleepiness. The rating were made on a printed form from the computerised Automated Neuropsychological Assessment Metrics test battery (ANAM). 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.

3.3.9 Mood

Fatigue and vigour were measured using the 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.

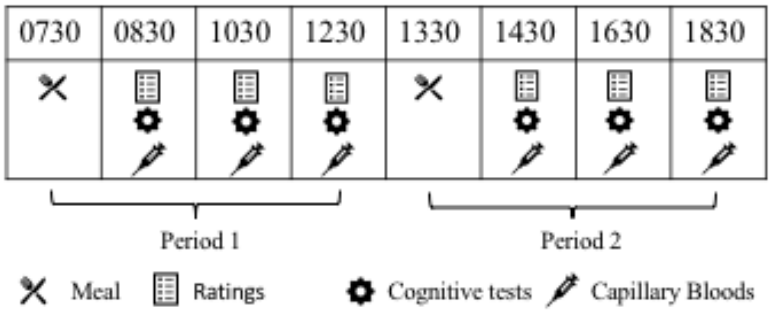

Figure 3.3.1 Schematic of the first two 6-hour periods of extended wakefulness protocol.

3.3.10 Statistical Analysis

All statistical analyses were produced in R version 3.6.0 with RStudio version 1.1463 (R Core Team, 2020). Data were analysed using a series of linear mixed models with restricted maximum likelihood and generalised linear mixed models using the R package "lme4". Fixed effects factors included diet (2 levels; KD and CHO-based diet), period (6 levels) and test (3 levels). A random intercept for participant was included to adjust for inter-individual variability. Diet order was also included as a fixed effect given the cross-over design, but was not reported here. Normality and homoscedasticity of the model's residuals were determined by visual inspection of Q-Q plot; 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 post-hoc pairwise comparisons were obtained using the Holm adjustment for multiplicity in the R package "emmeans". All differences were given as estimated marginal means for single comparisons or ranges of estimated marginal means for multiple comparisons, except for dietary intake, which was given as raw mean. Significance was inferred when $p \leq 0.05$. Graphs were produced in GraphPad Prism Version 9.1.1 (GraphPad Software, San Diego, California USA).

3.3.11 Comment from the author

This study was my first introduction to R and R Studio. Whilst I did not write the code, I executed each step and interpreted the outputs, learning the function of each R package along the way. Appendix G provides an example R script used for the analysis of outcome measures.

3.4 Results

3.4.1 Participants

Ten participants were recruited for the study; however, three withdrew due to operational demands, thus giving a total sample size of n = 7 men: age, 34.7 ± 7.0 years (range, 26-45 years); body mass, 84.1 ± 10.2 kg; height, 1.79 ± 0.03 m; body mass index, 26.2 ± 2.5 kg/m^2.

3.4.2 Dietary adaptation

3.4.2.1 Dietary intake

Table 4.1 summarises dietary intake during the dietary interventions. There were no differences between dietary interventions for energy intake; however, the KD was 21 g·day^{-1} higher in protein, 259 g·day^{-1} lower in carbohydrate and 100 g·day^{-1} higher in fat compared with the CHO-based diet.

Table 3.1 Dietary intake during the carbohydrate and ketogenic diet interventions.

	Energy (MJ)	Protein (g)	Fat (g)	Carbohydrate (g)
Carbohydrate diet	12.0 ± 3.5	131 ± 50	119 ± 41	285 ± 89
Ketogenic diet	11.4 ± 2.8	152 ± 47	220 ± 61	26 ± 13
p-value	0.41	**0.049**	**<0.001**	**<0.001**

Values are presented as raw mean ± SD. Abbreviations: *MJ*, megajoules; *g*, grams.

3.4.2.2 Capillary blood D-βHB concentration

There was a diet x week interaction for mean weekly D-βHB ($p < 0.001$), with post-hoc tests indicating D-βHB concentrations were higher in the KD during week-1 of adaptation compared with the CHO diet in week 1 by 0.37 mM ($p < 0.001$) and week-2 by 0.93 mM ($p < 0.001$). Mean D-βHB concentration was also higher during week-2 of adaptation compared with week-1 in the KD by 0.54 mM ($p = 0.001$). Days to reach D-βHB concentration ≥0.4 mM for participants in the KD was 3.9 ± 1.1.

Table 3.2 Capillary blood D-βHB concentration during the carbohydrate and ketogenic diet interventions.

	Baseline	Week-1 adapt	Week-2 adapt
Carbohydrate diet	0.11 ± 0.03	0.09 ± 0.03	0.07 ± 0.03
Ketogenic diet	0.09 ± 0.03	0.46 ± 0.07	1.00 ± 0.10
p-value	1.000	**<0.001**	**<0.001**

Values are presented as estimated marginal mean ± SEM.

3.4.2.3 Sleep duration

There were no differences in mean sleep duration during the 7 days prior to the extended wakefulness period for the KD (6.9 ± 0.9 hours·day^{-1}) and CHO-based diet (6.7 ± 1.1 hours·day^{-1}) (p = 0.20). For the night preceding the extended wakefulness, participants slept for 6.8 ± 1.0 hours (range, 5.2 to 8.2 hours) in the KD and 6.5 ± 0.9 hours (range, 4.8 to 7.1 hours) in the CHO-based diet (p = 0.63).

3.4.3 Extended wakefulness

3.4.3.1 Capillary blood D-βHB concentration

There was a diet x period interaction for capillary blood D-βHB concentration (Table 3.3), with post-hoc tests (Appendix A) indicating D-βHB was higher for all periods in the KD compared with the CHO-based diet by 0.78 to 1.49 mM (all $p < 0.001$). In the KD, D-βHB was higher in period 2 compared with periods 1, 3, 4 and 6 by 0.51 to 0.70 mM (all $p < 0.01$). D-βHB was higher in period 5 compared with periods 3,4 and 6 by 0.44 to 0.49 mM (all $p < 0.01$). In the CHO-based diet, D-βHB did not differ between periods (all $p > 0.05$).

Table 3.3 Main effects and interactions for capillary blood D-βHB concentration.

	F-statistic	*p*-value
Diet	$F_{(1, 209)} = 945.06$	**< 0.001**
Period	$F_{(5, 209)} = 6.35$	**< 0.001**
Test	$F_{(2, 209)} = 0.17$	0.872
Diet x period	$F_{(5, 209)} = 4.91$	**< 0.001**
Diet x test	$F_{(2, 209)} = 2.09$	0.091
Period x test	$F_{(10, 209)} = 1.19$	0.263
Diet x period x test	$F_{(10, 209)} = 0.58$	0.696

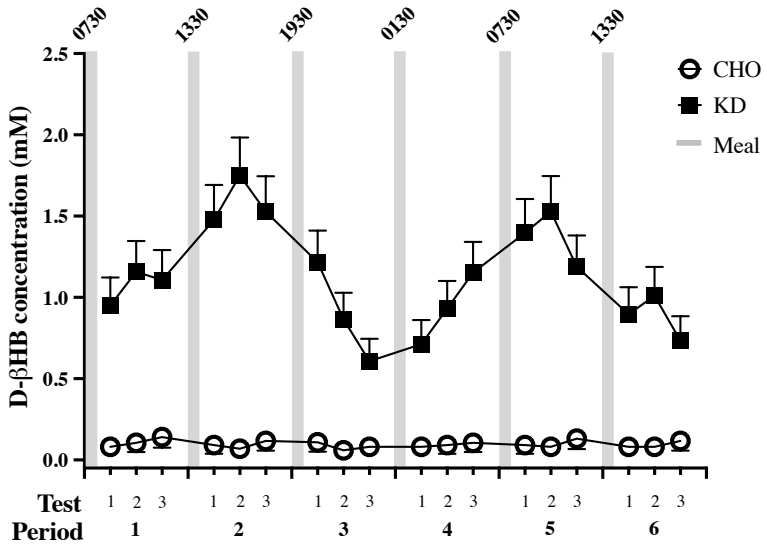

Figure 3.3.2 Capillary blood D-βHB concentration during 36 hours of extended wakefulness for the carbohydrate and ketogenic diet interventions. Values are presented as estimated marginal mean ± SEM.

3.4.3.2 Capillary blood glucose concentration

There was a diet x test interaction and a main effect of period for capillary blood glucose concentration (Table 3.4). In the CHO-based diet, glucose decreased from test 1 to 2 by 0.82 mM ($p < 0.001$) and from test 2 to 3 by 0.31 mM ($p = 0.002$). In the KD, glucose decreased from test 1 to 3 by 0.23 mM ($p = 0.038$). Moreover, glucose was overall higher in periods 3 and 4 compared with periods 1, 2 and 5 by 0.28 to 0.66 mM (all $p < 0.05$), and higher in period 6 than in periods 1,2 and 5 by 0.31 to 0.55 (all $p < 0.05$).

Table 3.4 Main effects and interactions for capillary blood glucose concentration.

	F-statistic	*p*-value
Diet	$F_{(1, 209)} = 117.74$	**< 0.001**
Period	$F_{(5, 209)} = 15.45$	**< 0.001**
Test	$F_{(2, 209)} = 48.15$	**< 0.001**
Diet x period	$F_{(5, 209)} = 1.34$	0.277
Diet x test	$F_{(2, 209)} = 20.64$	**< 0.001**
Period x test	$F_{(10, 209)} = 1.46$	0.127
Diet x period x test	$F_{(10, 209)} = 1.22$	0.382

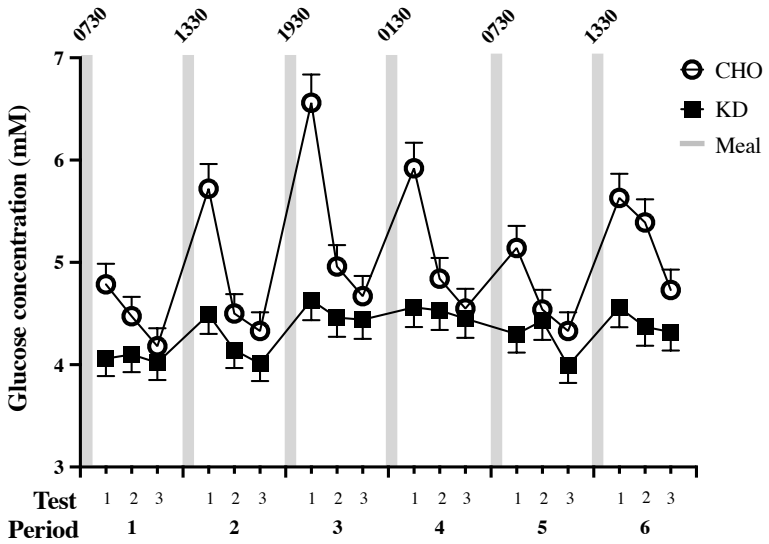

Figure 3.3.3 Capillary blood glucose concentration during 36 hours of extended wakefulness for the carbohydrate and ketogenic diet interventions. Values are presented as estimated marginal mean ± SEM.

3.4.4 PVT performance

3.4.4.1 Lapses

There was a main effect of diet and a period x test interaction for number of lapses (Table 3.5). The KD had 0.4 ± 0.2 fewer lapses compared with the CHO-based diet across 36 hours SD ($p < 0.001$). Post-hoc tests (Appendix A) indicated that in period 4, test 3 had 2.9 ± 0.9 more lapses than test 1 ($p < 0.001$) and 2.5 ± 0.8 more lapses than test 2 ($p < 0.001$). In period 6, test 3 had 0.9 ± 0.5 fewer lapses than test 1 ($p = 0.04$). Period 1 had 1.3 ± 0.6 fewer lapses compared with period 5 for tests 1 and 1.8 ± 0.7 fewer lapses for test 3 (all $p < 0.001$), whilst period 2 had 1.1 ± 0.5 fewer lapses than period 6 for test 1 ($p = 0.008$).

Table 3.5 Main effects and interactions for lapses.

	F-statistic	*p*-value
Diet	$F_{(1, 209)} = 15.32$	**< 0.001**
Period	$F_{(5, 209)} = 18.95$	**< 0.001**
Test	$F_{(2, 209)} = 1.24$	0.227
Diet x period	$F_{(5, 209)} = 1.49$	0.327
Diet x test	$F_{(2, 209)} = 0.19$	0.919
Period x test	$F_{(10, 209)} = 3.58$	**< 0.001**
Diet x period x test	$F_{(10, 209)} = 0.20$	1.000

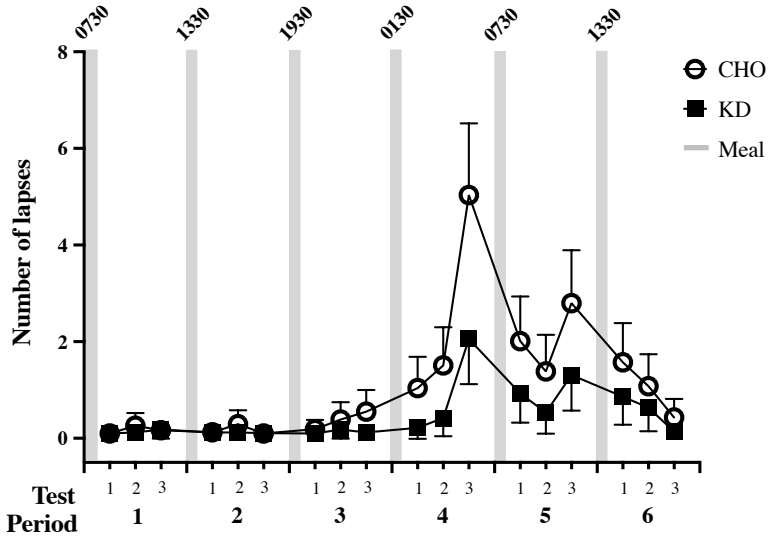

Figure 3.3.4 Number of lapses during 36 hours of extended wakefulness for the carbohydrate and ketogenic diet interventions. Values are presented as estimated marginal mean ± SEM.

3.4.4.2 Mean reciprocal response time

There was a main effect of diet and a period x test interaction for mean RRT (Table 3.6). The KD had a faster mean RRT by 0.14 ± 0.03 responses per second (responses s^{-1}) compared with the CHO-based diet ($p = 0.001$). Post-hoc tests (Appendix A) indicated that in period 4, mean RRT was slower for test 3 compared with test 1 by 0.41 ± 0.10 responses s^{-1} (p 0.001) and test 2 by 0.24 ± 0.10 responses s^{-1} ($p = 0.023$). In period 6, test 3 was faster than test 1 by 0.25 ± 0.10 responses s^{-1} ($p = 0.037$). Mean RRT in period 1 was faster than in period 5 for test 1 by 0.63 ± 0.10 responses s^{-1}, test 2 by 0.46 ± 0.10 responses s^{-1} and test 3 by 0.51 ± 0.10 responses s^{-1} (all $p < 0.001$). Mean RRT in period 2 was faster than period 6 for test 1 by 0.40 ± 0.10 responses s^{-1} ($p < 0.001$).

Table 3.6 Main effects and interactions for mean reciprocal response time.

	F-statistic	*p*-value
Diet	$F_{(1, 209)} = 16.73$	**< 0.001**
Period	$F_{(5, 209)} = 37.46$	**< 0.001**
Test	$F_{(2, 209)} = 1.79$	0.176
Diet x period	$F_{(5, 209)} = 1.80$	0.193
Diet x test	$F_{(2, 209)} = 0.35$	0.735
Period x test	$F_{(10, 209)} = 2.69$	**0.002**
Diet x period x test	$F_{(10, 209)} = 0.47$	0.908

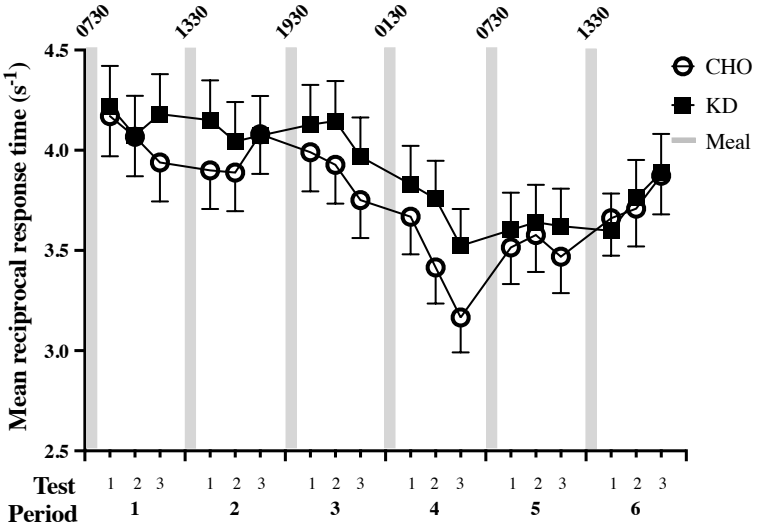

Figure 3.3.5 Mean reciprocal response time (responses s-1) during 36 hours of extended wakefulness for the carbohydrate and ketogenic diet interventions. Values are presented as estimated marginal mean ± SEM.

3.4.4.3 Mean slowest 10% response time

There was a main effect of diet and a period x test interaction for mean slowest 10% RT (Table 3.7). The KD had a faster mean slowest 10% RT by 58.5 ± 18.6 ms compared with the CHO-based diet ($p < 0.001$). Post-hoc tests (Appendix A) indicated that in period 4, test 3 was slower compared with test 1 by 255.6 ± 74.0 ms ($p < 0.001$) and test 2 by 192.2 ± 74.9 ms ($p = 0.014$). In period 6, test 1 was slower compared with test 2 by 160.2 ± 69.8 ($p = 0.034$) and test 3 by 209.3 ± 68.9 ms ($p = 0.003$). Mean slowest 10% RT in Period 1 was faster than in period 5 for test 1 by 246.6 ± 62.0 ms and test 3 by 221.9 ± 63.2 ms ($p <$ 0.001 and 0.001, respectively), whilst period 2 was faster than period 6 by 292.1 ± 68.7 ms for test 1 ($p = <0.0001$).

Table 3.7 Main effects and interactions for mean slowest 10% response time.

	F-statistic	*p*-value
Diet	$F_{(1, 209)} = 11.71$	**< 0.001**
Period	$F_{(5, 209)} = 21.77$	**< 0.001**
Test	$F_{(2, 209)} = 0.83$	0.350
Diet x period	$F_{(5, 209)} = 0.94$	0.568
Diet x test	$F_{(2, 209)} = 0.32$	0.857
Period x test	$F_{(10, 209)} = 3.06$	**0.001**
Diet x period x test	$F_{(10, 209)} = 0.56$	0.813

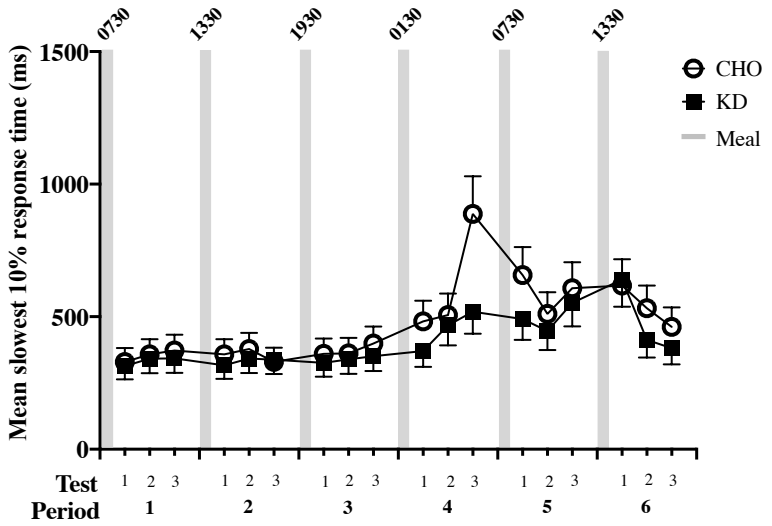

Figure 3.3.6 Mean slowest 10% response times during 36 hours of extended wakefulness for the carbohydrate and ketogenic diet interventions. Values are presented as estimated marginal mean ± SEM.

3.4.4.4 Mean fastest 10% response time

There were main effects of diet and period for mean fastest 10% RT (Table 3.8). The KD has a faster mean fastest RT by 3.5 ± 1.6 ms compared with the CHO-based diet ($p = 0.026$). Post-hoc tests (Appendix A) indicated that mean fastest 10% RT was faster in periods 1, 2 and 3 compared with periods 4 and 5 by 8.9 to 15.9 ± 2.7 ms (all $p < 0.05$), and faster in period 6 than period 4 by 10.2 ± 2.7 ms ($p = 0.002$).

Table 3.8 Main effects and interactions for mean fastest 10% response time.

	F-statistic	*p*-value
Diet	$F_{(1, 209)} = 4.72$	**0.026**
Period	$F_{(5, 209)} = 10.35$	**< 0.001**
Test	$F_{(2, 209)} = 2.76$	0.083
Diet x period	$F_{(5, 209)} = 0.38$	0.939
Diet x test	$F_{(2, 209)} = 0.53$	0.535
Period x test	$F_{(10, 209)} = 1.67$	0.105
Diet x period x test	$F_{(10, 209)} = 0.30$	0.865

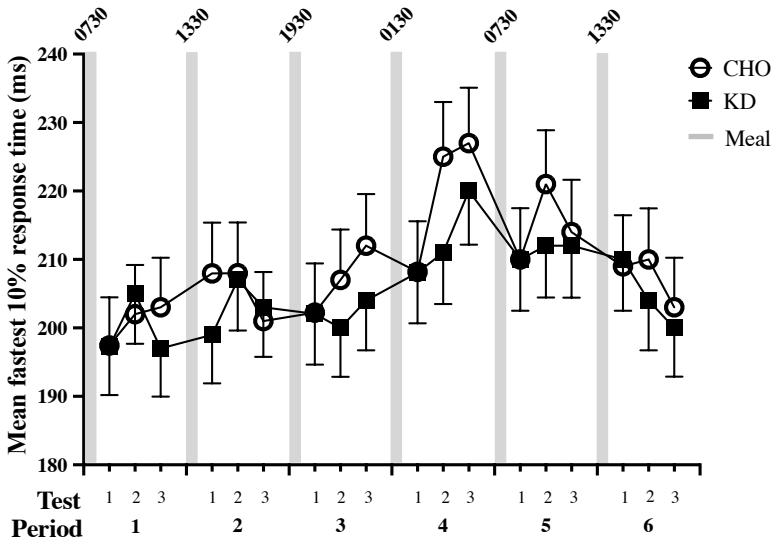

Figure 3.3.7 Mean fastest 10% response times during 36 hours of extended wakefulness for the carbohydrate and ketogenic diet interventions. Values are presented as estimated marginal mean ± SEM.

3.4.5 Mood

3.4.5.1 Vigour

There was a main effect of diet and period for vigour (Table 3.9). Vigour was higher in the KD compared with the CHO-based diet ($p < 0.001$). Post-hoc tests (Appendix A) indicated that in periods 1, 2 and 3, vigour was higher compared with periods 4, 5 and 6 (all $p < 0.001$) and vigour was higher in period 1 than in period 3 ($p = 0.008$).

Table 3.9 Main effects and interactions for vigour.

	F-statistic	*p*-value
Diet	$F_{(1, 209)} = 20.29$	**< 0.001**
Period	$F_{(5, 209)} = 48.83$	**< 0.001**
Test	$F_{(2, 209)} = 3.24$	0.0503
Diet x period	$F_{(5, 209)} = 0.91$	0.441
Diet x test	$F_{(2, 209)} = 0.16$	0.778
Period x test	$F_{(10, 209)} = 1.16$	0.291
Diet x period x test	$F_{(10, 209)} = 0.23$	0.998

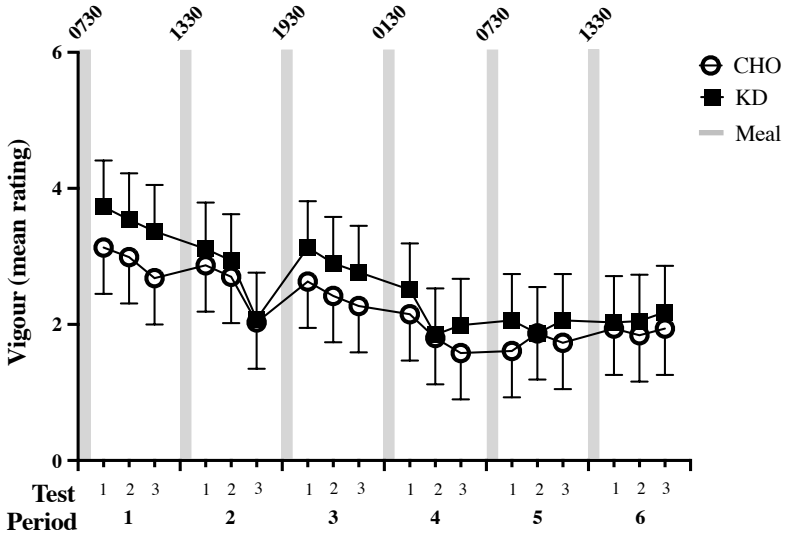

Figure 3.3.8 Vigour during 36 hours of extended wakefulness for the carbohydrate and ketogenic diet interventions. Values are presented as estimated marginal mean ± SEM.

3.4.5.2 Fatigue

There were main effects of diet, period and test for fatigue (Table 3.10). Fatigue was lower in the KD compared with the CHO-based diet ($p = 0.001$). Post-hoc tests (Appendix A) indicated that in periods 1, 2 and 3, fatigue was lower compared with periods 4, 5 and 6 (all $p < 0.001$), and fatigue was lower in period 1 compared with period 2 ($p = 0.021$) and period 3 ($p < 0.001$). Test 3 was higher compared with test 1 ($p = 0.035$).

Table 3.10 Main effects and interactions for fatigue.

	F-statistic	*p*-value
Diet	$F_{(1, 209)} = 10.80$	**0.001**
Period	$F_{(5, 209)} = 60.03$	**< 0.001**
Test	$F_{(2, 209)} = 2.46$	**0.036**
Diet x period	$F_{(5, 209)} = 0.32$	0.893
Diet x test	$F_{(2, 209)} = 0.09$	0.961
Period x test	$F_{(10, 209)} = 1.25$	0.332
Diet x period x test	$F_{(10, 209)} = 0.61$	0.909

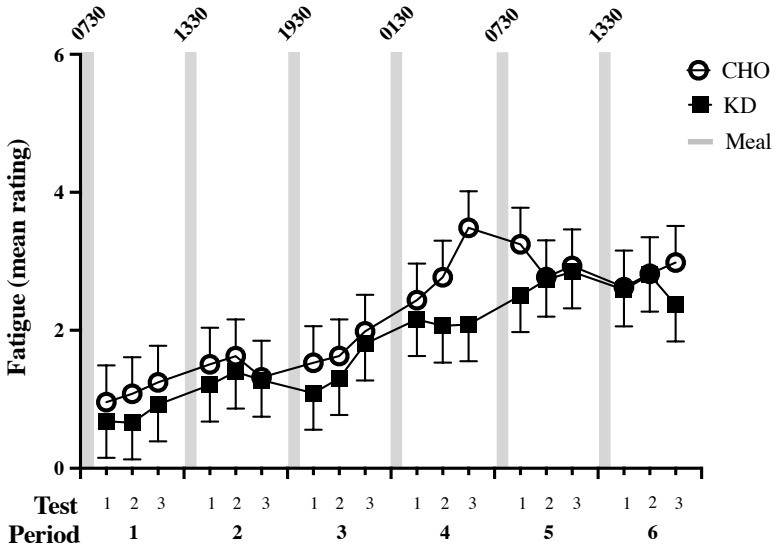

Figure 3.3.9 Fatigue during 36 hours of extended wakefulness for the carbohydrate and ketogenic diet interventions. Values are presented as estimated marginal mean ± SEM.

3.4.6 Subjective sleepiness

There was a main effect of diet and a period x test interaction for subjective sleepiness (Table 3.11). Sleepiness was lower in the KD compared with the CHO-based diet ($p < 0.001$). Post-hoc tests (Appendix A) indicated that in period 3, sleepiness was lower for test 1 compared with test 3 (p = 0.048) and, in period 4, sleepiness was lower for test 1 compared with test 2 ($p = 0.018$) and test 3 ($p = 0.002$). Sleepiness was lower in period 1 compared with period 5 for test 1, 2 and 3 (all $p < 0.001$) and lower in period 2 compared with period 6 in test 1, 2 and 3 (all $p < 0.001$).

Table 3.11 Main effects and interactions for subjective sleepiness.

	F-statistic	*p*-value
Diet	$F_{(1, 209)} = 17.33$	**< 0.001**
Period	$F_{(5, 209)} = 73.22$	**< 0.001**
Test	$F_{(2, 209)} = 1.43$	0.193
Diet x period	$F_{(5, 209)} = 1.88$	0.273
Diet x test	$F_{(2, 209)} = 0.17$	0.789
Period x test	$F_{(10, 209)} = 1.96$	**0.046**
Diet x period x test	$F_{(10, 209)} = 0.29$	0.984

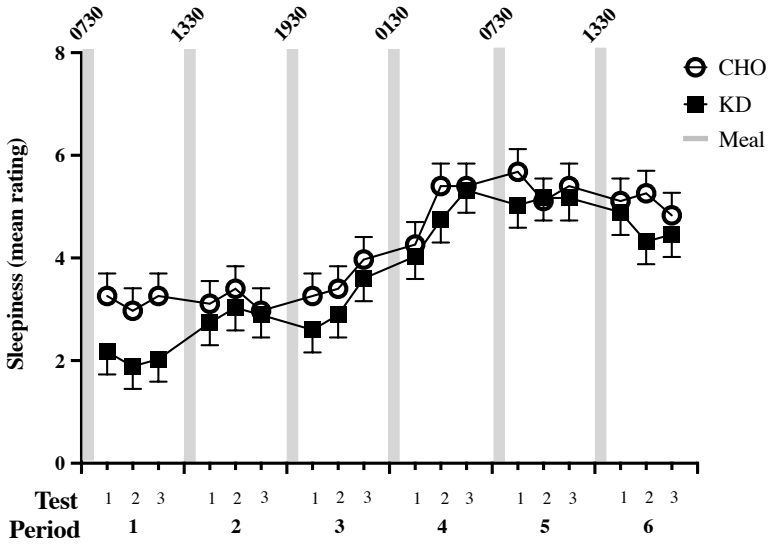

Figure 3.3.10 Subjective sleepiness during 36 hours of extended wakefulness for the carbohydrate and ketogenic diet interventions. Values are presented as estimated marginal mean ± SEM.

3.5 Discussion

This study investigated the effect of a 2-week KD compared with a CHO-based diet on cognitive performance, mood and subjective sleepiness during 36 hours of extended wakefulness in military personnel. The KD moderated the effects of SD-related impairments for all cognitive performance variables, mood (fatigue and vigour) and subjective sleepiness during the 36 hours; however, in contrast to our hypothesis, the absence of a diet x period interaction indicated these effects were not related to the degree of SD experienced. Also in contrast to our hypothesis, post-prandial effects were absent for both diets and appeared to be masked by the circadian influence for all variables except for fatigue, which increased across the post-prandial period independent of diet. These findings suggest the KD could mitigate SD-related impairments in cognitive performance, mood and sleepiness, with stable blood glucose and elevated KB concentrations appearing more beneficial for cognition than fluctuating blood glucose concentrations.

3.5.1 Effect of extended wakefulness

This study supports previous findings that SD of at least 24 hours produces a range of cognitive deficits and negative mood states (Durmer & Dinges, 2005; Goel et al., 2009; Lim & Dinges, 2010). Mean RRT, number of lapses, mean slowest 10% RT and subjective sleepiness increased between periods 1 and 5 (0730-1330 h) and periods 2 and 6 (1330-1930 h), which would have been due to an increase in the homeostatic drive for sleep as SD prolongs (Borbély et al., 2016). These periods covered the same 'clock time', allowing the evaluation of the effects of SD whilst controlling for the influence of circadian phase (Gabehart & Van Dongen, 2017). The effect of SD was also observed for fatigue and vigour, as mood decreased across the first 4 periods and remained low across periods 5 and 6.

The effects of SD can be confounded by inter- and intra-subject variability (Durmer & Dinges, 2005). For example, some individuals are more resilient to SD than others (Van Dongen, Maislin, et al., 2004); however, this inter-subject variability was accounted for in this study via cross-over design, with each participant acting as their own control. Furthermore, sleep duration did not differ between the two diet conditions for the 7 days prior and immediate night prior to commencing extended wakefulness. As the PVT exhibits negligible learning effects, improvements were not expected throughout the 36 hours (Durmer & Dinges, 2005). Whilst we did not measure intra-subject variability, we attempted to reduce any confounding effects

by controlling the testing environment; for example, participants wore ear muffs during testing to reduce distractions, light and temperature were held constant, physical activity was limited and caffeine was prohibited. Increased intra-subject variability is also due to an individual's performance becoming more variable as SD prolongs (Doran et al., 2001), which was accounted for by using linear mixed effects models when analysing the data.

3.5.2 Improved cognitive function, mood and sleepiness on the KD

Compared to the CHO-based diet, the KD moderated SD-related impairments for all cognitive variables during the 36 hours of extended wakefulness. Although there were no significant interactions between period and diet, differences between dietary conditions appeared greatest during period 4 (0130-0630 h) for lapses, mean RRT, fastest 10% RT and slowest 10% RT. A previous study comparing a non-ketogenic, high-fat diet and high-CHO diet over 24 hours of SD demonstrated a significant interaction between diet and period for irresistible sleepiness. Sleepiness was elevated for the high-CHO diet in period 2 (1200-1600 h) and 5 (2400-0400 h), and authors concluded a high-CHO diet reduces alertness (Lowden et al., 2004). It is possible that the sample size in the present study was not sufficient to detect diet interactions. Moreover, the seemingly beneficial effect of the KD (i.e. main effect of diet) is inconsistent with previous studies demonstrating unfavourable (Edwards et al., 2011) or no (Iacovides et al., 2019) effects following induction to a KD. Considering the KD intervention was 2 weeks, a duration which was in between those in the aforementioned studies, it is possible that the strict adherence to the KD in the present study to restrict CHO availability and increase ketogenesis potentially augmented cerebral adaptation. This may have underpinned improvements in cognitive performance and mood during SD when glucose metabolism was impaired (Thomas et al., 2000).

3.5.3 Post-prandial versus circadian effects

Irrespective of dietary condition, sleepiness, mean slowest 10% RT and lapses increased, and mean RRT declined across 0230-0630 h in period 4. This decline in performance suggests that the KD was not able to stabilise cognitive performance when the circadian drive for sleep was highest (Valdez, 2019), although it appeared cognitive performance whilst on the CHO-based diet declined more (see Figures 3.3-3.5). The reverse pattern was observed for mean RRT, slowest 10% RT and lapses across 1430-1830 h in period 6 as the circadian drive for wakefulness increased towards the early evening (Valdez, 2019). Post-prandial effects (main

effect of test) thus appeared to be masked by the circadian influence on performance (period x test interaction) with the exception of vigour.

The main effect of test for fatigue indicates a post-prandial effect, whereby fatigue was higher 5 hours after a meal compared with 1 hour after a meal, irrespective of dietary condition. This is consistent with prior research demonstrating decreased subjective feelings of energy two hours following a high-CHO meal (Benton, 2002) and increased subjective sleepiness towards the end of the post-prandial period for a high-CHO diet compared with a high-fat diet (Lowden et al., 2004). Decreasing blood glucose concentrations have also been associated with lower self-reported energy after performing cognitively demanding tasks (Owens et al., 1997), yet the stable blood glucose concentrations observed across the post-prandial period for the KD in the present study did not mitigate the decrease in vigour.

3.5.4 Capillary blood D-βHB and glucose concentrations

In the present study, the KD lowered blood glucose concentrations, although participants were not hypoglycaemic as concentrations remained >4.0 mM (Balijepalli et al., 2017). The prevention of hypoglycaemia, despite only consuming ~25g CHO per day, was possibly due to a reduction in CHO oxidation exceeding systemic glucose uptake (Harber, Schenk, Horowitz, & Barkan, 2005). Compared to the CHO-based diet, where there was an initial increase followed by incremental reductions in blood glucose concentration across the post-prandial period, the KD exhibited stable blood glucose concentrations (Figure 3.2). These differences in blood glucose concentration did not appear to differentiate dietary effects on cognition, mood or sleepiness as there was no diet x test interactions observed for these variables; therefore, the sustained increase in blood KB concentrations in the KD compared with the CHO-based diet across the extended wakefulness period, and presumably increased cerebral KB oxidation, provides a probable explanation.

Blood glucose concentration also appeared to display a circadian effect. For example, there was an increase in blood glucose concentration in the afternoon (1330-1830 h) and at night (1930-0630 h), independent of diet. Glucose tolerance and insulin sensitivity exhibit a circadian rhythm, whereby tolerance is highest in the morning and decreases in the afternoon and at night (Morgan, Aspostolakou, Wright, & Gama, 1999; Yamanaka, 2020). Whilst this pattern was observed for both dietary conditions, the increase in circulating KBs to support energy

production may have ameliorated potential cerebral metabolic impairments. Whilst blood D-βHB concentrations were higher in period 2 (1330-1930 h) and period 5 (0730-0130 h) in the KD, this pattern is inconsistent with prior studies. For example, a 6-week KD (74.3% EI fat, 19.5% EI protein and 6.2% EI CHO) measured blood D-βHB concentrations every hour over a 24-hour period and reported D-βHB to be highest at 0300 h (Urbain & Bertz, 2016). This was likely due to participants fasting over-night from 2200-0700 h; whereas, participants ingested isoenergetic meals every 6 hours in the present study. Whether circadian rhythms are present for D-βHB concentrations is uncertain and will be influenced by factors influencing ketogenesis and KB oxidation (e.g. exercise). Given SD also appears to alter cerebral gene expression of ketogenic and ketolytic enzymes (Chikahisa et al., 2014), more research is required to elucidate how KBs interact with the sleep-wake cycle and when this rhythm is disrupted by SD.

3.5.5 Adaptation process

The strict KD intervention throughout the 2-week adaptation phase, and the provision of meals during the 36-hour extended wakefulness period, ensured participants maintained elevated blood KB concentrations. This was demonstrated by daily measures of morning, fasted blood D-βHB >0.4 mM and weighed dietary records, which were validated by accompanying photos, meeting macronutrient and energy requirements during adaptation (Table 3.1). Whilst the optimal period for adaptation to a KD remains uncertain, a previous 4-day KD increased plasma KB concentrations (AcAc + D-βHB) to 4.8 mM, which increased cerebral KB metabolism from ~5% (on the CHO diet) to ~33% (Courchesne-Loyer et al., 2017). Although plasma KB concentrations were lower in the present study (i.e. D-βHB <2 mM), which would likely lower cerebral KB oxidation (Courchesne-Loyer et al., 2017), the longer dietary adaptation period may have augmented KB oxidation. Other studies have not employed such rigorous dietary intervention and monitoring (Edwards et al., 2011; Holloway et al., 2011; Lowden et al., 2004), which limits the extrapolation of their findings to KDs. Therefore, the strict protocol in the current study allows a confident assessment of the effects of the KD on cognitive performance, mood and subjective sleepiness during extended wakefulness.

3.5.6 Conclusion and implications for fatigue-management guidelines and future research

In conclusion, compared with the CHO-based diet, the 2-week KD demonstrated beneficial effects on cognitive performance, mood and sleepiness during 36 hours of extended wakefulness. Whilst adaptation to the KD has previously been associated with unfavourable effects on cognition, the present study's findings indicate that a KD could be considered by military personnel when periods of SD are anticipated. However, further research is required in simulated real-world settings with larger and more diverse sample sizes to assess effects on safety and performance as they relate to real-world military operations, prior to their implementation in this community.

4 Conclusion and recommendations

4.1 Study summary

This randomised controlled, cross-over trial was designed to examine the effect of a 2-week ketogenic diet (KD), versus a carbohydrate (CHO)-based diet, on cognitive performance, mood and subjective sleepiness during 36 hours of extended wakefulness in military personnel. To the authors knowledge, this is the first study to investigate the effect of a KD on cognition during sleep deprivation (SD).

This study was conducted under rigorous monitoring protocols to ensure participants adhered to dietary interventions. Participants' sleep duration prior to the extended wakefulness period did not differ between dietary conditions and was therefore not expected to have confounding effects on cognitive performance and subjective variables. This adaptation phase was difficult and time consuming for participants due to the dietary/lifestyle restrictions, but ensured participants physiological states were polarised. Moreover, during the extended wakefulness period, participants were unable to take part in any activity that may increase alertness such as exercising or consumption of caffeine. It should also be noted this study was conducted whilst the country was under COVID-related restrictions, impacting recruitment and assistance with data collection.

The study's objectives were:

1. To assess the effect of a 2-week KD, compared with a CHO-based diet, cognitive performance every 2 hours during a 36-hour period of extended wakefulness.
2. To assess the effect of a 2-week KD, compared with a CHO-based diet, on mood (fatigue and vigour) every 2 hours during a 36-hour period of extended wakefulness.
3. To assess the effect of a 2-week KD, compared with a CHO-based diet, on subjective sleepiness every 2 hours during a 36-hour period of extended wakefulness.

4.2 Main findings

4.2.1 Hypothesis 1: The KD would mitigate SD-related impairments in cognitive performance, mood and sleepiness compared with the CHO-based diet.

A main effect of diet and the absence of a diet x period interaction indicates that the KD moderated SD-related deficits in cognitive performance variables (lapses, mean reciprocal

response time, mean slowest 10% response time and mean fastest 10% response time), mood and subjective sleepiness during the 36-hour period of extended wakefulness, but that it was not related to the degree of SD experienced. The difference appeared greatest for period 4 (0230-0630 h), indicating a potential link between the provision of ketone bodies (KBs) and moderation of cognitive performance impairment during the biological night. This warrants further investigation as the sample size was too small to show any statistically significant interaction of diet and period within the statistical analyses.

4.2.2 Hypothesis 2: Differences in cognitive performance, mood and sleepiness between dietary conditions would increase towards the end of the post-prandial period, particularly in the latter stages of SD.

Any effect of post-prandial period was masked by circadian influence for all cognitive variables, except vigour. The effect of circadian phase on cognitive performance, independent of diet, was clear for period 4 (0230-0630 h), where performance declined across the period of circadian low, and period 6 (1300-1800 h), where performance improved during the circadian peak of performance, overriding the effects of increased homeostatic pressure for sleep (Borbély et al., 2016). In addition, the effect of post-prandial period on vigour was independent of diet and circadian phase. The absence of any significant differences between diets is surprising as falling blood glucose concentrations, observed across the post-prandial period for the CHO-based diet, have been associated with lower self-reported energy after performing cognitively demanding tasks (Owens et al., 1997).

4.3 Study strengths

1. The implementation of a cross-over design, where each participant acted as their own control, increased the power of the study to detect an effect as (multiple) confounders are controlled for, for example, one individual may be more vulnerable to the effects of SD than another (Van Dongen, Maislin, et al., 2004).
2. Use of the psychomotor vigilance task, based on simple reaction time, is a validated tool for the measurement of sustained and vigilant attention in SD studies. It is sensitive to the effects of SD and has minimal practice effects, thereby minimising intra-subject variability (Durmer & Dinges, 2005; Lim & Dinges, 2008). Whilst the assessment of cognitive performance in simulated real-life operational settings would be valuable, the psychomotor vigilance task has ecological validity (Goel, Basner, & Dinges, 2015), as

vigilance is a foundation for many tasks and operations in the military where rapid responses and sustained attention are required (Al-Shargie et al., 2019; Lieberman et al., 2006).

3. During extended wakefulness, light intensity and ambient temperature were held constant, physical activity was kept to a minimum and participants abstained from caffeine to minimise effects known to mask SD-related deficits (Gabehart & Van Dongen, 2017).
4. Participants were selected based on their habitual sleep duration meeting select criteria synonymous with optimal sleep duration and were monitored during the adaptation period. Participants' average sleep duration during the 7 days prior, and during the night prior to commencing the extended wakefulness period did not differ between dietary condition and was therefore not expected to have confounding effects on cognitive performance and subjective variables.
5. The strict inclusion and exclusion criteria ensured participants were healthy, within an age range of 18-50, had a normal BMI and were free from chronic illness and sleep disorders to reduce possible confounding effects on cognition (Murman, 2015).
6. The strict KD protocol, that involved reducing dietary CHO to <5% of energy intake for 2 weeks prior to SD, provided additional rigour and, therefore, confidence in the study's findings. Compliance to the protocol was high due to frequent measurement of capillary blood D-βHB concentrations, weighed image-assisted dietary reports and daily monitoring by a registered dietitian or student dietitian. This ensured participants remained in a state of nutritional ketosis (blood D-βHB >0.4mM) throughout the adaptation and extended wakefulness periods.
7. The use of linear mixed models allowed analysis of data both between subjects and within subjects by utilising fixed effects (diet, period and test) whilst accounting for non-independence in the data with the inclusion of a random intercept for participants ("Linear Mixed Effects Models," 2006). Linear mixed models allow repeated measures, increasing flexibility and providing greater control over sources of variability compared with linear models. Linear mixed effects models also accounted for the increased intra-variability resulting from prolonged SD.

4.4 Study limitations

1. The small sample size of n = 7 underpowered the study to detect potential differences between dietary conditions across the 36 hours of extended wakefulness. This meant that interactions between diet, circadian phase (period) and/or post-prandial period (test) were nonsignificant (i.e. $p > 0.05$), despite differences appearing in the data. Initially, we aimed to recruit a sample of n = 15; however, COVID-related restrictions, operational requirements and the strict eligibility/exclusion criteria were restraining. Given that low sample sizes can increase type 1 errors, the inferences from the analyses were interpreted cautiously and considered preliminary.
2. Despite asking participants to aim for 8 hours sleep the night prior to the period of extended wakefulness, sleep duration ranged from 4.8 to 8.2 hours. Thus, it is likely that at least some participants were sleep-restricted, which may have resulted in more pronounced performance deficits (Zhou et al., 2011); however, participants' preceding sleep duration did not differ between dietary conditions. Whilst the screening criteria required participants to habitually sleep for >7 hours per night, which was initially confirmed with participant reporting (i.e. not measured via actigraphy), participants not meeting this threshold during the study were not excluded as the sample size was already limited and participants' average sleep duration during the 7 days prior to the extended wakefulness period was consistent between dietary conditions. A shorter sleep duration is also more reflective of the wider working population, with many not achieving the recommended 7-9 hours of sleep per night (Caldwell et al., 2019).
3. This study based assumptions of cerebral substrate metabolism off blood KB concentrations, with a previous study observing a linear relationship between plasma ketone concentration and cerebral ketone metabolism (Courchesne-Loyer et al., 2017). As cerebral KB metabolism was not measured it cannot be assured this was the case in the present study. It was also assumed that increasing blood KB concentration via adherence to a KD would mitigate decreases in the cerebral metabolic rate of glucose during SD. Whilst SD appears to influence genes related to KB metabolism and increase blood KB concentration, at least in rodents (Chikahisa et al., 2014), the mechanisms by which adherence to the KD interacted with impaired cerebral metabolic rate of glucose were only speculative. It is possible that other mechanisms underpinned cognitive benefits observed following induction to the KD.
4. Participant recruitment included a strict eligibility/exclusion criteria that does not reflect all demographics within the New Zealand Defence Force or other occupations

at risk of SD-related incidents and accidents, such as the emergency services, healthcare and transport. For example, women, over 50 year olds, overweight-obese individuals, and Army and Navy personnel were not included. Therefore, extrapolation to other populations, particularly women, would be premature.

4.5 Final recommendations

4.5.1 Impact

The study's findings will contribute to understanding how dietary macronutrient manipulation affects cognition during SD. Given the increasing interest in KDs in various populations, particularly the military, ensuring guidance is evidenced based is essential, as it is a restrictive dietary pattern. Previous research in healthy individuals has attempted to examine adaptations to the KD largely for physical performance, yet little is known about its effect on cognition, particularly when exposed to stresses that alter cognitive performance. Since SD is likely to occur in the military, this study suggests that safety and performance are not compromised, albeit within the limitations of the study. Therefore, by working with the Aviation Medicine Unit at the Royal New Zealand Air Force Base, which is an operational unit involved in training aircrew on the medical and physiological risks of flight in the military, the findings will be directly integrated within their training courses. Moreover, the findings will be disseminated throughout the New Zealand Defence Force to key stakeholders, including the Defence Technology Agency, Army, Navy, and Special Forces, and will be integrated within New Zealand Defence Force Fatigue Management Guidelines. The study's findings will also aim to be disseminated at conferences and published in a peer reviewed journal. The study has already been presented at the 2021 Nutrition Society of New Zealand Conference and the abstract has been accepted for the 2021 Sport and Exercise Science New Zealand Conference (delayed to 2022).

For the participants involved, this study also provided an opportunity to not only feel the effects of fatigue caused by prolonged SD, but also objectively measure the deficits in their performance. This highlights that military personnel are just as susceptible as civilians to the effects of SD, which will aid in changing the ingrained 'can do' mindset that has many pilots and military personnel push through feelings of fatigue, when they should not. Feedback provided by one of the pilots participating in the study is as follows:

"In my role as a military pilot, a deeper understanding of diet, fatigue and its effect on cognitive performance improves personal management of my own health and wellbeing, thereby improving many areas of my individual human performance, e.g. decision making. As an Aircraft Commander, this ultimately contributes significantly to overall aircraft and crew safety, and mission effectiveness."

SQNLDR Marcus Hogan, 2021.

4.5.2 Recommendations

The following recommendations should be considered for future research examining the KD or alternative dietary strategies to induce ketosis, and its effect on cognition during SD:

1. At this time, recommendation of a KD to individuals in the military who experience periods of SD is not advised. If an individual chooses to pursue the diet for their own interest, the preliminary findings suggest this will not compromise cognitive performance and safety, but as this is a restrictive dietary pattern and difficult to adhere to in operational settings, more research is required to investigate the potential beneficial effects observed in the present study, and whether they outweigh potential adverse effects, before it's widespread recommendation. Research of exogenous ketone supplements could be considered, as nutritional ketosis can be reached in minutes, compared with days-to-weeks with a KD, and their effect on substrate metabolism may differ (Shaw et al., 2020).
2. Findings from the present study need to be confirmed with larger and more diverse population samples. Whilst measuring discrete cognitive domains that are sensitive to SD can detect subtle differences, it is difficult to relate this to how behaviour and decision making are influenced in real-world settings. Cognitive measures could employ simulators and situations reflective of true operational demands.
3. Future research should consider inclusion of a greater diversity of military personnel, including women and personnel from other services of the New Zealand Defence Force, such as the Army and Navy. Sleep deprivation-related deficits in cognitive performance not only pose a risk to military personnel, but also a number of other operational occupations; therefore, studies could also consider using participants from medical/emergency services, law enforcement and transport.
4. Future research should consider the effect of a KD on cognitive performance during chronic sleep restriction, due to its prevalence in modern society.

References

Achanta, L. B., & Rae, C. D. (2017). β-Hydroxybutyrate in the Brain: One Molecule, Multiple Mechanisms. *Neurochemical Research, 42*(1), 35-49. doi:10.1007/s11064-016-2099-2

Al-Shargie, F., Tariq, U., Mir, H., Alawar, H., Babiloni, F., & Al-Nashash, H. (2019). Vigilance Decrement and Enhancement Techniques: A Review. *Brain sciences, 9*(8), 178. doi:10.3390/brainsci9080178

Ancoli-Israel, S., Martin, J. L., Blackwell, T., Buenaver, L., Liu, L., Meltzer, L. J., . . . Taylor, D. J. (2015). The SBSM Guide to Actigraphy Monitoring: Clinical and Research Applications. *Behavioural Sleep Medicine, 13 Suppl 1*, S4-s38. doi:10.1080/15402002.2015.1046356

Balijepalli, C., Druyts, E., Siliman, G., Joffres, M., Thorlund, K., & Mills, E. J. (2017). Hypoglycemia: a review of definitions used in clinical trials evaluating antihyperglycemic drugs for diabetes. *Clinical epidemiology, 9*, 291-296. doi:10.2147/CLEP.S129268

Balkin, T. J., Bliese, P. D., Belenky, G., Sing, H., Thorne, D. R., Thomas, 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. doi:https://doi.org/10.1111/j.1365-2869.2004.00407.x

Balkin, T. J., & Wesensten, N. J. (2011). Differentiation of sleepiness and mental fatigue effects. In P. L. Ackerman (Ed.), *Cognitive fatigue: Multidisciplinary perspectives on current research and future applications.* (pp. 47-66). Washington, DC: American Psychological Association.

Banks, S., & Dinges, D. F. (2007). Behavioral and Physiological Consequences of Sleep Restriction. *JOURNAL OF CLINICAL SLEEP MEDICINE, 3*(5), 519-528. Retrieved from http://ezproxy.massey.ac.nz/login?url=https://search.ebscohost.com/login.aspx?direct=true&AuthType=ip,cookie,url,uid&db=edswsc&AN=000209776400013&site=eds-live&scope=site&authtype=sso&custid=s3027306

Banks, S., Dorrian, J., Basner, M., & Dinges, D. (2017). Sleep Deprivation. In *Principles and Practice of Sleep Medicine* (6 ed.).

Beersma, D. G. M., & Gordijn, M. C. M. (2007). Circadian control of the sleep–wake cycle. *Physiology & Behavior, 90*(2), 190-195. doi:https://doi.org/10.1016/j.physbeh.2006.09.010

Benington, J. H., & Heller, H. C. (1995). Restoration of brain energy metabolism as the function of sleep. *Prog Neurobiol, 45*(4), 347-360. doi:10.1016/0301-0082(94)00057-o

Benton, D. (2002). Carbohydrate ingestion, blood glucose and mood. *Neuroscience & Biobehavioral Reviews, 26*(3), 293-308. doi:https://doi.org/10.1016/S0149-7634(02)00004-0

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. doi:10.1111/jsr.12371

Bougard, C., Gomez-Merino, D., Rabat, A., Arnal, P., Van Beers, P., Guillard, M., . . . Chennaoui, M. (2018). Daytime microsleeps during 7 days of sleep restriction followed by 13 days of sleep recovery in healthy young adults. *Consciousness and Cognition, 61*, 1-12. doi:https://doi.org/10.1016/j.concog.2018.03.008

Burke, L. M. (2021). Ketogenic low-CHO, high-fat diet: the future of elite endurance sport? *The Journal of physiology, 599*(3), 819-843. doi:10.1113/JP278928

Burke, L. M., Ross, M. L., Garvican-Lewis, L. A., Welvaert, M., Heikura, I. A., Forbes, S. G., . . . Sharma, A. P. (2017). Low carbohydrate, high fat diet impairs exercise economy and negates the performance benefit from intensified training in elite race walkers. *The Journal of physiology, 595*(9), 2785-2807.

Buysse, D. J., Reynolds, C. F., 3rd, Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res, 28*(2), 193-213. doi:10.1016/0165-1781(89)90047-4

Cahill, G. F., Jr. (2006). Fuel metabolism in starvation. *Annu Rev Nutr, 26*, 1-22. doi:10.1146/annurev.nutr.26.061505.111258

Caldwell, J. A., & Caldwell, J. L. (2016). *Fatigue in Aviation : A Guide to Staying Awake at the Stick* (Vol. 2). [N.p.]: Routledge.

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

Carskadon, M., & Dement, W. (1989). Normal Human Sleep: An Overview. Principles and Practice of Sleep Medicine. M.H. Kryger (Ed.). *W.B. Saunders, Philadelphia*, 3-13.

Chaudhary, N. S., Taylor, B. V., Grandner, M. A., Troxel, W. M., & Chakravorty, S. (2021). The effects of caffeinated products on sleep and functioning in the military population: A focused review. *Pharmacology Biochemistry and Behavior, 206*, 173206. doi:https://doi.org/10.1016/j.pbb.2021.173206

Chikahisa, S., Shimizu, N., Shiuchi, T., & Séi, H. (2014). Ketone body metabolism and sleep homeostasis in mice. *Neuropharmacology, 79*, 399-404.

Chinoy, E. D., Cuellar, J. A., Huwa, K. E., Jameson, J. T., Watson, C. H., Bessman, S. C., . . . Markwald, R. R. (2020). Performance of seven consumer sleep-tracking devices compared with polysomnography. *Sleep, 44*(5). doi:10.1093/sleep/zsaa291

Cirelli, C., & Tononi, G. (2017). The Sleeping Brain. *Cerebrum : the Dana forum on brain science, 2017*, cer-07-17. Retrieved from https://pubmed.ncbi.nlm.nih.gov/28698776

Courchesne-Loyer, A., Croteau, E., Castellano, C.-A., St-Pierre, V., Hennebelle, M., & Cunnane, S. C. (2017). Inverse relationship between brain glucose and ketone metabolism in adults during short-term moderate dietary ketosis: A dual tracer quantitative positron emission tomography study. *Journal of Cerebral Blood Flow & Metabolism, 37*(7), 2485-2493. doi:10.1177/0271678X16669366

Czeisler, C., & Buxton, O. (2017). Human Circadian Timing System and Sleep-Wake Regulation. In (pp. 362-376.e365).

Dawson, D., & Reid, K. (1997). Fatigue, alcohol and performance impairment. *Nature, 388*(6639), 235-235. Retrieved from http://ezproxy.massey.ac.nz/login?url=https://search.ebscohost.com/login.aspx?direct=true&AuthType=ip,cookie,url,uid&db=edsovi&AN=edsovi.00006056.199707170.00010&site=eds-live&scope=site&authtype=sso&custid=s3027306

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. doi:10.1093/sleep/26.1.81

Dhillon, K. K., & Gupta, S. (2021). Biochemistry, Ketogenesis. In *StatPearls*. Treasure Island (FL): StatPearls Publishing. Copyright © 2021, StatPearls Publishing LLC.

Diekelmann, S., & Born, J. (2010). The memory function of sleep. *Nature Reviews Neuroscience, 11*(2), 114-126. doi:10.1038/nrn2762

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. doi:10.3758/BF03200977

Doran, S. M., Van Dongen, H. P., & Dinges, D. F. (2001). Sustained attention performance during sleep deprivation: evidence of state instability. *Arch Ital Biol, 139*(3), 253-267.

Durmer, J. S., & Dinges, D. F. (2005). Neurocognitive consequences of sleep deprivation. *Semin Neurol, 25*(1), 117-129. doi:10.1055/s-2005-867080

Edwards, L. M., Carter, E. E., Tyler, D. J., Robbins, P. A., Clarke, K., Holloway, C. J., . . . Brooker, H. (2011). Short-term consumption of a high-fat diet impairs whole-body efficiency and cognitive function in sedentary men. *FASEB Journal, 25*(3), 1088-1096. doi:10.1096/fj.10-171983

Frone, M. R., & Blais, A.-R. (2019). Work Fatigue in a Non-Deployed Military Setting: Assessment, Prevalence, Predictors, and Outcomes. *International journal of environmental research and public health, 16*(16), 2892. doi:10.3390/ijerph16162892

Gabehart, R., & Van Dongen, H. (2017). *Circadian Rhythms in Sleepiness, Alertness, and Performance, in Principles and Practice of Sleep Medicine*. Philadelphia: Elsevier.

Garbarino, S., Lanteri, P., Feeling, N. R., Jarczok, M. N., Quintana, D. S., Koenig, J., & Sannita, W. G. (2020). Circadian rhythms, sleep, and the autonomic nervous system: A position paper. *Journal of Psychophysiology, 34*(1), 1-9. doi:10.1027/0269-8803/a000236

Gip, P., Hagiwara, G., Ruby, N. F., & Heller, H. C. (2002). Sleep deprivation decreases glycogen in the cerebellum but not in the cortex of young rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 283*(1), R54-R59. doi:10.1152/ajpregu.00735.2001

Goel, N., Basner, M., & Dinges, D. F. (2015). Chapter Thirteen - Phenotyping of Neurobehavioral Vulnerability to Circadian Phase During Sleep Loss. In A. Sehgal (Ed.), *Methods in Enzymology* (Vol. 552, pp. 285-308): Academic Press.

Goel, N., Basner, M., Rao, H., & Dinges, D. F. (2013). Circadian rhythms, sleep deprivation, and human performance. *Progress in molecular biology and translational science, 119*, 155-190. doi:10.1016/B978-0-12-396971-2.00007-5

Goel, N., Rao, H., Durmer, J. S., & Dinges, D. F. (2009). Neurocognitive consequences of sleep deprivation. *Semin Neurol, 29*(4), 320-339. doi:10.1055/s-0029-1237117

Gupta et al., 2019. Altering meal timing to improve cognitive performance during simulated nightshifts. *Chronobiology International, 36*(12), 1–23. doi:10.1080/07420528.2019.1676256

Haghayegh, S., Khoshnevis, S., Smolensky, M. H., Diller, K. R., & Castriotta, R. J. (2019). Performance comparison of different interpretative algorithms utilized to derive sleep parameters from wrist actigraphy data. *Chronobiol Int, 36*(12), 1752-1760. doi:10.1080/07420528.2019.1679826

Halestrap, A. P., & Wilson, M. C. (2012). The monocarboxylate transporter family—Role and regulation. *IUBMB Life, 64*(2), 109-119. doi:https://doi.org/10.1002/iub.572

Harber, M. P., Schenk, S., Horowitz, J. F., & Barkan, A. L. (2005). Alterations in carbohydrate metabolism in response to short-term dietary carbohydrate restriction. *American Journal of Physiology - Endocrinology and Metabolism, 289*(2 52-2), E306-E312. doi:10.1152/ajpendo.00069.2005

Hirshkowitz, M., Whiton, K., Albert, S. M., Alessi, C., Bruni, O., DonCarlos, L., . . . Adams Hillard, P. J. (2015). National Sleep Foundation's sleep time duration recommendations: methodology and results summary. *Sleep Health, 1*(1), 40-43. doi:https://doi.org/10.1016/j.sleh.2014.12.010

Hoddes, E., Zarcone, V., Smythe, H., Phillips, R., & Dement, W. C. (1973). Quantification of Sleepiness: A New Approach. *Psychophysiology, 10*(4), 431-436. doi:https://doi.org/10.1111/j.1469-8986.1973.tb00801.x

Holloway, C. J., Cochlin, L. E., Emmanuel, Y., Murray, A., Codreanu, I., Edwards, L. M., . . . Lambert, B. (2011). A high-fat diet impairs cardiac high-energy phosphate metabolism and cognitive function in healthy human subjects. *American Journal of Clinical Nutrition, 93*(4), 748-755. doi:10.3945/ajcn.110.002758

Horne, J. A., & Ostberg, O. (1976). A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *Int J Chronobiol, 4*(2), 97-110.

Iacovides, S., Goble, D., Paterson, B., & Meiring, R. M. (2019). Three consecutive weeks of nutritional ketosis has no effect on cognitive function, sleep, and mood compared with a high-carbohydrate, low-fat diet in healthy individuals: A randomized, crossover, controlled trial. *American Journal of Clinical Nutrition, 110*(2), 349-357. doi:10.1093/ajcn/nqz073

International Civil Aviation Organization. (2016). *Manual for the oversight of fatigue management approaches*. Retrieved from https://www.icao.int

Jaworski, D. J., Roshan, Y. M., Tae, C. G., & Park, E. J. (2019). Detection of Sleep and Wake States Based on the Combined Use of Actigraphy and Ballistocardiography. *Conf Proc IEEE Eng Med Biol Soc, 2019*, 6701-6704. doi:10.1109/embc.2019.8857650

Johns, M. W. (1991). A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep, 14*(6), 540-545. doi:10.1093/sleep/14.6.540

Johnson, C. (2007). *The Systemic Effects of Fatigue on Military Operations* Paper presented at the International Conference on System Safety, London.

Johnson, D. R., Vincent, A. S., Johnson, A. E., Gilliland, K., & Schlegel, R. E. (2008). Reliability and construct validity of the Automated Neuropsychological Assessment Metrics (ANAM) mood scale. *Archives of Clinical Neuropsychology, 23*(1), 73-85. doi:10.1016/j.acn.2007.10.001

Johnston, J. D. (2014). Physiological links between circadian rhythms, metabolism and nutrition. *Experimental Physiology, 99*(9), 1133-1137. doi:https://doi.org/10.1113/expphysiol.2014.078295

Kales, A., & Rechtschaffen, A. (1968). *A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects: Allan Rechtschaffen and Anthony Kales, Editors*: U. S. National Institute of Neurological Diseases and Blindness, Neurological Information Network.

Kleitman, N., & Jackson, D. P. (1950). Body temperature and performance under different routines. *J Appl Physiol, 3*(6), 309-328. doi:10.1152/jappl.1950.3.6.309

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. *J Neurosci, 22*(13), 5581-5587. doi:10.1523/jneurosci.22-13-05581.2002

LaFountain, R. A., Miller, V. J., Barnhart, E. C., Hyde, P. N., Crabtree, C. D., McSwiney, F. T., . . . Volek, J. S. (2019). Extended Ketogenic Diet and Physical Training Intervention in Military Personnel. *Military Medicine, 184*(9/10), e538-e547. doi:10.1093/milmed/usz046

Lauritsen, K. M., Søndergaard, E., Luong, T. V., Møller, N., & Gormsen, L. C. (2020). Acute Hyperketonemia Does Not Affect Glucose or Palmitate Uptake in Abdominal Organs or Skeletal Muscle. *The Journal of Clinical Endocrinology & Metabolism, 105*(6), 1785-1790. doi:10.1210/clinem/dgaa122

Lieberman, H. R., Niro, P., Tharion, W. J., Nindl, B. C., Castellani, J. W., & Montain, S. J. (2006). Cognition during sustained operations: comparison of a laboratory simulation to field studies. *Aviat Space Environ Med, 77*(9), 929-935.

Lim, J., & Dinges, D. F. (2008). Sleep Deprivation and Vigilant Attention. *Annals of the New York Academy of Sciences, 1129*(1), 305-322. doi:https://doi.org/10.1196/annals.1417.002

Lim, J., & Dinges, D. F. (2010). A Meta-Analysis of the Impact of Short-Term Sleep Deprivation on Cognitive Variables. In (Vol. 136, pp. 375-389): Psychological Bulletin.

Lincoln, B. C., Rosiers, C. D., & Brunengraber, H. (1987). Metabolism of S-3-hydroxybutyrate in the perfused rat liver. *Archives of Biochemistry and Biophysics, 259*(1), 149-156. doi:https://doi.org/10.1016/0003-9861(87)90480-2

Linear Mixed Effects Models. (2006). In *Pharmacokinetic-Pharmacodynamic Modeling and Simulation* (pp. 181-204). Boston, MA: Springer US.

Lovibond, P. F., & Lovibond, S. H. (1995). The structure of negative emotional states: comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. *Behav Res Ther, 33*(3), 335-343. doi:10.1016/0005-7967(94)00075-u

Lowden, A., Holmbäck, U., Akerstedt, T., Forslund, J., Lennernäs, M., & Forslund, A. (2004). Performance and sleepiness during a 24 h wake in constant conditions are affected by diet. *Biol Psychol, 65*(3), 251-263. doi:10.1016/s0301-0511(03)00114-5

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. doi:https://doi.org/10.1016/j.neubiorev.2017.07.010

Mergenthaler, P., Lindauer, U., Dienel, G. A., & Meisel, A. (2013). Sugar for the brain: the role of glucose in physiological and pathological brain function. *Trends in neurosciences, 36*(10), 587-597. doi:10.1016/j.tins.2013.07.001

Miller, N., Shattuck, L., & Matsangas, P. (2011). Sleep and Fatigue Issues in Continuous Operations: A Survey of U.S. Army Officers. *Behavioral Sleep Medicine, 9*(1), 53-65. doi:10.1080/15402002.2011.533994

Minkel, J. D., Banks, S., Htaik, O., Moreta, M. C., Jones, C. W., McGlinchey, E. L., . . . Dinges, D. F. (2012). Sleep Deprivation and Stressors: Evidence for Elevated Negative Affect in Response to Mild Stressors When Sleep Deprived. *Emotion, 12*(5), 1015-1020. doi:10.1037/a0026871

Mitler, M. M., Carskadon, M. A., Czeisler, C. A., Dement, W. C., Dinges, D. F., & Graeber, R. C. (1988). Catastrophes, sleep, and public policy: consensus report. *Sleep, 11*(1), 100-109. doi:10.1093/sleep/11.1.100

Morgan, L. M., Aspostolakou, F., Wright, J., & Gama, R. (1999). Diurnal variations in peripheral insulin resistance and plasma non-esterified fatty acid concentrations: A possible link? *Annals of Clinical Biochemistry, 36*(4), 447-450. doi:10.1177/000456329903600407

Murman, D. L. (2015). The Impact of Age on Cognition. *Seminars in hearing, 36*(3), 111-121. doi:10.1055/s-0035-1555115

National Commission on Military Aviation Safety. (2020). *Military Aviation Losses FY2013 to FY2018.* Retrieved from https://www.militaryaviationsafety.gov/report/NCMAS_Final_Report.pdf

Owen, O. E., Morgan, A. P., Kemp, H. G., Sullivan, J. M., Herrera, M. G., & Cahill, G. F., Jr. (1967). Brain metabolism during fasting. *The Journal of clinical investigation, 46*(10), 1589-1595. doi:10.1172/JCI105650

Owens, D. S., Parker, P. Y., & Benton, D. (1997). Blood Glucose and Subjective Energy Following Cognitive Demand. *Physiology & Behavior, 62*(3), 471-478. doi:https://doi.org/10.1016/S0031-9384(97)00156-X

Paoli, A. (2014). Ketogenic diet for obesity: friend or foe? *International journal of environmental research and public health, 11*(2), 2092-2107. doi:10.3390/ijerph110202092

Paquet, J., Kawinska, A., & Carrier, J. (2007). Wake detection capacity of actigraphy during sleep. *Sleep, 30*(10), 1362-1369. doi:10.1093/sleep/30.10.1362

Pavón, S., Lázaro, E., Martínez, O., Amayra, I., López-Paz, J. F., Caballero, P., . . . Pérez-Núñez, P. (2021). Ketogenic diet and cognition in neurological diseases: a systematic review. *Nutrition Reviews, 79*(7), 802-813. Retrieved from http://ezproxy.massey.ac.nz/login?url=http://search.ebscohost.com/login.aspx?direct=true&AuthType=ip,cookie,url,uid&db=s3h&AN=150816832&site=eds-live&scope=site&authtype=sso&custid=s3027306

Phinney, S. D., Bistrian, B. R., Wolfe, R. R., & Blackburn, G. L. (1983). The human metabolic response to chronic ketosis without caloric restriction: physical and biochemical adaptation. *Metabolism, 32*(8), 757-768. doi:10.1016/0026-0495(83)90105-1

Pilcher, J. J., Band, D., Odle-Dusseau, H. N., & Muth, E. R. (2007). Human performance under sustained operations and 'acute sleep deprivation conditions: Toward a model of controlled attention. *Aviation Space and Environmental Medicine, 78*(5 II), B15-B24. Retrieved from http://ezproxy.massey.ac.nz/login?url=https://search.ebscohost.com/login.aspx?direct=true&AuthType=ip,cookie,url,uid&db=edselc&AN=edselc.2-52.0-34248206281&site=eds-live&scope=site&authtype=sso&custid=s3027306

R Core Team. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from http://www.r-project.org/index.html

Robinson, A. M., & Williamson, D. H. (1980). Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiol Rev, 60*(1), 143-187. doi:10.1152/physrev.1980.60.1.143

Roth, T. (2009). Slow wave sleep: does it matter? *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine, 5*(2 Suppl), S4-S5. Retrieved from https://pubmed.ncbi.nlm.nih.gov/19998868

Shaw, D. M., Maunder, E. D., Dulson, D. K., Merien, F., & Braakhuis, A. (2019). Effect of a Ketogenic Diet on Submaximal Exercise Capacity and Efficiency in Runners. *Medicine and Science in Sports and Exercise, 51*(10), 2135-2146. doi:10.1249/MSS.0000000000002008

Shaw, D. M., Merien, F., Braakhuis, A., Maunder, E., & Dulson, D. K. (2020). Exogenous Ketone Supplementation and Keto-Adaptation for Endurance Performance: Disentangling the Effects of Two Distinct Metabolic States. *Sports Medicine, 50*(4), 641-656. doi:10.1007/s40279-019-01246-y

Signal, T. L., Gale, J., & Gander, P. H. (2005). Sleep measurement in flight crew: comparing actigraphic and subjective estimates to polysomnography. *Aviat Space Environ Med, 76*(11), 1058-1063.

Teo, L., Crawford, C., Yehuda, R., Jaghab, D., Bingham, J. J., Gallon, M. D., . . . Berry, K. (2017). Whole dietary patterns to optimize cognitive function for military mission-readiness: a systematic review and recommendations for the field. *Nutrition Reviews, 75*(suppl_2), 73-88. doi:10.1093/nutrit/nux009

Tharumalay, R. D., Din, N. S. B., & Ahmad, M. (2020). The Effects of Circadian Rhythm Disruption towards Metabolic Stress and Mental Health: A Review. *Kesan Gangguan Ritma Sirkadian terhadap Stres Metabolik dan Kesihatan Mental: Satu Tinjauan Kepustakaan., 18*(1), 47-61. doi:10.17576/JSKM-2020-1801-07

Thomas, M., Sing, H., Belenky, G., Holcomb, H., Mayberg, H., Dannals, R., . . . Redmond, D. (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. doi:https://doi.org/10.1046/j.1365-2869.2000.00225.x

Urbain, P., & Bertz, H. (2016). Monitoring for compliance with a ketogenic diet: what is the best time of day to test for urinary ketosis? *Nutrition & metabolism, 13*, 77-77. doi:10.1186/s12986-016-0136-4

Valdez, P. (2019). Homeostatic and circadian regulation of cognitive performance. *Biological Rhythm Research, 50*(1), 85-93. Retrieved from http://ezproxy.massey.ac.nz/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=s3h&AN=133728261&site=eds-live&scope=site

Van Dongen, H., 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.

Van Dongen, H., Maislin, G., & Dinges, D. F. (2004). Dealing with inter-individual differences in the temporal dynamics of fatigue and performance: Importance and techniques. *Aviation Space and Environmental Medicine, 75*(SUPPL.1), A147-A154. Retrieved from http://ezproxy.massey.ac.nz/login?url=https://search.ebscohost.com/login.aspx?direct=true&AuthType=ip,cookie,url,uid&db=edselc&AN=edselc.2-52.0-84871913996&site=eds-live&scope=site&authtype=sso&custid=s3027306

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. doi:10.1093/sleep/26.2.117

Veech, R. L. (2004). The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 70*(3), 309-319. doi:https://doi.org/10.1016/j.plefa.2003.09.007

Volek, J. S., Freidenreich, D. J., Saenz, C., Kunces, L. J., Creighton, B. C., Bartley, J. M., . . . Phinney, S. D. (2016). Metabolic characteristics of keto-adapted ultra-endurance runners. *Metabolism, 65*(3), 100-110. doi:10.1016/j.metabol.2015.10.028

Volek, J. S., Noakes, T., & Phinney, S. D. (2015). Rethinking fat as a fuel for endurance exercise. *European Journal of Sport Science, 15*(1), 13-20. doi:10.1080/17461391.2014.959564

Webber, R. J., & Edmond, J. (1977). Utilization of L(+)-3-hydroxybutyrate, D(-)-3-hydroxybutyrate, acetoacetate, and glucose for respiration and lipid synthesis in the 18-day-old rat. *Journal of Biological Chemistry, 252*(15), 5222-5226. doi:https://doi.org/10.1016/S0021-9258(19)63335-1

Wheless, J. W. (2008). History of the ketogenic diet. *Epilepsia, 49*(s8), 3-5. doi:https://doi.org/10.1111/j.1528-1167.2008.01821.x

Williamson, A., & Feyer, A.-M. (2000). Moderate sleep deprivation produces impairments in cognitive and motor performance equivalent to legally prescribed levels of alcohol intoxication. *Occupational and Environmental Medicine, 57*(10), 649-655. doi:10.1136/oem.57.10.649

Williamson, A., Lombardi, D. A., Folkard, S., Stutts, J., Courtney, T. K., & Connor, J. L. (2011). The link between fatigue and safety. *Accident Analysis & Prevention, 43*(2), 498-515. doi:https://doi.org/10.1016/j.aap.2009.11.011

Wu, J. C., Gillin, J. C., Buchsbaum, M. S., & Hershey, T. (1991). The effect of sleep deprivation on cerebral glucose metabolic rate in normal humans assessed with positron emission tomography. *Sleep: Journal of Sleep Research & Sleep Medicine, 14*(2), 155-162. Retrieved from http://ezproxy.massey.ac.nz/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=psyh&AN=1991-32534-001&site=eds-live&scope=site&authtype=ip,sso&custid=s3027306

Yamanaka, Y. (2020). Basic concepts and unique features of human circadian rhythms: implications for human health. *Nutrition Reviews, 78*(Supplement_3), 91-96. doi:10.1093/nutrit/nuaa072

Zhou, X., Ferguson, S. A., Matthews, R. W., Sargent, C., Darwent, D., Kennaway, D. J., & Roach, G. D. (2011). Sleep, Wake and Phase Dependent Changes in Neurobehavioral Function under Forced Desynchrony. *Sleep, 34*(7), 931-941. doi:10.5665/sleep.1130

Zinn, C., McPhee, J., Harris, N., Williden, M., Prendergast, K., & Schofield, G. (2017). A 12-week low-carbohydrate, high-fat diet improves metabolic health outcomes over a control diet in a randomised controlled trial with overweight defence force personnel. *Applied Physiology, Nutrition and Metabolism, 42*(11), 1158-1164. doi:10.1139/apnm-2017-0260

Appendices

Appendix A: Supplementary results

D-βHB post-hoc analysis

Comparison of period within diets

Diet = CHO		
Contrast	t ratio	*p* value
2-1	-0.434	1.0000
3-1	-0.724	1.0000
3-2	-0.291	1.0000
4-1	-0.415	1.0000
4-2	0.018	1.0000
4-3	0.309	1.0000
5-1	-0.205	1.0000
5-2	0.229	1.0000
5-3	0.520	1.0000
5-4	0.211	1.0000
6-1	-0.415	1.0000
6-2	0.018	1.0000
6-3	0.309	1.0000
6-4	0.000	1.0000
6-5	-0.211	1.0000
Diet = KD		
Contrast	t ratio	*p* value
2-1	3.788	**0.0020**
3-1	-1.636	0.6892
3-2	-5.424	**< 0.0001**
4-1	-1.242	0.8626
4-2	-5.030	**< 0.0001**
4-3	0.394	1.0000
5-1	2.290	0.1840

5-2	-1.498	0.6892
5-3	3.926	**0.0013**
5-4	3.532	**0.0046**
6-1	-1.660	0.6892
6-2	-5.448	**< 0.0001**
6-3	-0.024	1.0000
6-4	-0.418	1.0000
6-5	-3.950	**0.0013**

Comparison of diets with periods

Contrast	t ratio	*p*-value
Period 1 KD-CHO	11.942	**<0.0001**
Period 2 KD-CHO	16.157	**<0.0001**
Period 3 KD-CHO	11.033	**<0.0001**
Period 4 KD-CHO	11.117	**<0.0001**
Period 5 KD-CHO	14.433	**<0.0001**
Period 6 KD-CHO	10.700	**<0.0001**

Glucose post-hoc analysis

Comparison of diets with tests

Contrast	t ratio	*p*-value
Test 1 KD-CHO	-11.031	**<0.0001**
Test 2 KD-CHO	-4.517	**<0.0001**
Test 3 KD-CHO	-2.832	**0.0032**

Comparison of tests within diets

Contrast	t ratio	*p*-value
CHO 2-1	-7.539	**<0.0001**
CHO 3-1	-10.738	**<0.0001**
CHO 3-2	-3.199	**0.0016**
KD 2-1	-1.002	0.3177
KD 3-1	-2.511	**0.0384**
KD 3-2	-1.509	0.2657

Main effect of period

Contrast	t ratio	*p*-value
2-1	2.587	**0.0622**
3-1	6.681	**<0.0001**
3-2	4.094	**0.0007**
4-1	5.488	**<0.0001**
4-2	2.901	**0.0288**
4-3	-1.193	0.9365
5-1	1.996	0.2364
5-2	-0.591	1.0000
5-3	-4.685	**0.0001**
5-4	-3.492	**0.0053**
6-1	5.734	**<0.0001**
6-2	3.147	**0.0151**
6-3	-0.947	1.000
6-4	0.246	1.0000
6-5	3.738	**0.0024**

Lapses post-hoc analysis

Comparison of tests within periods

Contrast	t ratio	*p*-value
Period 1		
2-1	1.014	0.9349
3-1	1.014	0.9349
3-2	0.000	1.000
Period 2		
2-1	0.544	0.9446
3-1	-0.544	1.0000
3-2	-1.087	0.8348
Period 3		
2-1	1.007	0.9446
3-1	0.991	0.9446

3-2	-0.017	0.9867
Period 4		
2-1	0.845	0.3988
3-1	4.398	**0.0001**
3-2	3.553	**0.0009**
Period 5		
2-1	-0.952	0.6841
3-1	0.852	0.6841
3-2	1.805	0.1450
Period 6		
2-1	-0.699	0.4855
3-1	-2.504	**0.0391**
3-2	-1.805	0.1450

Comparison of periods within tests

Contrast	t ratio	*p*-value
Test 1		
2-1	0.544	1.0000
3-1	0.544	1.0000
3-2	0.000	1.0000
4-1	2.485	0.1235
4-2	1.942	0.4280
4-3	1.942	0.4280
5-1	4.360	**0.0003**
5-2	3.816	**0.0023**
5-3	3.816	**0.0023**
5-4	1.874	0.4280
6-1	3.967	**0.0014**
6-2	3.424	**0.0082**
6-3	3.423	**0.0082**
6-4	1.481	0.7001
6-5	-0.393	1.0000
Test 2		

2-1	0.073	1.0000
3-1	0.537	1.0000
3-2	0.464	1.0000
4-1	2.316	0.2980
4-2	2.244	0.3031
4-3	1.790	0.6126
5-1	2.393	0.2638
5-2	2.320	0.2980
5-3	1.857	0.5831
5-4	0.077	1.0000
6-1	2.254	0.3031
6-2	2.181	0.3031
6-3	1.717	0.6126
6-4	-0.063	1.0000
6-5	-0.140	1.0000
Test 3		
2-1	-1.014	1.0000
3-1	0.520	1.0000
3-2	1.534	0.7588
4-1	5.869	**< 0.0001**
4-2	6.884	**< 0.0001**
4-3	5.349	**< 0.0001**
5-1	4.198	**0.0004**
5-2	5.212	**< 0.0001**
5-3	3.678	**0.0024**
5-4	-1.671	0.6729
6-1	0.448	1.0000
6-2	1.463	0.7588
6-3	-0.072	1.0000
6-4	-5.421	**< 0.0001**
6-5	-3.740	**0.0021**

Mean reciprocal response time post-hoc analysis

Comparison of tests within periods

Contrast	t ratio	*p*-value
Period 1		
2-1	-1.167	0.6039
3-1	-1.282	0.6039
3-2	-0.115	0.9087
Period 2		
2-1	-0.587	1.000
3-1	0.489	1.000
3-2	1.076	0.8499
Period 3		
2-1	-0.220	0.8264
3-1	-1.955	0.1558
3-2	-1.735	0.1684
Period 4		
2-1	-1.672	0.0960
3-1	-4.217	**0.0001**
3-2	-2.545	**0.0233**
Period 5		
2-1	0.519	1.0000
3-1	-0.142	1.0000
3-2	-0.661	1.0000
Period 6		
2-1	1.073	0.2942
3-1	2.528	**0.0366**
3-2	1.455	0.2942

Comparison of periods within tests

Contrast	t ratio	*p*-value
Test 1		
2-1	-1.598	0.5578
3-1	-1.283	0.8043

3-2	0.315	0.9346
4-1	-4.339	**0.0002**
4-2	-2.741	**0.0465**
4-3	-3.057	**0.0202**
5-1	-6.280	**<0.0001**
5-2	-4.682	**0.0001**
5-3	-4.998	**<0.0001**
5-4	-1.941	0.3218
6-1	-5.552	**<0.0001**
6-2	-3.954	**0.0009**
6-3	-4.269	**0.0003**
6-4	-1.212	0.8043
6-5	0.728	0.9346
Test 2		
2-1	-1.018	1.0000
3-1	-0.335	1.0000
3-2	0.683	1.0000
4-1	-4.844	**< 0.0001**
4-2	-3.827	**0.0019**
4-3	-4.509	**0.0001**
5-1	-4.594	**0.0001**
5-2	-3.576	**0.0043**
5-3	-4.259	**0.0004**
5-4	0.251	1.0000
6-1	-3.312	**0.0098**
6-2	-2.294	0.1596
6-3	-2.977	**0.0261**
6-4	1.533	0.7612
6-5	1.282	1.0000
Test 3		
2-1	0.173	1.0000
3-1	-1.955	0.2594
3-2	-2.128	0.2375

4-1	-7.275	**<0.0001**
4-2	-7.448	**<0.0001**
4-3	-5.319	**<0.0001**
5-1	-5.140	**<0.0001**
5-2	-5.313	**<0.0001**
5-3	-3.185	**0.0134**
5-4	2.135	0.2375
6-1	-1.742	0.2594
6-2	-1.914	0.2594
6-3	-0.214	1.0000
6-4	5.533	**<0.0001**
6-5	3.398	**0.0073**

Slowest 10% response time post-hoc analysis

Comparison of tests within periods

Contrast	t ratio	*p*-value
Period 1		
2-1	0.688	1.0000
3-1	0.864	1.0000
3-2	0.176	1.0000
Period 2		
2-1	0.546	1.0000
3-1	-0.064	1.0000
3-2	-0.610	1.0000
Period 3		
2-1	0.183	1.0000
3-1	0.724	1.0000
3-2	0.541	1.0000
Period 4		
2-1	1.143	0.2546
3-1	3.866	**0.0004**
3-2	2.724	**0.0140**
Period 5		

2-1	-1.431	0.3410
3-1	0.158	0.8747
3-2	1.589	0.3410
Period 6		
2-1	-2.405	0.0341
3-1	-3.310	**0.0033**
3-2	-0.905	0.3663

Comparison of periods within tests

Contrast	t ratio	*p*-value
Test 1		
2-1	0.369	1.0000
3-1	0.515	1.0000
3-2	0.146	1.0000
4-1	2.237	0.1846
4-2	1.867	0.3797
4-3	1.722	0.4332
5-1	4.654	**0.0001**
5-2	4.284	**0.0003**
5-3	4.139	**0.0005**
5-4	2.417	0.1320
6-1	5.479	**< 0.0001**
6-2	5.110	**< 0.0001**
6-3	4.964	**< 0.0001**
6-4	3.243	**0.0124**
6-5	0.825	1.0000
Test 2		
2-1	0.228	1.0000
3-1	0.010	1.0000
3-2	-0.218	1.0000
4-1	2.691	0.1154
4-2	2.463	0.1604
4-3	2.681	01154

5-1	2.535	0.1556
5-2	2.307	0.1789
5-3	2.525	0.1556
5-4	0.156	1.0000
6-1	2.387	0.1789
6-2	2.159	0.2242
6-3	2.376	0.1789
6-4	-0.305	1.0000
6-5	-0.149	1.0000
Test 3		
2-1	-0.558	1.0000
3-1	0.375	1.0000
2-3	0.934	1.0000
4-1	5.238	**<0.0001**
4-2	5.797	**<0.0001**
4-3	4.863	**<0.0001**
5-1	3.947	**0.0012**
5-2	4.506	**0.0001**
5-3	3.572	**0.0040**
5-4	-1.291	1.000
6-1	1.305	1.000
6-2	1.863	0.4468
6-3	0.930	1.0000
6-4	-3.933	**0.0012**
6-5	-2.642	0.0708

Fastest 10% response time post-hoc analysis

Main effect of period

Contrast	t ratio	*p*-value
2-1	1.496	0.6802
3-1	1.593	0.6758
3-2	0.097	1.0000
4-1	5.934	**< 0.0001**

4-2	4.437	**0.0002**
4-3	4.341	**0.0003**
5-1	4.891	**< 0.0001**
5-2	3.394	**0.0082**
5-3	3.298	**0.0103**
5-4	-1.043	1.000
6-1	2.166	0.2200
6-2	0.670	1.0000
6-3	0.573	1.0000
6-4	-3.768	**0.0024**
6-5	-2.725	0.0559

Vigour post-hoc analysis

Main effect of period

Contrast	t ratio	*p*-value
2-1	-2.186	0.1495
3-1	-3.260	**0.0078**
3-2	-10.74	1.000
4-1	-9.749	**< 0.0001**
4-2	-7.563	**< 0.0001**
4-3	-6.489	< 0.0001
5-1	-10.640	**< 0.0001**
5-2	-8.454	**< 0.0001**
5-3	-7.380	**< 0.0001**
5-4	-0.891	1.0000
6-1	-9.627	**< 0.0001**
6-2	-7.441	**< 0.0001**
6-3	-6.367	**< 0.0001**
6-4	0.122	1.0000
6-5	1.013	1.0000

Fatigue post-hoc analysis

Main effect of period

Contrast	t ratio	*p*-value
2-1	2.890	**0.0213**
3-1	3.925	**0.0007**
3-2	1.036	0.9044
4-1	10.544	**< 0.0001**
4-2	7.654	**< 0.0001**
4-3	6.618	**< 0.0001**
5-1	11.860	**< 0.0001**
5-2	8.971	**< 0.0001**
5-3	7.935	**< 0.0001**
5-4	1.317	0.7576
6-1	11.061	**< 0.0001**
6-2	8.171	**< 0.0001**
6-3	7.135	**< 0.0001**
6-4	0.517	0.9044
6-5	-0.799	0.9044

Main effect of test

Contrast	t ratio	*p*-value
2-1	0.779	0.4369
3-1	2.540	**0.0354**
3-2	1.761	0.1593

Sleepiness post-hoc analysis

Comparison of tests within periods

Contrast	t ratio	*p*-value
Period 1		
2-1	-0.810	1.000
3-1	-0.202	1.0000
3-2	0.607	1.0000
Period 2		

2-1	0.810	1.0000
3-1	0.000	1.0000
3-2	-0.810	1.0000
Period 3		
2-1	0.607	0.5444
3-1	2.429	**0.0480**
3-2	1.822	0.1399
Period 4		
2-1	2.631	**0.0183**
3-1	3.441	**0.0021**
3-2	0.810	0.4191
Period 5		
2-1	-0.607	1.0000
3-1	-0.202	1.0000
3-2	0.405	1.0000
Period 6		
2-1	-0.607	1.0000
3-1	-1.012	0.9382
3-2	-0.405	1.0000

Comparison of periods within tests

Contrast	t ratio	*p*-value
Test 1		
2-1	0.607	1.0000
3-1	0.607	1.0000
3-2	0.000	1.0000
4-1	4.048	**0.0007**
4-2	3.441	**0.0056**
4-3	3.441	**0.0056**
5-1	7.489	**< 0.0001**
5-2	6.881	**< 0.0001**
5-3	6.881	**< 0.0001**

5-4	3.441	**0.0056**
6-1	6.477	**< 0.0001**
6-2	5.869	**< 0.0001**
6-3	5.869	**< 0.0001**
6-4	2.429	0.0800
6-5	-1.012	1.0000
Test 2		
2-1	2.226	0.1624
3-1	-2.024	0.2213
3-2	-0.202	1.000
4-1	7.489	**< 0.0001**
4-2	5.262	**< 0.0001**
4-3	5.465	**< 0.0001**
5-1	7.691	**< 0.0001**
5-2	5.465	**< 0.0001**
5-3	5.667	**< 0.0001**
5-4	0.202	1.0000
6-1	6.679	**< 0.0001**
6-2	4.453	**0.0001**
6-3	4.655	< 0.0001
6-4	-0.810	1.0000
6-5	-0.1012	1.0000
Test 3		
2-1	0.810	0.8382
3-1	3.238	**0.0098**
3-2	2.429	0.0960
4-1	7.691	**< 0.0001**
4-2	6.881	**< 0.0001**
4-3	4.453	0.0001
5-1	7.489	**< 0.0001**
5-2	6.679	**< 0.0001**
5-3	4.250	**0.0003**
5-4	-0.202	0.8398

6-1	5.667	**< 0.0001**
6-2	4.857	**< 0.0001**
6-3	2.429	0.0960
6-4	-2.024	0.1770
6-5	-1.822	0.2099

Appendix B: Participant information sheet

The effect of a short-term ketogenic diet on cognitive function, sleep, fatigue, heart rate variability and mood

Researchers Introduction

We are investigating the effects of a short-term ketogenic diet on cognitive function, sleep, fatigue and the autonomic nervous system. The findings of this research will contribute towards dietary recommendations to manage fatigue within the New Zealand Defence Force (NZDF), a Masters of Nutrition and Dietetics research thesis and academic journals and conferences. There is a signed deed in place between the NZDF and Massey University for conducting this research, which has also been approved by the NZDF Research Ethics Committee and command.

The research will be conducted by Dr. David Shaw, Dr. Margo Van Den Berg and Lydia Henderson. Please ensure you read and understand all pages. If you have any queries, please contact Dr. David Shaw (lead researcher) (see details below).

Dr. David Shaw | PhD, NZRD
Aerospace Physiologist, Aviation Medicine Unit, Royal New Zealand Air Force Base Auckland
School of Sport, Exercise and Nutrition, Massey University, Auckland

Dr. Margo Van Den Berg | PhD
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Lydia Henderson | BSc
Student Dietitian, School of Sport, Exercise and Nutrition, Massey University, Auckland

Project Description and Invitation

Fatigue is defined as "*a physiological state of reduced mental or physical performance capability resulting from sleep loss, extended wakefulness, circadian phase, and/or workload (mental and/or physical activity) that can impair a person's alertness and ability to perform safety-related operational duties*". This increases the risk of incident, accident and, potentially, loss of life. Lifestyle factors, such as diet, influence fatigue and sleep. Nevertheless, New Zealand Defence Force (NZDF) fatigue management strategies do not incorporate dietary macronutrient recommendations.

A very low-carbohydrate (CHO), ketogenic diet (KD) has gained attention for its effects on physical performance and health; however, little is known about its effects on cognitive function (e.g. reaction time and attention), sleep and fatigue. A KD switches the body's fuel preference away from CHO and towards fat and ketone bodies, with the latter providing an alternative energy source for the brain and central nervous system (CNS). It is possible to measure the CNS response via heart rate variability (HRV; i.e. the variability in time between beats of your heart), which may help identify individuals who respond favourably to a KD. Considering a KD is widely employed within various populations, including military, it is important to assess its effectiveness to inform future dietary recommendations within the NZDF.

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 method.*

Recruitment for this study is via word-of-mouth and internal advertising at the New Zealand Defence Force.

- *Method of obtaining participant names.*

Participation for this study is on a voluntary basis. Your name will only be obtained following your expression of interest.

- *Selection criteria.*

To be deemed eligible, you will need to meet all of the following inclusion criteria: 1) aged 18-50 years; 2) non-obese (i.e. BMI less than 27); 3) healthy; 4) consuming a mixed-diet comprising more than 45% energy intake (EI) from CHO (determined by a 3-day diet record, including 2 weekdays and 1 weekend day); and 5) habitually going to bed between 2100-0000 h and waking between 0600-0900 h.

- *Exclusion criteria.*

You will be deemed ineligible if you meet one of the following exclusion criteria: 1) habitually consumed a KD or exogenous ketone supplements in the previous 2 years; 2) smoker; 3) average caffeinated beverage consumption more than 3 cups/day; 4) have a medical, psychiatric or sleep disorder; 5) habitually sleep less than 7 hours or more than 9 hours; 6) regularly consume medications or medications acting on the central nervous system; 7) history of drug or alcohol abuse; 8) food allergies or engaging in restrictive dietary patterns; 9) trans meridian travel or shift-work in 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 ascertained using standardised questionnaires.

If during the screening process you are identified as a poor sleeper and, therefore, deemed ineligible to take part in the study, you will be provided with information on good sleep practices. If you have any concerns about your sleep, we will recommend that you follow up with your GP or NZDF physician.

- *Number of participants to be involved and the reason for this number.*

The aim is to recruit 15 participants in total. Selection for participating will occur on a first-in, first-served basis.

- *Details of compensation/reimbursement of expenses/payments offered for participation (where relevant).*

You will be provided with all resources to complete the study. However, you will be required to purchase and prepare your own diet during the dietary adaptation period for both conditions. Participants will be offered to enter a prize draw, to win one (1) prize, valued at $300, in recognition of their time and effort spent in the study.

- *Description of discomforts or risks to participants as a result of participation.*

The discomforts you may experience during the study are: 1) possible lack of energy following induction to the KD; 2) the finger prick during measurement of capillary blood glucose and ketones; and 3) tiredness during the extended wakefulness period. All other study procedures will be undertaken as part of your normal day 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

If eligible, you will be asked to perform the cognitive test battery and complete the questionnaires on 5 occasions to familiarise yourself with the study's requirements. Then, you will undertake a 7-day *baseli*ne testing period, followed by a *dietary intervention* period to a KD of CHO diet (CHO-D), then a 36-hour period of *extended wakefulness*. A washout period of 12.5 days will separate the two trial arms, then you will repeat the process with the alternative diet (i.e. 8 weeks in total; Figure 1). Data collection will occur during January to March 2021. The first diet you are required to consume will be randomly allocated. During the *dietary adaptation* period, you will be 'free-living', whereas the *extended wakefulness* period will be conducted at the Aviation Medicine Unit, Royal New Zealand Air Force Base in Whenuapai, Auckland, New Zealand. This study will occur during work time; therefore, you will require consent from your line of command to participate.

Baseline testing and dietary adaptation

You will continue consuming your habitual, high-CHO diet during the 7 days of *baseline testing* and then will be required to consume, in a randomised order, a CHO-D or a very-low-CHO, high-fat, KD for 14 days during the *dietary adaptation* period (Figure 2).

The *dietary adaptation* period requires:

- Avoidance of over-the-counter medications (e.g. anti-histamines), dietary supplements, alcohol and napping.
- Limit caffeine intake to no more than 100 mg ingested prior to 1200 h.
- Maintain your normal sleep-/wake cycle and physical activity levels.
- Avoidance of exercise in the 2 hours prior to bed.
- All other lifestyle choices will be allowed to vary naturally as per your normal habits.

Daily measurements take at home during the *baseline testing* and *dietary adaptation* periods includes:

- Sleep using the actigraph watch and sleep diary.
- HRV (measured upon waking).

- Capillary blood glucose and ketones (measured in the morning immediately after HRV).
- Daily subjective sleepiness (measured at 1600 h).
- Daily mood (measured at 1600 h).

Weekly measurements taken at the Aviation Medicine Unit during the *baseline testing* and *dietary adaptation* periods includes:

- Cognitive performance (measured on days 7 of *baseline testing* and days 7 and 14 of *dietary adaptation* between 0800 and 1000 h prior to breakfast and exercising).

All measurements will be specified on a personal to-do list and will occur within ± 0.5 hours of the first measure.

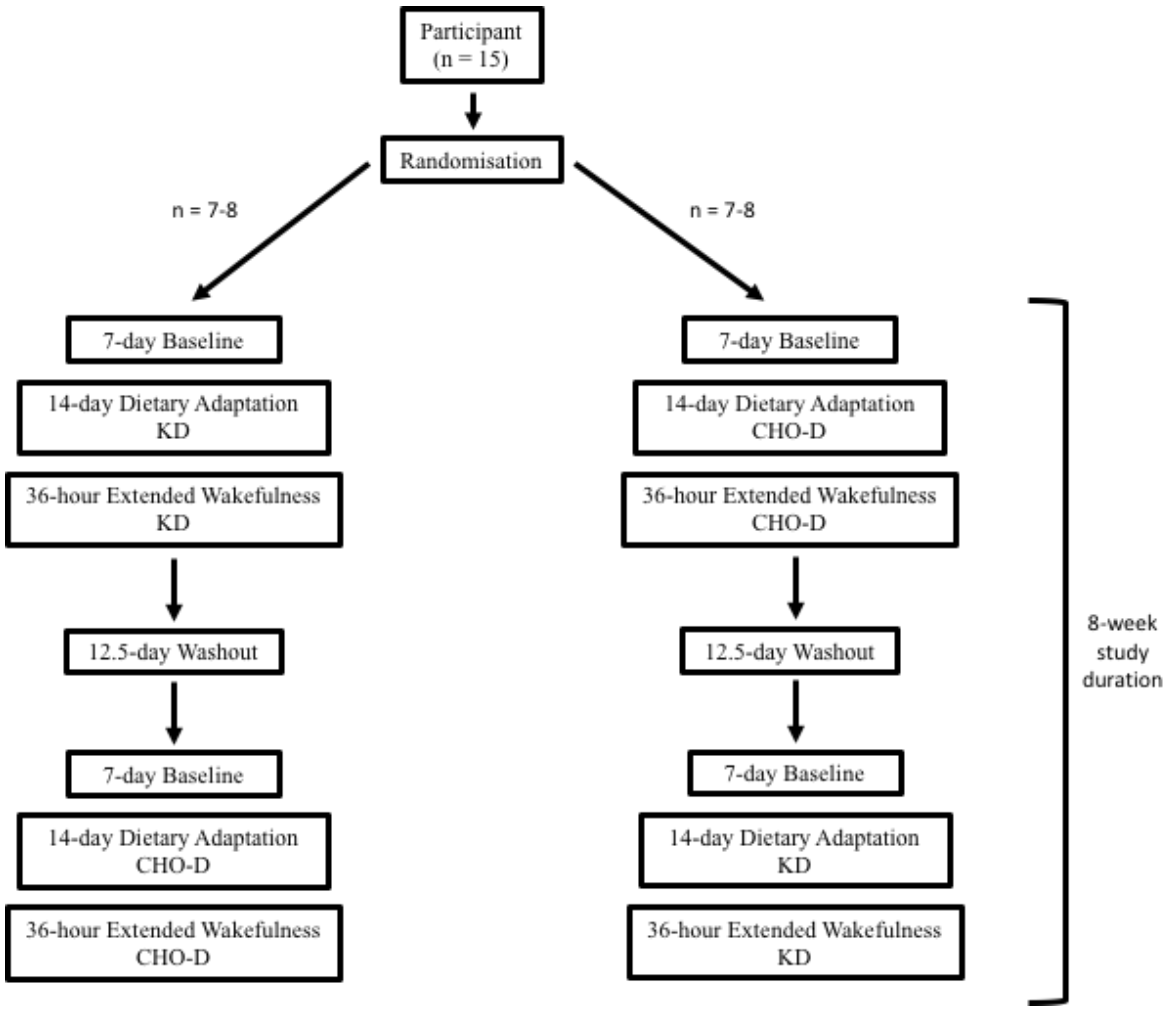


Figure 1. Overview of study design.

Extended wakefulness

On the last night of each *dietary adaptation* period, you will require an 8 hour sleep in preparation for the period of *extended wakefulness*. If you do not get sufficient sleep, you may be excluded from the study.

You will be woken by a phone call at 0630 h and will not be allowed to fall back to sleep or consume caffeine after waking. You will present to the Aviation Medicine Unit at 0700-0715 h to commence a 36-hour period of extended wakefulness from 0730 h. This period will comprise of 6 x 6-hour blocks commencing with a meal. You will be allowed 30 min to consume each meal (Figure 2). Cognitive performance, subjective sleepiness, mood, hunger, capillary blood glucose and ketones will be measured 1, 3 and 5 hours following each meal (i.e. 2-hours between tests).

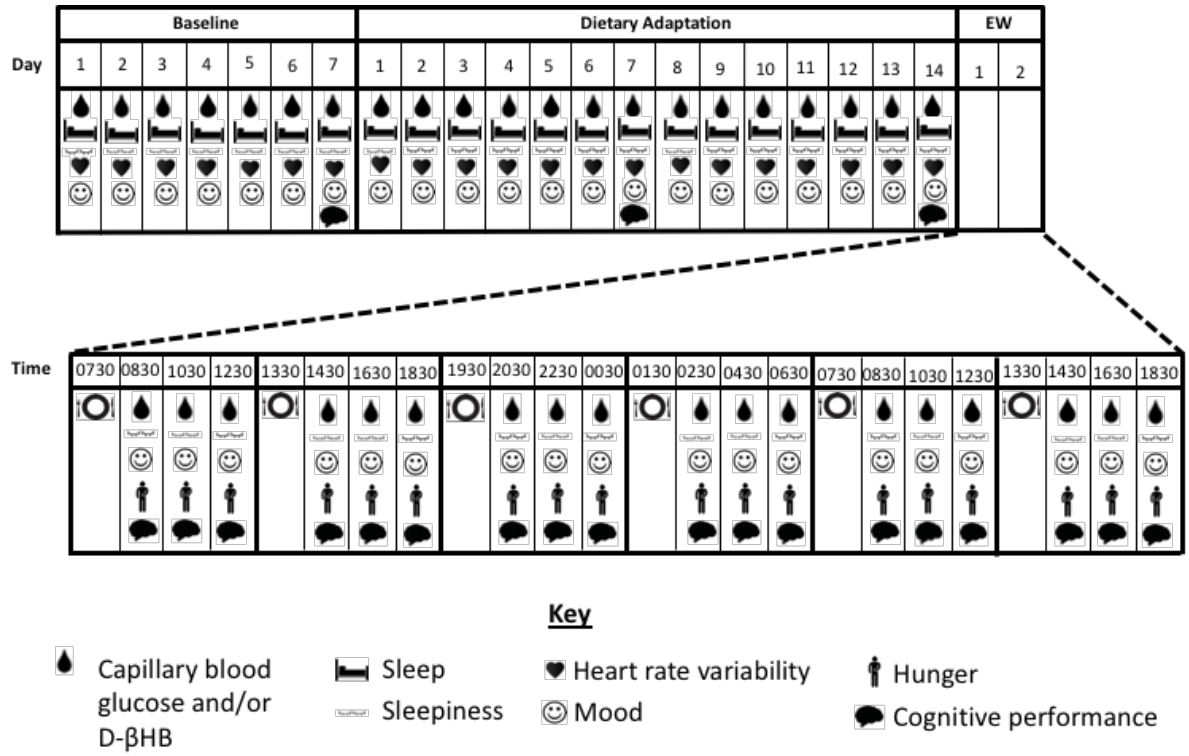
You will remain awake in the laboratory and will be continuously monitored. The environment will be strictly controlled and you will be with 6 or7 other participants. You will have free time to read, watch movies, play board games, and interact with other participants and research staff; however, no vigorous physical activity, interaction with people external to the study, or use of phones will be allowed. You will also not be informed of the time. Following completion, you will be provided with transport home and requested to refrain from duty for a 24-hour period to allow for sufficient recovery.

Dietary intervention

For each dietary condition, you will be provided with comprehensive education from a registered dietitian, including an individualised meal plan specific to your estimated energy requirements. The KD will comprise less than 40 g per day of CHO, 15-20% energy intake (EI) from protein and more than 75% EI from fat and you will be required to ingest an electrolyte capsule twice daily (i.e. morning and afternoon) and prescribed high-sodium fluids to maintain blood electrolytes. The CHO-D will comprise more than 45% EI from CHO, 15-20% EI from protein and less than 40% EI from fat. It is important you stay hydrated by consuming sufficient water throughout the study. You will also be required to purchase and prepare your own food and fluid during the 7-day *baseline testing* and 14-day *dietary adaptation* period. A registered dietitian will be available at all times, either via phone, email or in person, to provide guidance and support.

During the 36-h period of *extended wakefulness*, you will be provided meals by the research team in accordance with your dietary allocation. Meals will be provided at 0730 h (breakfast), 1330 h (lunch), 1930 h (dinner) and 0130 h (night), with each providing 25% of your daily energy requirements. For the KD, you will ingest a salt capsule three times daily (i.e. 0800, 1600 and 0000 h). No caffeine or alcohol will be allowed.

Figure 2. Overview of study procedures.



Dietary monitoring

To monitor dietary compliance, you will be trained in dietary reporting and asked to provide an image-assisted, weighed dietary record in real-time to a registered dietitian via a mobile phone application (WhatsApp). Diet records will be required on:

- 3 non-consecutive days during *baseline testing*;
- Days 1, 3, 6, 8, 10 and 12 during the *dietary adaptation* period; and
- 3 non-consecutive days during the washout period.

Compliance to the KD will be via morning capillary blood ketone concentrations equal or more than 0.4 mmol/L prior to breakfast and exercising. If blood capillary ketones concentration is less than 0.4 mmol/L, the registered dietitian will assess your dietary intake and provide recommendations; however, if concentrations are less than 0.4 mmol/L on 2 consecutive days between days 5-14 during the *dietary adaptation period*, you will be excluded from the study due to incompliance.

During the 36-hour period of *extended wakefulness*, you will only be able to consume food provided by the research team. For the KD, you will be required to have capillary blood ketone concentrations equal or more than 0.4 mmol/L for more than 50% of tests to allow for natural biological variation; otherwise, you will be excluded from the study.

Measurement of capillary blood glucose and ketone concentration

Capillary blood glucose and ketone concentration will be measured from a fingertip blood sample using a point-of-care, handheld device and standardised techniques. You will be trained on how to use the device.

Measurement of cognitive performance

Cognitive performance will be assessed with a test battery, including sustained attention using the handheld Psychomotor Vigilance Task (PVT)-192, and working memory and choice reaction speed using the computer-based Automated Neuropsychological Assessment Metrics test battery (ANAM).

Measurement of sleep

You will wear a wrist actigraphy monitor (the size of a wrist watch) on your non-dominant wrist at all times during the study, except when showering or swimming, and complete a sleep diary to record your bedtime and risetime. The actigraph 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.

Measurement of subjective sleepiness

Subjective sleepiness will be determined using a printed form during the *baseline testing* and *dietary adaptation* period and from the ANAM test battery during the *extended wakefulness* period.

Measurement of heart-rate variability (HRV)

HRV will be measured using a commercially available smartphone application. You will be provided with a demonstration of how to use the smartphone application prior to the study You will be required to measure your HRV immediately upon wakening, whilst remaining lying down.

Measurement of mood

Mood will be measured from the ANAM mood scale using a printed form during the *baseline testing* and *dietary adaptation* period and from the ANAM test battery during the *extended wakefulness* period.

Measurement of hunger

Hunger will be measured using a visual analogue scale of hunger.

- *Your time involved in this study.*

Baseline and dietary adaptations period

Dietary reporting: 2 hours

Heart rate variability recording: 1 hour

Questionnaires: 1 hour

Capillary blood tests: 1 hour
Cognitive tests: 1.5 hours

Extended wakefulness period
Extended wakefulness: 72 hours

- *Any conflict of financial interest and/or role.*

The researchers have no potential conflicts of interest. All research findings will be screened prior to publication by the Office of the Chief of Defence Force and Defence Security of the NZDF, who have the right to embargo material.

- *Any support processes in place to deal with adverse physical or psychological risks (where relevant).*

You will be provided with the contact details of the research team in case you have any queries or concerns. You will also have access to a registered dietitian to guide you through each dietary intervention. If there is a medical issue, a medic will be available to intervene. 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 or NZDF physician.

During the 36-hour period of extended wakefulness, SQNLDR (Dr) Gus Cabre, who is Officer Commanding Aviation Medicine Unit and Aviation Medical Officer will be on call. If needed, he will provide treatment in accordance with standard medical and military procedures.

Data Management

- *What will happen to the data when it is obtained.*

During the data collection period, Dr David Shaw and Lydia Henderson will have information linking participants' names to their study identification number. As soon as all study data has been collected and de-identified, any personal identifying information will be immediately destroyed.

The researchers have signed a confidentiality agreement, agreeing to keep confidential all information concerning this project. Data will then be analysed by researchers at the New

Zealand Defence Force and Massey University. The findings of the study will be published in peer-reviewed scientific journals, conference proceedings, and NZDF briefings and reports. All publications, except internal / NZDF reports, will be embargoed until provided consent by the Office of the Chief of Defence Force and Defence Security of the NZDF.

- *Storage and disposal of data.*

Hard copy data will be stored in a locked cabinet at the Aviation Medicine Centre, Royal New Zealand Air Force Base Auckland. All electronic data will be stored on the NZDF and Massey University password protected networks. Hard copy data will be destroyed after 2 years or earlier once it has been converted to an electronic form.

- *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 of the data will be stored, as previously described, to protect your privacy. Only de-identified, aggregated data will be published. No material that could personally identify you will be used in any reports on the study. Your employer will not be notified of your responses.

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.

Committee Approval Statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 20/47. 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 humanethicssoutha@massey.ac.nz.

Project Contacts

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Appendix C: KD guide

The Ketogenic Diet

This handbook is for participants in the study investigating the effects of a short-term ketogenic diet on cognitive function, sleep, fatigue, heart rate variability and mood. It is not for use by the general public.

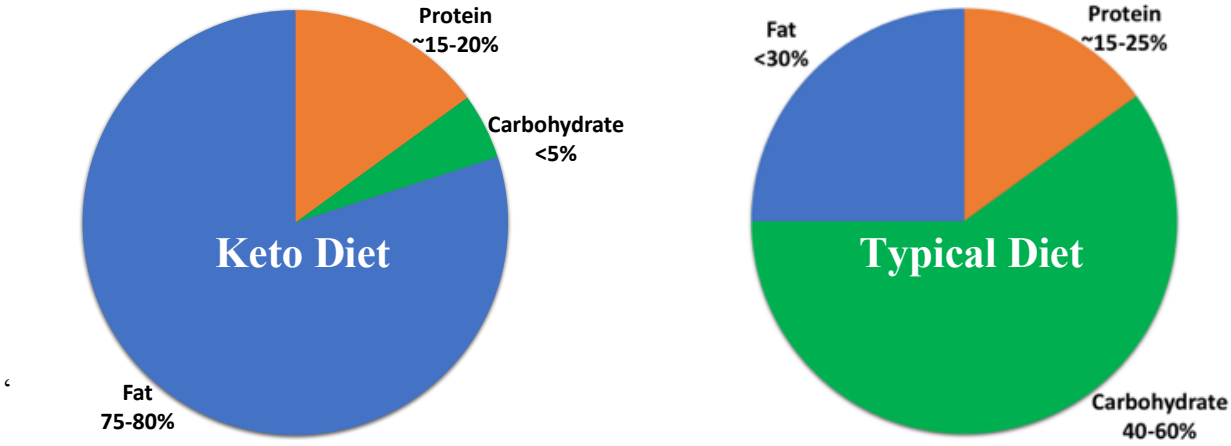
Introduction:

Thank you for participating in this study investigating the effects of a short-term ketogenic diet on cognitive function, sleep, fatigue, heart rate variability and mood. As the ketogenic diet gains popularity within mainstream society, it is important that we understand its effects on military personnel. By participating in this study, you are contributing to the collection of valuable data that will help guide decisions made within the NZDF regarding fatigue management and diet.

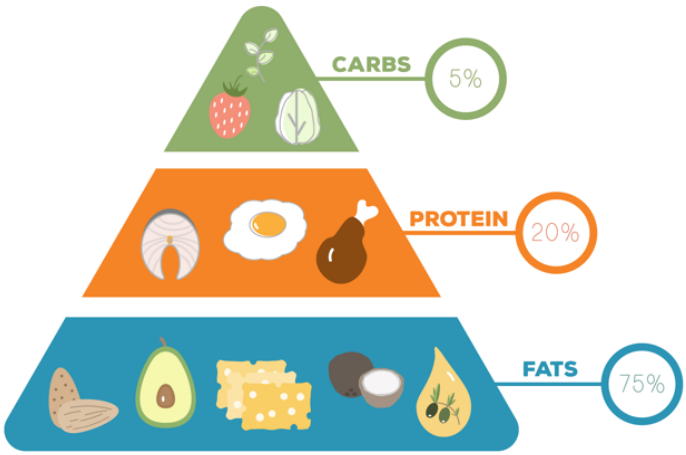
This guide is designed to help you successfully complete the dietary adaptation phase of the study where you are asked to follow a ketogenic diet for 14 days. To ensure the data we collect from you is accurate and reliable, it is critical you follow these guidelines. If you have any queries please contact Dr David Shaw or Lydia Henderson (student dietitian).

What is a Keto Diet?

A ketogenic diet is very low in carbohydrates (<5%), moderate in protein (15-20%) and high in fat (75-90%). In contrast, the Ministry of Health recommends New Zealanders consume a diet consisting of 40-60% carbohydrates, 15-25% protein and <30% fat.



The purpose of the ketogenic diet is to change the type of fuel the body uses to create energy. When consuming a diet very low in carbohydrate, body stores become low and our brain loses its main energy source, glucose. The brain, therefore, requires an alternative energy source. Adapting to a ketogenic diet increases the reliance on fat for energy, rather than carbohydrate. As fat cannot bypass the blood-brain-barrier, the liver produces ketones as a new fuel source. Increased concentrations of ketones in the blood indicates nutritional ketosis, as measured by a capillary blood test, and depends on strict dietary adherence. Even one carbohydrate snack will switch the body back to using carbohydrate as a fuel.



Carbohydrates

Typically, less than 5% of energy intake from carbohydrate is approximately 30-50g, which is equivalent to a couple of slices of bread.

Avoid foods and drinks high in carbohydrates

Foods to avoid

Grains and cereals
Including wheat, corn, pasta, rice, oats, buckwheat, quinoa, rye, cereals, breakfast cereals, bread, baked products etc.

Sugar
Including honey, golden syrup, maple syrup, date syrup, table sugar, chocolate, ice-cream, basked products, lollies etc.

Fruit
Apples, bananas, plums, nectarines, oranges etc. (however, some berries can be consumed in moderation)

Starchy vegetables
Potato, kumara, sweet potato, yams, taro, pumpkin, corn etc.

Legumes
Lentils, chickpeas, kidney beans etc

Processed meat
Sausages, flavoured / glazed ham and bacon etc.

Low fat foods
These tend to higher in carbohydrate and sugar than full-fat versions. You also need the additional fat to help you retain energy balance.

Drinks to avoid

Alcohol
Beer, wine, RTDs, liqueurs etc. However, some spirits may be fine in very small quantities.

Soft drink
Cola, lemonade etc. However, you may have small servings of zero sugar and diet options.

Juice
Any sort including freshly pressed

Sports drink and coconut water

Powerade, Gatorade, Horleys Replace etc.
Energy drinks
V, Mother, Redbull etc.

Flavoured milk and milk
Plain (cows) milk contains 3-5 g of carbohydrate per 100 ml; therefore, very small portions are allowed.

Foods low in carbohydrates

Non starchy fruits and vegetables including:

Fresh, raw	Carbohydrate per 100g	Carbohydrate per cup (g)
Asparagus	1.4	2
Bamboo shoots	1.3	1.8
Broccoli	0.4	0.4
Brussel sprouts	2.1	1.9
Carrot	5	5.8
Celery	1.2	1.5
Courgette	1.7	3
Cucumber	1.5	3
Cauliflower	2	2.3
Cabbage	2.4	2.5
Capsicum	2.3	2
Eggplant	2.6	2.3
Green beans	2.4	3.3
Kale	3.4	3.9
Leek	3.7	3.5
Mushrooms	0	0
Lettuce, cos	1.8	0.9
Onions	4.3	6
Radish	1.9	3
Rhubarb	1.7	4
Spinach	0.6	0.3
Spring onion	4.6	4.8
Tomato	2.4	5.8

Meat, chicken, fish, liver, kidney and eggs
These options provide almost no carbohydrate when raw but it is important to remember that marinades and sauces may contain added sugar and carbohydrates.

Nuts and seeds
Nuts and seeds contain small amounts of carbohydrate. **Limit your intake to 60 g per day** of nuts and/or seeds. Additionally, be careful of sweetened versions e.g. honey roasted/glazed.

Berries, tomato, avocado and olives
Most fruits should be avoided; however, you can eat berries, tomatoes, avocado and olives in limited amounts. **Please consume less than 50 g per day of each**, however, if you do not eat one you may have slightly more of another. 50 grams of avocado/berries/olives is around 1/3 cup.

Protein

It is important to eat enough protein to prevent the muscle breakdown, but equally important to not over eat protein. Our bodies use excess protein to produce carbohydrate (i.e. glucose) that can be used for energy; therefore, preventing adaptation to the ketogenic diet.

Below are some examples of high protein foods to include in your day.

Raw	Protein per 100g	Protein per serve
Beef (with fat)	21.4g	38.3g / small steak
Lamb (with fat)	20.4g	16.3g / small chop
Pork (with fat	21.5g	27.1g / small chop
Chicken (with skin)	20.1g	36g / small breast
Salmon	21.3g	25.2g / small fillet
Liver	20g	---
Kidney	18.2g	---
Eggs	12.6g	5.5g / regular egg

Aim to spread protein rich foods throughout the day rather than having one meal that is very high in protein.

Protein supplements:
Protein powders, bars and drinks can include large amounts of carbohydrate. If you are currently using a protein supplement, please check with Dave that it is suitable.

Fat

Fat will make up the majority of daily energy intake. It is important to consume enough fat to meet energy needs and prevent weight loss.

Fats and oils:

	Fat per 100g	Fat per serve
Butter	70.1g	13.3g / Tbs
Lard	100g	17g / Tbs
Cream, fluid	35.9g	6g / Tbs
Olive oil	100g	18.4g / Tbs
Coconut oil	99.9g	18.4g / Tbs
Coconut cream	18.9g	3.9g / Tbs

High fat foods	Fat per 100g	Fat per serve
Salmon	13.9g	16.4g / small fillet
Olives, green	20.5g	5.4g / ¼ cup
Avocado	21.6g	17.2g / ½ avocado
Almonds	55.1g	16.9g / ¼ cup
Seeds, mix	49.1g	18.7g / ¼ cup
Brie (cheese)	32g	1.6g / small slice
Peanut butter	54.3g	13.6g / Tbs

Fat boosting tips

- Drizzle olive oil over your meals and salads
- Eat the fat / skin / rind of meats
- Add cream to your hot drinks

Please avoid

- Margarine
- Vegetable oils

Fluids and electrolytes

Electrolytes are lost during the initial weeks of adapting to a ketogenic diet. **You will need to increase your salt intake by 1-2g/day to prevent this.**

Here are some suggestions to boost your salt intake

- Season food with salt and add more at the table.
- Consume a (low carbohydrate) salty soup or broth.
- Purchase salted nuts instead of unsalted.
- If you cook meat, don't discard the fluid, use it in other dishes, such soups.

What can you drink?

- Water
- Tea
- Coffee
- Salty soups
- Diet / zero sugar soft drink
- Vegetable juice

Tips on reading food labels

It is important to understand how to read foods labels as many processed foods contain carbohydrate and hidden sugars. You will find information about fat, protein and carbohydrate in the nutrition information section of a packaged food (shown below).

It is important to note that not all foods will have this information panel. If you are unsure about how much carbohydrate it contains, ask Dave.

NUTRITION INFORMATION		
Servings per can: 2		
Serving size: 210g		
	Average Quantity Per serving	Average Quantity Per 100g
ENERGY	895kJ	425kJ
PROTEIN	10.8g	5.1g
FAT: TOTAL	1.2g	0.6g
-SATURATED	0.2g	0.1g
CARBOHYDRATE	33.7g	16.1g
-SUGARS	15.5g	7.4g
DIETARY FIBRE	11.9g	5.7g
SODIUM	1300mg	620mg
POTASSIUM	650mg	310mg
IRON	2.7mg	1.3mg

Make sure the total carbohydrate is less than 2 grams per 100g or 100ml.

The amount carbohydrate per serving can be misleading as many manufacturers manipulate the size of this serving to make it appear low in sugar or carbohydrate. Your portion size is likely to be larger than the specified serving. It is more informative to use the per 100g/ml column for accurate information.

7-day example meal plan

Below is an example of a weekly plan to provide ideas for meals and snacks whilst following a ketogenic diet. Please note that **this is not a personalised plan** specific to your energy requirements.

	Mon	Tue	Wed	Thurs	Fri	Sat	Sun
B/fast	Vegetable omelette including mushrooms, spinach and courgette	LCHF muesli, unsweetened Greek yoghurt, unsweetened almond milk	Bacon, avocado, tomato, spinach	Cauliflower rosti with egg / bacon and hollandaise sauce	Berry and coconut parfait	Low CHO bread toasted with salmon and avocado	Cream cheese pancakes
Snack	Veggie sticks and guacamole	Peanut butter cookies	Nuts and brie cheese	Kale chips	Low carbohydrate muffin	Boiled eggs	Pork rind
Lunch	Turkey and brie sandwich with low CHO bread	Caesar salad with chicken and olive oil	Bunless burger	Eggplant pizza	Veggie and tofu stir-fry with peanut sauce	Frittata with veggies, avocado and olive oil	Creamy mushroom and bacon soup
Snack	Keto crackers and cheese	Creamy berry protein shake	Nuts and cherry tomatoes	Jerky	Tin of tuna	Pepperoni slices	Salty soup
Dinner	Pizza with cauliflower base	Courgette noodles with chicken and pesto	Chicken curry with cauliflower rice	Salmon with salad	Beef and cauliflower fried rice	Meatballs with tomato sauce and salad	Lettuce wrap enchiladas
Snack	Berries and whipped cream	Avocado pudding	Keto lava cake	Keto chocolate mouse	Fat bomb	Keto cookie	90% dark chocolate
Fluid	Water Salty soup Electrolyte drink	Water Salty soup Electrolyte drink	Water Salty soup Electrolyte drink	Water Salty soup Electrolyte drink	Water Salty soup Electrolyte drink	Water Salty soup Electrolyte drink	Water Salty soup Electrolyte drink

Dining out

Eating out is still possible on a ketogenic diet as most cafes and restaurants have low-carbohydrate options.

- View the menu before you go so you know what to ask for when you arrive.
- Avoid places where you may be tempted by high carbohydrate foods and drinks.
- Avoid fast food restaurants, in general, as they typically provide highly processed, high(er) carbohydrate options.
- Replace carbohydrate-based sides, such as chips and rice, with a green salad.
- Avoid milky coffees and instead, ask for milk or cream on the side with no added sugar.
- Avoid deep fried and battered options.
- Avoid gravy.
- Ask for the bread to be removed (e.g. eggs with salmon, avocado and mushrooms , rather than eggs on toast).
- Avoid sweet sauces and dressings or ask for the ingredient list (many sauces and dressings contain sugar, such as Thai curries and salad dressings).
- Avoid processed meat.

What options do you have?

- Salad with meat / chicken / fish with an oil based dressing or vinaigrette. These can be acquired from a variety of places, such as Pita Pit, Subway and Tank.
- Vegetable omelette.
- Steak / chicken breast or thighs / salmon or tuna fillet with salad.
- Bunless burger with premium mince or chicken breast and an unsweetened sauce.
- Add fat by asking for butter or olive oil for your meal or salad.

Consider carrying a small bottle of olive oil with you in case where you dine out doesn't have any in stock.

Snack Ideas

- Eggs (e.g. boiled)
- Cheese
- Cold cuts
- Avocado (limit to 50 g per day)
- Olives (limit to 50 g per day)
- Nuts and seeds (limit to 60 g per day)
- Celery
- Cucumber
- Capsicum
- Low carbohydrate dip / sauce (e.g. guacamole or salsa)
- Berries (limit to 50 g per day)
- Heavy whipping cream
- 90% dark chocolate (limit to 20 g per day)
- Pork rhind
- Beef jerky
- Kale chips

Low-carbohydrate alternatives for high-carbohydrate foods

Keto Bread (Diet Doctor webpage)
Ingredients

- 5 tbsp ground psyllium husk powder
- 1¼ cups almond flour (this is finer than almond meal)
- 2 tsp baking powder
- 1 tsp sea salt
- 1 cup water
- 2 tsp cider vinegar
- 3 egg whites
- 2 tbsp sesame seeds (optional)

Method

1. Preheat the oven to 350°F (175°C).
2. Mix the dry ingredients in a large bowl. Bring the water to a boil.
3. Add vinegar and egg whites to the dry ingredients, and combine well. Add boiling water, while beating with a hand mixer for about 30 seconds. Don't over mix the dough.
4. Moisten hands with a little olive oil and shape dough into 6 separate rolls. Place on a greased baking sheet. Top with optional sesame seeds.
5. Bake on lower rack in the oven for 50–60 minutes, depending on the size of your bread rolls. They're done when you hear a hollow sound when tapping the bottom of the bun.
6. Serve with butter and toppings of your choice.

Keto Crackers (Diet Doctor webpage)
Ingredients

- ⅓ cup almond flour
- ⅓ cup unsalted sunflower seeds
- ⅓ cup unsalted pumpkin seeds
- ⅓ cup flaxseed or chia seeds
- ⅓ cup sesame seeds
- 1 tbsp ground psyllium husk powder
- 1 tsp salt
- ¼ cup melted coconut oil
- 1 cup boiling water

Method

1. Preheat the oven to 300°F (150°C).
2. Mix all dry ingredients in a bowl. Add boiling water and oil. Mix together with a wooden fork.
3. Keep working the dough until it forms a ball and has a gel-like consistency.
4. Place the dough on a baking sheet lined with parchment paper. Add another paper on top and use a rolling pin to flatten the dough evenly.
5. Remove the upper paper and bake on the lower rack for about 40-45 minutes, check occasionally. Seeds are heat sensitive so pay close attention towards the end.
6. Turn off the oven and leave the crackers to dry in the oven. Once dried and cool, break into pieces and spread a generous amount of butter on top.

Cream cheese pancakes:
Ingredients: Makes 6

- 2 large eggs
- 1 tablespoon water
- 2 oz cream cheese, cubed
- 2/3 cup almond flour
- 1 teaspoon baking powder
- 2 teaspoons vanilla extract/ or the zest of one lemon
- 1/2 teaspoon cinnamon

Method

1. Add all ingredients to blender. Start with eggs and water and cream cheese so you don't have anything get stuck at bottom.
2. Blend until smooth, scraping down the sides if needed. Let batter sit for 2 minutes.
3. Heat a non-stick skillet to medium heat. For each pancake, pour 3 to 4 tablespoons of batter onto skillet.
4. Once you start to see little bubbles form, flip and continue to cook until pancake is browned on each side.
5. Serve pancakes topped with butter and a few berries

Chocolate Fat Bomb recipe (What The Fat book) (makes 20 x ~30 g serves)
Ingredients

- 1 cup (250 ml) Cream
- ⅕ cup (50 g) Butter
- ⅖ cup (100 g) Coconut oil
- ⅘ cup (200 g) 85% dark Chocolate, broken in pieces
- 1/3 cup (20 g) desiccated coconut
- Cocoa or coconut threads for rolling

Method

1. In a pot add the cream, butter and coconut oil and slowly heat until it begins to bubble.
2. Pour the hot mixture over the chocolate pieces and whisk vigorously to incorporate the ingredients are nicely mixed together.
3. Add to the mixture the desiccated coconut, mix well and set in a container or in individual moulds like an ice cube tray.
4. Chill the mix completely in the fridge
5. Once chilled cut into bite size pieces or roll into bite-sized balls.
6. At this point you can roll them in some coconut threads or cocoa – or simply transfer them as they are into a container with a tight fitting lid and storing them in the fridge for up to 2 weeks.

For more recipes, simply search Keto or Ketogenic options. If you are ever unsure if the recipe is truly low carb, send it to one of us to check.
Some good websites are:
https://ketosummit.com/ketogenic-diet-recipes/ and https://www.dietdoctor.com/low-carb/keto/recipes

Appendix D: Caffeine handout

During this study, please limit your caffeine intake to **100mg 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 (30mls)	45-90
Instant coffee (1 cup)	60-80
Cappuccino or Latte (250mls)	45-75
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 icecream (1/2 cup)	2

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

Appendix E: Example meal plan during EW (CHO diet)

Total kJ per day: 9900kJ
kJ per meal: ~2500 (590kcal)
20% protein, 50% carbohydrate, 30% fat

Meal	**Food**
Breakfast	120g muesli 200g yogurt 70g fruit
Lunch	150g noodles/potato/rice 1 cup salad 120g main meat meal 40g cake/biscuit
Dinner	150g noodles/potato/rice 1 cup of salad 120g main meat meal 1 biscuit/cake
Snack	4 slices freyas bread Lettuce 10g chutney 6 slices of champagne ham (~70g) 1 Tim Tam
Breakfast	120g Muesli 200g yogurt 70g fruit
Lunch	150g noodles/potato/rice 120g main meat meal 1 cup salad 1 Tim Tam

Appendix F: Participant study calendar

Participant ID

Period	Date	Weight	Actigraphy and sleep diary	HRV	Capillary blood	Sleepiness, hunger and mood	Cognitive tests	Diet record
Baseline	14th Jan							
	15th Jan							
	16th Jan							
	17th Jan							
	18th Jan							
	19th Jan							
	20th Jan							
Week 1 Ketogenic diet adaptation	21st Jan							
	22nd Jan							
	23rd Jan							
	24th Jan							
	25th Jan							
	26th Jan							
	27th Jan							
Week 2 Ketogenic diet adaptation	28th Jan							
	29th Jan							
	30th Jan							
	31st Jan							
	1st Feb							
	2nd Feb							
	3rd Feb							
Extended Wakefulness	4th Feb							
	5th Feb							
Washout	6th Feb							
	7th Feb							
	8th Feb							
	9th Feb							
	10th Feb							
	11th Feb							
	12th Feb							
	13th Feb							
	14th Feb							
	15th Feb							
	16th Feb							
	17th Feb							

Baseline	18th Feb							
	19th Feb							
	20th Feb							
	21st Feb							
	2nd Feb							
	23rd Feb							
	24th Feb							
Week 1 High carbohydrate adaptation	25th Feb							
	26th Feb							
	27th Feb							
	28th Feb							
	1st March							
	2nd March							
	3rd March							
Week 2 High carbohydrate adaptation	4th March							
	5th march							
	6th March							
	7th March							
	8th March							
	9th March							
	10th March							
Extended Wakefulness	11th March							
	12th March							

Appendix G: Simplified R script for data analyses

1. Set working directory

2. Load packages

```
# for example;
library(here) # read in data
library(tidyverse) # data manipulation
library(janitor) # cleaning data
library(lme4) # linear mixed effects models
library(car) # anovas
library(emmeans) # marginal effects
```

3. Read in data

4. Check dataframe

```
# for example;
str(dataframe)
head(dataframe)
tail(dataframe)

# clean names and specify factors
data <- dataframe %>%
  clean_names() %>%
  mutate(
    diet = as_factor(diet),
    period = as_factor(period),
    test = as_factor(test),
    order = as_factor(order),
    id = as_factor(id))
```

5. Create linear mixed model

```
# Including fixed effects and accounting for diet order and including a random intercept for participa
nt id
mod1 <- lmer(dv ~ diet*period*test + order + (1|id), data, REML = TRUE)
```

6. Visually inspect distribution of the model's residuals with a Q-Q plot

```
qqnorm(resid(mod1))
qqline(resid(mod1))
```

7. If normality or heteroscedacity is violated, consider transforming the data or using a generalised linear mixed model

```
# for example;
mod2 <- lmer(sqrt(dv) ~ diet*period*test + order + (1|id),
             data, REML = TRUE) # square-root transformed

mod3 <- lmer(log(dv) ~ diet*period*test + order + (1|id),
             data, REML = TRUE) # log transformed

mod4 <- glmer(dv ~ diet*period*test + order + (1|id),
              data,
              family = Gamma(link = "log")) # generalised lmm using gamma family with log link
```

```
mod5 <- glmer(dv ~ diet*period*test + order + (1|id),
              data,
              family = Gamma(link = "inverse")) # generalised lmm using gamma family with inverse lin
k
```

8. Assess model fit

```
anova(mod1, mod2, mod3, mod4, mod5)
```

9. Visually inspect distribution of new model's residuals with a Q-Q plot

```
qqnorm(resid(mod2))
qqline(resid(mod2))
```

10. Determine p-values for model using Type-II Wald F tests and Kenward Rogers degrees of freedom

```
Anova(mod2, type = "II", KR = TRUE, test.statistic = "F")
```

11. Post-hoc analyses for significant interactions or main effects

```
# for example, a diet x period interaction;
emms <- emmeans(mod2, ~ diet*period) # create emm_grid for comparisons
pairs(emms, by = "diet", reverse = TRUE, adjust = "holm") # pairwise comparisons for period
within diets with Holm adjusted p-values
pairs(emms, by = "period", reverse = TRUE, adjust = "holm") # pairwise comparisons for peri
od within diets with Holm adjusted p-values

# effect sizes for within and between diet comparisons
vc <- VarCorr(mod2) # determine random effects and residual error for model
totSD <- sqrt(vc)
eff_size(emms, sigma = totSD, edf = df.residual(mod2))
```