SLEEP DEPRIVATION AND ULTRA-ENDURANCE PERFORMANCE: ASSESSMENT AND COUNTERMEASURES

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"El explorador de la Naturaleza debe considerar la investigación como un sport incomparable, en el cual todo, desde los procederes técnicos hasta la elaboración doctrinal, constituye un perenne manantial de gratas satisfacciones. Quien en presencia de un arduo problema no sienta crecer su entusiasmo, a medida que el entendimiento redobla sus esfuerzos; quien, al aproximarse el solemne momento del fiat lux, impacientemente esperado, no tenga el alma inundada por la emoción precursora del placer, debe abandonar las empresas científicas."

Santiago Ramón y Cajal (de su discurso durante la ceremonia de admisión en la Real Academia de Ciencias Exactas y Naturales, Madrid, el 5 de diciembre de 1897)

"The explorer of nature must view research as the best of all possible sports, whose every facet—from the technical procedures to the elaboration of theories—is a never-ending source of indescribable satisfaction. You should abandon science if you don't feel growing enthusiasm and an increasing sense of power when working with a difficult problem—if your soul isn't flooded with the emotion of anticipated pleasure— when approaching the long-awaited and solemn moment of the fiat lux."

Santiago Ramón y Cajal (from his speech at the ceremony of admission to the Spanish Royal Academy of Sciences, Madrid, the 5th of December 1897).

Abstract

Considered a psychobiological state similar to mental fatigue, sleep deprivation can be defined as a period of extended wakefulness for, at least, 24 hours. Ultra-endurance events are often described as exercise bouts lasting more than six hours, or running distances covered longer than a traditional marathon.

The first aim of this thesis was to explore the sleep and performance of athletes in ultra-marathons longer than 161 km (100 mi). A moderate association between sleep duration and race time was found. Before the first sleep episode, athletes remained awake for more than 24 h. Ratings of perceived exertion (RPE), sleep bouts, sleep time, and sleepiness increased over the course of the race, whereas running speed decreased. As a consequence of the psychophysiological demands of ultra-endurance exercise and the exacerbated sleep loss, it has been suggested that perception of effort plays a key role in pace regulation during ultra-marathon events.

The second aim was to investigate the use of caffeine as a countermeasure for the detrimental effects of sleep deprivation on endurance performance. After one night of sleep deprivation, 6 $mg \cdot kg^{-1}$ of caffeine improved 30-min running time trial performance by 5.54%. Caffeine reduces perception of effort, allowing participants to sustain a higher running speed at a lower RPE. Therefore, we propose that caffeine can be used to counteract the negative effects associated with sleep deprivation on endurance performance.

The third aim was to study the effects training for a mountain ultra-marathon on sleep deprivation tolerance. Three bouts of exercise after one night of sleep deprivation over a 14-week period did not improve tolerance to the negative effects of sleep deprivation on endurance performance. One night of sleep deprivation reduces time to exhaustion by 28% when running at 75% of peak treadmill speed. Better sleep deprivation tolerance was associated with better chances to finish a ~340-km mountain ultra-marathon race.

The main findings presented in this doctoral research thesis are: 1) a moderate positive association between sleep-related parameters and race time, suggesting that faster runners sleep less and spend less time in activities (i.e., restoring food/drinks at the checkpoints, resting, sleeping) other than moving towards the finish line. 2) The use of caffeine as a measure to counteract the negative effects of sleep deprivation on endurance performance. 3) Three exercise bouts in a sleep deprived state over a 14-week period did not reduce the negative effects of sleep deprivation. These findings might be particularly useful for athletes and/or coaches in the field of ultra-marathon and ultra-endurance performance. Sleep assessment during a mountain-ultra marathon and the countermeasures provided might help to plan their races accordingly.

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List of Abbreviations

AAT	Alpha attenuation test
ACC	Anterior cingulate cortex
AFz	EEG electrode labelling between the prefrontal and frontal zero
Ag/AgCl	Silver/silver chloride
AM	Ante meridiem
ANOVA	Analysis of variance
AU	Arbitrary units
BET	Brain endurance training
BF	Breathing frequency
$B[La]^-$	Blood lactate concentration
BRUMS	Brunel mood scale
CAFF	Caffeine trial
CI	Confidence Interval
CO_2	Carbon dioxide
CWL	Constant workload
EEG	Electroencephalography
FP2	EEG electrode labelling prefrontal right side
FS	Rating of feelings
Fz	EEG electrode labelling frontal zero
gmbH	From German, *Gesellschaft mit beschränkter Haftung*, limited liability company.
GMT	Greenwich mean time
GPS	Global positioning system
HR	Heart rate
IQR	Interquartile range

ISMS	Intrinsic and success motivation scales
KSS	Karolinska sleepiness scale
LED	Light-emitting diode
LTd	Private company limited by shares
MAP	Maximal aerobic power
MDN	Median
MEQ	Morningness-eveningness questionnaire
N	Number
NASA	National aeronautics and space administration
NBA	National basketball association
NREM	Non-rapid eye movement
NS	Normal sleep
O_2	Oxygen
PAR-Q	Physical activity readiness questionnaire
$PETCO_2$	End-tidal carbon dioxide partial pressure
$PETO_2$	End-tidal oxygen partial pressure
PLA	Placebo trial
PM	Post meridiem
POMS	Profile of mood states
PTS	Peak treadmill speed
PSQI	Pittsburgh sleep quality index
PVT	Psychomotor vigilance test
RER	Respiratory exchange ratio
RH	Relative humidity
RPE	Rating of perceived exertion
RT	Reaction time
S.C. a r.l.	From Italian *società cooperativa a responsabilità limitata*, limited liability cooperative entity

S.C.p.a	From Italian *società cooperativa per azioni*, public limited cooperative entity
SD	Standard deviation, or sleep deprivation when specified
SWA	Slow wave activity
SPSS	Statistical package of social sciences
S.r.l.	From Italian *società a responsabilità limitata*, limited liability company
SSP	Signal-space projection
SPM	Statistical parametric mapping
SVD	Singular value decomposition
TLX	Task load index
TT	Time trial
TTE	Time to exhaustion
UK	United Kingdom
USA	United States of America
UTC	Coordinated universal time
VAS	Visual analogue scale
$\dot{V}CO_2$	Rate of Carbon dioxide production
$\dot{V}E$	Minute ventilation
$\dot{V}E/\dot{V}O_2$	Ventilatory equivalents for oxygen
$\dot{V}E/\dot{V}CO_2$	Ventilatory equivalents for carbon dioxide
$\dot{V}O_2$	Rate of oxygen uptake
$\dot{V}O_{2\ max}$	Maximum oxygen uptake
$\dot{V}O_{2\ peak}$	Peak oxygen uptake
VT	Ventilatory threshold or tidal volume when appropriate
VT1	First ventilatory threshold
VT2	Second ventilatory threshold
yr	Years
α	Alpha

Δ or δ Delta

Units of Measure

bpm	Beats per minute
cm	Centimetres
hPa	Hectopascals
h	Hours
HH:MM:SS	Hours, minutes, and seconds
Hz	Hertzs
g	Grams
km	Kilometres
kg	Kilograms
$km \cdot h^{-1}$	Kilometres per hour
µL	Microlitre
mg	Milligrams
$mg \cdot kg^{-1}$	Milligrams per kilogram
mL	Millilitres
$mL \cdot kg^{-1}$	Millilitres per kilogram
$mL \cdot kg^{-1} \cdot min^{-1}$	Millilitres per kilogram per minute
min	Minutes
mmol·L	Millimoles per litre
ms	Milliseconds
s	Seconds
yr	Years
°C	Degree Celsius

Statement of Original Authorship

No part of this thesis has been submitted in support of an application for any degree or other qualification of the University of Kent, or any other University or Institution of learning.

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Preface

This thesis includes work that has been written in a manuscript format, aiming to be submitted for publication. Therefore, there may be some repetition in the report of theories and past research in each introduction, and the description of the same methods that have been used in various chapters, which have been adjusted from the prepared article.

Oral presentations:

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Martinez-Gonzalez. B., and Marcora, S.M. (2020). The effects of caffeine on running performance after one night of total sleep deprivation. 5th International Congress on Soldiers' Physical Performance. Quebec City, Canada.

Ethical Approval Reference Numbers

Ethical approval was granted by the University of Kent, School of Sport and Exercises Sciences Research Ethics and Advisory Group for chapters 3, 4, and 5. In favour of the structure of the thesis and its readability, Chapter 5, which was already included in the approval of Chapters 3 and 4, has been considered independently rather than a part of these chapters.

Table i - *Ethical Approval Reference Numbers*

	Original Ethics Reference Number	Amendment Ethics Reference Number(s)
Chapter 3	Prop 136_2016_17	Prop 32_2018_19 Prop 53_2018_19
Chapter 4	Prop 129_2016_17	Prop 41_2017_18 Prop 89_2017_18 Prop 81_2018_19 Prop 82_2018_19 Prop 84_2018_19
Chapter 5	Prop 74_2018_19	

Chapter 1: General Introduction.

1.1 Background

Despite the reason why humans sleep is still being debated in the scientific community, sleep is recognised as a biological process involved in the recovery and maintenance of several physiological and cognitive functions (Cirelli and Tononi, 2008; Mignot, 2008; Roth, Rattenborg and Pravosudov, 2010). Every 24 h, 7-9 h is the recommended sleep duration for adults according to the National Sleep Foundation (Hirshkowitz *et al.*, 2015). Recently, it has been observed an exponential growth in the number of peer-reviewed publications in relation to sleep and athletes, aiming to better understand how to optimise sleep to enhance health and performance. Furthermore, in an expert consensus statement published lately (Walsh *et al.*, 2020), authors have emphasised the importance of sleep in athletes, particularly in those at the highest level, exposed to insufficient sleep (< 7 hours of sleep per night) and sub-optimal sleep quality (e.g., sleep fragmentation) due to several factors associated with elite sports, such as anxiety, competitions, nutritional supplements, stress, training and travelling amongst others.

A prolonged period of at least 24 h of wakefulness can be defined as sleep deprivation and considered a psychobiological state similar to mental fatigue, mainly manifested as sleepiness (Wesensten, 2012). Sleep loss has a detrimental effect on cell growth and repair (Czeisler and Klerman, 1999), cognition (Belenky *et al.*, 2003), glucose metabolism (Spiegel, Leproult and Van Cauter, 1999), immune response (Spiegel, Sheridan and Van Cauter, 2002), learning and memory (Walker and Stickgold, 2005), mental well-being (Haack and Mullington, 2005), and resistance to respiratory infections (Cohen *et al.*, 2009). Amongst all the forms of sleep loss, sleep deprivation is considered as one of the main determinants of mental fatigue (Owens, 2007). Frequently, mental fatigue and sleep deprivation definitions are reciprocally used. However, it is important

to emphasise that they are two different phenomena. Alterations in mood, tiredness, and performance are induced by both psychobiological states, mental fatigue is commonly linked to demanding cognitive activity, whereas sleep deprivation is connected to lack of sleep (Ackerman, 2010).

Numerous research has explored sleep deprivation and cognitive performance. Several constraints have been found in the context of endurance performance and sleep deprivation (Fullagar *et al.*, 2015).

Ultra-endurance performance can be defined as a bout of exercise that exceeds six hours in duration (Zaryski and Smith, 2005), or a running distance longer than the traditional marathon (42.195 km). Furthermore, ultra-marathons are an extraordinary scenario to assess the acute consequences and responses of extreme loads and stress (Millet and Millet, 2012). Sleep has a meaningful impact in ultra-endurance events where athletes minimise their sleep time in order to decrease their finishing time, resulting in a worse performance (Poussel *et al.*, 2015). Despite the limited role of sleep deprivation on the development of central fatigue, during ultra-endurance exercise, ratings of perceived exertion are increased (Millet, Martin and Temesi, 2018). In ultra-endurance events between 36 and 60 h, sleep duration has been positively correlated to finish time (Martin *et al.*, 2018) However, sleep in longer events has not been fully investigated yet.

In this thesis, sleep and ultra-endurance performance have been both investigated in the context of fatigue.

1.2 General aims and outline of the thesis

In recent years, a growing research interest in sleep and sports has been observed in the literature. Mainly, due to its importance for ensuring an optimal performance, and to investigate sport-related factors (i.e., caffeine intake, jetlag, physiological and psychological stress, training / competition times) that could play a part in potential negative effects associated with poor sleep. Furthermore, participation in ultra-endurance events has increased considerably. As a result of the duration of these races (e.g., distances longer than 42.195 km, up to several hundreds of kilometres), sleep management may be

a key factor of ultra-endurance performance, not only as part of the race strategy, but also because of the negative effects of sleep deprivation. However, little is known about sleep and ultra-endurance performance, particularly in events longer than 161 km. Potential countermeasures to counteract the negative effects of sleep deprivation on ultra-endurance performance have not been fully explored. Therefore, the main purpose of this thesis was to investigate the sleep of athletes competing at a ~431 km mountain ultra-marathon, and to explore interventions that could potentially reduce the negative effects of sleep deprivation on ultra-endurance performance.

This thesis is divided into six chapters: a literature review on sleep and endurance performance (Chapter 2), exploring the sleep of endurance athletes, the effects of sleep deprivation, primarily in endurance performance, but also in cognitive performance, and finally potential countermeasures to reduce the negative effects of sleep deprivation. As part of this doctoral research program, three experiments were conducted: a field observational study conducted over several editions of a race (Chapter 3). The aim of that study was to investigate sleep parameters of runners during a ~430 km mountain ultra-marathon (Aim 1). A randomised double-blind placebo-controlled crossover study (Chapter 4), aiming to explore the effects of caffeine supplementation on running endurance performance after one night of total sleep deprivation (Aim 2). Using an innovative testing protocol to simulate an ultra-endurance event (Chapter 5), aiming to describe the negative effects of sleep deprivation on endurance performance (Aim 3). Additionally, as part of the preparation for a mountain ultra-marathon, casual exercise bouts in a sleep deprived state were conducted with the purpose of investigating a potential increase in sleep deprivation tolerance, and therefore, improving endurance performance (Aim 4). To conclude this thesis, a general discussion of the findings is presented in Chapter 6.

The studies conducted during this doctoral research program should contribute adding new knowledge on the topic. Additionally, findings may help athletes to better understand sleep management during an ultra-marathon, and potential measures to counteract the negative effects of sleep deprivation on ultra-endurance performance.

1.3 Thesis format

This thesis contains a review of the literature and three experimental studies. Written in a conventional paper format in individual chapters, the above-mentioned experiments are treated separately. All the manuscript is written in the formatting adopted by the School of Sports and Exercise Science at the University of Kent. All the references cited are included at the end of the thesis in a single list. Figures and tables are numbered chronologically, but separately within each chapter.

Chapter 2: Sleep Loss and Endurance Performance.

2.1 Introduction

Despite the role of sleep in humans is still being debated in the scientific community, sleep is considered to play a key role in many physiological and psychological functions (Fullagar *et al.*, 2015). Lack of sleep is considered as one of the state variables that can influence mental fatigue. Sleep deprivation can be defined as a psychobiological state caused by a prolonged period of wakefulness (Boonstra *et al.*, 2007; Ackerman, 2010). However, athletes' sleep may not be as optimal as desired, particularly in elite athletes. High sport-related physiological and psychological demands (i.e., anxiety, jetlag, muscle soreness, training load) may contribute to impair athletes' sleep (Halson *et al.*, 2022). In recent years, an increase in the number of publications about sleep and athletic performance have been observed. Considering that it remains unclear whether the detrimental effects of sleep deprivation on performance are applicable to the athletic population that very rarely stay one night without sleep (Walsh *et al.*, 2020), it is also true that sleep deprivation is highly relevant for certain groups, such as soldiers in sustained operations (McLellan, Caldwell and Lieberman, 2016), or athletes participating in non-stop competitions lasting several days (Martin *et al.*, 2018). To the best of our knowledge, sleep loss in ultra-endurance performance has not been widely investigated, especially in distances longer than 161 km.

The aim of the present review is to summarise the current state of the literature about sleep in endurance athletes, underlying its importance on endurance performance. The first part of this chapter covers the definition of sleep loss and the effects of sleep deprivation on endurance performance primarily, as it is the main interest of this thesis, and also shortly on cognitive performance. A brief overview of the role of sleep in endurance athletes, including ultra-endurance events, sleep variables of interest, and current tools available for sleep assessment in athletes. In the last part, potential

countermeasures to reduce the negative impact of sleep loss on endurance performance have been reported. Due to the main area of interest of this doctoral research program, sleep deprivation has been the main focus of research, other types of sleep loss have not been fully explored in this chapter.

2.2 The sleep-wake cycle in humans

In order to explain the time course of sleepiness and wakefulness, the two-process model of sleep regulation was postulated (Borbély, 1982; Daan, Beersma and Borbély, 1984). A scheme of the model can be found in Figure 2.1. Sleep is regulated by the interaction between a process controlled by the circadian pacemaker (Process C) and a homeostatic process involving sleep and wake (Process S). Process C can be entrained by an external zeitgeber, such as the light-dark cycle, modulating sleep in an approximately 24-hour period (Putilov, 1995). Core body temperature and melatonin circadian rhythms are markers of C. Sleep pressure regulates Process S. During wakefulness, for approximately 16 hours, sleep pressure increases until the upper bound is reached, then sleep is triggered. During sleep, sleep pressure decreases until the lower bound is reached, then awakening is triggered. EEG activity, non-rapid eye movement (NREM), and slow wave activity (SWA) are markers of S (Borbély, 1982). A progressive decline in alertness is caused by a rapid increase in sleep pressure, particularly during the night. In the following morning, an increase in the level of alertness is stimulated by the circadian pacemaker. Under standard conditions this happens two times per circadian cycle (Borbély *et al.*, 2016).

In humans, a sleep-wake cycle out of the range of entrainment of the circadian pacemaker (i.e., one full night of sleep deprivation) will result in sleep happening at different circadian phases. Therefore, a deterioration of alertness and cognitive performance is experienced by most people after one night of sleep deprivation (Putilov *et al.*, 2009).

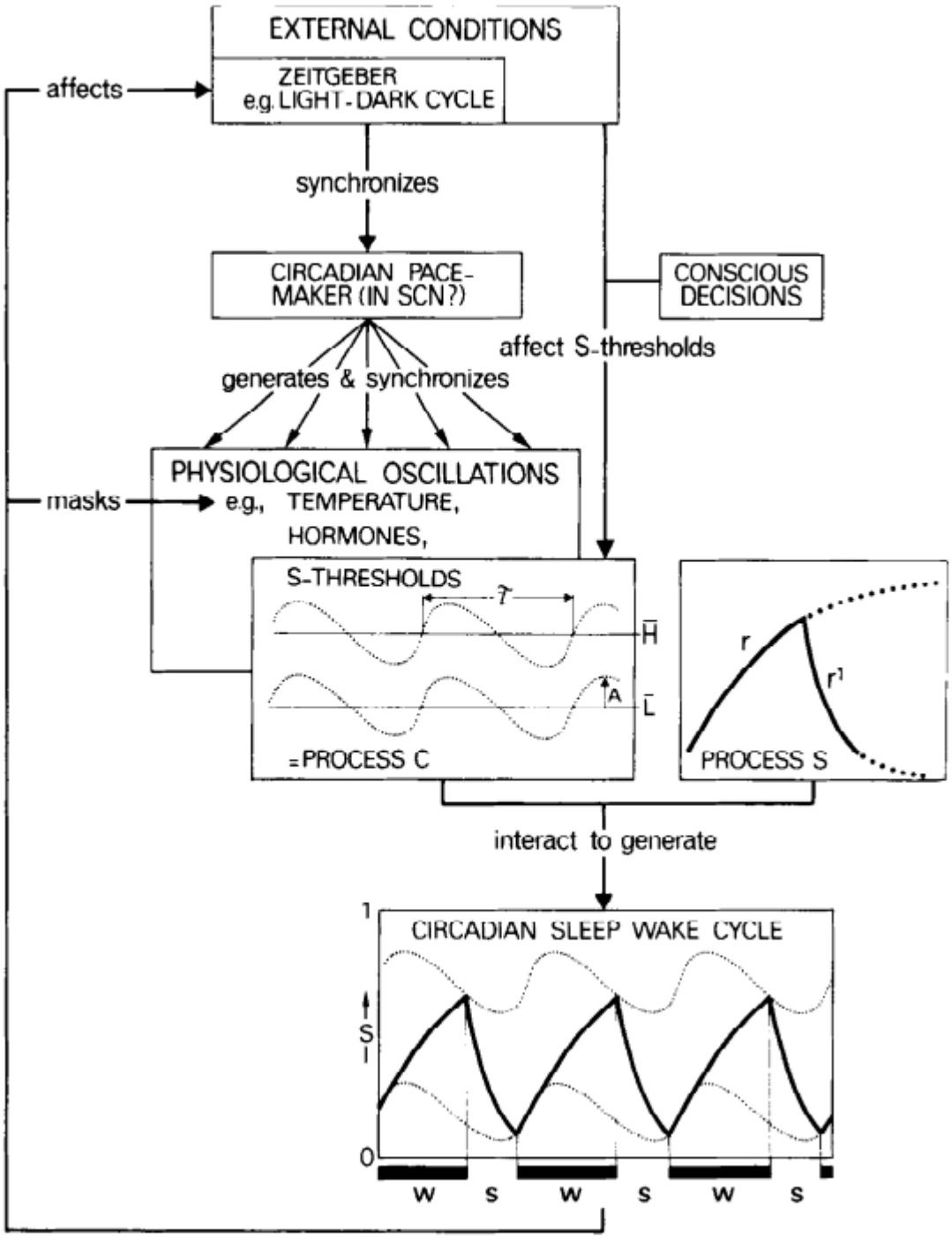


Figure 2.1. Scheme of the two-process model of sleep regulation. From Daan, Beersma and Borbély (1984).

Van Dongen and Dinges (2001) mentioned three parameters in particular related to the decline in alertness and performance due to sleep deprivation and sleep disruption: 1) the timing and/or rate of adjustment of the circadian process, (i.e., circadian phase), 2) the

amount of sleep needed per day (i.e., sleep need), and 3) the rate of impairment per hour of sleep loss (i.e., vulnerability to sleep deprivation).

In order to account for individual differences in sleep and wakefulness, (Van Dongen, Vitellaro and Dinges, 2005) further developed three factors: 1) chronotype (from Greek word *chronos* for time), phase difference in the timing of sleep and wakefulness (i.e., "morning type", "evening type"), 2) somnotype (from Latin word *somnos* for sleep), daytime and night-time patterns of sleep propensity, and 3) trototype (from Greek *trotos* for vulnerability), vulnerability to sleep loss.

Trototype can be quantified objectively via EEG: one of the effects of sleep deprivation on the regulation of the level of alertness/sleepiness is a rise of slow theta combined with a drop of fast alpha power density (Putilov *et al.*, 2009). Several genes have been investigated in relation to vulnerability to sleep deprivation: a *PER3* polymorphism has been suggested as a potential candidate to predict susceptibility to impairments in performance during sleep deprivation. However, it is still unclear the percentage of trototypic variability in performance responses to sleep deprivation explained by the *PER3* polymorphism (King, Belenky and Van Dongen, 2009). Susceptibility to performance impairment due to sleep deprivation may be affected by *ADORA2A* polymorphisms (King, Belenky and Van Dongen, 2009). Further research is required to identify potential markers of individual vulnerability to sleep loss.

2.3 Sleep loss

The term 'sleep loss' refers to a shortened period of sleep time. Three main types of sleep loss can be found in the literature: 'sleep deprivation' can be defined as a prolonged period of continuous wakefulness (i.e., more than 24 hours without sleep); 'sleep restriction' is considered a period of partial lack of sleep (i.e., less sleep time than usual, either as a consequence as a late bedtime, or an early wake up time); and 'sleep fragmentation', described as a period of wakefulness between sleep intervals (Jones and Harrison, 2001; Boonstra *et al.*, 2007; Fullagar *et al.*, 2015). From a clinical perspective, diverse pathologies are linked to sleep disorders directly: insomnia (Baglioni *et al.*, 2010);

and obstructive sleep apnoea (Lévy *et al.*, 2015). Or indirectly: chronic pain (Nijs *et al.*, 2018); depression (Murphy and Peterson, 2015); and restless-leg syndrome (Wetter *et al.*, 1998). Nevertheless, external factors can put individuals in a state of sleep deprivation, such as competing in a non-stop ultra-endurance event (Martin *et al.*, 2018); night work shifts (Kecklund and Axelsson, 2016); sustained military operations (McLellan, Bell and Kamimori, 2004; McLellan *et al.*, 2004, 2005, 2007; Kamimori *et al.*, 2015); and travelling across multiple time zones (Sack *et al.*, 2007). Despite being used as synonyms in the literature, 'sleepiness' and 'mental fatigue', from a conceptual and experimental perspective, both terms are differentiated: sleepiness refers to lack of sleep, and mental fatigue entails effort. However, both play a part in related performance decrements (Ackerman, 2010). Similar effects on sleepiness and cognitive performance have been reported in all of these types of sleep loss (Bonnet and Arand, 2003).

Sleep loss in athletes

Considering that the optimal sleep duration for adults has been established between seven and nine hours (Hirshkowitz *et al.*, 2015). In a recent consensus statement, (Walsh *et al.*, 2020) have revealed that elite athletes are particularly exposed to insufficient sleep (< 7 hours of sleep per night) and sub-optimal sleep quality (e.g., sleep fragmentation). In terms of sleep quantity, as an example, 75% of elite South African athletes stated that their average sleep was 6-8 h per night (Venter, 2012). A group of athletes from individual and team sports reported a total sleep time ranging from 6.1 to 7.5 hours (Lastella *et al.*, 2015), less sleep than the general target (8 hours of sleep per night). In a recent systematic review, athletes slept 7.2 hours per night (Vlahoyiannis *et al.*, 2021). Furthermore, elite athletes spent more time in bed than non-elite, but no differences were found in sleep time (Wilson and Baker, 2021), concluding that the increase in time in bed was not translated into more sleep time. Compared to rest days, elite swimmers slept 1.6 hours less than during training days (Sargent, Halson and Roach, 2014). Similarly, sleep times below 7 hours have been found during both training periods, and the night of competition (Roberts, Teo and Warmington, 2019).

Regarding sleep quality, prior to a competition, sleep has been described as poor by 64% of Australian athletes (Juliff, Halson and Peiffer, 2015); 83.3% of Brazilian paralympic athletes (Silva *et al.*, 2012); a group of British elite athletes from different sport disciplines (Leeder *et al.*, 2012); endurance sport athletes from Canada (Wilson and Baker, 2021); and 65.8% of German athletes (Erlacher *et al.*, 2011). Furthermore, systematic reviews about sleep quality and elite sports, longer sleep latencies and greater sleep fragmentation have been reported in elite athletes (Gupta, Morgan and Gilchrist, 2017; Roberts, Teo and Warmington, 2019; Vlahoyiannis *et al.*, 2021).

Several elements have been described as sleep disruptors (Figure 2.2): exposure to high altitude disrupted sleep of sea-level athletes, in comparison to high-altitude athletes (Sargent *et al.*, 2013); caffeine consumption increased sleep latency, therefore, sleep efficiency is reduced, and tend to be in relation to a reduction in sleep duration (Dunican *et al.*, 2018); after an elite football night match, sleep duration was reduced 157 min compared to a day match, and 181 min in comparison to a training day (Fullagar *et al.*, 2016b); sleep duration was impaired during long-haul international travel in elite footballers (Fullagar *et al.*, 2016a); late night social media activity of NBA players was associated with a reduction in next-day game performance: less points and rebounds (Jones *et al.*, 2019); bright light exposure in stadiums during a night game may affect subsequent sleep (Nédélec *et al.*, 2015); participation in ultra-endurance events is characterised by a reduction in sleep, compared to the 8 hours target in adults (Léger *et al.*, 2008; Hurdiel *et al.*, 2012, 2014, 2018; Poussel *et al.*, 2015); 65% of a cohort of 632 german athletes experienced poor sleep linked to pre-competition anxiety the night before a competition (Erlacher *et al.*, 2011). Similarly, in a group of 27 paralympic athletes that participated in Beijing 2008 Paralympic Games, the vast majority reported poor sleep quality the night before a competition (Silva *et al.*, 2012); 40 minutes of high-intensity intensity exercise between 21:20 and 22:00 delayed sleep by 14 mins (Oda and Shirakawa, 2014); training schedule dictates the sleep duration of elite athletes (Sargent, Halson and Roach, 2014); a high volume of training disrupted sleep, due to an increase in the number of movements during sleep in elite female swimmers (Taylor, Rogers and Driver, 1997); An overtraining state reduced the sleep efficiency of high school and university swimmers, in comparison to non-overreaching swimmers (Wall *et al.*, 2003).

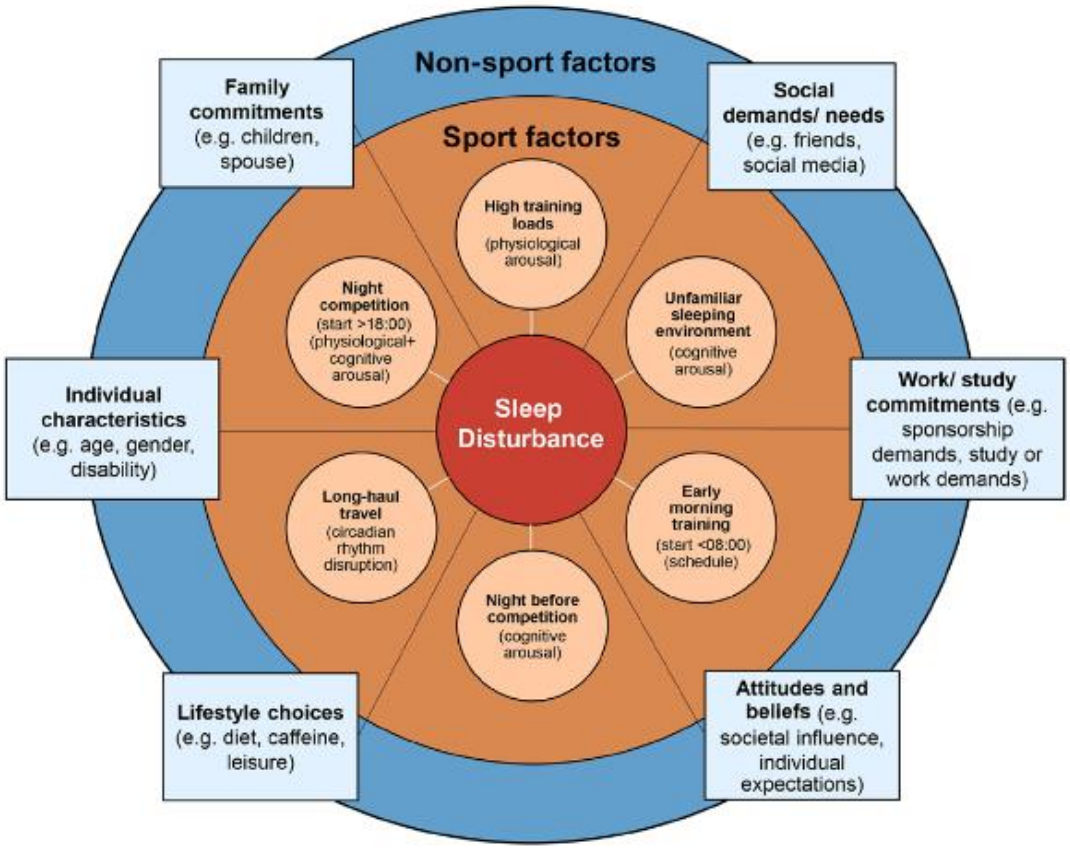


Figure 2.2 Elements that contribute to sleep disruption in athletes. (Orange) Sport-specific components. (Blue) Components not related to sport. From Walsh *et al.* (2020).

Despite the numerous studies found in the literature about the sub-optimal levels of sleep quantity and quality in elite athletes, only a few experiments have compared their results with a control non-athlete group. Additionally, various study designs and variables have been reported, further high-quality evidence is required.

2.4 Sleep assessment

Numerous sleep-related parameters can be monitored in order to assess sleep, the most commonly reported are perceived recovery; perceived sleep quality; sleep architecture (sleep stages); sleep duration (quantity); sleep efficiency (quality); sleep-onset latency (time to fall asleep); sleepiness; time in bed; and wake after sleep onset.

Instruments to measure sleep parameters can be divided into two main categories: objective and subjective.

Objective tools to measure of sleep

The gold standard method to monitor sleep is Polysomnography (PSG), used as a diagnostic tool in medicine. In this type of sleep study, conducted overnight, several subjects' signals are continuously monitored during sleep including brain activity (EEG), breathing rate, eye movement (EOG), heart electrical activity (ECG), muscle activation (EMG), and oxygen saturation. Using this method, the different sleep stages can be investigated (i.e., REM sleep, non-REM sleep). However, PSG is an overly complex, expensive, and obstructive technique. Traditionally, PSG is performed in a laboratory or hospital, mainly used to assess sleep disorders or in research. However, Portable PSG options have been developed recently. In the context of sport-related research it might be a potential tool to assess sleep architecture in athletes. Nevertheless, Portable PSG cannot replace traditional PSG (Hof Zum Berge *et al.*, 2020).

A non-invasive alternative to PSG is actigraphy. Using a 3-axis accelerometer, sleep/wake parameters can be determined based on recorded movement and the use of proprietary algorithms (Lee and Suen, 2017). Two types of devices can be found: research-grade equipment, such as wrist-worn actigraphy devices, validated against PSG (Kosmadopoulos *et al.*, 2014), and wearable activity monitors, a less expensive option. Nonetheless, actigraphy devices generally overestimate sleep duration and sleep efficiency, in comparison to PSG. Furthermore, measurements are influenced by the threshold applied in the proprietary software (Fuller *et al.*, 2017). Therefore, in order to obtain reliable data, the use of devices validated against PSG is strongly recommended. It has been also suggested to choose a threshold sensitivity based on previous research (Sargent *et al.*, 2016).

Another non-invasive, affordable alternative to PSG is a commercially available headband (The Dreem Headband, Dreem, Paris, France) that combines five dry EEG electrodes with a triaxial accelerometer and a pulse oximeter. Using a deep learning algorithm, sleep stages assessment can be performed, and physiological responses can be

recorded. However, further longitudinal sleep studies using this device are required (Arnal *et al.*, 2020). Wearable technology has increased exponentially over the last decade, sleep monitoring devices using EEG electrodes might be a feasible alternative to measure sleep during field studies. The sleep behaviour of an ultra-endurance cyclist during a 24-day simulated tour was measured using the mentioned headband (Nédélec, Chauvineau and Guilhem, 2022).

Subjective tools to measure sleep

A cost-effective, easy method to collect sleep data is via sleep logs. On a daily basis, individuals can report several sleep-related data, such as: bedtime; caffeine intake; daytime naps; perceived sleep quality; wake episodes during the night and wake time. Self-report sleep diaries have been widely used in several studies, in combination with actigraphy, as a method to cross-check data in athletes (Halson *et al.*, 2014; Sargent *et al.*, 2014, 2021; Lastella *et al.*, 2015a, 2015b; Caia *et al.*, 2017; Lastella *et al.*, 2020, 2022). Nevertheless, when using this type of questionnaire, recall bias, a systematic error that occurs when individuals cannot rigorously remember previous experiences or details are omitted, should be addressed (Raphael, 1987).

In sleep research, the use of questionnaires is very extended due to its low cost and simple administration. Various sleep-related questionnaires have been found in the literature. For instance, The Pittsburgh Sleep Quality Index Questionnaire (PSQI) has been used to assess sleep quality (Buysse *et al.*, 1989), and The Athlete Sleep Screening Questionnaire (ASSQ), developed to detect clinical sleep disturbances (Bender *et al.*, 2018). However, in addition to recall bias, the disposition of a person to answer a question not reflecting their true responses accurately, called response bias, must be taken into consideration when using data from questionnaires (Clausen and Ford, 1947). Additionally, questionnaires in this context may not have been validated in athletes.

2.5 Sleep deprivation assessment

As postulated in the two-process model of sleep regulation, sleep and wakefulness are part of a homeostatic mechanism that increases or decreases sleep propensity in response to insufficient or excess sleep, respectively (Achermann and Borbély, 2011). Mathematical models of sleepiness and performance have been developed based on this model (Van Dongen *et al.*, 2003). In the context of sleep deprivation, sleepiness has been discussed in several studies as an effect of sleep loss (Froeberg *et al.*, 1971; Jewett *et al.*, 1999; Van Dongen and Dinges, 2003). Tools to measure sleepiness can be organised in two main categories: objective and subjective.

Objective tools to measure sleepiness

The gold standard test to measure sleepiness is The Multiple Sleep Latency Test (MLST), designed by Richardson *et al.* (1978), consisting of an overnight PSG, followed by 4-5 naps of 20-30 minutes every 2 hours during the day, in a quiet darkened room. EEG and EOG measurements are recorded (Carskadon *et al.*, 1986). Sleep latency and sleep propensity are assessed during MLST (Thorpy, 1992). MLST has been shown as a sensitive test to several types of sleep loss (Janjua *et al.*, 2003; Punjabi, Bandeen-Roche and Young, 2003), highly reliable in both clinical and healthy populations (Zwyghuizen-Doorenbos *et al.*, 1988; Drake *et al.*, 2000).

In order to provide an objective measure of sleep propensity, and therefore, assess the ability to maintain wakefulness, the Maintenance of Wakefulness test was proposed by Mitler, Gujavarty and Browman (1982). This validated test is based on PSG and requires participants not to fall asleep for 20-40 minutes during 4-5 sessions.

To evaluate objective decreases in alertness due to sleep deprivation, The Alpha Attenuation Test (AAT) was created by Stampi, Stone and Michimori (1995). In a quiet and illuminated room, while remaining awake and relaxed, individuals are required to be seated down and to look at a fixation point on a computer (López Zunini *et al.*, 2013). ATT consists of alternating two minutes with eyes closed and two minutes of eyes opened for a total of twelve minutes, while EEG is recorded. Power alterations in the EEG alpha-

band frequency are expected due to sleepiness: a decrease when eyes are closed, and an increase when eyes are opened.

In order to assess decreases in performance related to sleepiness, The Psychomotor Vigilance Test (PVT), developed by Dinges and Powell (1985), has been proposed as the most sensitive cognitive test (Balkin *et al.*, 2004). However, other cognitive tests using different tasks have been used to detect sleepiness-related impairments in performance, such as the 10-Choice Reaction Time (Thorne *et al.*, 1985), the Serial Addition/Subtraction Task (Thorne *et al.*, 1985), and the Wilkinson 4-Choice Reaction Time (Wilkinson and Houghton, 1975).

Subjective tools to measure sleepiness

Rating scales are used to evaluate subjective sleepiness. The Epworth Sleepiness Scale (ESS), designed by Johns (1991), aims to assess daytime sleepiness, A question regarding the chances of falling asleep in eight different scenarios is presented to the participants. The Karolinska Sleepiness Scale (KSS), developed by Akerstedt and Gillberg (1990), is a 9-point Likert scale that has been validated against EEG (Kaida *et al.*, 2006). The Sleep-Wake Activity Inventory evaluates daytime sleepiness uses fifty-nine items about four different domains (Rosenthal, Roehrs and Roth, 1993), The Stanford Sleepiness Scale (SSS), created by Hoddes *et al.* (1973), consists of seven statements that denote degrees of sleepiness. Visual Analogue Scales (VAS) have been used to measure subjective feelings of sleepiness (Herbert, Johns and Doré, 1976).

Furthermore, as part of the assessment of mood, sleepiness can also be evaluated using The Brunel Mood Scale (BRUMS) designed by Terry, Lane and Fogarty (2003) or The Profile of Mood States (POMS) created by McNair, Lorr and Droppleman (1992).

Recall and response biases should be considered when collecting and analysing data using scales and/or questionnaires (Clausen and Ford, 1947; Raphael, 1987).

2.6. Sleep during ultra-endurance events

Due to the focus of this thesis on non-stop mountain ultra-marathon events, multi-day competitions, such as cycling events, mountain expeditions, *Marathon des Sables*, The Trans Europe Footrace Project (a 64-stage 4,486 km ultramarathon) or ultra-endurance triathlons, have not been considered in this section.

Several studies have investigated the sleep of participants in different ultra-endurance competitions. During 2013 edition of *Ultra-Trail du Mont Blanc* (a 168 km mountain ultra-marathon), using a self-reported electronic questionnaire, 72% of the participants in the study that completed the race did not sleep at all, finishing the race faster than the sleepers (Poussel *et al.*, 2015). In another study conducted in the same, seventeen participants reported 12 ± 17 minutes of sleep (measured via actigraphy) in race times ranging from 27 to 44 hours (Hurdiel *et al.*, 2015). In the 2015 edition, ninety-two runners reported 23 ± 22 minutes (self-reported via sleep diary) for a total race duration ranging from 29 to 46 hours (Hurdiel *et al.*, 2018). In the 2019 edition, nineteen participants that completed the race with a total time between 34 and 45 hours, reported a total sleep time (assessed using actigraphy) between 0 and 80 minutes (Baron *et al.*, 2022).

During the *Badwater Ultramarathon* (216 km) two participants finished in 46 and 54 hours, respectively. However, sleep times were not very well documented (Doppelmayr, Finkernagel and Doppelmayr, 2005).

In the 2018 edition of The Run Across Taiwan Ultra-Marathon (246 km), eight participants did report less than 30 minutes of sleep (via self-reported questionnaires) in 34 to 44 hours of competition (Huang *et al.*, 2021).

Four runners competing at The Irrational S.O.U.T.H (a 326 km ultra-marathon) reported 4.7 ± 3.0 hours of sleep during 82.5 ± 7.1 hours of competition (Bianchi, Miller and Lastella, 2022). Sleep was measured using a wrist-worn actigraphy device.

In *Tor des Géants* 2011 (a 330 km ultra-marathon) fifteen runners reported 9.1 ± 5.4 h of sleep in 122.4 ± 17.2 h of race time (Saugy *et al.*, 2013). In 2013, during the same event, one participant reported 11.7 hours of sleep for a total race duration of 125.6 hours

(Savoldelli *et al.*, 2017). During the same race, sixteen finishers reported 7.7 ± 2.9 h of sleep during 125 ± 7 of race time (Spring *et al.*, 2022). Unfortunately, the methods used to collect sleep data in all of these studies were not disclosed. During 2013 and 2014 editions, another study was conducted (Tonacci *et al.*, 2016), sleep was estimated by a wearable wristband (Fitbit) during the competition. 28 finishers reported 9.2 ± 3.4 hours of sleep in 86.7 ± 51.1 hours of competition.

A survey conducted by Martin *et al.* (2018) to ultra-marathon runners, an electronic questionnaire revealed one the main strategies to prepare for an ultramarathon was sleep extension (an increase in typical total sleep time) before the event, and micro naps during the race. However, only 21% of the participants disclosed a strategy to manage sleep during ultra-marathons. Sub-analyses have shown that sleep duration during an ultra-marathon was associated with faster finish time for races lasting between 36 and 60 hours.

2.7 Effects of sleep deprivation on endurance performance

Endurance performance can be defined as a whole-body exercise lasting more than 75 s and requiring continuous effort (McCormick, Meijen and Marcora, 2015). Limited research has been conducted investigating the effects of sleep deprivation on endurance performance, findings are still in dispute (Fullagar *et al.*, 2015).

Various reductions in endurance performance have been reported across different sleep deprivation durations: 20% when walking at speed eliciting 160 bpm after 30 h of sleep deprivation (Martin and Chen, 1984); 20% in a time to task failure running at 85% of V̇O2 max after 28 h of sleep deprivation (McLellan, Bell and Kamimori, 2004); 11% in a time to task failure test walking to exhaustion test at 80% of V̇O2 max after 36 h of sleep deprivation (Martin, 1981); 9% in an incremental time to task failure test cycling at 65% of MAP after one night of sleep deprivation (Temesi *et al.*, 2013); 8% in a 8-km time trial run after 26 h of sleep deprivation (Khcharem *et al.*, 2022); 5% in volleyball players that performed an incremental time to task failure cycling test after 25-30 h of

sleep deprivation (Azboy and Kaygisiz, 2009); 5% in an incremental time to task failure cycling test after 30 h of sleep deprivation (Chen, 1991); 4.4% on a 5-km running time trial after two nights of sleep deprivation (Oliver *et al.*, 2015); 2.9% in a 30-min running time trial after one night of sleep deprivation (Oliver *et al.*, 2009).

Nonetheless, no statistically significant performance impairments have been observed in several studies: in an incremental time to task failure cycling test after 60 h of sleep deprivation (Vaara *et al.*, 2018); in an incremental time to task failure cycling test after 60 h of sleep deprivation (Goodman *et al.*, 1989); in a shuttle run after 30 h of sleep deprivation (Racinais *et al.*, 2004); in a 6.3 km time trial run after 27 h of sleep deprivation (McLellan *et al.*, 2005); in runners that performed an incremental task to time failure cycling test after 25-30 h of sleep deprivation (Azboy and Kaygisiz, 2009); in a Yo-Yo test after 24 h of sleep deprivation (Donald *et al.*, 2017); in an incremental task to time failure cycling test after one night of sleep deprivation (Hill *et al.*, 1994). One potential partial explanation for these conflicting results could be the use of incremental protocols that may not elicit a significant decrease in performance in a sleep-deprived state, in comparison to normal sleep conditions.

In the light of the results shown, it has been provided ample evidence that endurance performance is impaired by sleep deprivation, particularly in exercise lasting more than 30 minutes, despite some opposite results. However, further research is needed to gain a better understanding of the effects of sleep deprivation in other endurance exercise protocols and sport disciplines. Given the rare occasions when athletes would be exposed to one or more nights of total sleep deprivation, the relevance of the results reported above to the athletic population remains questionable.

The rationale behind the negative effects of sleep deprivation on endurance performance is still unsettled. Regarding cardio-respiratory parameters, small changes have been described in heart rate, oxygen uptake, respiratory exchange ratio, and ventilation (Martin, 1981; Martin and Haney, 1982; Plyley *et al.*, 1987; Mougin *et al.*, 1991; Azboy and Kaygisiz, 2009; Oliver *et al.*, 2009). Limited effects of sleep deprivation on thermoregulation have been described (Sawka, Gonzalez and Pandolf, 1984; Oliver *et al.*, 2009). In time to task failure tests at fixed intensities and steady state exercise, an increase in ratings of perceived exertion have been related to a decrease in endurance

performance (Martin, 1981; Martin and Gaddis, 1981; Bond *et al.*, 1986; Symons, VanHelder and Myles, 1988; Temesi *et al.*, 2013).

Nevertheless, no differences were found in ratings of perceived exertion during a 30-min time trial run after 30 h of sleep deprivation, despite a significant decrease in performance (Oliver *et al.*, 2009). It can be argued that, if running speed would have been similar, an increase in perception of effort could have been observed. In addition to that, it has been suggested that an increase in central and peripheral fatigue is unlikely to explain decreases in endurance performance in a sleep deprived state. Nonetheless, opposing results have been shown: partial sleep deprivation did not impair intermittent aerobic exercise (Mejri *et al.*, 2014). Potential explanations for this lack of impairment could be the test used (Yo-Yo intermittent recovery test level 1); the nature of the exercise (intermittent); and the population tested (Taekwondo players). In order to further investigate the reasons why sleep deprivation impairs endurance performance after sleep deprivation, future studies are necessary.

2.8 Effects of sleep deprivation on cognitive performance

Sustained attention and vigilance seem to be the cognitive functions more impacted by sleep deprivation (Doran, Van Dongen and Dinges, 2001; Dorrian and Dinges, 2005; Lim and Dinges, 2008, 2010; Goel *et al.*, 2013). Reaction time, response accuracy, and increments in performance variability are testing variables related to sustained attention sensitive to sleep deprivation (Samkoff and Jacques, 1991; Dinges *et al.*, 1997; Jewett *et al.*, 1999; Belenky *et al.*, 2003; Durmer and Dinges, 2005). Other cognitive functions are also affected by sleep deprivation, such as decision making (Harrison and Horne, 2000), learning (Gosselin, De Koninck and Campbell, 2005), logical reasoning (Harrison and Horne, 2000), planning (Nilsson *et al.*, 2005), verbal fluency (Jones and Harrison, 2001), and working memory (Chee *et al.*, 2006).

Sleep deprivation has an effect on mood and emotions. Higher levels of confusion, depression, stress, subjective fatigue and subjective sleepiness (Dinges *et al.*, 1997).

Lower levels of vigour (Martin, Bender and Chen, 1986; Meney *et al.*, 1998). Sleep deprivation has been associated with hallucinations (Kahn-Greene *et al.*, 2007).

In the context of athletic performance, the effects of sleep deprivation on cognitive performance have been reported (Fullagar *et al.*, 2015). Despite ample evidence of the cognitive performance during exercise in a sleep deprived state (Angus, Heslegrave and Myles, 1985; Martin, Bender and Chen, 1986; Meney *et al.*, 1998; Scott, McNaughton and Polman, 2006), only a modest number of studies have reported the effects of sleep deprivation during sport events (Fullagar *et al.*, 2015). Sleep deprivation has a detrimental effect on cognitive performance during solo sailing races (Hurdiel *et al.*, 2014). However, in an adventure race lasting 96-125 h, no effect on decision making Stroop Task performance was observed after 100 h of sleep deprivation (Lucas *et al.*, 2009). The motivation to perform a certain task may be decreased due to lack of sleep, resulting in less effort invested, and therefore, disengaging from the task (Lim and Dinges, 2008; Massar *et al.*, 2019).

Individual variability has been commonly reported in the literature regarding the effects of sleep deprivation on cognitive performance, some individuals may be especially vulnerable to the negative effects of sleep deprivation, whereas others may be more resilient (Van Dongen *et al.*, 2004; Van Dongen, Maislin and Dinges, 2004; Lim, Choo and Chee, 2007; Rupp, Wesensten and Balkin, 2012). Future research may explore the rationale behind individual differences in cognitive performance.

2.9 Countermeasures to reduce the negative effect of sleep deprivation

Caffeine

Contained in many beverages and foods, caffeine is a wakefulness-promoting natural substance, mainly consumed as coffee, and one of the most widely used psychoactive substances in the world (Kushida, 2006). The role of caffeine as an antagonist of A_1 and A_{2a} adenosine receptors in the central nervous system is the most suggested mechanism of action (Graham, 2001; Doherty and Smith, 2005; Ganio *et al.*, 2009; Spriet, 2014; McLellan, Caldwell and Lieberman, 2016; Southward, Rutherfurd-

Markwick and Ali, 2018). Interactions with dopamine receptors have also been proposed (Cauli and Morelli, 2005; Rossi *et al.*, 2010). It has been suggested that adenosine receptors on GABA neurons, blocked by caffeine, may contribute to the inhibition of those hypothalamic neurons involved in sleep promotion (Strecker *et al.*, 2000).

The effects of caffeine have been widely investigated since the early 1990s (Rivers and Webber, 1907) in several laboratory and field studies. In a sleep deprived state, caffeine improves alertness and mood (Penetar *et al.*, 1993), enhances choice reaction time (Lorist, Snel and Kok, 1994), cognitive performance (Lagarde *et al.*, 2000), reduces subjective levels of sleepiness (Keister and McLaughlin, 1972), increases sustained attention (Dinges *et al.*, 2000; Rogers *et al.*, 2001), vigilance (Zwyghuizen-Doorenbos *et al.*, 1990), working memory (Dinges *et al.*, 2000). and wakefulness (Kamimori *et al.*, 2000). After one night of sleep deprivation, caffeine, in addition to naps, have been demonstrated to have greater positive results in cognitive performance (Bonnet and Arand, 1994a, 1994b, 1995), than caffeine alone (Dinges *et al.*, 2000; Rogers *et al.*, 2001).

In order to overcome the negative effects of one night of sleep deprivation, 100-150 mg of caffeine is an adequate dose (Lagarde *et al.*, 2000; Kamimori *et al.*, 2015). For longer periods of sleep deprivation, 200-600 mg are required (Lieberman *et al.*, 2002; Tharion, Shukitt-Hale and Lieberman, 2003). However, larger doses (300-600 mg) do not improve cognitive performance, when compared to lower doses (Lagarde *et al.*, 2000). As sleep deprivation continues over time, higher doses are needed to sustain the beneficial effects of caffeine on cognitive performance (Bonnet and Arand, 1995; Wesensten *et al.*, 2002).

The use of caffeine as an ergogenic aid in sports is extended (Del Coso, Muñoz and Muñoz-Guerra, 2011). Caffeine is allowed in sports. However, this substance is currently in the World Anti-doping Agency (WADA) Monitoring Program, a list of substances that are not included in the Prohibited List, monitored to detect any potential misuse in sport. Several studies have reported a beneficial effect of caffeine on endurance performance (McLellan, Caldwell and Lieberman, 2016). Limited research has been found in the effects of caffeine on endurance performance after sleep deprivation. In a military context, 400 mg of caffeine, followed by two consecutive 100 mg doses

improved time to task failure run at 85% of V̇O2 max after 28 h of sleep deprivation by 25% (McLellan, Bell and Kamimori, 2004). 200 mg of caffeine improved 6.3-km running time trial performance after 27 h of sleep deprivation by 1% (McLellan *et al.*, 2005). Nevertheless, caffeine dosages were not normalised according to the subjects' body weight. Only one paper has been found in relation to caffeine, sleep deprivation and endurance performance in a non-military setting: After 26 h of sleep deprivation, 5 $mg \cdot kg^{-1}$ of caffeine improved 8-km running time trial performance by 5.5% (Khcharem *et al.*, 2022). However, environmental and genetic factors seem to be responsible for inter-individual variability responses to caffeine (Pickering and Kiely, 2018). Additionally, from an ecological validity perspective, concerns are raised regarding the fact that athletes are rarely exposed to prolonged periods of sleep deprivation, whereas they seem to be more affected by sleep disruptions. Future investigations are required to gain further knowledge about the effects of caffeine on endurance performance after sleep deprivation.

Methylphenidate and Dextroamphetamine

Mainly used to treat attention deficit hyperactivity disorder, emotional instability associated with a history of substance abuse (i.e., alcohol, drugs), and narcolepsy. Executive function and vigilance can be improved due to the effect of these wakefulness-promoting drugs (Caldwell *et al.*, 1995, 2000; Pigeau *et al.*, 1995; Magill *et al.*, 1998). Nevertheless, these drugs are highly addictive. Therefore, the use of these substances is not approved to treat the deficits linked to sleep deprivation (Rogers, Dorrian and Dinges, 2003).

Modafinil

Modafinil is a psychoactive synthetic stimulant developed as a treatment of addictions, attention deficit disorders, depression, fatigue-induced diseases, obstructive sleep apnoea and narcolepsy (Wesensten, 2012). Despite its mechanism of action not being fully established, the stimulating effects of modafinil on the adrenergic system,

specifically the Alpha-1 adrenoceptor has been proposed (Duteil *et al.*, 1990). A possible role of modafinil in an *ad hoc* potentiation of the central nervous system catecholaminergic signalling has been suggested (Beck *et al.*, 2008). Furthermore, in sleep-promoting neurons, the blockade of noradrenaline reuptake, and a weak inhibition of dopamine uptake have been proposed as the rationale behind the stimulating effects of modafinil on alertness (Volkow *et al.*, 2009). Nevertheless, modafinil is not a dopamine receptor antagonist.

Positive changes in mood and feelings, alongside an euphoric effect of modafinil have been revealed (Jasinski, 2000; Rush *et al.*, 2002; Makris *et al.*, 2007). In healthy individuals, a repeated administration of 100-400 mg of modafinil per dose, increases alertness and cognitive performance after prolonged sleep deprivation (Wesensten, 2012). Similar to caffeine, in a sleep deprived state, modafinil improves objective and subjective alertness, reaction time, and sustained attention (Wesensten *et al.*, 2002). Consequently, modafinil reduces objective and subjective sleepiness, and subjective feelings of fatigue (Wesensten *et al.*, 2002). Several doses of 100 mg of modafinil increases the cognitive functions mentioned above (Baranski *et al.*, 2002; Bodenmann *et al.*, 2009). However, in order to maintain baseline levels of cognitive performance. Repeated doses of 200 mg or more are needed (Lagarde and Batejat, 1995; Batéjat and Lagarde, 1999; Caldwell *et al.*, 2000). A single dose of 200 mg of modafinil has been proposed in order to return to baseline levels of cognitive performance after one night of sleep deprivation (Wesensten *et al.*, 2002; Batéjat *et al.*, 2006; Dagan and Doljansky, 2006). For two or more nights of sleep deprivation, a single dose of 400 mg has been suggested (Wesensten *et al.*, 2002; Huck *et al.*, 2008; Killgore *et al.*, 2008). Nevertheless, similar subjective and objective effects have been found in caffeine and amphetamines (Warot *et al.*, 1993). It has been reported that 600 mg of caffeine have the same positive effect on cognitive performance than 200 and 400 mg of modafinil in sleep deprived healthy individuals (Wesensten *et al.*, 2002). However, modafinil effects seem to last for a longer period of time (Killgore *et al.*, 2008).

Regarding the side effects of modafinil, it has been linked to less side effects, in comparison to other synthetic stimulants (Rogers, Dorrian and Dinges, 2003). In specific divisions in the United States army, during prolonged sustained operations, the use of

modafinil has been authorised to maintain alertness. Nonetheless, in a sport context, the use of modafinil is prohibited in- competition by the WADA, listed as a non-specified stimulant substance.

Sleep

In the context of sleep deprivation, several sleep-related interventions have been proposed to enhance cognitive and endurance performance.

Naps seem to have a beneficial effect on cognitive and physical performance (Lovato and Lack, 2010; Blanchfield *et al.*, 2018). Naps lasting between fifteen and 120 minutes after 36-88 h of sleep deprivation increases cognitive performance (Dinges *et al.*, 1988, 2000). However, conflicting results have been found regarding the effects of napping on subjective sleepiness: beneficial (Naitoh, 1981; Helmus *et al.*, 1997) and no effects (Dinges *et al.*, 1988, 2000) have been reported. Moreover, more beneficial effects of napping are suggested, when taking place earlier in a prolonged period of sleep deprivation, before having accumulated an ample sleep debt (Dinges *et al.*, 1988). In order to investigate the effect of naps on endurance performance in a sleep deprived state, further research is crucial to identify effective nap durations and timing during the day.

Sleep extension, defined as a deliberately longer sleep duration than habitual with the aim of banking sleep, has been proposed in the literature as a potential tool to reduce the negative effects of sleep deprivation, and therefore, improve endurance performance (Walsh *et al.*, 2020). In a sleep deprivation context, a beneficial effect of sleep extension has been observed in mood (Mah, 2008), ratings of perceived exertion (Arnal *et al.*, 2016), sustained attention (Arnal *et al.*, 2015) and mood. In the preparation for an ultra-marathon, sleep extension is the main sleep strategy reported (Martin *et al.*, 2018)

However, several caveats have been suggested regarding the use of sleep extension: sleep inertia (i.e., grogginess on waking) may have a negative effect on performance (Thompson *et al.*, 2014), or a potential ceiling effect with no further performance improvements. (Walsh *et al.*, 2020). Nevertheless, due to the novelty of sleep extension as a potential method to counteract the negative effects of sleep

deprivation, future research is needed to fully explore its potential mechanisms of action, and performance benefits.

After a period of sleep deprivation (54-64 h), short periods of time (5-20 min) used as breaks from cognitive-demanding activities and work, improves alertness and cognitive performance (Heslegrave and Angus, 1985; Pigeau *et al.*, 1995). However, effects seem to be small and transient (Rogers, Dorrian and Dinges, 2003).

Other countermeasures

Bright light, cold air, noise, and physical activity have also been described in the literature as countermeasures for sleep deprivation. But effects seem to be temporary and unclear (Kushida, 2006).

Creatine is an organic compound found in food such as meat or fish. It is also produced in the liver. In humans, phosphocreatine (PCr) acts as an energy buffer, facilitating the maintenance of adenosine triphosphate (ATP). However, results reported in the literature are mainly related to cognitive performance: 20 g of creatine monohydrate per day during 7 days improves cognitive performance and mood after 24 h (McMorris *et al.*, 2006) and 36 h of sleep deprivation (McMorris *et al.*, 2007). Furthermore, only skill execution has been investigated regarding sleep deprivation and athletic performance (Cook *et al.*, 2011). Additionally, creatine supplementation has been shown to reduce the homeostatic sleep pressure in rats (Dworak *et al.*, 2017). Nevertheless, the use of creatine supplementation to reduce the negative effects of sleep deprivation on endurance performance have not been fully explored yet. Differences in supplementation protocols, inter-individual variability in response to creatine loading, PCr concentrations in the tissue, and its relevance to endurance performance require further investigation, The potential mechanisms of action and evidence in the context of sleep deprivation and endurance performance are scarce at this stage.

2.10 Summary and conclusions

There is a growing concern in relation to the sleep of the athletic population. Particularly in elite athletes, more prone to sleep disturbances resulting in a reduction in sleep and poorer quality. There is ample evidence about the detrimental effect of sleep deprivation on cognitive performance and sleepiness. The effects of sleep deprivation on endurance performance have not been fully explored yet. It seems that sleep deprivation in particular, impairs exercise performance longer than 30 min. Similar to the findings in relation to mental fatigue, despite no alterations in cardio-respiratory parameters, decreases in endurance performance after sleep deprivation are associated with higher ratings of perceived exertion.

Diverse countermeasures, such as caffeine, modafinil, and sleep have been proposed in order to counteract the negative effects of sleep deprivation on both cognitive and endurance performance.

Further research is required to better understand the role of sleep deprivation on endurance performance and potential mechanisms to counteract its negative effects. In the following chapters, based on the gaps presented in the current literature, several aspects have been explored: due to the impact of sleep time on non-stop ultra-endurance performance events, and the lack of data in competitions longer than 161 km in particular, a descriptive study was proposed to better understand the sleep management of athletes during these races, and its performance implications. The use of caffeine as a countermeasure to reduce the negative effects of sleep deprivation on running performance have been studied in a randomised controlled experiment. As part of the preparation for a mountain ultra-marathon race, the negative effects of sleep deprivation on endurance performance have been investigated to explore the concept of sleep deprivation tolerance and its role in ultra-endurance performance.

Chapter 3: Sleep During Mountain Ultra-Marathon Events.

Main findings: There was a moderate association between race time and sleep duration. Throughout the race, sleep bouts, rest, and sleep times increased. Before the first sleep episode, participants were awake for 24.6 ± 4.9 h. Years of experience competing in ultra-marathons and a more morning chronotype were amongst the differences between finishers and non-finishers.

3.1 Abstract

In non-stop ultra-marathon events sleep is a key factor for both race performance and psychophysiological aspects. Sleep duration is positively correlated to finish time in events lasting between 36 and 60 h. However, sleep in longer events has not been fully investigated yet. For this reason, the aim of our study was to investigate sleep and performance during a ~431 km ultra-marathon. Data collection took place in three editions. From the twenty-seven athletes registered in the race that participated in the study, seventeen completed the event. Actigraphy watches were used to monitor sleep/wake, and activity during the race. At each of the five main checkpoints established, participants were asked about their sleepiness, perceived exertion, and sleep. Finishers (N = 17) completed the race in 141.8 ± 19.1 h with a total rest time of 26.3 ± 8.6 h. Participants spent 13.9 ± 4.9 h in bed with 11.2 ± 3.6 h of sleep in 6 ± 2 sleep episodes. An 81.6 ± 13.3% sleep efficiency was reported. Athletes stayed awake for 24.6 ± 10.3 h before their first sleep bout. Throughout the race, rest time, the number of sleep periods, and sleep time increased ($p < 0.001$), particularly during the second half of the race. Sleep efficiency did not change ($p = 0.913$). Several associations were found between race time and other variables: a positive high association with number of sleep bouts (r = 0.789), Moderate positive associations with rest time (r = 0.655), time in bed (r = 0.611), and sleep time (r = 0.532), respectively. A positive moderate association with sleepiness (r_p =

0.513). A low moderate positive association with perceived exertion (p = 0.409). The results of this study suggested that faster runners rest and sleep less. Furthermore, it seems that the most adopted sleep strategy was not to sleep at the first quarter of the race. Longer rest and sleep times in the second half of the race proposed an increase in sleep pressure, possibly as a result of the accumulated sleep loss, and the psychophysiological requirements of ultra-endurance exercise. Our findings may have helped runners interested in such long events to optimise their rest and sleep strategies during the race accordingly.

3.2 Introduction

Running events longer than a marathon (42.195 km) are commonly named as ultra-marathon races, lasting several days, and performed on a wide range of ground surfaces, such as flat roads or tracks (Hoffman, 2020), or on mountain trails with significant positive and negative elevation changes, and at high altitude (Savoldelli *et al.*, 2017). Ultra-marathons are an outstanding scenario to explore human physical and psychological responses to the stress induced by an extreme environment that cannot be performed in a laboratory (Millet and Millet, 2012). In the literature, several studies during ultra-marathons has been reported: Western States Endurance Run - 161 km (Nieman *et al.*, 2003; Utter *et al.*, 2009; Hoffman *et al.*, 2012), *Ultra-Trail du Mont Blanc* - 168 km (Hurdiel *et al.*, 2015; Poussel *et al.*, 2015; Suter *et al.*, 2020), *Tor des Géants* - 330 km (Saugy *et al.*, 2013; Degache *et al.*, 2016; Savoldelli *et al.*, 2017), are just a few examples of this phenomenon.

Self-regulation of intensity (i.e., speed, power output) during endurance exercise is known as pacing strategy, a key component for optimal performance. Despite the fact that the mechanisms underlying regulation of exercise intensity are still strongly debated, pacing is regulated by the central nervous system (Marcora, 2010; Gibson *et al.*, 2018), nor only fatigue and/or perceived exertion, but also behavioural responses (i,e., motivation, previous experience, knowledge of distance/time covered/remaining, opponents behaviour). For a review on regulation of exercise intensity during competition see Konings and Hettinga (2018). Pacing during ultra-endurance events may be regulated

by fatigue resistance (Abbiss and Laursen, 2008). A constant pace (even pacing) has been proposed as the optimal strategy for prolonged exercise, under stable environmental conditions. However, the nature of ultra-marathon events includes a myriad of unstable factors (i.e., changes in elevation, environmental conditions, competition dynamics, terrain) making even pacing difficult to adopt as a strategy during these events. Therefore, a progressive decrease in exercise intensity (positive pacing) have been reported during 100 to 161 km ultramarathons (Lambert *et al.*, 2004; Parise and Hoffman, 2011; Hoffman, 2014; Knechtle *et al.*, 2015; Renfree, Crivoi do Carmo and Martin, 2016; Tan, Tan and Bosch, 2016). So far, the pacing strategy of an ultra-marathon longer than 160 km has not been studied yet.

Seven to nine hours of sleep every twenty-four hours is an optimal sleep duration for adults between eighteen and sixty-four years old, according to the National Sleep Foundation (Hirshkowitz *et al.*, 2015). Similar to mental fatigue, for a more detailed definition of mental fatigue, see Van Cutsem *et al.* (2017), sleep deprivation can be defined as a psychobiological state caused by a prolonged period of wakefulness (Boonstra *et al.*, 2007; Ackerman, 2010), that could have a negative effect on many aspects: in a consensus statement published recently, it has been pointed out that there is evidence to suggest that sleep deprivation (one night or more without any sleep) has a negative effect on physical performance (Walsh *et al.*, 2020). In sleep deprived subjects, a reduction in performance during high-intensity running due to an increased perceived exertion has been proposed (Oliver *et al.*, 2009). From a cognitive perspective, the negative effects of sleep deprivation are quite clear, for a review see Fullagar *et al.* (2015).

In ultra-marathon events, it is well known that runners are aware of the importance of sleep management strategies, increasing sleep time before the start of the race (Poussel *et al.*, 2015; Martin *et al.*, 2018), and trying to sleep as little as possible during the race, in order to minimise the time spent not moving, since the race clock never stops (Millet *et al.*, 2011; Savoldelli *et al.*, 2017). It is thought that the fastest runners would not sleep at all, but beyond some running time, participants would have to spend some time sleeping. In a 168 km ultra-marathon, individuals with a finish time longer than 36 h had some type of sleep, longer sleep times positively correlated with race time (Hurdiel *et al.*, 2015). Participants in this study reported 0.2 ± 0.3 h of sleep (measured via actigraphy)

in 36.0 ± 7.3 h of race. A case study done in a 330 km ultramarathon described that the participant slept 11.7 h in 125.6 h of race (Savoldelli *et al.*, 2017). In the same race, fifteen runners disclosed 9.1 ± 5.4 h of sleep in 122.4 ± 17.2 h of race (Saugy *et al.*, 2013). For races longer than 36 h, sleep is correlated with race duration (Martin *et al.*, 2018). However, little is known about sleep in non-stop events longer than 330 km.

The aim of the present study was to investigate the sleep of ultra-marathon runners during an event longer than 330 km. Additionally, sleepiness and ratings of perceived exertion were measured. We hypothesised that the faster runners would report lower sleep times.

3.3 Methods

Participants

Athletes registered in the events (see *Event*s section) were eligible to take part of the study. Participants were recruited and fully briefed about the research purposes before the start of the race. They were asked to sign a written informed consent. The study was approved by the University of Kent, School of Sport and Exercises Sciences Research Ethics and Advisory Group, which conformed to the standards set by the Declaration of Helsinki (2003). Further details about the participants can be found in Table 3.1. Peak oxygen uptake ($\dot{V}O_{2\ peak}$) was estimated by applying validated gender-specific multiple regression equations (Jackson *et al.*, 1990).

Table 3.1. Participants' normative data.

	TSR1	TSR2	TSF
Number of participants	5 (1F)	14 (1F)	8 (1F)
Age (yr)	46.8 ± 6.9	46.4 ± 5.5	45.9 ± 11.6
Stature (m)	1.74 ± 0.30	1.75 ± 0.75	1.78 ± 0.83
Body mass (kg)	69.2 ± 3.8	75.4 ± 8.5	77.3 ± 10.4
Estimated $\dot{V}O_{2\ peak}$ ($mL \cdot kg \cdot min^{-1}$)	43.6 ± 6.1	43.7 ± 3.9	43.5 ± 4.4
Experience (yr)	9.4 ± 4.4	6.1 ± 3.4	8.6 ± 4.7
Training ($h \cdot week^{-1}$)	10.0 ± 2.9	9.9 ± 4.3	11.0 ± 3.2
Sleep Quality	3.80 ± 2.38	5.21 ± 2.01	4.50 ± 2.51
Chronotype	53.6 ± 10.6	60.9 ± 9.6	59.0 ± 6.6

The Spine Race 2018 (TSR1), The Spine Race 2019 (TSR2), The Spine Fusion 2019 (TSF). F = female. Data are expressed as mean ± SD.

Study design

In order to investigate how athletes sleep during ultra-marathon events, a series of field studies were conducted. Participants were approached by the researcher before the start of the race, at each checkpoint, and at the finish line. Participants were monitored on the route using a GPS tracker provider by the race organisers. A summary of the protocol can be found in Figure 3.1

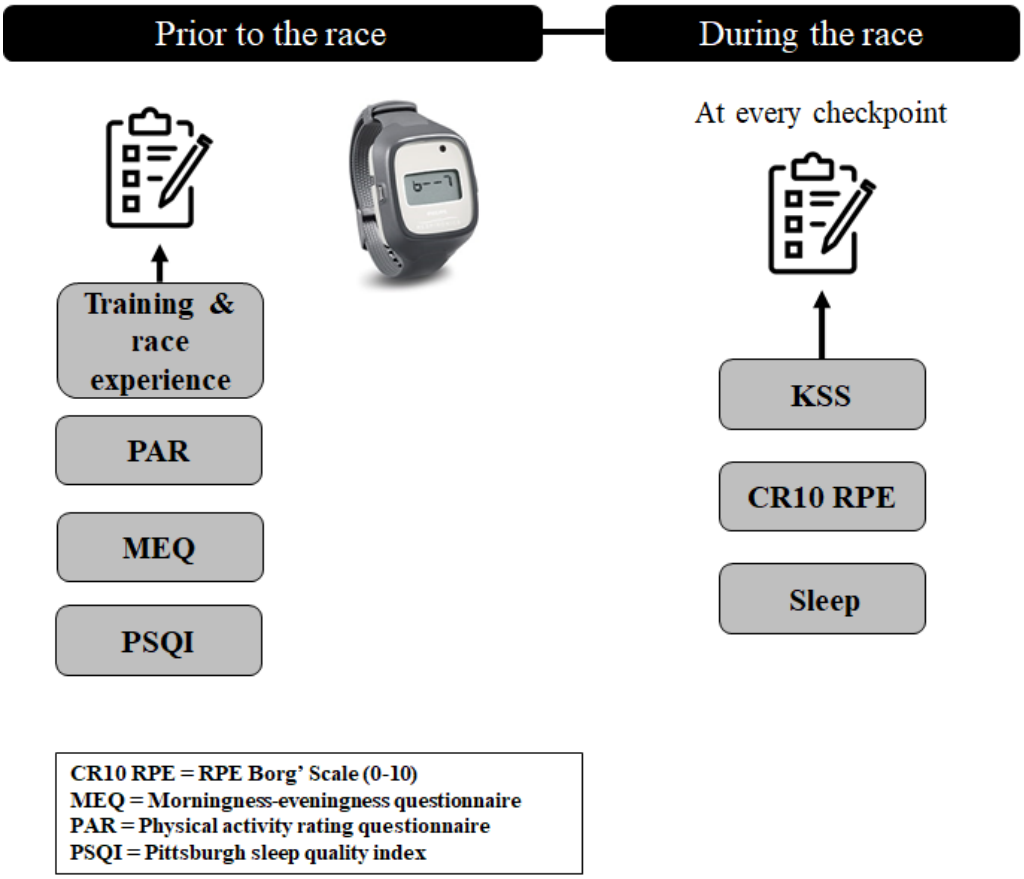


Figure 3.1 Study protocol

Testing procedures

Preliminary visit

The day before the start of the race, traditionally, race organisers require participants to go to a designated hall in order to complete several activities such as register, collect their race numbers, attend a pre-race safety, and/or pass the compulsory equipment check.

In addition to that, participants in the study were introduced to the researcher. After having the consent form signed, participants were asked to complete several questionnaires: training and race experience, physical activity, chronotype, and sleep quality (see *Questionnaires* section for further details). Participants were also familiarised with the scales used during the study, standardised written instructions were supplied to

the participants, including an explanation of the nature and use of the scales. The researcher reinforced the content of the instructions and answered questions after participants had read the instructions (Noble and Robertson, 1996). Finally, participants were briefed about the nature and the correct use of the actiwatch during the study and given some written information. After that, the corresponding device was given to the participants.

Actigraphy

Actigraphy watches (Actiwatch Spectrum PRO, Philips Respironics, Murrysville, Pennsylvania, USA) were used in order to have an objective measure of sleep. This device continuously records light exposure and quantity of body movement (30-second epochs for this study) with a piezo-electric accelerometer. Default sleep/wake threshold (activity counts above 40) was used. Using a proprietary algorithm and software (Philips Respironics Actiware version 6.0.9) sleep parameters are calculated. This device and method have been used to assess sleep/wake behaviour in athletes several times (Halson *et al*., 2014; Sargent *et al*., 2014; Lastella *et al*., 2015a; Lastella *et al*., 2015b). For the purpose of this study, the following variables were reported: time in bed; total sleep time; total sleep bouts; sleep efficiency (percentage of time spent in bed sleeping); activity counts per minute; activity counts per minute asleep; and number of sleep bouts out of the main checkpoints. Participants were instructed to wear the watch continuously and properly fitted on their non-dominant hand. This device has a built-in feature that triggers an automatic function to exclude data when it is off wrist. Nevertheless, to adjust or exclude data when appropriate, visual inspection of the actigraphy plot (Figure 3.2) by the researcher permits consistency assessment, in addition to data recalled from participants verbally at each checkpoint.

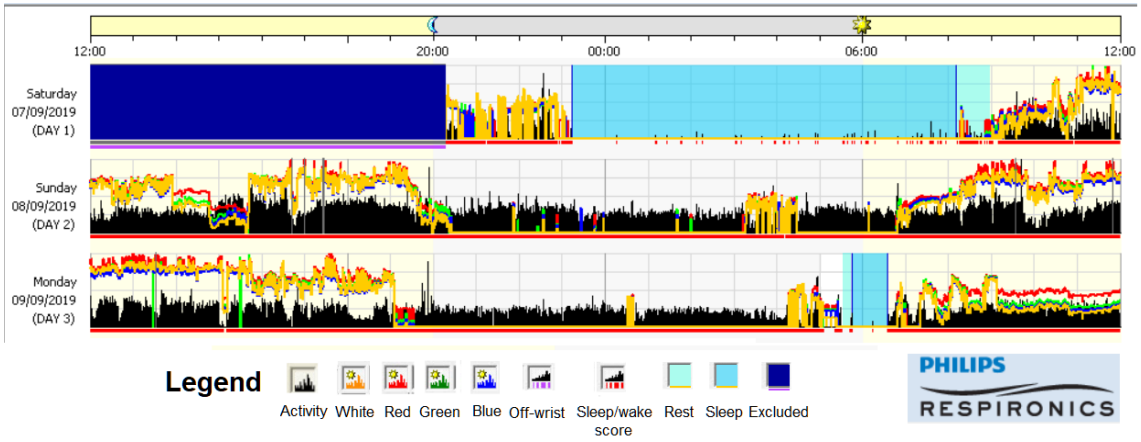


Figure 3.2 Actigraphy plot.

Sleepiness

In order to measure subjective levels of sleepiness, the Karolinska Sleepiness Scale (KSS), proposed by Akerstedt and Gillberg (1990) was used. Participants were asked to rate their sleepiness immediately before the start of the race, at every checkpoint, and at the finish line using a 9-point Likert scale (1 = extremely alert; 2 = very alert; 3 = alert; 4 = rather alert; 5 = neither alert nor sleepy; 6 = some signs of sleepiness; 7 = sleepy but no effort to keep awake; 8 = sleepy, some effort to keep awake; and 9 = very sleepy, great effort to keep awake, fighting sleep).

Perceived exertion

Overall ratings of perception of effort (RPE) were measured using the CR10 (0-10) RPE Scale (Borg, 1998) modified by Foster *et al.* (1995). At each checkpoint and the finish line, participants were asked to rate their RPE.

Questionnaires

As part of the data collected to estimate $\dot{V}O_{2\ peak}$ such as gender, age, stature, and body mass, the Physical Activity Rating, PAR (Jackson *et al.,* 1990) was administered to determine participant's level of physical activity. Additionally, for normative purposes, participants were also asked to answer questions related to their experience in competition and their weekly hours.

The main purpose of The Morningness-Eveningness Questionnaire, MEQ (Horne and Ostberg, 1976) is to estimate the time of the day (morning, evening, in between) corresponding to peak alertness produced by the individual circadian rhythms. Interpretation of the scores can be found in Table 3.2.

Table 3.2 Interpretation of the Morningness-Eveningness Questionnaire (MEQ).

MEQ score	**16-30**	**31-41**	**42-58**	**59-69**	**70-86**
Chronotype	Definite evening	Moderate evening	Intermediate	Moderate morning	Definite morning

As a way to assess sleep quality, The Pittsburgh Sleep Quality Index Questionnaire, PSQI (Buysse *et al.*, 1989) was administered to the participants. A total score of 5 or greater is indicative of poor sleep quality.

Race performance

Time of the day, race time, distance, and speed were collected using data provided by GPS trackers that race organisers give to the participants, in order to monitor them

during the event. Each race had a live tracking website associated, open to the public. This service was provided by Open Tracking LTd. (Ulverston, Cumbria, UK).

Events

The Spine Race (TSR)

TSR is a ~431 km (268 miles) non-stop winter mountain ultra-marathon on The Pennine Way National Trail, tracking the backbone of England. From Edale in Derbyshire (England) to Kirk Yetholm (Scotland), the race takes place in some iconic National Parks such as the Peak District, Yorkshire Dales, and The North Pennines. Over Hadrian's Wall, a World Heritage-listed Roman stone wall, it crosses the outlying Cheviot Hills to finish in the Scottish Borders with a total elevation gain of 13,347 metres. Despite being a single-stage mountain-ultramarathon, five checkpoints are established by race organisers where participants can rest, change their clothes, get some food and drink, have a shower, get medical support, go to sleep, and refill their hydration options. At each checkpoint participants have access to a resupply bag in order to pick up or drop items from the backpacks they must carry at all times during the race with all the compulsory equipment. There are also several secondary checkpoints where participants can get food and refill their hydration options. No crew or outside support is allowed. Participants have 168 hours to complete the race, with additional cut-off times at each checkpoint. The winter weather conditions, with temperatures below zero, intense winds on exposed hills and waist-deep snow drifts, TSR is considered as 'Britain's most brutal race'. The current course records are 87:53:57 and 83:13:23 for men and women, respectively. We collected data in 2018 (TSR1) and 2019 (TSR2) editions.

The Spine Fusion (TSF)

TSF is the summer version of TSR. Route, distance, number of checkpoints, and cut-off times are the same. There is a slight variation on the location of some checkpoints

and the mandatory kit. The current course records are 78:04:14 and 81:19:07 for men and women, respectively. Data was collected in the 2019 edition.

Further details of the events in Table 3.3

Table 3.3 Main checkpoints and cumulative distance.

CP1 (km)	CP2 (km)	CP3 (km)	CP4 (km)	CP5 (km)	Finish (km)
74	172	226	289	353	421

Statistical Analysis

Due to the nature of field studies, sample sizes were too small to analyse the events independently, only descriptive statistics (mean ± standard deviation) were used to report data for each race individually. Two participants took part in TSR1 and TSR2. Therefore, their data from TSR1 were excluded. Then, data were pooled into finishers and non-finishers groups. Two-tailed paired-samples t-tests were used to assess differences in finishers only for: time awake between the start of the race and the first sleep bout; time awake between the end of the race and the last sleep bout. Finishers data were normalised for race distance, four points were calculated as percentage of race completed (25%, 50%, 75% and 100%). Data were checked for assumptions of all statistical tests. Some variables did not meet the normality assumption; therefore, Friedman tests (χ^2) were used to further investigate differences in finishers only. Wilcoxon signed-rank tests were used for follow-up pairwise comparisons with a Bonferroni correction. Pearson's product-moment correlation (r), or Spearman rank order correlation (r_ρ) tests when assumptions were not met, were used to assess associations between variables from finishers only. The strength of the associations between variables are defined as low (> 0.3), moderate (> 0.5), high (> 0.7), and very high (> 0.9), respectively (Hinkle, Wiersma and Jurs, 2003). Data not normally distributed are presented as median (*Mdn*) and interquartile range (*IQR*).

Independent-samples t-tests were used to assess differences in normative data between finishers and non-finishers. Statistical significance was accepted at $p < 0.05$ (two-tailed). Statistical analyses were performed using SPSS Statistics 28.0 (IBM, Armonk, New York, USA).

3.4 Results

Individual analysis of the races

Weather conditions and day lengths are summarised in Table 3.4. An overview of the main variables grouped by race are presented in Figure 3.3. Since races took place in different time zones, Coordinated Universal Time (UTC) standard was used for descriptive purposes. To compare, times of the day are all reported in the Greenwich Meridian Mean Time (GMT).

Table 3.4. Weather conditions and day length during the events.

		Min Temp (ºC)	Max Temp (ºC)	RH (%)	Wind speed ($km \cdot h^{-1}$)	Gust speed ($km \cdot h^{-1}$)	Day length (h)
TSR1	**Start**	1	3	89	11	17	8.03
	Finish	-2	3	77	13	26	8.01
TSR2	**Start**	5	9	89	41	65	7.98
	Finish	0	4	92	9	19	7.94
TSF	**Start**	9	18	67	10	11	17.00
	Finish	11	14	86	32	41	17.44

The Spine Race 2018 (TSR1), The Spine Race 2019 (TSR2), The Spine Fusion 2019 (TSF).

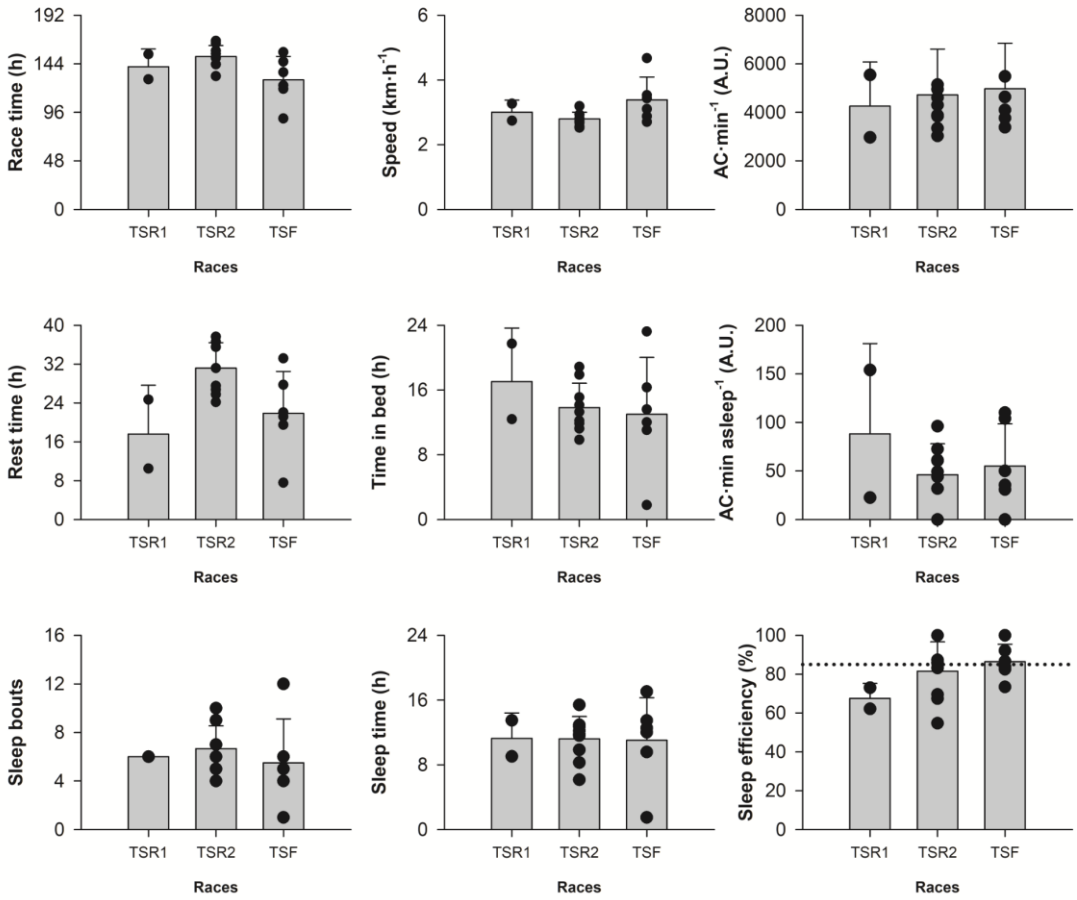


Figure 3.3. Performance, activity, and sleep parameters grouped by race. The Spine Race 2018 (TSR1). The Spine Race 2019 (TSR2). The Spine Fusion 2019 (TSF). Data in bars are presented as mean ± SD. Circles depict individual data. Dotted line represents 85% of sleep efficiency.

The Spine Race 2018 (TSR1)

TSR1 took place from 14th to 21st January 2018. Race started at 8 am (UTC +0). Due to extreme adverse weather conditions outside the race was held for ten hours from 17th January at 8 pm, 18th January at 6 am (UTC +0). Participants were told to remain indoors. Those who were outside were told to move to the nearest checkpoint / indoor place. Next-day cut-off time was modified in accordance with the stoppage, but cut-off at the finish line remained unaltered. Fifty-three out of 118 athletes finished the race (44.9%).

Race performance

Two out of five participants (40%) completed the race in 141.1 ± 17:5 h at an average speed of 3.00 ± 0.37 km·h^{-1}. Finishers (N = 2) ranked from 4th to 31st place. Total rest time was 17.6 ± 10.0 h. Total number of activity counts per minute was 4254 ± 1818. Cumulative data are presented in Figure 3.4.A. (race time), Figure 3.4.B. (rest time), Figure 3.5.A. (running speed), Figure 3.5.B. (activity counts per minute).

Sleep data

Finishers had 6 sleep bouts during the race. 4 ± 2 of them were out of the main checkpoints. Total time in bed was 17.1 ± 6.6 h, with a total sleep time of 11.3 ± 3.2 h. Average sleep efficiency was 67.6 ± 7.7%. During sleep, total activity counts per minute was 88 ± 93. Cumulative data can be found in Figure 3.6.A. (sleep bouts), Figure 3.6.B. (sleep bouts out of the main checkpoints), Figure 3.7.A. (time in bed), Figure 3.7.B. (sleep time), Figure 3.8.A. (sleep efficiency), and Figure 3.8.B. (activity counts per minute asleep). Until the first sleep bout, finishers were awake for 25.4 ± 15.4 h. After the last sleep bout, they were awake for 17.3 ± 11.2 h until they crossed the finish line (Figure 3.9.A). 12.1 ± 5.6% of race time was rest time. 70.4 ± 22.3% of rest time was sleep time. 7.91 ± 1.25% of race time was sleep time (Figure 3.9.B).

Sleepiness

Sleepiness score (out of 9) before the start of the race was 2. Sleepiness at the end of the race was 8 ± 1. Further details of sleepiness can be seen in Figure 3.10.A.

Perceived exertion

RPE (out of 10) at the first checkpoint (74 km) was 4 ± 1. RPE at the end of the race was 9 ± 1. Further details of RPE are presented in Figure 3.10.B.

The Spine Race 2019 (TSR2)

TSR2 took place from 13th to 20th January 2019. Race started at 8 am (UTC +0). Seventy-three out of 126 athletes finished the race (57.9%).

Race performance

Nine out of fourteen participants (64%) completed the race in 151.1 ± 10.9 h at an average speed of 2.8 ± 0.2 km·h^{-1}. Finishers (N = 9) ranked from 21st to 72nd place. Total rest time was 31.2 ± 5.3 h. Total activity counts per minute was 4718 ± 1883. Cumulative data are presented in Figure 3.4.A. (race time), Figure 3.4.B. (rest time), Figure 3.5.A. (running speed), Figure 3.5.B. (activity counts per minute).

Sleep data

Finishers had 7 ± 2 sleep bouts during the race. 2 ± 1 of them were out of the main checkpoints. Total time in bed was 13.8 ± 3.0 h, with a total sleep time of 11.2 ± 2.8 h. Average sleep efficiency was 79.4 ± 20.0%. During sleep, total activity counts per minute was 60 ± 21. Cumulative data can be found in Figure 3.6.A. (sleep bouts), Figure 3.6.B. (sleep bouts out of the main checkpoints), Figure 3.7.A. (time in bed), Figure 3.7.B. (sleep time), Figure 3.8.A. (sleep efficiency), and Figure 3.8.B. (activity counts per minute asleep). Until the first sleep bout, finishers were awake for 21.2 ± 7.9 h. After the last sleep bout, they were awake for 16.4 ± 8.1 h until they crossed the finish line (Figure 3.9.A). 20.7 ± 3.7% of race time was rest time. 35.7 ± 5.5% of rest time was sleep time. 7.47 ± 1.94% of race time was sleep time (Figure 3.9.A).

Sleepiness

Sleepiness score (out of 9) before the start of the race was 2 ± 1. Sleepiness at the end of the race was 5 ± 3. Further details of sleepiness can be seen in Figure 3.10.A.

Perceived exertion

RPE (out of 10) at the first checkpoint (74 km) was 5 ± 2. RPE at the end of the race was 8 ± 2. Further details of RPE are presented in Figure 3.10.B.

The Spine Fusion 2019 (TSF)

TSF took place from 22nd to 29th June 2019. Race started at 7 am (UTC +1). Twenty-seven out of forty athletes finished the race (67.5%).

Race performance

Six out of eight participants (75%) completed the race in 128.1 ± 23.2 h at an average speed of 3.4 ± 0.2 km·h^{-1}. Finishers (N = 6) ranked from 2nd to 26th place. Total rest time was 21.9 ± 8.6 h. Total activity counts per minute was 4974 ± 1870. Cumulative data are presented in Figure 3.4.A. (race time), Figure 3.4.B. (rest time), Figure 3.5.A. (running speed), Figure 3.5.B. (activity counts per minute).

Sleep data

Finishers had 6 ± 4 sleep bouts during the race. 2 ± 3 of them were out of the main checkpoints. Total time in bed was 13.0 ± 7.0 h, with a total sleep time of 11 ± 5.3 h. Average sleep efficiency was 86.4 ± 9.0%. During sleep, total activity counts per minute was 66 ± 38. Cumulative data can be found in Figure 3.6.A. (sleep bouts), Figure 3.6.B. (sleep bouts out of the main checkpoints), Figure 3.7.A. (time in bed), Figure 3.7.B. (sleep

time), Figure 3.8.A. (sleep efficiency), and Figure 3.8.B. (activity counts per minute asleep). Until the first sleep bout, finishers were awake for 29.5 ± 11.8 h. After the last sleep bout, they were awake for 15.7 ± 16.6 h until they crossed the finish line (Figure 3.9.A). 16.6 ± 6.4% of race time was rest time. 47.5 ± 15.9% of rest time was sleep time. 8.18 ± 3.42% of race time was sleep time (Figure 3.9.B.).

Sleepiness

Sleepiness score (out of 9) before the start of the race was 3 ± 2. Sleepiness at the end of the race was 6 ± 3. Further details of sleepiness can be seen in Figure 3.10.A.

Perceived exertion

RPE (out of 10) at the first checkpoint (after 74 km) was 7 ± 2. RPE at the end of the race was 7 ± 2. Further details of RPE are presented in Figure 3.10.B.

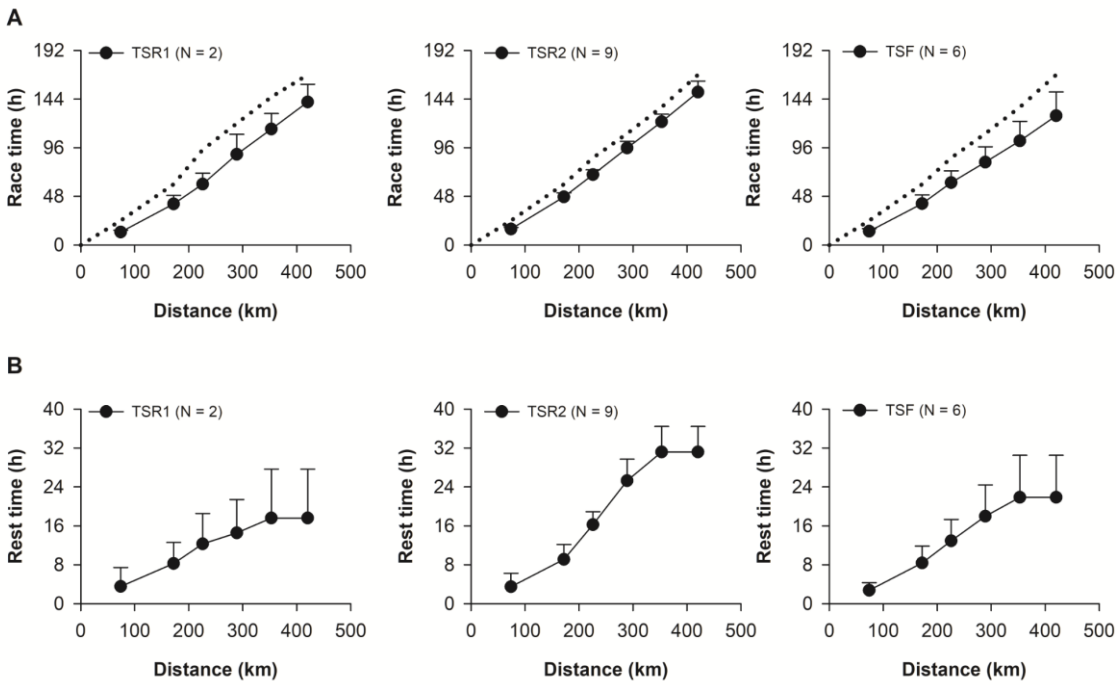


Figure 3.4 Cumulative data: race time (A), rest time (B). The Spine Race 2018 (TSR1). The Spine Race 2019 (TSR2). The Spine Fusion 2019 (TSF). Data are presented as mean ± SD. Dotted lines depict cut-off times.

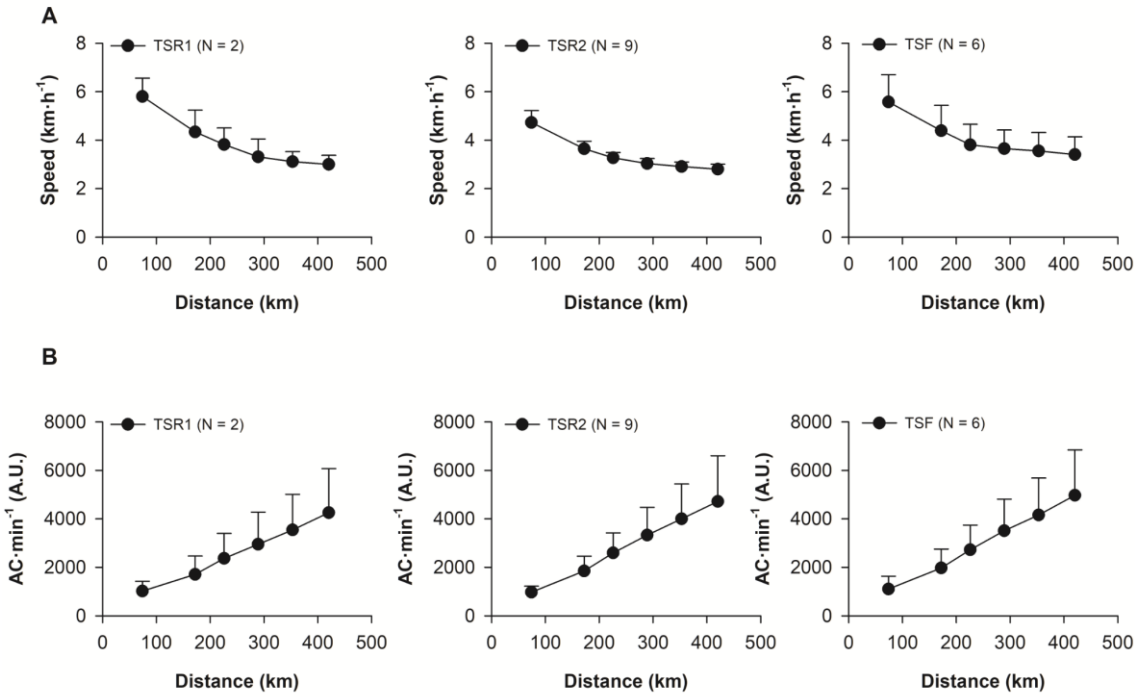


Figure 3.5 Cumulative data: running speed (A), activity counts per minute (B). The Spine Race 2018 (TSR1). The Spine Race 2019 (TSR2). The Spine Fusion 2019 (TSF). Data are presented as mean ± SD. Dotted lines depict mean running speed.

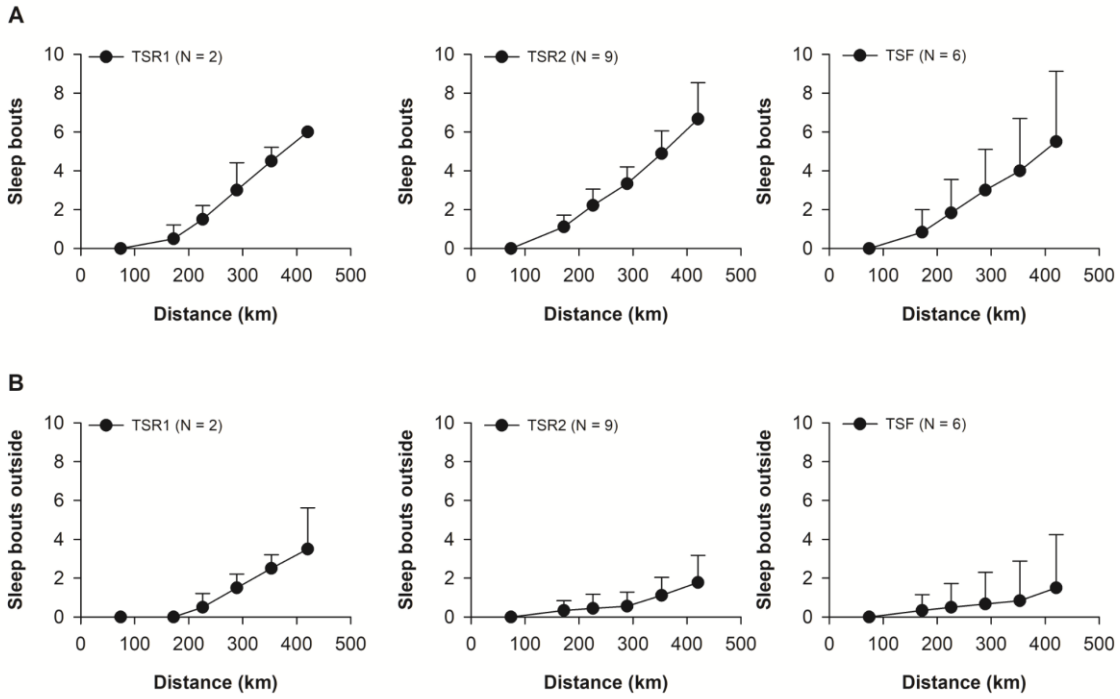


Figure 3.6 Cumulative data: number of sleep bouts (A), number of sleep bouts out of the main checkpoints (B). The Spine Race 2018 (TSR1). The Spine Race 2019 (TSR2). The Spine Fusion 2019 (TSF). Data are presented as mean ± SD.

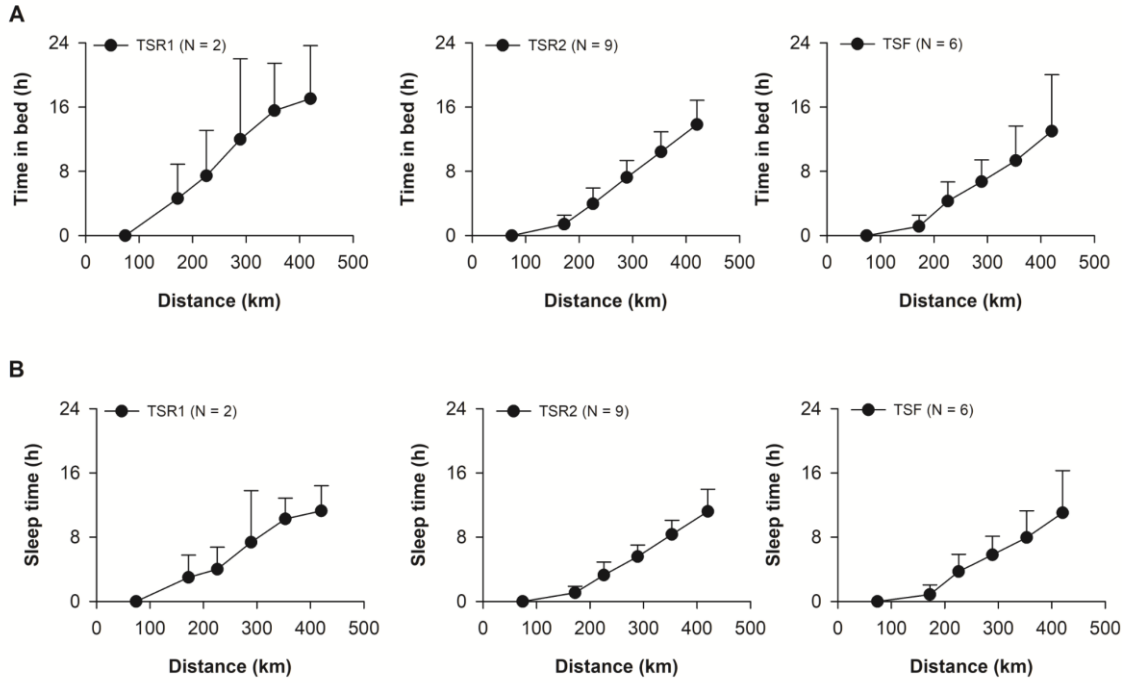


Figure 3.7 Cumulative data: time in bed (A). Sleep time (B). The Spine Race 2018 (TSR1). The Spine Race 2019 (TSR2). The Spine Fusion 2019 (TSF). Data are presented as mean ± SD.

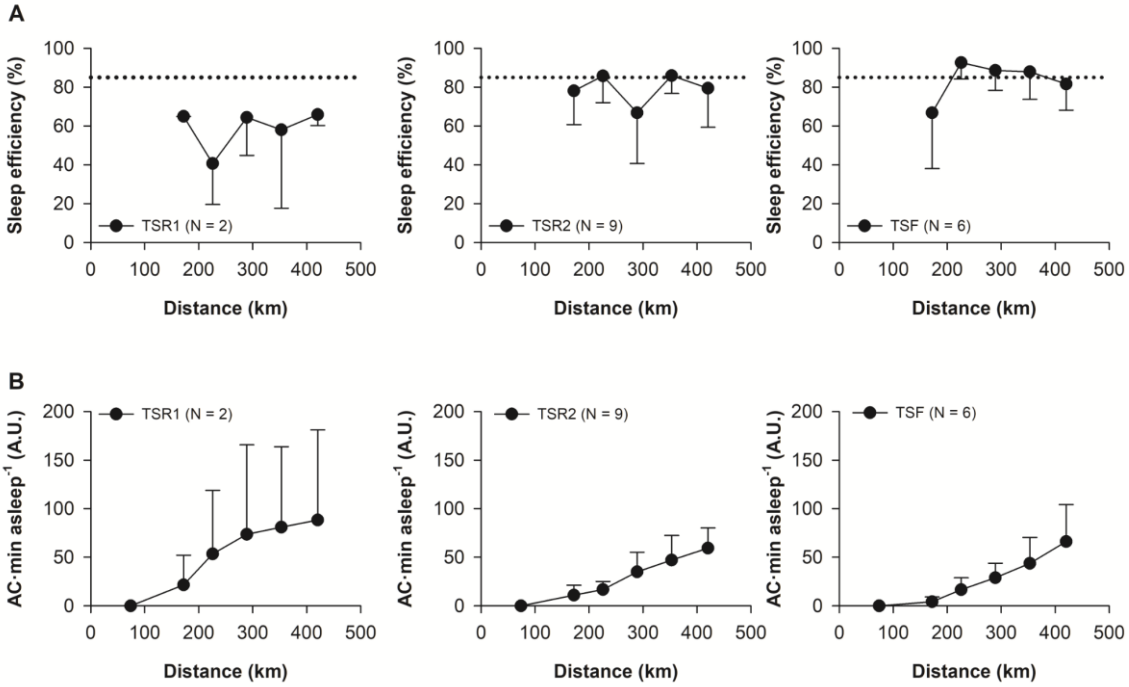


Figure 3.8 Cumulative data: sleep efficiency (A). activity counts per minute asleep (B). The Spine Race 2018 (TSR1). The Spine Race 2019 (TSR2). The Spine Fusion 2019 (TSF). Data are presented as mean ± SD. Dotted line represents 85% of sleep efficiency.

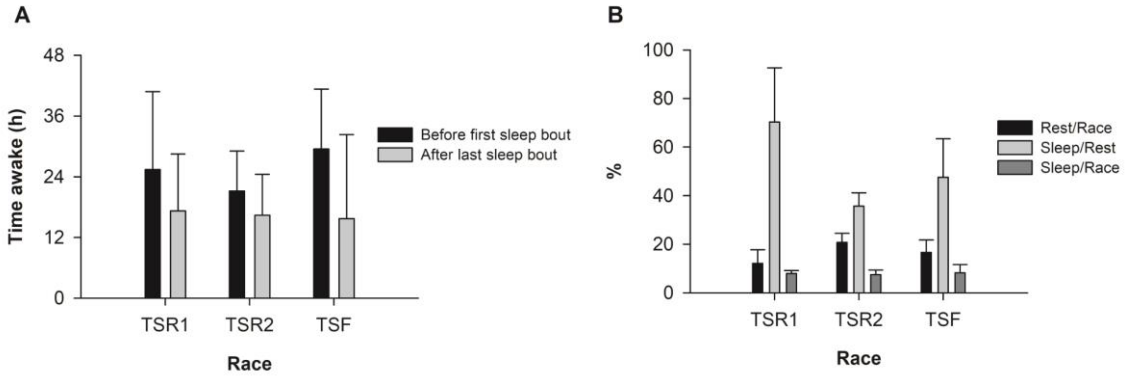


Figure 3.9 Time awake at the start and the end of each race (A). Ratios of Rest/Race, Sleep/Rest, and Sleep/Race (B). The Spine Race 2018 (TSR1). The Spine Race 2019 (TSR2). The Spine Fusion 2019 (TSF). Data are presented as mean ± SD.

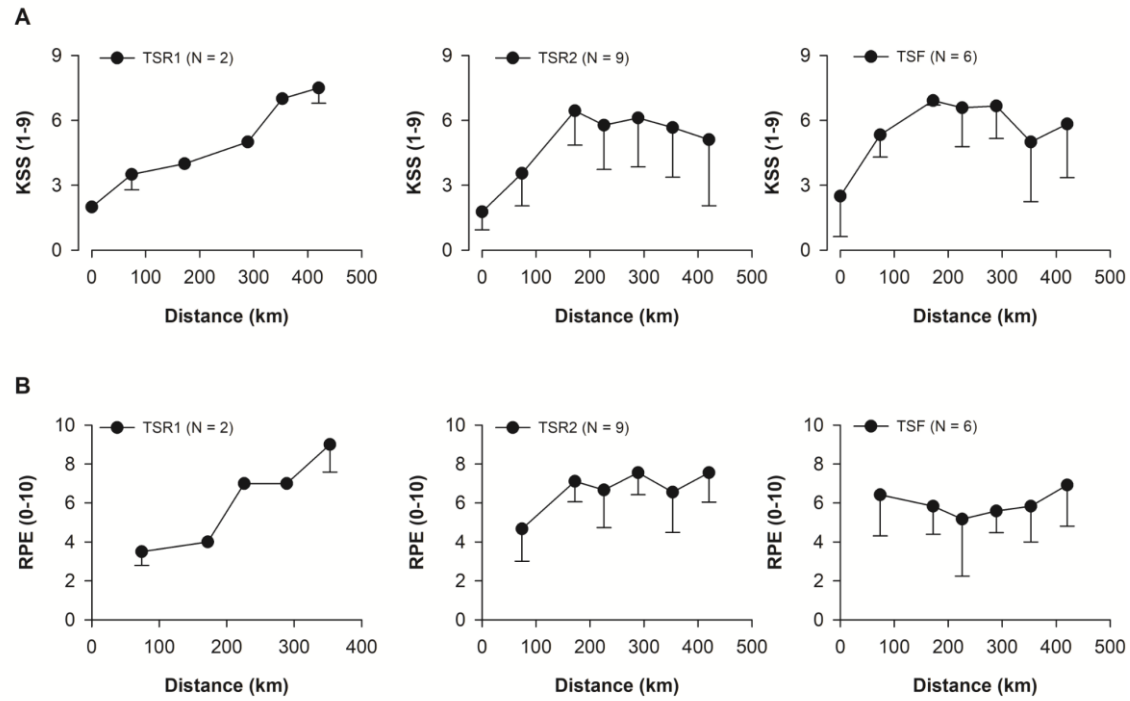


Figure 3.10 Subjective sleepiness (A), ratings of perceived exertion (B). The Spine Race 2018 (TSR1). The Spine Race 2019 (TSR2). Data are presented as mean ± SD.

Pooled data: finishers

Race performance

Data from finishers (N = 17, 1 female) were normalised corresponding to their race time (%).

Split time was different over the course of the race. $\chi^2(3) = 49.871$, $p < 0.001$. Follow-up analysis revealed that participants completed the first quarter of the race distance (*Mdn* = 15.9 h; *IQR* = 3.3 h) faster than the third (*Mdn* = 47.7 h; *IQR* = 7.9 h; $p < 0.001$), and the last one (*Mdn* = 53.7 h; *IQR* = 9.9 h; $p < 0.001$). The second quarter of the race (*Mdn* = 30.8 h; *IQR* = 5.8 h) was faster than the last quarter ($p < 0.001$). No significant differences were found between other points Figure 3.11.A.

Split speed was different over the course of the race. $\chi^2(3) = 46.059$, $p < 0.001$. Post-hoc analysis indicated that participants ran faster in the first quarter of the race (*Mdn* = 4.65 km·h^{-1}; *IQR* = 1.12 km·h^{-1}) than in the third (*Mdn* = 2.45 km·h^{-1}; *IQR* = 0.45 km·h^{-1}; $p < 0.001$), and the last one (*Mdn* = 2.45 km·h^{-1}; *IQR* = 0.53 km·h^{-1}; $p < 0.001$). Split speed was faster in the second quarter (*Mdn* = 3.18 km·h^{-1}; *IQR* = 0.65 km·h^{-1}) than in the third ($p = 0.009$), and the last one ($p = 0.002$). No significant differences were found between other points Figure 3.11.B.

Rest time was different over the course of the race. $\chi^2(3) = 36.692$, $p < 0.001$. Follow-up analysis disclosed that rest time in the third quarter (*Mdn* = 12.07 h; *IQR* = 5.68 h) was higher than in the first (*Mdn* = 3.45 h; *IQR* = 2.62 h; $p < 0.001$), second (*Mdn* = 5.27 h; *IQR* = 1.87 h; $p = 0.001$), and last (*Mdn* = 5.30 h; *IQR* = 3.20 h; $p = 0.001$). No significant differences were found between other points Figure 3.11.C.

Activity counts per minute was different over the course of the race. $\chi^2(3) = 31.800$, $p < 0.001$. Post-hoc analysis indicated that activity counts (A.U.) in the third quarter (*Mdn* = 1395; *IQR* = 533) were higher than in the first (*Mdn* = 999; *IQR* = 346; $p = 0.021$), and second (*Mdn* = 672; *IQR* = 325; $p < 0.001$). In the last quarter (*Mdn* = 1238;

IQR = 382), activity counts were higher than in the second ($p < 0.001$). No significant differences were found between other points. Figure 3.11.D.

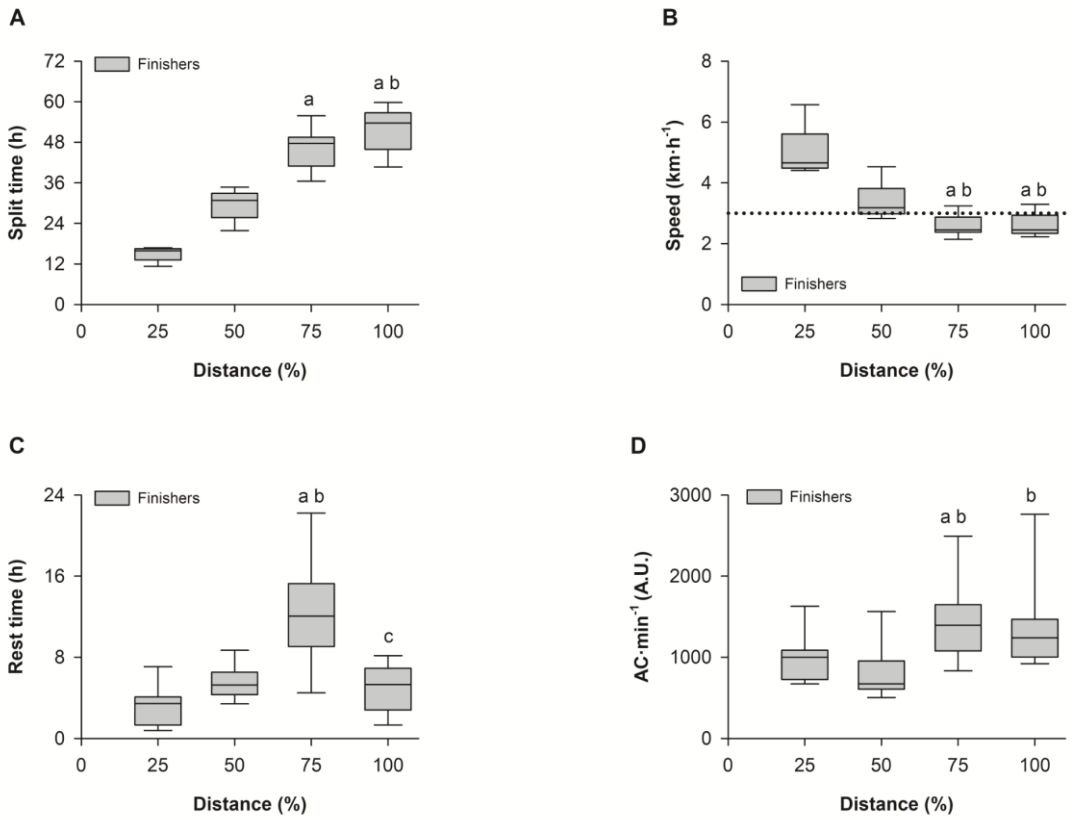


Figure 3.11 Pooled data from TSR1, TSR2, and TSF finishers: race time (A), rest time (B), speed (C), activity counts per minute (D). a denotes difference from 25%, b denotes difference from 50%, c denotes difference from 75%. Dotted line depicts mean average speed. Boxplot shows medians and interquartile ranges. Whiskers indicate min and max.

Sleep data

Number of sleep bouts was different over the course of the race. $\chi^2(3) = 44.143$, $p < 0.001$. Follow-up analysis revealed that the number of sleep episodes in the first quarter (*Mdn* = 0; *IQR* = 0) was lower than in the third (*Mdn* = 2; *IQR* = 0; $p < 0.001$), and last (*Mdn* = 3; *IQR* = 2; $p < 0.001$). The number of sleep bouts in the second quarter (*Mdn* = 1; *IQR* = 1) was lower than in the third ($p = 0.021$), and last ($p = 0.001$). No significant differences were found between other points Figure 3.12.A.

The number of times that sleep bouts happened out of the main checkpoints was different over the course of the race. $\chi^2(3) = 22.043$, $p < 0.001$. Post-hoc analysis disclosed that this was more frequent in the last quarter of the race (*Mdn* = 1; *IQR* = 2) than in the first one (*Mdn* = 0; *IQR* = 0; $p = 0.005$). No significant differences were found between other points Figure 3.12.B.

Time in bed was different over the course of the race. $\chi^2(3) = 39.810$, $p < 0.001$. Follow-up analysis indicated that participants spent less time in bed during the first quarter of the race (*Mdn* = 0; *IQR* = 0) than in the third (*Mdn* = 5.63 h; *IQR* = 3.27 h; $p < 0.001$), and last (*Mdn* = 6.51 h; *IQR* = 3.45 h; $p < 0.001$). Time in bed in the second quarter of the race (*Mdn* = 1.31 h; *IQR* = 1.83 h) was lower than in the third ($p = 0.026$), and last ($p = 0.004$). No significant differences were found between other points Figure 3.12.C.

Sleep time was different over the course of the race. $\chi^2(3) = 40.178$, $p < 0.001$. Post-hoc analysis revealed that participants slept less in the first quarter (*Mdn* = 0; *IQR* = 0) than in the third (*Mdn* = 5.33 h; *IQR* = 1.75 h; $p < 0.001$), and last (*Mdn* = 5.89 h; *IQR* = 3.45 h; $p < 0.001$). Sleep time was lower in the second quarter (*Mdn* = 0.98 h; *IQR* = 0.83 h) of the race than in the third ($p = 0.039$), and last ($p = 0.003$). No significant differences were found between other points Figure 3.12.D.

Participants stayed awake 8.33 h [95 CI: +1.57, +15.10] more between the start of the race and the first sleep bout (24.6 ± 4.9 h), than between the last sleep bout and the end of the race (16.3 ± 11.3 h), $t(16) = 2.612$, $p = 0.019$.

No significant differences were found in sleep efficiency over the course of the race ($p = 0.913$). Figure 3.12.E.

No significant differences were found in activity counts per minute asleep over the course of the race ($p = 0.905$). Figure 3.12.F.

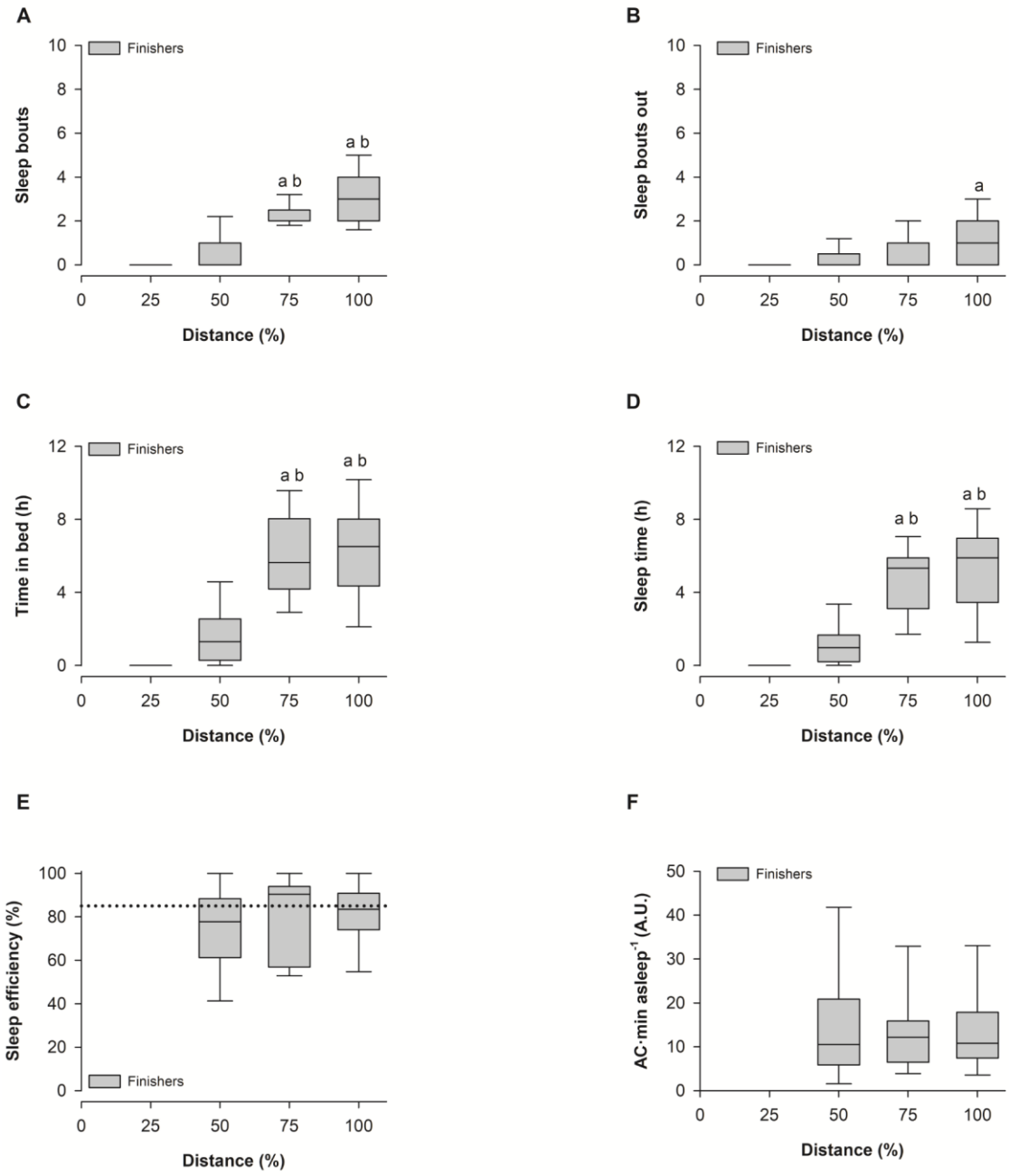


Figure 3.12 Pooled data from TSR1, TSR2, and TSF finishers: number of sleep bouts (A), number of sleep bouts out of the main checkpoints (B), time in bed (C), sleep time (D), sleep efficiency (E), activity counts per minute asleep (F). a denotes difference from 25%, b denotes difference from 50%. Dotted line depicts 85% sleep efficiency. Boxplot shows medians and interquartile ranges. Whiskers indicate min and max.

Sleepiness

Subjective sleepiness was different over the course of the race. $\chi^2(4) = 33.940$, $p < 0.001$. Follow-up analysis revealed that participants reported lower scores of subjective sleepiness (1-9) was at the start of the race (*Mdn* = 2; *IQR* = 1) than in the second (*Mdn* = 7; *IQR* = 1; $p < 0.001$), third (*Mdn* = 7; *IQR* = 3; $p < 0.001$), and last quarters (*Mdn* = 8; *IQR* = 2; $p = 0.003$). No significant differences were found between other points. Figure 3.13.A.

Perceived exertion

RPE was different over the course of the race. $\chi^2(3) = 7.976$, $p = 0.047$. Post-hoc analysis did not disclose significant differences between points. Figure 3.13.B.

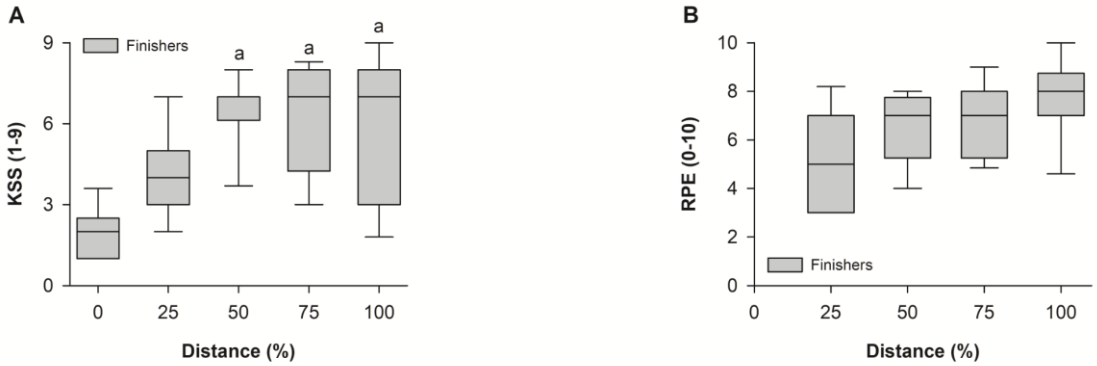


Figure 3.13 Pooled data from TSR1, TSR2, and TSF finishers: subjective sleepiness (A), ratings of perceived exertion (B). a denotes difference from 0%, b denotes difference from 25%, c denotes difference from 50%, d denotes difference from 75%.

Finishers' race time associations with other variables

Normative data

No significant associations were found between race time and age ($p = 0.305$), height ($p = 0.790$), body mass ($p = 0.348$), BMI ($p = 0.254$), $\dot{V}O_{2\ peak}$ ($p = 0.786$), years

competing (p = 0.985), number of sessions per week (p = 0.387), hours per week (p = 0.273), sleep quality (p = 0.163), and chronotype (p = 0.305).

Race performance

A moderate positive association was found between race time and total rest time (r = 0.655, p = 0.004).

No significant associations were found between race time and activity counts per minute (p = 0.786).

Sleep data

A high positive association was found between race time and total number of sleep bouts (r = 0.789, p < 0.001), Moderate positive associations were found between total number of sleep bouts out of the main checkpoints (r_p = 0.600, p = 0.011), total time in bed (r = 0.611, p = 0.009), and total sleep time, (r = 0.532, p = 0.028).

No significant associations were found between race time and average sleep efficiency (p = 0.227), and total activity counts per minute asleep (p = 0.313).

Sleepiness

A high positive association was found between race distance and sleepiness (r_p = 0.513, p < 0.001).

Perceived exertion

A low positive association was found between race distance and RPE (r_p = 0.409, p < 0.001). A low negative association between RPE and running speed was found (r_p = -0.436, p < 0.001).

Pooled data: finishers and non-finishers

In order to further investigate some variables, data were pooled into finishers (N = 17, 1 female) and non-finishers (N = 10, 2 females) groups.

Finishers (9 ± 4 years) had 5 years of experience more than non-finishers (5 ± 2 years), p = 0.003. Finishers' MEQ score (62 ± 7) was 7 points higher than non-finishers (55 ± 11), p = 0.046. The higher the MEQ score, the more morning-type chronotype.

No significant differences between groups were found for age (p = 0.617), height (p = 0.470), body mass (p = 0.889), BMI (p = 0.604), $\dot{V}O_{2\ peak}$ (p = 0.210), sessions per week (p = 0.925), hours per week (p = 0.318), PSQI score (p = 0.084), and subjective sleepiness at the start (p = 0.632).

3.5 Discussion

In the first half of the race, the highest running speed and lowest rest, sleep, sleepiness, and RPE values were observed, agreeing with the idea that athletes are fresh at the beginning of the race, particularly, in terms of sleep, during the first quarter of the race, the strategy adopted was not to sleep at all. Participants were awake for more than 24 hours before their first sleep bout. However, in the second half, it was the opposite, speed was reduced while rest, sleep, and activity counts increased. Accumulated fatigue and cognitive impairments might explain this phenomenon, RPE increased over the course of the race, but no significant differences between points were found. Subjective sleepiness reported increased significantly, compared to the start of the race. Interestingly, in terms of sleep quality, described as good when sleep efficiency is higher than 85%,

was not affected, no differences in sleep efficiency or activity counts per minute asleep were found. It seems that race performance was strongly related to rest and sleep. The lower the rest and sleep times the lower the race duration, and therefore, better race performance.

From all the normative data collected, the main differences between finishers and non-finishers were years of experience participating in ultra-marathons, and chronotype classification: finishers were predominantly "moderately morning type", while non-finishers were "intermediate type". Compared to offshore solo sailors during a 4021-mile race, a cohort that faced sleep deprivation during a competition, 40% of the participants (N = 42), were classified as "morning type" chronotype (Filardi, Morini and Plazzi, 2020). Furthermore, 71% of ultra-marathon swimmers (N = 24) that participated in the study conducted by Dunican *et al.* (2022) reported a "morning type" or "moderately morning type" chronotype. It seems that in ultra-endurance competition, morning chronotypes are predominant. However, further investigation is required to better understand this phenomenon and any potential relation to performance.

Race performance

Seventeen out of twenty-seven participants (63%) reached the finish line. The sample size obtained in our study is in line with the total drop-out rates in this particular ultra-marathon, ranging from 44.9 to 67.5%, calculated according to the race results published by the race organisers. Finishers completed the event in 141.8 ± 19.1 h at an average speed of 3.0 ± 0.5 $km \cdot h^{-1}$. An overall positive pacing was observed, supporting the data reported in the literature in events ranging from 100 to 161 km (Lambert *et al.*, 2004; Parise and Hoffman, 2011; Hoffman, 2014; Knechtle *et al.*, 2015; Renfree, Crivoi do Carmo and Martin, 2016; Tan, Tan and Bosch, 2016). Speed decreased from the start of the race until the end. In the third and last quarter of the race, speed was maintained while race time and activity counts increased in this period. However, rest time only increased in the third quarter, followed by a reduction in the last quarter of the race. Despite no increase in running speed, athletes might deliberately decide to reduce their rest time influenced by the proximity of the end of the race. In addition, as explained in

the following section, sleep parameters increased in the third and last quarter of the race. A potential explanation might be the fact that, due to the length of the event, as time went by, accumulated fatigue required more time to rest and recover, as well as more effort and activity counts to carry on, despite the reduction in speed. Rest time reported was 26.3 ± 8.6 h. In other words, 18.3 ± 5.1% of race time was used for activities (i.e., restoring food/drinks at the checkpoints, resting, sleeping) other than moving towards the finish line. It could be hypothesised that, the less time you spent in these actions the better your finish time will be. A moderate positive association between rest time and race time was found (r = 0.655)

Sleep data

In the present study, participants went to sleep 6 ± 2 times. Out of these sleep episodes, 2 ± 2 were outside the main checkpoints. Participants did not stop to sleep during the first quarter of the race (105 km). The number of sleep bouts significantly increased after the middle of the race. The number of sleep episodes increased notability during the last quarter of the race, with an increase in the number of sleep episodes out of the main checkpoints. This might be influenced by several factors: Hurdiel *et al.* (2018) suggested that the sleep debt accumulated during the event may not be compensated by small amounts of sleep, therefore an increased number of sleep episodes could be a symptom of exertion. In addition to that, the proximity of the finish line may also have contributed to explain the increased number of sleep episodes outside the main checkpoints during the last quarter of the race. In light of the high positive association between number of sleep bouts and race time (r_p = 0.826), our findings are in the same direction as data reported in shorter events, such as *Ultra-Trail du Mont Blanc* (168 km), where most finishers did not sleep and completed the race faster than those who slept (Hurdiel *et al.*, 2015; Poussel *et al.*, 2015). Nevertheless, this sleep strategy (no sleep) might not be feasible for slower runners, or during events lasting several days.

Participants spent 13.9 ± 4.9 h in bed (either asleep or awake). Time in bed progressively increased over the course of the race, particularly during the second half of the race.

Athletes slept 11.2 ± 3.6 h during the race, 7.8 ± 2.4% of race time. Our results are in the range of the results reported in studies during a 330 ultra-marathon: 7.43% in a study with fifteen runners (Saugy *et al.*, 2013); 9.32% in a case study (Savoldelli *et al.*, 2017). In a 168 km ultra-marathon: 1.33% in a study with ninety-two runners (Hurdiel *et al.*, 2018); 0.56% in a study with seventeen participants (Hurdiel *et al.*, 2015). A moderate positive relationship between sleep time and race time was found (r = 0.532). In races longer than 60 h, a positive relationship (r = 0.440) between self-reported sleep duration and finish time has been found (Martin *et al.*, 2018).

No changes in sleep efficiency were observed during the race, averaging 81.6 ± 13.3%. Lower sleep efficiency indicates poor sleep, across all age groups, the National Sleep Foundation considers good sleep quality when sleep efficiency ≥ 85% (Ohayon *et al.*, 2017). However, that threshold was calculated under normal sleep conditions and with a standard population. During exceptional circumstances, such as an ultra-marathon, further research should investigate whether it is a valid sleep efficiency threshold or a more specific one is required. An increase in time in bed and sleep time may have been a response to an accumulated poor sleep quality, in addition to the increase in fatigue and recovery needs caused by ultra-endurance exercise. Our data did not support that hypothesis. But participants were not asked to rate their perceived sleep quality, which is different from sleep efficiency (total sleep time divided by time in bed). Future studies can easily address this question using a visual analogue scale. No differences were found in activity counts per minute asleep. Body movements asleep are often related to Stage 1 NREM and REM sleep (Gori *et al.*, 2004), but the equipment used to monitor sleep did not have a feature to analyse and interpret actigraphy data in relation to sleep phases. Little is known regarding sleep phases during ultra-marathon.

Athletes stayed awake for 24.6 ± 10.3 h before their first sleep bout, and 16.3 ± 11.3 h between their last sleep bout and the end of the race. One of the two runners that took part in a study during a 216 km ultra-marathon reported a period of wakefulness of about 25 h (Doppelmayr, Finkernagel and Doppelmayr, 2005). In shorter events, where some athletes reported no sleep at all, longer periods of wakefulness have been described. Mainly because of the significant shorter race durations, between 29.4 and 46.2 hours in a 168 km event (Hurdiel *et al.*, 2018). The longer the time awake, the worse the cognitive

performance (Doppelmayr, Finkernagel and Doppelmayr, 2005; Hurdiel *et al.*, 2018). Furthermore, cognitive performance follows a circadian rhythm, peaking in the early evening and reaching the lowest point in the early morning hours (Goel *et al.*, 2013). This is particularly important in events where navigation is required: impaired vigilance and sustained attention may have an impact on the ability of runners to follow tracks, calculate directions, or encounter off-track distractions. The circadian process of cognitive performance may be considered when planning sleep strategies for ultra-marathons. Our results propose that runners sustained a longer period of wakefulness at the start than at the end of the event.

Sleepiness

Compared to the start of the race, subjective sleepiness increased. A significant moderate positive association was found between sleepiness and distance run ($r_p = 0.513$). This is consistent with the results reported by Hurdiel *et al.* (2018). These authors further investigated the effect of the time of the day on sleepiness values. Sleepiness followed the same circadian modulation as cognitive performance, being sensitive to the time of the day. Unfortunately, that was out of the scope of our study. The same considerations mentioned in the previous section would be applicable for sleepiness too.

Perceived exertion

RPE increased during the race with no significant differences between points. Our findings are aligned with the literature: RPE increased across the course of a 68 km ultra-marathon (Utter *et al.*, 2003); 73 km ultra-marathon (Chapman and Mickleborough, 2009); 80.5 km treadmill run (Howe *et al.*, 2019); 87 km ultra-marathon (Mann *et al.*, 2015), and in 24-h running competitions (Martin *et al.*, 2010). A significant low positive association was found between RPE and distance run ($r_p = 0.409$). A significant low negative association between RPE and split speed was found ($r_p = -0.436$). Similar associations have been observed in Chapman and Mickleborough (2009). In that study, moderate and high associations were reported between RPE and distance ($r = 0.737$), and

between RPE and split speed (r = -0.687) respectively. These relationships suggest that runners may regulate their speed according to their perceived exertion. RPE increased at lower running speeds in runners after one night of sleep deprivation (Oliver *et al.*, 2009).

Differences between finishers and non-finishers

In the present study, finishers had more experience competing in ultra-marathon events than those who did not finish. Unsurprisingly, experience in ultra-endurance events has been pointed out as key to success in ultra-marathons (Knechtle *et al.*, 2009; Knechtle, 2012; Hoffman and Parise, 2015).

Finishers' MEQ score (62 ± 7) was 7 points higher than non-finishers (55 ± 11). Given the fact that "early morning" and "intermediate" types are the most common chronotypes in elite athletes across different sports (Lastella *et al.*, 2016), competitive marathon male runners were more morning-oriented than physically active men (Henst *et al.*, 2015). Consequently, differences found between groups in our study are not relevant *per se*. (Brager *et al.*, 2020) has hypothesised that, in theory, "early morning" chronotypes might deal better with ultra-marathon demands, due to a higher pain tolerance (Jankowski, 2013), and better thermoregulatory control (Muginshtein-Simkovitch *et al.*, 2015) than "evening types" under sleep loss conditions.

Limitations

The main weakness of the present study is the lack of data regarding the use of stimulant substances during the events. Caffeine is used as an ergogenic aid in sports (Del Coso, Muñoz and Muñoz-Guerra, 2011), and it is known to reduce RPE (Doherty and Smith, 2005). Furthermore, caffeine is widely used to enhance wakefulness (McLellan, Caldwell and Lieberman, 2016). Moreover, in a study investigating sleep habits and strategies of ultra-marathon runners, 12.7% reported the use of stimulants to resist the sleep pressure during competitions (Martin *et al.*, 2018).

Polysomnography is the gold standard method for sleep monitoring. Sadly, it is not very suitable or ecologically valid in a field environment. For these reasons, actigraphy has been proposed as an alternative, a wrist-worn actigraphy device is a low-cost, non-invasive option, commonly used by sleep and sport scientists. In relation to polysomnography, actigraphy devices used in this study were validated against this method, but it is known that actigraphy commonly overestimates total sleep time and sleep efficiency (Walsh *et al.*, 2020). Despite having recalled sleep data with participants at each checkpoint to cross-check actigraphy data, this technology cannot record the so-called microsleep (lasting between 1 to 15 s). Hurdiel *et al.* (2015) has suggested that athletes may experience several microsleep episodes over the course of an ultra-marathon, helping them to maintain performance. Unfortunately, the devices used lacked sleep stages measurement. Additionally, anxiety around sleep may be caused by the use of the watches during competition. In addition to that, several sleep-related factors were not studied, such as daytime sleepiness, sleep management strategies before and during the race, and previous experience with sleep deprivation. That information could have helped to better understand the behavioural approach of the participants to sleep during an ultra-marathon.

Given the fact that data were collected from three different races over the same route, several aspects should be taken into consideration when looking at the findings of the present study. Due to the nature of field studies, and the drop-out rates observed in ultra-marathons, pooled data is not an uncommon practice in ultra-marathon research (Parise and Hoffman, 2011; Knechtle, 2012; Hoffman, 2014; Hoffman and Parise, 2015; Knechtle *et al.*, 2015; Tan, Tan and Bosch, 2016; Tonacci *et al.*, 2016). The events related in this study took place under various times of the year. Therefore, weather conditions and day lengths were different (further details in Table 3.4.). This was particularly noticeable in TSR1, when the race was held for ten hours, due to extreme adverse weather conditions outside. Stoppage happened in the late evening and the race was restarted in the early hours of the following day, involving all the participants, who were told to remain indoors. Race / sleep strategies may have been affected because of this. No differences with the rest of the data were observed, so it is very unlikely that TSR1 data (N = 2) might have had a considerable influence in our results (N = 17). Our data

comprised performances from athletes that completed the event within 53.5% and 99.1% of the allowed time, ranking from 2nd to 72nd.

It is well established that the integration of several signals are involved in perception of effort (Millet *et al.*, 2011). Many factors that could have influenced RPE were not taken into consideration in our study: cognitive performance, muscle-related factors regarding central and peripheral fatigue; self-efficacy; emotion regulation; motivation; mood.

Fluid and caloric intake were not monitored in this study. Gastrointestinal distress is among the most common symptoms experienced by ultra-marathon runners (Hoffman, 2016). These two factors may have an influence in exercise regulation during ultra-marathon events.

3.6 Conclusions

In conclusion, the present study described several sleep-related parameters during a ~431 km ultra-marathon race. In the 141.8 ± 19.1 h that took participants to complete the event, 11.2 ± 3.6 h of sleep in 6 ± 2 sleep episodes were recorded. Coupled with an extended period of wakefulness before the first sleep bout, it seems that most of the runners deliberately chose not to sleep at the initial stages of the race. However, just as performance decreased throughout the race, rest and sleep times increased, suggesting an increased sleep pressure, potentially, as a consequence of the psychophysiological demands of ultra-endurance exercise, and the exacerbated sleep loss. Sleep efficiency and movement while asleep were not worsened over the course of the race. In agreement with the current literature in shorter ultra-marathons, sleep was positively related to finish time, in other words, the better the performance, the less sleep. Nevertheless, from a logistical point of view, less time spent at rest implies less time at the main checkpoints doing other activities than sleep (i.e., eating, refuelling hydration / nutritional options, chatting, looking for equipment in their backpacks). This could be an improvement area for athletes to be more time-efficient at the checkpoints, in particular during self-supported events.

Further research is needed to better understand optimal sleep strategies in ultra-marathon events that, due to its length, not to sleep at all might not be a feasible strategy for the vast majority of athletes. Potential interventions should aim to reduce the sleep pressure. Caffeine is a widely available substance that promotes wakefulness and improves endurance performance. Some ultra-marathon runners had already reported the use of caffeine as part of their sleep strategy. But little is known about the effects of caffeine during competitions lasting several days. Timing and dosage are also a key factor to take into account, due to its detrimental effect on sleep. Despite being the most adopted strategy at the start of the race, sleep deprivation has a negative effect on both cognitive and endurance performance. Nevertheless, no studies had investigated yet the effects of different sleep allocation over consecutive days on endurance performance (i.e., over a 3-day period, sleep deprivation on the first night followed by four hours of sleep on the second night, versus two hours of sleep during the first and second nights). Another pathway to further explore is to potentially reduce the circadian misalignment induced by total sleep deprivation, which has a detrimental effect on several areas, such as cognition; immune response; metabolic function; inflammatory response; bone health; and cardiovascular function.

Chapter 4: The Effects of Caffeine on Running Performance after One Night of Total Sleep Deprivation.

Main findings: 6 mg·kg^{-1} of caffeine, after one night of total sleep deprivation, improved time trial performance by 5.54%. Caffeine supplementation after prolonged periods of wakefulness could be an effective measure to counteract the negative effects associated with sleep deprivation.

4.1 Abstract

The purpose of the study was to investigate the effects of caffeine on endurance performance after one night of sleep deprivation. Ten subjects visited our laboratory on three separate occasions: a preliminary visit to perform a $\dot{V}O_{2\ max}$ test and to familiarise with the protocol, equipment and questionnaires used; two experimental visits, in a randomised order, a pill containing 6 mg·kg^{-1} of caffeine or placebo were given to the participants after a night of sleep deprivation. The Alpha Attenuation Test (AAT), the Psychomotor Vigilance Test (PVT), and the Stroop Test were administered to assess sleepiness, vigilance, and inhibitory control, respectively. Participants run for 30 minutes at a constant workload at 60% $\dot{V}O_{2\ max}$ (CWL) followed by a 30-minute time trial (TT). During the running bouts, cardiopulmonary response, blood lactate, ratings of perceived exertion (RPE), and ratings of feelings (FS) were measured. Questionnaires revealed that, in comparison to placebo, subjective sleepiness was lower (p = 0.011), perceived performance was higher (p = 0.024), with no differences in motivation (p = 0.668) nor success in task (p = 0.522). Compared to placebo, despite no differences found in ATT (p = 0.784), caffeine enhanced PVT performance (reaction time, p = 0.001; number of lapses, p = 0.008), and Stroop test performance (incongruent stimuli: reaction time, p = 0.008; accuracy, p = 0.022). During CWL, RPE was lower in caffeine condition (p =

0.034). Caffeine increased minute ventilation ($p = 0.009$) and tidal volume ($p < 0.001$). Caffeine improved TT performance ($p = 0.008$) by 319 m (5.54%). Consequently, running speed was higher in caffeine ($p = 0.008$). At minute 10, RPE was lower in caffeine than in placebo ($p = 0.018$). Physiological parameters were greater in caffeine (heart rate, $p = 0.013$; blood lactate, $p = 0.005$; $\dot{V}O_2$, $p = 0.005$; $\dot{V}CO_2$, $p = 0.012$; minute ventilation, $p = 0.002$; tidal volume, $p = 0.002$). No differences were found in FS neither during CWL ($p = 0.073$) nor during TT ($p = 0.112$). Our results provide evidence that, after one night of sleep deprivation, caffeine can improve running performance. An enhancement in cognitive functions, such as vigilance and inhibitory control, followed by a reduction in RPE during exercise, might have allowed participants to sustain a higher speed during TT. As a result of that, increases in physiological parameters, such as heart rate, $\dot{V}O_2$, $\dot{V}CO_2$, VT, and blood lactate were observed. Caffeine supplementation could be an effective measure to counteract the negative effects associated with prolonged periods of wakefulness in both cognitive and running performance.

4.2 Introduction

As part of the conclusions of the study about sleep during mountain ultra-marathon events (Chapter 3), one potential intervention to reduce the sleep pressure is the use of caffeine. Mainly consumed as coffee, present in many foods and beverages, caffeine is one of the most popular psychoactive substances in the world. The effects of caffeine ingestion have been investigated since the early 1990s (Rivers and Webber, 1907). Athletes from a wide variety of sporting disciplines use caffeine as an ergogenic aid (Del Coso, Muñoz and Muñoz-Guerra, 2011). In a recent systematic review and meta-analysis, a small but significant effect of caffeine on endurance performance has been reported, suggesting that caffeine doses between 3 and 6 $mg \cdot kg^{-1}$ has much the same ergogenic effect on endurance performance (Southward, Rutherfurd-Markwick and Ali, 2018). Depending on the exercise protocol and modality, diverse increases in performance have been found. Caffeine improved performance in 3.2 ± 4.3% across different exercise modalities (Ganio *et al.*, 2009). In running, compared to placebo, caffeine increased time trial performance by 2.2 ± 5.59% (Southward, Rutherfurd-Markwick and Ali, 2018). In a previous meta-analysis, including time to exhaustion

protocols, a 19.0 ± 13.6% improvement in running performance was found (Doherty and Smith, 2005). Differences in performance improvement may be explained by the use of different exercise protocols.

Potential mechanisms of action to explain the ergogenic effects of caffeine for endurance performance have been proposed. The most widely accepted factor is the role of caffeine as an antagonist of A_1 and A_{2a} adenosine receptors (Graham, 2001; Doherty and Smith, 2005; Ganio *et al.*, 2009; Spriet, 2014; McLellan, Caldwell and Lieberman, 2016; Southward, Rutherfurd-Markwick and Ali, 2018). During whole-body exercise, caffeine induces a near 6% reduction in ratings of perceived exertion, RPE (Doherty and Smith, 2005). A possible explanation for that is a reduction in the activity of cortical areas related to the proposed sensory areas for perception of effort. In submaximal isometric knee-extension, compared to placebo, after caffeine ingestion, a decrease in the activity of the premotor and motor areas of the cortex with a consequent reduction of perception of effort was reported (de Morree, Klein and Marcora, 2014). From a cognitive perspective, vigilance, attention, and mood could be enhanced due to the action of caffeine as an adenosine receptor antagonist (McLellan, Caldwell and Lieberman, 2016).

Sleep is a biological process involved in the recovery and maintenance of several physiological and cognitive functions (Cirelli and Tononi, 2008; Mignot, 2008; Roth, Rattenborg and Pravosudov, 2010). According to the National Sleep Foundation, for adults between 18 and 64 years old, 7-9 h of sleep every 24 hours is the optimal sleep duration (Hirshkowitz *et al.*, 2015). Sleep deprivation can be considered as a prolonged period of wakefulness (i.e., 24 hours without sleep). Concentrations of adenosine play a key role in sleep/wake control (Porkka-Heiskanen *et al.*, 1997). Sleep deprivation increases adenosine in the basal forebrain and cortex (Porkka-Heiskanen, Strecker and McCarley, 2000). Caffeine blocks adenosine receptors on GABA neurons that might take part in an inhibition of hypothalamic neurons involved in the sleep process (Strecker *et al.*, 2000). The use of caffeine to increase wakefulness has been studied extensively, for a review see McLellan, Caldwell and Lieberman (2016). From a neurobiological point of view, sleep deprivation decreases activity between the medial prefrontal cortex (PFC) and ventral anterior cingulate cortex (ACC) with the amygdala (Yoo *et al.*, 2007; Motomura

et al., 2013). Regions of the stratum implicated in emotion regulation and motivation (e.g., the caudate, putamen) are highly sensitive to sleep (Gujar *et al.*, 2011).

In a review about the effect of sleep loss on athletic performance, Fullagar *et al.* (2015) have pointed out evidence to sustain that sleep deprivation has a negative effect on exercise performance: Total distance covered in a 30-min self-paced running time trial was reduced by ~3% after one night of sleep deprivation (Oliver *et al.*, 2009); time to exhaustion in a walking test decreased ~11% after 36 hours of sleep deprivation (Martin, 1981) . However, the mechanisms behind this remain uncertain. It has been hypothesised that reductions in performance could be due to the influence of perception of effort in high-intensity exercise, with no changes observed in cardiopulmonary parameters (Oliver *et al.*, 2009). Blood lactate accumulation was not altered by ~50 hours of sleep deprivation after a time to exhaustion walking test (Martin and Chen, 1984).

It is well established that several cognitive functions are impaired by sleep deprivation (Durmer and Dinges, 2005; Killgore, 2010; Lim and Dinges, 2010). It has been suggested that sleep deprivation may notably affect the prefrontal cortex (PFC) due to its use during long periods of wakefulness (Harrison, Horne and Rothwell, 2000). Furthermore, inhibitory control, an executive function crucial to behavioural self-regulation, seems to be modulated by the dorsolateral prefrontal cortex (DLPFC). Inhibitory control may play an important role for the regulation of exercise, restraining the unpleasant sensations commonly associated with exercise, such as muscle pain or thermal discomfort (Hagger *et al.*, 2010). Inhibitory control was impaired over the course of two nights of sleep deprivation (Drummond, Paulus and Tapert, 2006).

Summarising, normal affective processing is altered by sleep deprivation. Killgore (2010) suggested that, perhaps, debilitated prefrontal inhibitory systems would not allow appropriate integration of emotions, leading to a negative emotional bias in mood, perception, decreased tolerance to frustration, and impaired emotion regulation for decision making.

In the literature, several studies have investigated the use of caffeine as a way to reduce the negative effects of sleep deprivation on endurance performance. In military personnel, after 28 h of sleep deprivation, 400 mg of caffeine, followed by 2 doses of 100

mg during the night, improved 25% time to exhaustion in a running test at 85% of $\dot{V}O_{2\ max}$ with lower RPE values reported (McLellan, Bell and Kamimori, 2004). In soldiers after 27 h of sleep deprivation, three subsequent doses of 200 mg of caffeine during the night improved a 6.3 km running time trial by 3.47% (McLellan *et al.*, 2005). In recreational runners, 5 $mg \cdot kg^{-1}$ of caffeine improved 8 km running performance by 2.8% after 26 h of sleep deprivation (Khcharem *et al.*, 2022). On the other hand, 6 $mg \cdot kg^{-1}$ of caffeine did not have a significant effect in the Yo-Yo test after 24 h of without sleep (Donald *et al.*, 2017).

The aim of our study was to investigate the effect of 6 $mg \cdot kg^{-1}$ of caffeine on running performance after one night of total sleep deprivation. Our hypothesis was that time trial running performance would be improved after caffeine ingestion due to a reduction in RPE. Furthermore, we would expect cognitive performance to be enhanced by caffeine due to a reduction in sleepiness.

4.3 Methods

Participants

Ten recreationally active male runners (Age: 30.3 ± 5.6 years; Stature: 1.79 ± 0.05 m; Body mass: 76 ± 8.1 kg; $\dot{V}O_{2\ max}$: 51.4 ± 5.2 $mL \cdot kg^{-1} \cdot min^{-1}$; Peak treadmill speed: 17.3 ± 2.4 $km \cdot h^{-1}$; 5.2 ± 3.2 hours per week; 5-km personal best in the last 12 months: 22.3 ± 5 min; Self-reported caffeine intake: 186 ± 159 $mg \cdot day^{-1}$) took part in the study. Only non-smoker participants, free of a history of cardiovascular, metabolic, neurological, and pulmonary conditions, without a trunk or lower-limb soft tissue or bone injury or surgery in the last three months were included. Additionally, participants were assessed for chronotype and quality of sleep, using the Morningness-Eveningness Questionnaire, MEQ (Horne and Ostberg, 1976), and the Pittsburgh Sleep Quality Index Questionnaire, PSQI (Buysse *et al.*, 1989) respectively. Participants with extreme chronotypes (MEQ score < 30 or MEQ score > 70) and/or a PSQI score > 5 were excluded. Prior to being involved in the study, all participants were fully briefed about the study procedures. After that, they were asked to voluntarily give written informed

consent to participate in the study. Ethical approval was granted by the University of Kent, School of Sport and Exercises Sciences Research Ethics and Advisory Group. The study was conducted in accordance with the Declaration of Helsinki (2013).

Study Design

A randomised, double-blind, placebo-controlled, counterbalanced, crossover experimental design was used. Participants were required to visit the University of Kent, School of Sport and Exercise Sciences on three separate occasions. After a preliminary visit, the order of caffeine and placebo trials was randomised for the two experimental visits. Testing was conducted in the Psychobiology Laboratory. Participants spent the night of sleep deprivation in a room of the same building with one member of the research staff. Preliminary visit was completed between 3 and 10 days prior to the first experimental visits, and seven days between the first and the second experimental visit. A summary of the protocol can be found in Figure 4.1.

Participants were encouraged to keep their habitual routines during the whole duration of the experiment: food and sleep were monitored using 3-day diaries prior to each experimental visit. Participants' physical activity behaviour throughout the study was monitored using a daily and physical activity diary, and the leisure and occupational physical activity level scales (Saltin and Grimby, 1968). Experimental visits took place during the time frame (08:00 am - 11:00 am) when lack of sleep seems to have the most negative impact on cognitive function (Mollicone *et al.*, 2010). To control the effect of meals and hydration status on sleep deprivation (Smith, Maben and Brockman, 1993), participants were instructed to maintain their habitual diet and meal times and to drink an amount of water equal to 35 $mL \cdot kg^{-1}$ of body mass the day prior to each visit; 24 hours prior to each visit participants were asked to refrain any alcohol intake and avoid strenuous exercise; sport drinks, tea or caffeinated substances were forbidden within 12 hours before each visit; participants were encouraged to sleep at least seven hours prior to each visit. Prior to each experimental visit, participants were provided with a standardised breakfast to avoid the risk of hypoglycaemia during exercise (Sedliak *et al.*, 2007) and to control inter-individual variability among participants (Bougard *et al.*,

2009). A £100 Amazon voucher was given to all participants who completed the experiment as a monetary reward.

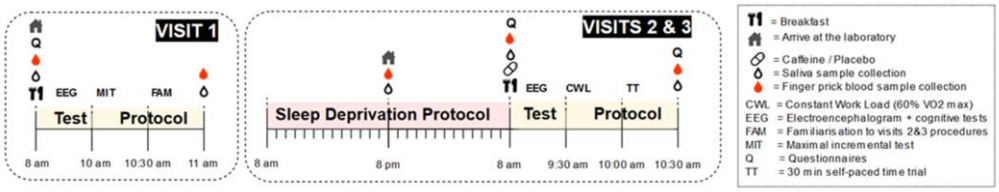


Figure 4.1 Timeline of the study

Testing Procedures

Preliminary visit and familiarisation

Participants were briefed about the testing procedures and were asked to complete a health questionnaire (PAR-Q). In order to check adherence to the instructions given, participants completed a pre-test checklist with questions related to any recent acute illness, infection and/or injury, and any medication/drug taken. Suitable participants were invited to voluntarily sign an informed consent form. Participants were given several questionnaires to complete regarding and competition history; caffeine consumption; motivation; and expectations. Participants were familiarised to the scales used during the experiment; Karolinska Sleepiness Scale, KSS (Akerstedt and Gillberg, 1990); intrinsic and success motivation scales, ISMS (Matthews, Campbell and Falconer, 2001), the Feeling Scale, FS (Hardy and Jack Rejeski, 1989), and the Ratings of Perceived Exertion Scale (RPE). Additionally, anchoring procedures were used to establish low and high RPE anchor points (Noble and Robertson, 1996). Further details in *Psychological Questionnaires* section.

Participants were asked to wear an electroencephalography (EEG) wireless cap prior to the start of cognitive testing (further details in the *EEG procedures* section). Participants were assessed for sleepiness using a valid method to measure decreases in alertness due to sleep loss, the 12-min Alpha Attenuation Test, ATT (Stampi, Stone and Michimori, 1995), After that, in order to measure cognitive performance, participants

were asked to complete a 10-min Psychomotor Vigilance Test (PVT) and a 3-min Stroop Test. Both tests have been used previously as valid and sensitive tools to assess decline in cognitive performance caused by sleep loss (Balkin *et al.*, 2004; Basner and Dinges, 2011). Further details in the *Cognitive Tests* section.

Height and weight were measured to the nearest 0.1 cm and 0.1 kg using a stadiometer and weight scale (Seca, Birmingham, UK) with the participant in bare or stocking feet. After a 5 minute warm-up at 8 $km \cdot h^{-1}$ participants performed a maximal incremental test on a motorised treadmill (Pulsar 3P; h/p/cosmos Sports and Medical, Nussdorf-Traunstein, Germany) with a fixed gradient of 1% to replicate the energetic cost of running outdoors in a flat surface (Jones and Doust, 1996). Test starting speed was set at 9 $km \cdot h^{-1}$ with 1 $km \cdot h^{-1}$ increments every minute. Test ended when participants reached volitional exhaustion. Heart rate (HR) response was recorded continuously during the test using a chest band and a monitor (Polar V800, Polar Instruments, Kempele, Finland). Expired gases were measured on a breath-by-breath basis using a metabolic cart throughout the entire test (MetaLyzer; Cortex Biophysik GmbH, Leipzig, Germany), calibrated before the test according to the manufacturer's specifications. Immediately after the test, a 5 µL capillary blood sample was taken from one of participants' fingers and blood lactate ($B[La^-]$) concentration was measured (Biosen; EFK Diagnostics, London, UK). Overall RPE was obtained every minute using the 15-point Borg's RPE scale following standard instructions (Borg, 1998). Criteria for achieving maximal effort was accepted when two of the following were attained: heart rate within 10 $beats \cdot min^{-1}$ of age-predicted maximum; RER ≥ 1.10; RPE ≥ 17; and $B[La^-] \geq 8$ $mmol \cdot l^{-1}$ (American College of Sports Medicine, 2014). $\dot{V}O_{2\,max}$ was determined as the highest 30-s average oxygen uptake. Peak Treadmill Speed (PTS) was calculated using the following formula (Kuipers *et al.*, 2003):

PTS ($km \cdot h^{-1}$) = last competed stage speed + [(seconds at last uncompleted stage / 60 s]

After test completion, participants rested for 30 minutes (seated, walking around the laboratory, and doing stretching exercises *ad libitum*). Then, participants were familiarised with all the testing procedures, questionnaires, scales, and protocols used in the experimental visits.

Experimental visits

For each experimental visit testing procedures were the same: in order to measure participants' sleep/wake activity, they were required to wear continuously, on their non-dominant hand, an actigraphy watch (Actiwatch Spectrum PRO, Philips Respironics, Murrysville, Pennsylvania, USA) from the day prior to the experimental visit until the start of the laboratory testing procedures after the sleep deprivation night. Participants were asked to maintain their usual get-up time, avoiding naps, keeping their regular diet, and physical activity routines during the day (further details in *Actigraphy* section).

At 08:00 pm participants were required to arrive at the School of Sport and Exercise Sciences to spend the night of total sleep deprivation. A member of the research staff stayed in the designated room monitoring the participants to ensure participants remained awake and to avoid any napping, food consumption and/or use of caffeinated substances. Activities such as holding a conversation with the researcher, listening to the radio or music, playing video games, reading, and/or watching movies or series were allowed during the night (Plukaard, 2015; Shortz *et al.*, 2015). To better control for inter-individual differences, apart from ambulatory movement (e.g., walking), activities that could influence fatigue were not allowed (e.g., light exercise). In order to control fluid intake during the night, participants were given an empty water bottle, with measurement marks in mL, they could refill *ad libitum* during the sleep deprivation night. The following morning, the total amount of water consumed was written down for the purpose of replication during the following sleep deprivation night. In order to control food intake during the night, at 02:00 am participants were allowed to eat two snack bars (130 kcal per bar, Special K, Kellogg Company, Battle Creek, Michigan, USA) and a 150 mL glass of orange juice (60 kcal per 150 mL, ASDA Orange Juice, Asda stores Limited, Leeds, UK).

At 08:00 am, participants were transferred to the laboratory where they were asked to complete a pre-test checklist to check adherence to the instructions given beforehand, after that, participants were assessed for sleepiness and motivation using the KSS and ISMS, respectively. Then, participants consumed a single capsule containing 6 $mg \cdot kg^{-1}$

body mass of caffeine (Food Grade; Sigma-Aldrich, Missouri, USA) during the caffeine trial (CAFF), and an identical-looking single capsule with 6 $mg \cdot kg^{-1}$ body mass of microcrystalline cellulose during the placebo trial (PLA). The capsule was swallowed with 150 mL of water. After that, participants consumed a standardised breakfast, composed of one 150 mL glass of orange juice and two snack bars as mentioned previously.

Participants performed AAT, PVT, and Stroop tests while wearing the EEG cap, as explained in the previous section.

Then, after a 5-min warm-up at a speed corresponding to 50% of their $\dot{V}O_{2\ max}$, participants performed a 30-min running constant workload test (CWL) at a fixed speed corresponding to 60% of their $\dot{V}O_{2\ max}$ followed by a 30-min self-paced time trial (TT), with a 5-min rest period between bouts. The only information that participants were able to see was the time remaining to finish the bout, displayed on a screen with a digital timer. HR was recorded continuously during both tests using a chest band and a monitor. Expired gases were measured using a breath-by-breath gas analyser throughout the entire duration of both tests. In order to measure $B[La^-]$ concentrations, a 5 µL finger capillary blood sample was taken from participants at three time points: prior to the start of CWL, at the end of CWL, and immediately after TT. Overall RPE and FS were collected every 5 minutes during each test. Participants were told to give their best performance prior to the start of the TT.

Lastly, after TT completion, participants were asked to complete the validated multidimensional scale NASA-TLX (Hart and Staveland, 1988) to estimate the subjective workload perceived.

Perceptual responses during exercise

Affective response: for the purpose of measuring exercise-related affect during the running tasks, the Feeling Scale, FS (Hardy and Jack Rejeski, 1989) was used. This is a bipolar scale of pleasure and displeasure ranging from -5 (“very bad”) to +5 (“very

good"). At the beginning of each visit, standardised written instructions were provided with an explanation and nature of the scale.

Perceived exertion: overall ratings of perception of effort (RPE) were measured using a 6-20 RPE Scale (Borg, 1998). At the beginning of each visit, standardised written instructions were supplied to the participants, including an explanation of the nature and use of the scale, a definition of perception of effort as "how effortful, heavy, and strenuous the exercise feels", (Marcora, 2010), and definitions of scale anchors (e.g., "13 Somewhat hard. It feels OK to continue"). The researcher reinforced the content of the instructions and answered questions after participants had read the instructions (Noble and Robertson, 1996).

Psychological questionnaires

Sleepiness: in order to measure subjective levels of sleepiness, the Karolinska Sleepiness Scale (KSS), developed by Akerstedt and Gillberg (1990), was used before and after each experimental visit. Using a 9-point Likert scale (1 = extremely alert; 2 = very alert; 3 = alert; 4 = rather alert; 5 = neither alert nor sleepy; 6 = some signs of sleepiness; 7 = sleepy but no effort to keep awake; 8 = sleepy, some effort to keep awake; and 9 = very sleepy, great effort to keep awake, fighting sleep).

Workload: the multidimensional rating scale NASA-TLX (Hart and Staveland, 1988) was used to evaluate the subjective workload experienced during the running task. This scale has six subscales: Mental Demand; Physical Demand; Temporal Demand; Performance; Effort; and Frustration. For each subscale, two descriptors are anchored at its end points: "very low" on the left end, and "very high" on the right one, with the exemption of Performance, which has "perfect" on the left endpoint and "failure" on the right one. Participants were required to complete this at the end of each experimental visit.

Cognitive tests

AAT: in order to assess objective decrements in alertness due to sleep loss, the Alpha Attenuation Test (ATT), created by Stampi, Stone and Michimori (1995), was used. In sleepiness conditions, EEG power alterations in the alpha band frequency (8-12 Hz) are expected to decrease with eyes closed and increase with eyes open. Test was performed in a laboratory lit (100-150 lux), participants were asked to remain seated, in front of a computer screen displaying a white asterisk on a black background as a fixation point. During the preliminary visit, the distance from the chair to the screen was established according to participants' preference and replicated during the experimental visits. The tests involved alternate 2-min cycles of eyes closed / opened for 12 minutes. EEG was continuously registered during the test. Participants were asked to avoid blinking, and to refrain from contracting facial muscles and moving, as a way of avoiding noise in the EEG signal.

PVT: as a tool to evaluate a decrease in vigilance caused by sleep loss (Balkin *et al.*, 2004; Basner and Dinges, 2011), a computerised version of the Psychomotor Vigilance Test (PVT) was used (E-Prime 2.0, Psychology Software Tools, Sharpsburg, Pennsylvania, USA). Participants were instructed to focus on the screen for the whole test duration and, as soon as a visual stimulus appeared in the centre of the computer screen, to press the keyboard spacebar button with their preferred hand. After each response, reaction time was shown for 1,000 ms in the centre of the screen prior to the next stimulus. Participants were encouraged to respond as fast and as possible. During the preliminary visit, the distance from the chair to the screen, and keyboard position on the table were established according to participants' preference and replicated during the experimental visits. A graphic bullseye on a white background was fixed as the visual stimulus. Inter-stimulus interval period was randomised with a range from 2,000 to 10,000 ms. Only responses ≥ 150 ms were considered valid. Responses < 150 ms (i.e., errors of commission) were considered false starts and a message was displayed on the screen. Responses ≥ 500 ms (i.e., errors of omission) were computed as lapses. Other responses, such as wrong responses (i.e., pressing another keyboard button), and lack of responses (≥ 30,000 ms) were also considered as errors. The following parameters were evaluated

as sensitive measures to sleep loss: mean reaction time (calculated excluding false starts and lapses), and number of lapses (Basner and Dinges, 2011).

Stroop Test: in order to assess decrements in vigilance due to sleep loss (Balkin *et al.*, 2004), the 3-min Stroop Test was used (E-Prime 2.0, Psychology Software Tools, Sharpsburg, Pennsylvania, USA). Participants were instructed to focus on the screen for the whole test duration, and to press the button on the response pad (RB-730, Cedrus Corporation, San Pedro, California, USA), corresponding to the printed colour of a word with the name of a colour displayed, with their preferred hand. Five colours were used in this test: blue, green, red, white, and yellow. Twenty-five randomly selected trials were included in this test. The visual stimulus used was either an incongruent colour word (i.e., 20 trials of words with colour names not corresponding to the printed colour of the words displayed; the word "blue" written in "green" colour) or a congruent colour (i.e., 5 trials of words matching the colour name with the printed colour of the word displayed; the word "blue" written in "blue" colour). Five aligned asterisks (Arial Rounded MT font, 36 font size, in bold) displayed in the same position on a black background were used as a fixation point preceding the stimulus. The duration of the fixation point on screen was 500 ms. Words (Arial Rounded MT font, 18 font size, in bold) were shown in the centre of the screen on a black background. The duration of the stimulus displayed was infinite. Participants completed a practice trial (i.e., 25 randomly selected trials) prior to the start of the test. Participants were encouraged to respond as fast and accurately as possible. During the preliminary visit, the distance from the chair to the screen, and response pad position on the table were established according to participants' preference and replicated during the experimental visits. Mean RT, numbers of errors and accuracy (total errors divided by total responses, expressed as a percentage) were evaluated as variables.

EEG procedures

Using a wireless 32-channel cap (g.Nautilus EEG system, g.tec Medical Engineering GmbH, Schiedlberg, Austria) with an active, gel-based Ag/AgCl reference electrode technology (g.LADYbird, g.tec Medical Engineering GmbH, Schiedlberg, Austria) EEG data were collected. Electrode placement in the cap was done according to

the 10-20 international system (Jasper, 1958). Reference electrode was placed on the right earlobe, and the ground electrode on the AFz site. Sampling frequency was set at 500 Hz with 12-bit resolution. Following manufacturer instructions, a 0.5 Hz - 30 Hz bandpass and a 48 - 52 Hz notch filter were also applied. Electrode impedances were checked prior to testing and maintained to < 30 kΩ. Using a wireless receiver connected to a computer, EEG data was transmitted via the 2.4 GHz band. In order to acquire and analyse EEG data Matlab R2014a (Mathworks, Natick, Massachusetts, USA) was used.

EEG data analysis

EEG data were analysed using SPM v12 (statistical parametric mapping, Wellcome Trust, London, UK). EEG analysis was done at the sensor level. Data was filtered for 0.5-30 Hz using 7th order Butterworth filter, downsampled to 128 Hz and montaged based on average electrode average activity. Then, noise artefacts (i.e., eye-blinks) were removed using FP2 electrode activity. Based on Singular Value Decomposition (SVD) mode, spatial confounds were denoted. Using Signal-Space Projection (SSP) correction mode, sensor data was revised. A maximum of two components of spatial confounds were removed from the EEG data.

EEG data analysis was focused, particularly, on Fz electrode activity and Alpha frequency band (8-12 Hz). Using Morlet wavelet transformation with seven wavelet cycles, time-frequency analysis was conducted on the 0.5-15 Hz frequency range. Then, time-frequency data was rescaled to log-ratio based on 100 ms activity prior to the beginning of each data section. Lastly, data was averaged over time and frequency band. An alpha attenuation coefficient (AAC) was calculated as the ratio of eyes closed/open in the Fz channel. The higher the alertness, the higher the AAC. Additionally, AAC method aims to minimise inter-individual variability (Stampi, Stone and Michimori, 1995).

Actigraphy

As a way to have an objective measure of sleep, an actigraphy watch (Actiwatch Spectrum PRO, Philips Respironics, Murrysville, Pennsylvania, USA) was used. This device continuously records light exposure and quantity of body movement (30-second epochs for this study) with a piezo-electric accelerometer. Default sleep/wake threshold (activity counts above 40) was used. Using a proprietary algorithm and software (Philips Respironics Actiware version 6.0.9) sleep parameters are calculated. This device and method have been used to assess sleep/wake behaviour in athletes several times (Halson *et al*., 2014; Sargent *et al*., 2014; Lastella *et al*., 2015a; Lastella *et al*., 2015b). For the purpose of this study, only total sleep times were reported.

Statistical analysis

Data were checked for assumptions of all statistical tests. Equivalent non-parametric tests were used when data failed to meet any assumption. Two-tailed paired-samples t-tests were used to analyse differences between trials for: distance covered in TT; motivation; sleep time the night before each trial; and workload perceived. With the purpose of checking the effects of sleep deprivation only (manipulation check), two-tailed paired-samples t-tests were used to compare placebo and preliminary visit (normal sleep) for AAT; PVT; sleepiness, and Stroop task. Two-way repeated measures ANOVAs were conducted to investigate differences between trials and six time points (5-min splits) during exercise, for physiological (i.e., HR, $\dot{V}O_2$) and perceptual parameters (i.e., affect, RPE). When the assumption of sphericity was not met, Greenhouse-Geisser correction was applied. Post-hoc analyses using pairwise comparisons were performed using Bonferroni correction, when significant interactions were found. To identify outliers (i.e., ± 3 SD) or input errors, studentized residuals were checked, if confirmed, outliers were not excluded.

As a measure of magnitude of significant differences in the most relevant variables, Cohen's d (d) effect sizes were calculated for paired-samples t-tests. Values greater than 0.2, 0.5, and 0.8 depict small, medium, and large effects, in that order (Cohen, 1988). For ANOVAs, partial eta squared (ηp^2) effect sizes were reported. Values greater than 0.01,

0.06 and 0.14, were anchored to small, moderate, and large effect sizes, respectively (Cohen, 1988). Data are presented as means ± SD unless otherwise stated. Data from variables not following a normal distribution are presented as medians (*Mdn*) preceded by the interquartile range (*IQR*). Statistical significance was accepted at $p < 0.05$ (two-tailed). Statistical analyses were performed using SPSS Statistics 28.0 (IBM, Armonk, New York, USA).

4.4 Results

Normative data

Using a 11-Likert Scale (from "-5 = very negative" to "+5 very positive"), participants rated their expectations regarding the effect of sleep deprivation (-4 ± 1), and caffeine (2 ± 1) on running performance, respectively. Six out of ten participants guessed correctly for the caffeine trial. The first experimental visit took place 5 ± 2 days after the preliminary visit. Experimental visits were separated by 8 ± 2 days. Two nights before each trial, participants slept 7.3 ± 1.4 h in caffeine, and 7.2 ± 1.6 h in placebo. Participants did not sleep the night before each trial, being awake for 23.3 ± 0.9 h in caffeine, and 23.6 h ± 1.7 h in placebo. Further descriptive data could be found in Table 4.1.

Table 4.1. Normative data

	Capsule ingestion (time)	Start of cognitive tests (min after capsule ingestion)	Start of exercise protocol (min after capsule ingestion)	Laboratory temperature (ºC)	Laboratory humidity (% RH)	Laboratory barometric pressure (hPA)
PLA	07:31-08:07 AM	59.5 ± 20.0	75.0 ± 16.7	20.2 ± 1.1	36.9 ± 6.4	1011 ± 12
CAFF	07:48-08:07 AM	52.2 ± 7.1	70.1 ± 10.3	19.8 ± 1.0	37.2 ± 7.0	1012 ± 14

Caffeine (CAFF), placebo (PLA). Data reported as range for time of capsule ingestion. And mean ± SD for start of cognitive tests, start of exercise protocol, and laboratory environmental conditions. Data are presented as mean ± SD.

Manipulation check: sleep deprivation

Subjective sleepiness (out of 9) was 2.4 points higher in placebo (6.70 ± 2.00) than in normal sleep (4.30 ± 1.25). t(9) = 3.032, p = 0.014. Figure 4.2.A.

Alertness (expressed as Alpha Attenuation ratio) was 1.1 points lower in placebo (1.12 ± 0.37) than in normal sleep (2.22 ± 1.15). t(8) = -3.461, p = 0.009. Figure 4.2.B.

PVT reaction time was 53.69 ms slower in placebo (342.19 ± 45.66 ms) than in normal sleep (288.49 ± 36.96 ms). t(9) = 3.129, p = 0.012. Number of lapses were higher in placebo (*Mdn* = 11, *IQR* = 4-33) than in normal sleep (*Mdn* = 0.5, *IQR* = 0-3.5). Z = 2.668, p = 0.008. Figures 4.2.C and 4.2.D.

For congruent stimuli in the Stroop test, reaction time was not significantly different between placebo and normal sleep (p = 0.280). No differences were observed neither in the number of errors (p = 0.396) nor accuracy (p = 0.327).

For incongruent stimuli in the Stroop test, reaction time was 59.32 ms slower in placebo (852.02 ± 179.09 ms) than in normal sleep (719.49 ± 116.36 ms). $t(9) = 2.711$, $p = 0.024$. No differences were observed neither in the number of errors ($p = 0.137$) nor accuracy ($p = 0.139$). Figures 4.2.E to 4.2.G.

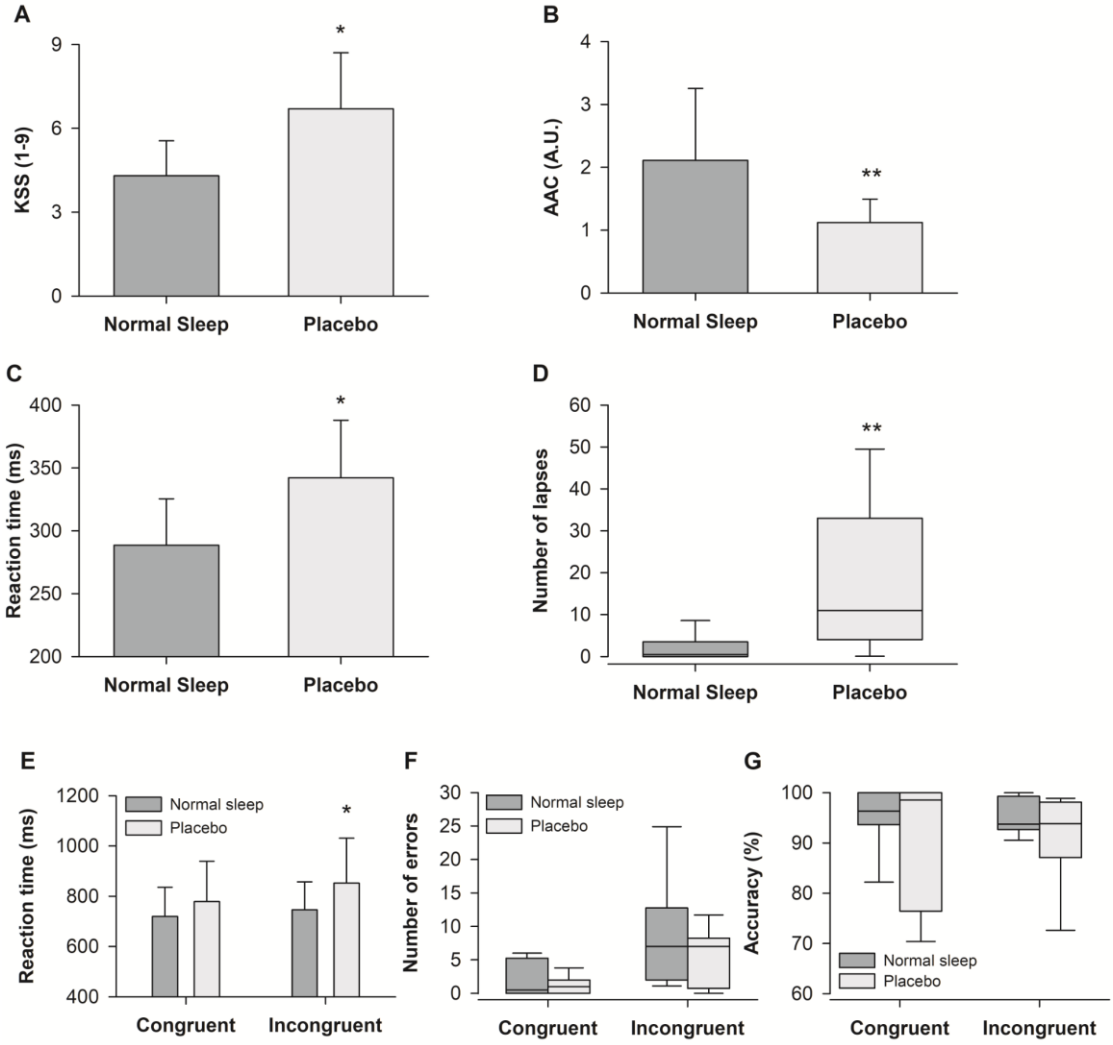


Figure 4.2 Manipulation checks: Sleep deprivation, Karolinska Sleepiness Scale (A). Alpha Attenuation Coefficient (B). Psychomotor Vigilance Test: reaction time (C), number of lapses (D). Stroop test: reaction time (E), number of errors (F), accuracy (G). Data presented as mean ± SD. Boxplot shows medians and interquartile ranges. Whiskers indicate min and max. * denotes difference between trials ($p < 0.05$). ** ($p < 0.01$).

Sleepiness

Subjective sleepiness (out of 9) was 1.6 points lower in caffeine (5.10 ± 1.97) than in placebo (6.70 ± 2.00). t(9) = -3.207, p = 0.011, d = 0.81. Figure 4.3.A.

Workload

Perceived performance (out of 100, inverted scale), was 16.5 points higher in caffeine (34.5 ± 21.1) than in placebo (51.0 ± 25.6). t(9) = -2.703, p = 0.024, d = 0.70. No differences were found in mental demand (p = 0.343), physical demand (p = 0.823), temporal demand (p = 0.309), effort (p = 0.302), and frustration (p = 0.337). Figure 4.3.B.

Motivation

No significant differences were found between caffeine and placebo in intrinsic motivation (p = 0.668), nor success on task (p = 0.522). Figure 4.3.C and 4.3.D.

Alertness

EEG data from participant number 2 were excluded due to technical reasons (bad EEG recording with too many artefacts). No significant differences were found in the Alpha Attenuation Test between placebo and caffeine, p = 0.784. Figure 4.3.E.

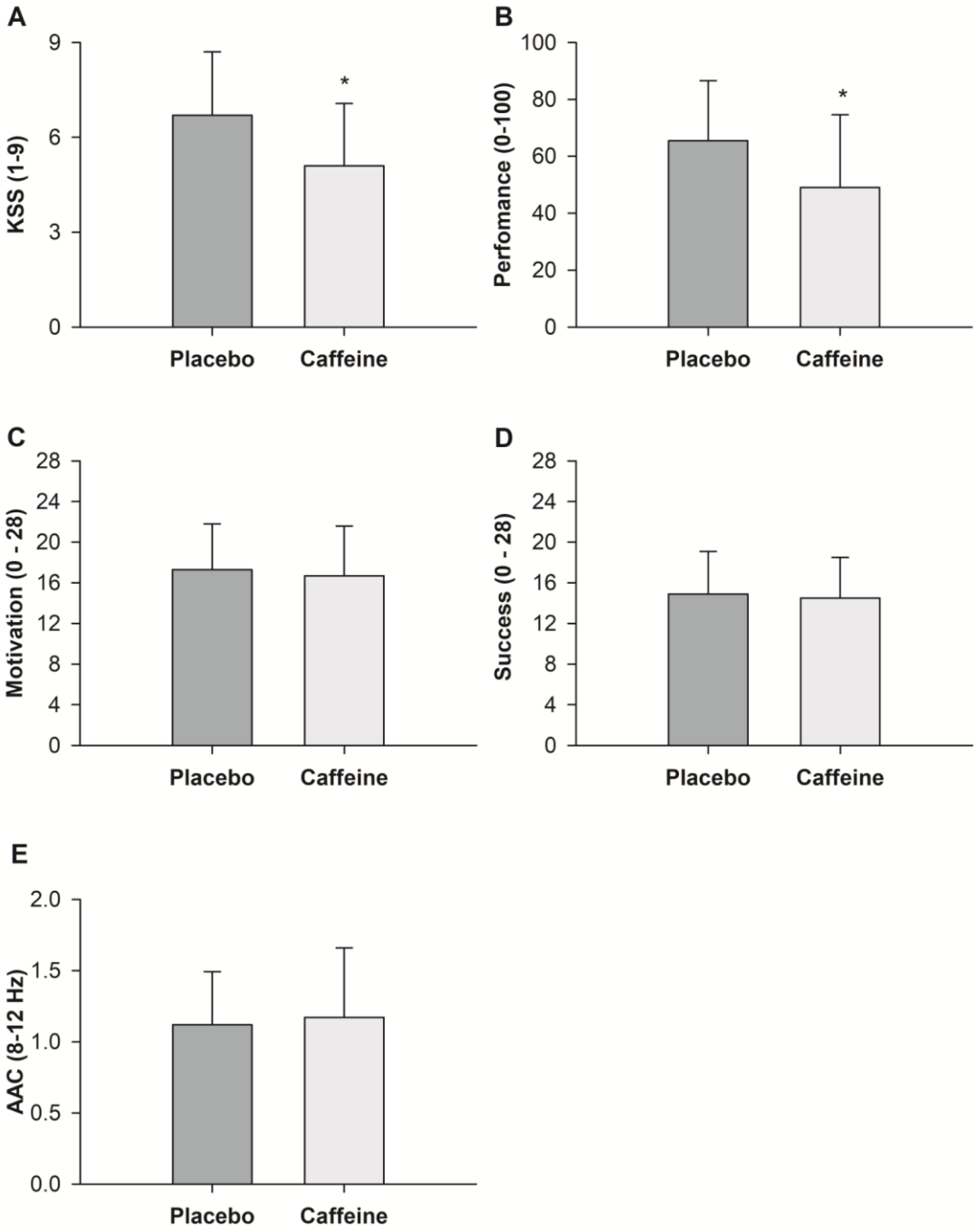


Figure 4.3 Changes in the Karolinska Sleepiness Scale (A), NASA-TLX workload perceived performance (B), intrinsic motivation (C), and success on task (D). Changes of channel Fz in the alpha power activity ratio expressed as Alpha Attenuation Coefficient (E). Data presented as mean ± SD. * denotes difference between trials ($p < 0.05$).

Cognitive performance

As shown in Figure 4.4. In the PVT, reaction time was 39.48 ms faster in caffeine (302.71 ± 37.77 ms) than in placebo (342.19 ± 45.66 ms). t(9) = -5.194, p = 0.001, d = 0.94. Number of lapses was lower in caffeine (*Mdn* = 1.00, *IQR* = 0-6.25) than in placebo (*Mdn* = 11.0, *IQR* = 4-33). Z = -2.668, p = 0.008.

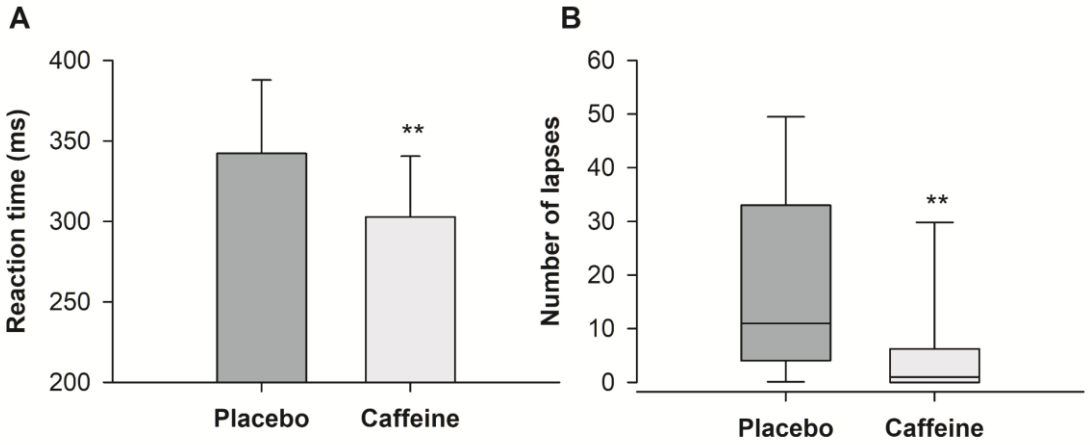


Figure 4.4. Changes in the Psychomotor Vigilance Test. Reaction time (A), presented as mean ± SD. Number of lapses (B), boxplot shows medians and interquartile ranges. Whiskers indicate min and max. * denotes difference between trials ($p < 0.05$). ** ($p < 0.01$).

As presented in Figure 4.5. In the Stroop Test, for congruent stimulus, reaction time was 103.43 ms faster in caffeine (675.38 ± 104.18 ms) than in placebo (778.81 ± 159.78). t(9) = 0.020, p = 0.020, d = 0.77. No differences were found for the number of errors between caffeine and placebo (p = 0.670). No differences were found for accuracy between caffeine and placebo (p = 0.401).

For incongruent stimulus, reaction time was 146.33 ms faster in caffeine (705.70 ± 96.23 ms) than in placebo (852.02 ± 179.09 ms). t(9) = -3.997, p = 0.003, d = 1.02.

No differences were found for the number of errors between caffeine and placebo (p = 0.078). Accuracy in caffeine (*Mdn* = 96.57%, *IQR* = 91.22-98.78) was higher than in placebo (*Mdn* = 93.85%, *IQR* = 87.11-98.12). Z = -2.293, p = 0.022.

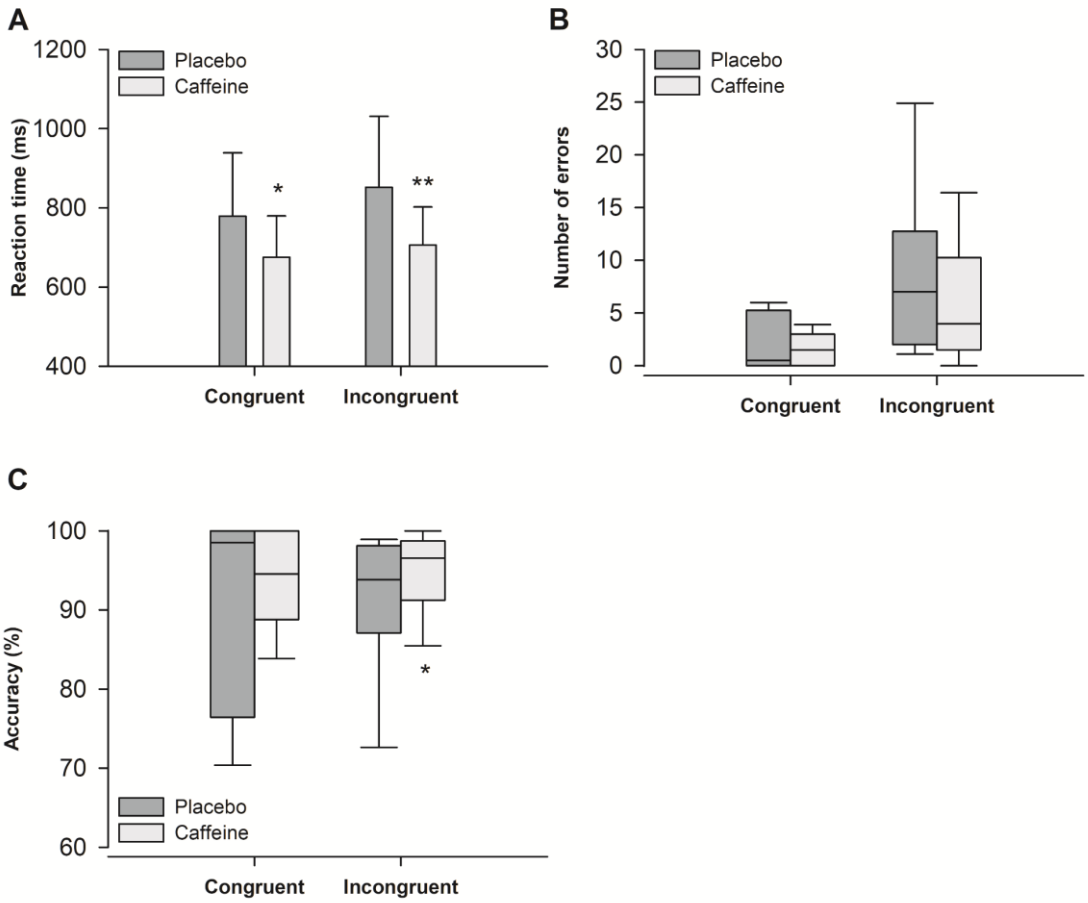


Figure 4.5 Changes in the Stroop Task. Reaction time (A) presented as mean ± SD. Number of errors (B). Accuracy (C). Boxplots showed medians and interquartile ranges. Whiskers indicate min and max. * denotes difference between trials (p < 0.05). ** (p < 0.01).

Perceptual responses to 30-min constant workload

As shown in Figure 4.6.A no significant interaction between trial and time in RPE was found (p = 0.323). Main effects of trial and time were revealed, RPE was lower in

caffeine, compared to placebo ($F_{1,1}$ = 6.237, p = 0.034, ηp^2 = 0.409), RPE increased over time ($F_{1,1.125}$ = 8.238, p = 0.015).

No significant interaction between trial and time in affect was revealed (p = 0.642). Ratings of feeling state decreased over time ($F_{1,1.185}$ = 5.157, p = 0.040), but no difference between trials was found (p = 0.073). Figure 4.6.B.

Physiological responses to 30-min constant workload

As presented in Figure 4.7.A no significant interaction between trial and time in HR was found (p = 0.280), HR increased over time (p < 0.001), but no difference between trials was revealed (p = 0.224).

Neither a significant interaction between trial and time (p = 0.423), nor a main effect of trial (p = 0.356) nor time (p = 0.071) were found in B[La^-] concentrations. Figure 4.7.B.

As reported in Figure 4.8.A no significant interaction between trial and time on $\dot{V}O_2$ was found (p = 0.303). $\dot{V}O_2$ increased over time. ($F_{1,1.942}$ = 46.754, p < 0.001), but no difference between trials was revealed (p = 0.070).

No significant interaction between trial and time on $\dot{V}CO_2$ was found (p = 0.301). $\dot{V}CO_2$ increased over time ($F_{1,2.192}$ = 57.366, p < 0.001), but no difference between trials was disclosed (p = 0.605). Figure 4.8.B.

Neither a significant interaction between trial and time on RER (p = 0.644), nor a main effect of trial (p = 0.160) nor time (p = 0.098) were found. Figure 4.8.C.

As shown in Figure 4.9.A no significant interaction between trial and time on $\dot{V}E$ was found (p = 0.226). $\dot{V}E$ was 5 $L \cdot min^{-1}$ higher in caffeine (81.5 ± 8.1 $L \cdot min^{-1}$) than in placebo (76.5 ± 6.3 $L \cdot min^{-1}$), $F_{1,2.048}$ = 11.108, p = 0.009, ηp^2 = 0.552, and increased over time ($F_{1,2.048}$ = 37.254, p < 0.001).

No significant interaction between trial and time on VT was found (p = 0.578). VT was 0.116 L higher in caffeine (2.07 ± 0.29 L) than in placebo (1.96 ± 0.28 L), $F_{1,2.712}$ =

31.794, $p < 0.001$, $\eta p^2 = 0.779$, and increased over time ($F_{1,2.712} = 6.585$, $p < 0.001$). Figure 4.9.B.

No significant interaction between trial and time on BF was found ($p = 0.234$). BF increased over time ($F_{1,1.950} = 21.971$, $p < 0.001$), but no difference between trials was revealed (p = 0.989). Figure 4.9.C.

30-min time trial performance

A paired-samples *t*-test revealed that, compared with placebo (5756 ± 1366 m), caffeine increased the total distance covered (6075 ± 1243 m) by 319 m [95CI +107, +532], t(9) = 3.396, $p = 0.008$, d = 0.24. Individual performances can be seen in Figure 4.10.A. No interaction between trial and time for running speed was found ($p = 0.387$). However, running speed was 0.633 km·h^{-1} higher in caffeine (12.1 ± 2.5 km·h^{-1}) than in placebo (11.5 ± 2.7 km·h^{-1}), $F_{1,1.927} = 11.413$, $p = 0.008$, $\eta p^2 = 0.559$, and increased over time ($F_{1,1.927} = 9.340$, $p = 0.002$, $\eta p^2 = 0.509$). Figure 4.10.C.

Perceptual responses to 30-min time trial

As reported in Figure 4.6.A an interaction between trial and time for RPE was found ($F_{1,5} = 3.125$, $p = 0.017$, $\eta p^2 = 0.258$). RPE increased over time ($F_{1,1.390} = 95.144$, $p < 0.001$), but no main effect of trial was found ($p = 0.279$). Follow up analysis revealed that, at min 10, RPE (6-20) was 0.75 points lower in caffeine (13.2 ± 0.8), than in placebo (13.9 ± 1.2), $p = 0.018$. No differences were found between trials at min 5 ($p = 0.052$), min 15 ($p = 0.625$), min 20 ($p = 0.405$), min 25 ($p = 1.000$), and min 30 ($p = 0.343$).

No interaction between trial and time was determined for affect ($p = 0.206$), feeling state decreased over time ($F_{1,1.679} = 30.991$, $p < 0.001$), but no difference between trials was unveiled ($p = 0.112$). Figure 4.6.B.

Physiological responses to 30-min time trial

As shown in Figure 4.7.A no interaction between trial and time for HR was found (p = 0.568). However, HR was 6 bpm higher in caffeine (169 ± 12 bpm) than in placebo (163 ± 14 bpm), $F_{1,2.146}$ = 9.546, p = 0.013, ηp^2 = 0.515. HR increased over time ($F_{1,2.146}$ = 42.625, p < 0.001).

An interaction between trial and time for B[La^-] was found ($F_{1,1}$ = 18.796, p = 0.010, ηp^2 = 0.539). B[La^-] was higher in caffeine than in placebo ($F_{1,1}$ = 13.255, p = 0.005, ηp^2 = 0.596). Post-hoc pairwise comparisons revealed that, after the test, B[La^-] was 3.02 mmol·L^{-1} higher in caffeine (8.00 ± 3.11 mmol·L^{-1}) than in placebo (4.99 ± 2.95 mmol·L^{-1}), p = 0.005, d = 0.99. No difference in B[La^-] between trials before the test was found (p = 0.362). In caffeine, B[La^-] was 4.34 mmol·L^{-1} higher after the time trial (8.00 ± 3.11 mmol·L^{-1}), compared to before the start (3.66 ± 1.78 mmol·L^{-1}), p = 0.007, d = 1.71). No difference was unveiled in placebo (p = 0.220). Figure 4.7.B.

As it can be seen in Figure 4.8.A no interaction between trial and time for $\dot{V}O_2$ was found (p = 0.334). Nevertheless. $\dot{V}O_2$ was 0.188 L·min^{-1} higher in caffeine (3.06 ± 0.43 L·min^{-1}) than in placebo (2.87 ± 0.43 L·min^{-1}), $F_{1,1.434}$ = 18.281, p = 0.005, ηp^2 = 0.608, and increased over time ($F_{1,1.434}$ = 18.281, p < 0.001).

No interaction between trial and time for $\dot{V}CO_2$ was found (p = 0.273). However, $\dot{V}CO_2$ was 0.179 L·min^{-1} higher in caffeine (2.92 ± 0.56 L·min^{-1}), compared to placebo (2.74 ± 0.53 L·min^{-1}), $F_{1,1.802}$ = 9.697, p = 0.012, ηp^2 = 0.519, and increased over time ($F_{1,1.802}$ = 23.110, p < 0.001). Figure 4.8.B.

Neither an interaction between trial and time for RER (p = 0.611), nor a main effect of trial (p = 0.861) were found. RER increased over time ($F_{1,2.471}$ = 29.863, p < 0.001). Figure 4.8.C.

As reported in Figure 4.9.A an interaction between trial and time for $\dot{V}E$ was found ($F_{1,2.173}$ = 4.003, p = 0.032, ηp^2 = 0.308). $\dot{V}E$ was 12 L·min^{-1} higher in caffeine (105 ± 22 L·min^{-1}), compared to placebo (93 ± 24 L·min^{-1}), p = 0.002, ηp^2 = 0.686, and increased over time (p < 0.001). $\dot{V}E$ was higher in caffeine than in placebo at min 5 (p = 0.016),

min 15 (p = 0.031), min 20 (p < 0.001), min 25 (p = 0.001), and min 30 (p < 0.001). No differences were found at min 10 (p = 0.119).

No interaction between trial and time for VT was found (p = 0.073). However, VT was 0.164 L higher in caffeine (2.20 ± 0.40 L), compared to placebo (2.04 ± 0.39 L), $F_{1,2.168}$ = 19.930, p = 0.002, ηp^2 = 0.689, and increased over time ($F_{1,2.168}$ = 15.062, p < 0.001). Figure 4.9.B.

Neither an interaction between trial and time for BF (p = 0.260), nor a main effect of trial (p = 0.051) were found. BF increased over time ($F_{1,2.013}$ = 28.780, p < 0.001). Figure 4.9.C.

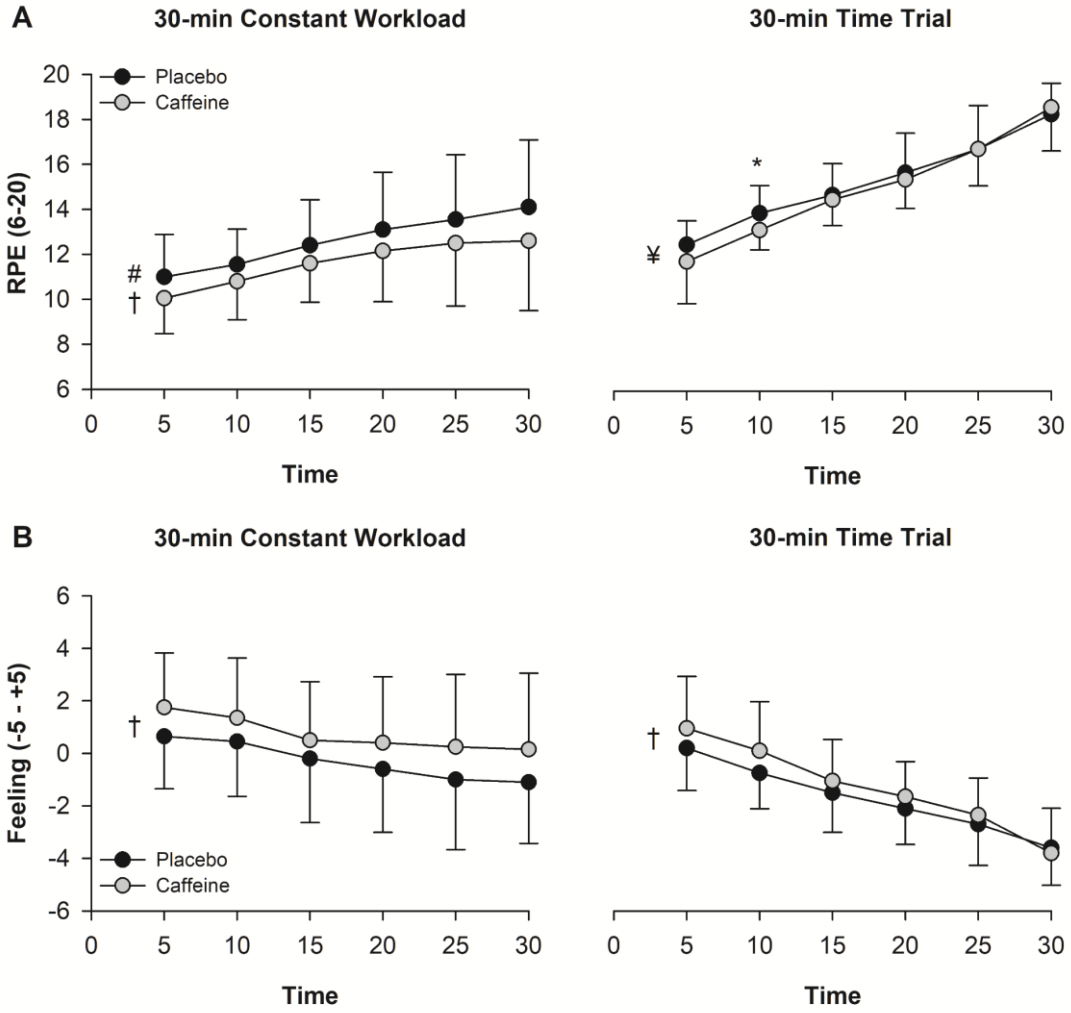


Figure 4.6 Changes in perceptual variables in Constant Workload and Time Trial. Ratings of perceived exertion (A), and feeling state (B). Data presented as mean ± SD. # denotes main effect of trial ($p < 0.05$). † denotes main effect of time ($p < 0.05$). ¥ denotes interaction between trial and time ($p < 0.05$). * denotes difference between trials ($p < 0.05$).

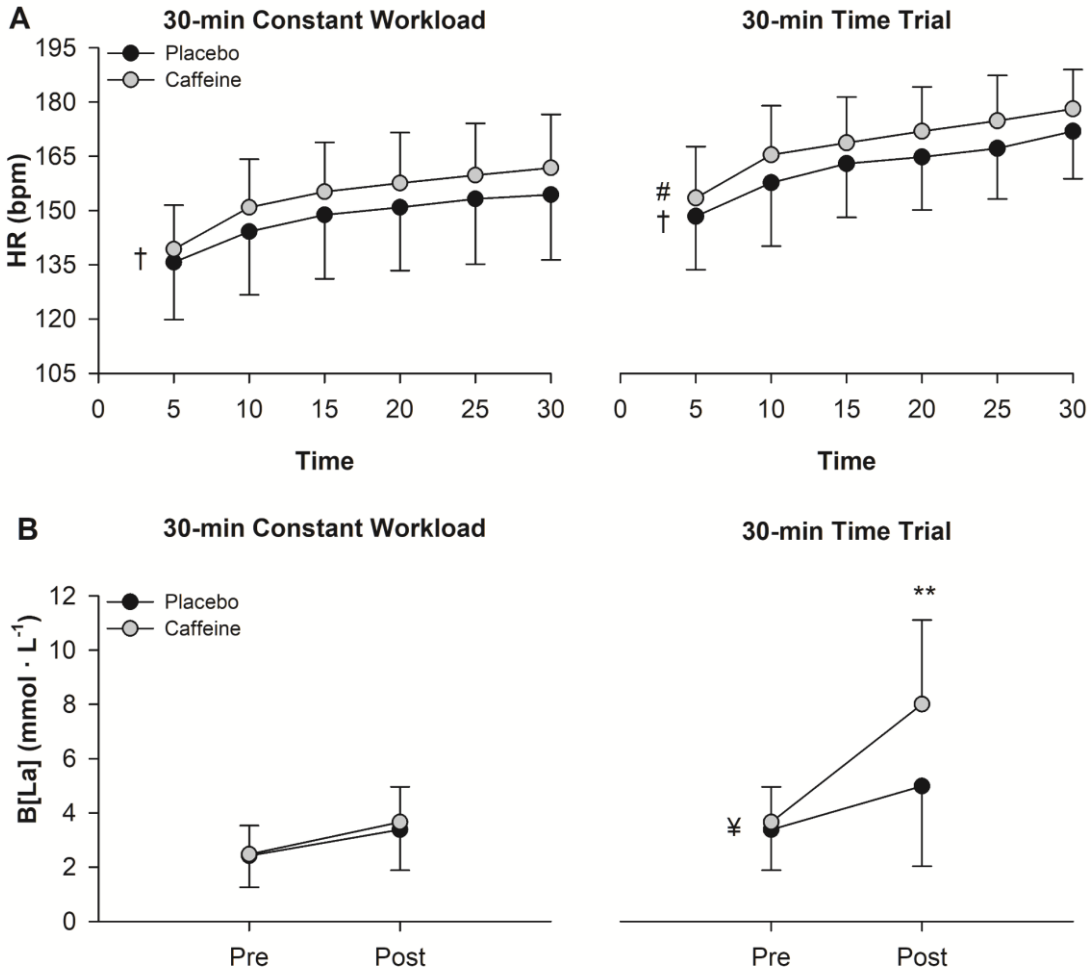


Figure 4.7 Changes in physiological variables in Constant Workload and Time Trial. Heart rate (A), and blood lactate concentration (B). Data presented as mean ± SD. # denotes main effect of trial ($p < 0.05$). † denotes main effect of time ($p < 0.05$). ¥ denotes interaction between trial and time ($p < 0.05$). ** denotes difference between trials ($p < 0.01$).

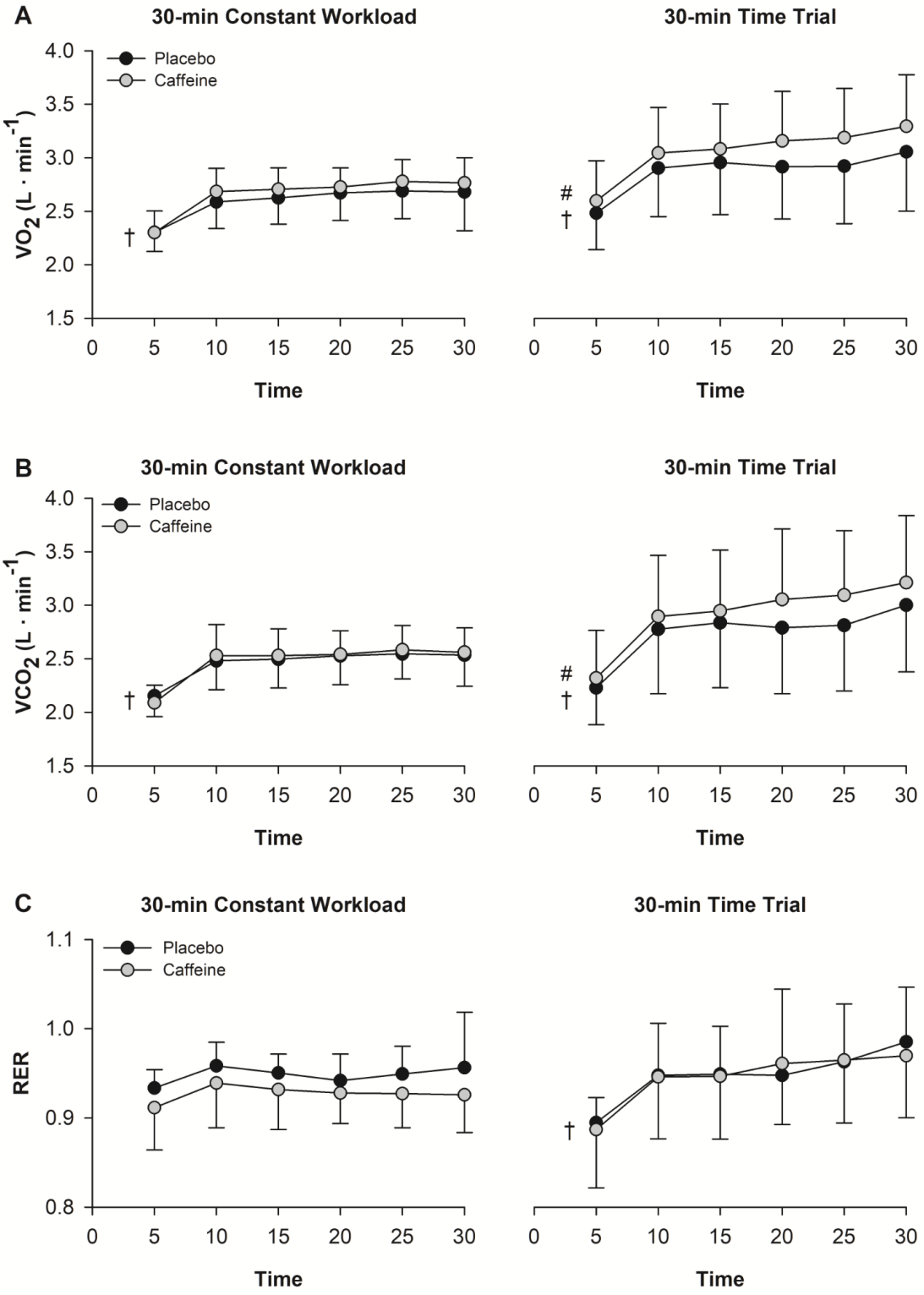


Figure 4.8 Changes in respiratory variables in Constant Workload and Time Trial. Oxygen consumption (A), carbon dioxide production (B), and respiratory exchange ratio (C). Data presented as mean ± SD. # denotes main effect of trial ($p < 0.05$). † denotes main effect of time ($p < 0.05$).

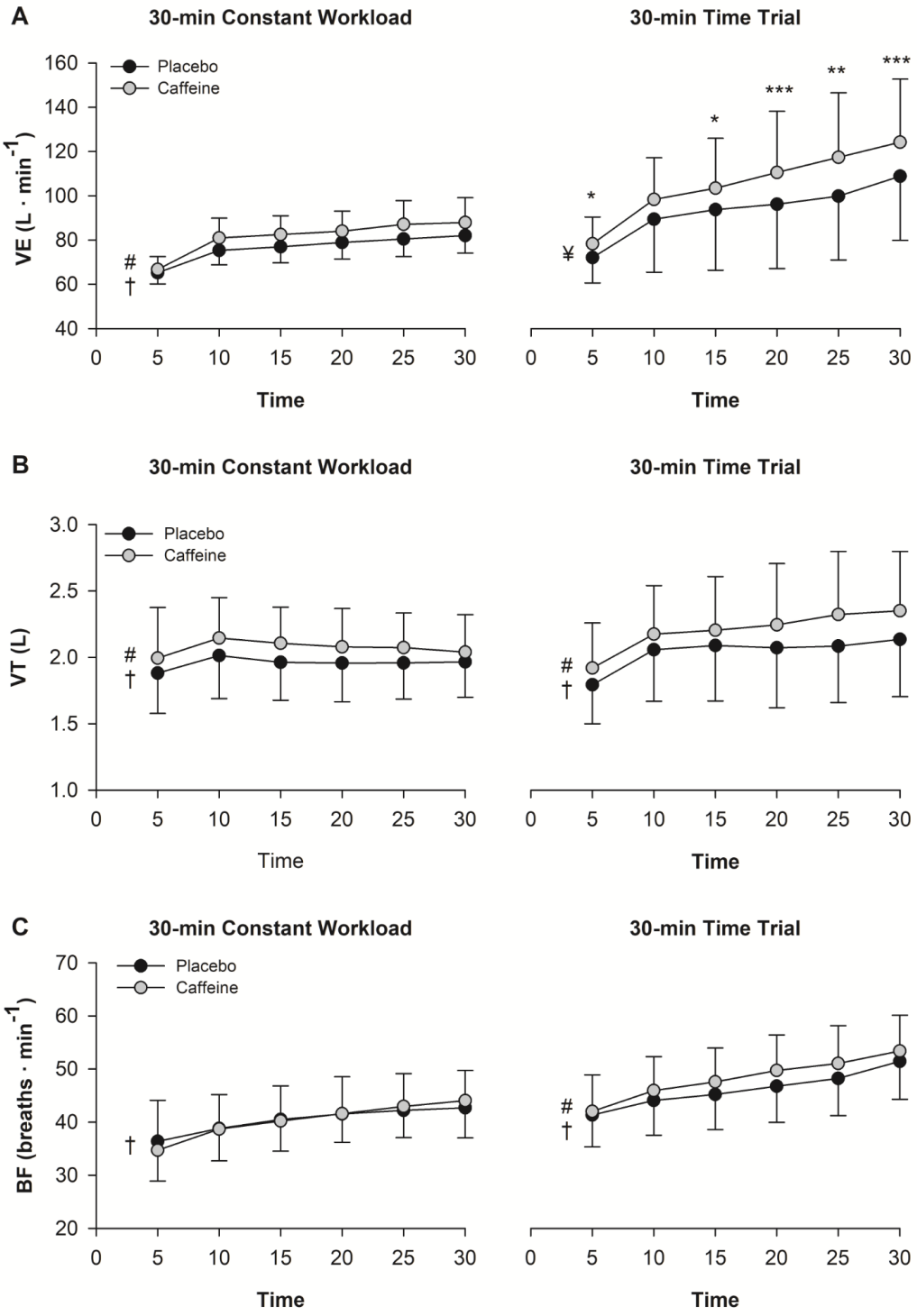


Figure 4.9 Changes in respiratory variables in Constant Workload and Time Trial. Minute ventilation (A), tidal volume (B), and breathing frequency (C). Data presented as mean ± SD. # denotes main effect of trial ($p < 0.05$). † denotes main effect of time ($p < 0.05$). ¥ denotes interaction between trial and time ($p < 0.05$). * denotes difference between trials ($p < 0.05$). ** ($p < 0.01$). *** ($p < 0.001$).

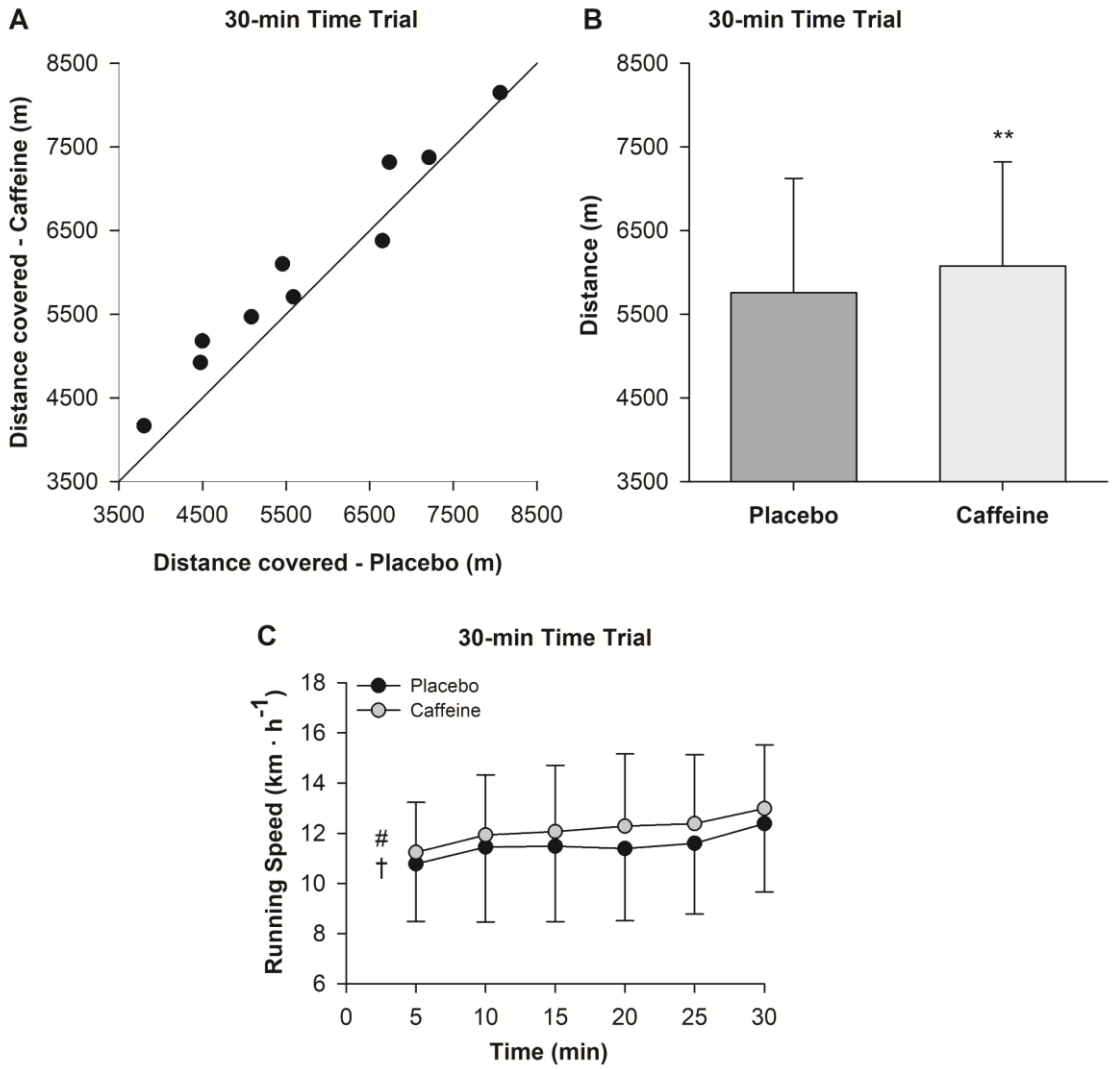


Figure 4.10 Scatterplot of individual performances in the 30-min Time Trial. Points above the line depict a better performance in caffeine compared to placebo (A). Changes in distance covered in the 30-min Time Trial (B). Changes in running speed in the 30-min Time Trial (C). Data presented as mean ± SD. # denotes main effect of trial ($p < 0.05$). † denotes main effect of time ($p < 0.05$). ** denotes difference between trials ($p < 0.01$).

4.5 Discussion

The main finding of the present study is that 6 mg·kg^{-1} of caffeine, after one night of total sleep deprivation, improved time trial performance. Participants were able to sustain a higher running speed at lower RPE values. As a result of that, cardiopulmonary and blood lactate responses were higher in caffeine than in placebo. At a cognitive level,

caffeine increased sustained attention and vigilance. The role of caffeine as an adenosine receptor antagonist may explain our results due to its ergogenic effects on cognitive and exercise performance after sleep deprivation.

30-min time trial

In support of our main hypothesis, caffeine improved distance covered in a 30-min time trial run by 5.54%. Our results are in the same direction as similar investigations reported in the literature. 4.9% in 8 km time trial (Khcharem *et al.*, 2022), and 3.43% in 6.3 km time trial (McLellan *et al.*, 2005). The effect size of our study (0.24) is lower than in the studies mentioned above (0.53, and 1.13, respectively). However, it is within the effect sizes range (0.22-0.61) reported in a recent review of 21 published meta-analyses of caffeine supplementation and aerobic endurance (Grgic *et al.*, 2020). In studies investigating caffeine and running time trial performance in non-sleep deprived participants, improvements in performance are ranging from 0.1% to 2.35%, and effect sizes from 0.01 to 0.32 (Cohen *et al.*, 1996; O'Rourke *et al.*, 2008; Church *et al.*, 2015; Khcharem *et al.*, 2021, 2022). Consequently, it can be argued that caffeine can be used to countermeasure the negative effects of sleep deprivation on running performance. It seems that caffeine intake after sleep deprivation could maintain performance at levels observed in control, where subjects were not sleep deprived (McLellan, Bell and Kamimori, 2004; Khcharem *et al.*, 2022). However, a limitation of our study is the lack of data in a non-sleep deprived state as a control. In addition to that, several aspects must be taken into consideration regarding the efficacy of caffeine: exercise protocol; status; caffeine dosage; caffeine source, timing of ingestion; and timing of ingestion (Burke, 2008; McLellan, Caldwell and Lieberman, 2016), and genetic polymorphisms (Grgic *et al.*, 2021). Another limitation of our study is that plasma caffeine concentrations were not reported. Due to the exceptional circumstances of a global pandemic, we were unable to analyse the blood samples on time for this thesis. Despite participants being instructed to refrain from caffeine sources in any form 12 hours before each trial, our study lacked a reliable measure of caffeine levels before and after experimental trials.

A potential mechanism that could explain this improvement in performance is a reduction in RPE. Our results are in agreement with a previous study (McLellan, Bell and Kamimori, 2004), where, in a run to exhaustion test after a 24 h of wakefulness, RPE was significantly lower in caffeine, compared to placebo. Unfortunately, the rest of the studies investigating caffeine and endurance performance in sleep deprived subjects did not record RPE values. An increase in RPE during exercise after sleep deprivation has been well documented (Martin, 1981; Myles, 1985; Symons, VanHelder and Myles, 1988; Rodgers *et al.*, 1995; McLellan, Bell and Kamimori, 2004; Oliver *et al.*, 2009). Central processing sensory inputs responsible for RPE production during exercise might be negatively affected by a mental fatigued state, such as sleep deprivation (Marcora, 2009), The ergogenic effect of caffeine, on exercise, by blocking adenosine receptors in the central nervous system, promotes a decrease in RPE at the constant workload (i.e., running speed, cycling power output), and/or an increase in the workload at a given RPE (Doherty and Smith, 2005).

At a cardiovascular level, heart rate response was higher in caffeine than in placebo, contrary to the findings of (McLellan, Bell and Kamimori, 2004). Other relevant studies did not report heart rate response. Differences in caffeine dosage and exercise protocol may explain why our results diverged from those reported previously in a sleep deprived setting.

To the best of our knowledge, in the context of endurance exercise and caffeine in sleep deprived subjects, this is the first study where cardiopulmonary parameters were reported. $\dot{V}O_2$, $\dot{V}CO_2$, minute ventilation, tidal volume, and breathing frequency increased in the caffeine trial. It has been suggested that sleep deprivation has a minimal effect on cardiopulmonary variables during exercise (Fullagar *et al.*, 2015). $\dot{V}O_2$ during a 1500 m time trial increased after 150-200 mg of caffeine (Wiles *et al.*, 1992). Increases in minute ventilation during maximal and submaximal exercise after caffeine ingestion has been reported for 8 $mg \cdot kg^{-1}$, but not in lower doses < 6 $mg \cdot kg^{-1}$ (Chapman and Mickleborough, 2009). A summary of studies about the effects of caffeine on ventilation can be found in (Chapman, Wilhite and Mickleborough, 2012). These authors hypothesise about the ergogenic effect of caffeine on ventilation, increasing alveolar partial pressure and oxygen saturation. But only in scenarios where pulmonary gas exchange is

compromised (e.g., altitude, exercise induced arterial hypoxemia), and at submaximal workloads. Furthermore, questions have been raised from the same authors, whether the stimulating effects of caffeine on the central nervous system ventilation increases the work of the breathing muscles, or decreases blood flow of the locomotor muscles involved during exercise, reducing any potential benefit from increased alveolar oxygenation. In any case, there is not a clear explanation for the disparity of results in the literature, such as factors regarding caffeine efficacy and the use of different exercise protocols.

At a perceptual level, the feeling state was not different between trials. Interestingly, participants perceived in the NASA-TLX scale that, in caffeine, they achieved a higher level of performance. Considering that during the test, participants were not able to see anything but time remaining, it seems that lower RPE reported, in addition to an increased alertness and vigilance (see *Cognitive performance* section below) may have influenced their perceived performance.

30-min constant workload

RPE was lower in caffeine in comparison to placebo. In the context of submaximal exercise and sleep deprivation, mixed results have been reported in the literature: some research have reported no differences were found in 30 min running at 60% of $\dot{V}O_{2\ max}$ (Oliver *et al.,* 2009); Whilst other have reported an increase in RPE during 50 min walking at 28% of $\dot{V}O_{2\ max}$ (Myles, 1985); during one hour walking at 28% of $\dot{V}O_{2\ max}$ (Plyley *et al.,* 1987); and during 40 min of submaximal cycling (Temesi *et al.,* 2013). If we take sleep deprivation out of the equation, during constant rate exercise, a 6% decrease in RPE has been shown in caffeine, compared to placebo (Doherty and Smith, 2005).

From all the physiological parameters measured, only minute ventilation and tidal volume were higher in caffeine than in placebo. Our results are partially in agreement with (Birnbaum and Herbst, 2004), 7 $mg \cdot kg^{-1}$ of caffeine increased tidal volume and decreased RPE in a 30 min run at 70% of $\dot{V}O_{2\ max}$. However, in that study no significant differences were found for breathing frequency (p = 0.074) or minute ventilation (p =

0.104). 3.3 mg·kg^{-1} of caffeine increased tidal volume and reduced breathing frequency in a 50 min walk at 50% $\dot{V}O_{2\,max}$ (Brown *et al.*, 1991).

Cognitive performance

As hypothesised, PVT performance after sleep deprivation was better in caffeine than in placebo. Caffeine improved sustained attention and vigilance due to a faster mean reaction time and lower errors made, in comparison to placebo. These results are in accordance with previous studies (McLellan *et al.*, 2005; Killgore and Kamimori, 2020; Khcharem *et al.*, 2022). In the context of ultra-endurance exercise, a better vigilance and attention can be translated into less chances of making mistakes when following a GPS track during a race, particularly under sleep deprivation conditions. It seems that genetic variation of *ADORA2A* modulates the effects of caffeine on psychomotor vigilance in sleep deprivation (Bodenmann *et al.*, 2012). Unfortunately, our study did not analyse participants' *ADORA2A* genotype.

In line with the findings in PVT, caffeine improved reaction time in the Stroop task for both congruent and incongruent stimuli. No differences were found in the number of errors between trials. Accuracy was worse only to incongruent stimuli. Despite the fact that several studies have shown that sleep deprivation impaired reaction times and number of errors in the Stroop task (Lingenfelser *et al.*, 1994; McCarthy and Waters, 1997; Stenuit and Kerkhofs, 2008), other authors disclosed that response inhibition is not compromised by sleep deprivation (Sagaspe *et al.*, 2006; Cain *et al.*, 2011). The findings of the present study are partially aligned with the literature: worsened reaction time, but no changes in the number of errors. This might be due to the use of a shorter version of the Stroop Task (25 trials vs. 50-200 trials reported in the literature mentioned above). The fact that neutral stimuli (i.e., non-word stimulus) were not included in the task, as in previous studies (Sagaspe *et al.*, 2006; Cain *et al.*, 2011) might represent a possible limitation of the present study.

In contrast to our hypothesis, no differences regarding alertness and EEG activity in the alpha band (8-12 Hz) were found. A significant increase in alpha activity was

observed in the caffeine group after sleep deprivation. (Deslandes *et al.*, 2006). However, in this study subjects were sat with eyes closed.

Perceptual responses to caffeine and sleep deprivation

As a result of sleep deprivation, subjective sleepiness increased. Alertness and vigilance decreased, as shown in the AAT, PVT, and Stroop tests, when comparing normal sleep and placebo.

In agreement with our hypothesis, Subjective sleepiness was reduced by caffeine. Our results are consistent with the results published investigating caffeine and subjective sleepiness using the Karolinska Sleepiness Scale in both, sleep and non-sleep deprived subjects (Biggs *et al.*, 2007; Beaven and Ekström, 2013; Hansen *et al.*, 2019).

No differences in the affective response during exercise were revealed between trials. An increase in negative affective responses was found in studies investigating sleep loss (Franzen, Siegle and Buysse, 2008; Minkel *et al.*, 2012). An increase in positive affective responses during cycling after caffeine supplementation have been reported (Backhouse *et al.*, 2006, 2011). It might be plausible that the combination of both factors, showing opposite responses, could explain our results in terms of affective response.

Participants expected that sleep deprivation would negatively affect their running performance. Their expectations towards running performance improved by caffeine was slightly positive. Positive and negative expectations may influence the effectiveness of an intervention (Ross and Olson, 1981; Benedetti *et al.*, 2006). The placebo and nocebo effect on sports performance have been reviewed recently, showing moderate placebo effects of caffeine (Hurst *et al.*, 2020). However, no studies in running time trials and caffeine were included in that review. Changes in motivation associated with caffeine supplementation expectations may influence endurance performance (Shabir *et al.*, 2018). Although, in our study, no differences were found between caffeine and placebo in intrinsic motivation nor success on task, suggesting that caffeine did not alter participants' motivation, essential to achieve high levels of performance (Matthews, Campbell and Falconer, 2001). In caffeine study designs, the placebo effect is often neglected. In order

to address this methodological issue, it has been suggested to control several aspects, such as expectations; regular caffeine intake; motivation; side effects; baseline trial (no ingested substance), correct treatment identification rate (De Salles Painelli *et al.*, 2020). In our study the correct guessing rate was 60%. Unfortunately, in the studies reviewed by De Salles Painelli *et al.* (2020) running studies did not disclose the guessing rate. Nevertheless, our results are within the range reported for other sports (11-75%). However, we did not control for potential side effects that could have influenced participants' performance. This could have been easily checked asking the participant to complete a checklist with the most common symptoms.

No differences were found in the amount of sleep two nights before trials, showing that participants were rested, and not under a sleep restriction that could have worsened the effects of one night of total sleep deprivation. Sleep below optimal levels can impair neurobehavioral aspects, such as alertness, attention, and mood (Banks and Dinges, 2007). Subjective sleepiness was lower in caffeine than in placebo. In a study investigating caffeine supplementation during 48 h of total sleep deprivation (Hansen *et al.*, 2019), a similar pattern was found: there were significant differences for subjective sleepiness scores between caffeine and placebo after 48 h of sleep deprivation.

4.6 Conclusions

To conclude, 6 $mg \cdot kg^{-1}$ of caffeine, after one night of total sleep deprivation, improved time trial performance by 5.54%. Cognitive aspects such as vigilance and alertness were also enhanced by caffeine. Caffeine decreased RPE in both submaximal and maximal workloads. Therefore, being able to sustain a higher running speed at a lower RPE might have led to an increase in other physiological parameters, such as ventilation or blood lactate production during the time trial.

Moreover, caffeine supplementation after prolonged periods of wakefulness could be an effective measure to counteract the negative effects associated with sleep deprivation. This could be particularly useful in environments where sleep opportunities are not optimal, such as ultra-endurance events or military operations. However, more

research is needed in cohorts of people regularly exposed to sleep deprivation (i.e., night shift workers, ultramarathon runners, special operations soldiers) in order to investigate whether the results are similar with a population used to work in a sleep deprived state.

Further investigations using EEG and neuroimaging are needed in order to better understand the neurobiological mechanisms underlying the relation between sleep deprivation, caffeine, and RPE.

Chapter 5: The Effects of Training for a Mountain Ultra-Marathon on Sleep Deprivation Tolerance.

Main findings: Three bouts of exercise after one night of sleep deprivation over a 14-week period did not improve tolerance to the negative effects of sleep deprivation, neither in endurance nor in cognitive performance. One night of sleep deprivation reduced time to exhaustion running at ~75% of peak treadmill speed by ~28%, and mean reaction time by ~17%. A better sleep deprivation tolerance was associated with a better performance in a ~340 km mountain ultra-marathon.

5.1 Abstract

In ultra-marathon events, sleep management is a crucial factor, runners try to sleep as little as possible, putting themselves in a sleep deprived state that has negative effects on cognitive and physical performance. Several strategies have been used in order to minimise or counteract these effects, such as caffeine consumption or napping. Based on an innovative intervention, called Brain Endurance Training, aiming to improve endurance performance decreasing perceived effort as a result of an increased resistance to mental fatigue, the purpose of the study was to investigate the effects of three bouts of exercise in a sleep deprived state over a 14-week period in a group of soldiers, as part of their preparation for a mountain ultra-marathon. Ten participants were tested six times in total: three prior and three after the training phase. The protocol consisted of one preliminary visit to perform a $\dot{V}O_{2\,max}$ test and to familiarise with the protocol, equipment and questionnaires used. And two consecutive experimental visits: a 10-min Psychomotor Vigilance Test (PVT) followed by a time to exhaustion test (TTE) on a treadmill at a Δ speed, calculated from the results of the $\dot{V}O_{2\,max}$ test, after one night of normal sleep and one night of sleep deprivation, respectively. Mood, sleepiness, fatigue, and other variables

were measured using questionnaires during each experimental visit. After the training phase, participants took part in a ~340 km mountain ultra-marathon race. Despite an increase in training parameters, no differences were found in cardio-respiratory fitness after the training. Sleep deprivation had a negative effect on both endurance and cognitive performance. As a result of sleep deprivation, TTE was impaired by 28.3 ± 19.9% (439 ± 311 s). PVT mean reaction time was increased by 17.4 ± 23.5% (48.1 ± 70.3 ms). Three bouts of exercise under sleep deprivation over a 14-week period training for a mountain ultra-marathon did not improve tolerance to sleep deprivation. Moderate and high positive associations between sleep deprivation tolerance and distance covered in the race were found. Future investigations need to address the limitations found in this study that may have influenced the results of this study. Nevertheless, further research is required to investigate the length and number of exercise bouts per week in a sleep deprived state to potentially reduce the negative effects of sleep deprivation.

5.2 Introduction

In the context of mountain ultra-marathon competitions, sleep management is a key factor (Chapter 3). The use of caffeine as a potential tool to reduce the negative effects of sleep deprivation on performance has been explored (Chapter 4). Sleep deprivation is often defined as a state of mental fatigue caused by a prolonged period of wakefulness (Boonstra *et al.*, 2007; Ackerman, 2010). A detrimental effect of sleep deprivation on endurance performance has been reported (Martin, 1981; Martin and Gaddis, 1981; Symons, VanHelder and Myles, 1988; Oliver *et al.*, 2009; Temesi *et al.*, 2013). However, findings are still controversial, mainly due to the fact that elite athletes rarely spent one night of complete sleep deprivation. Furthermore, different results have been found in activities shorter than 30 min (Fullagar *et al.*, 2015).

In events shorter than 36 h, it seems that the best strategy is not to sleep at all (Martin *et al.*, 2018). In a 168 km ultra-marathon, longer sleep times positively correlated with race time (Hurdiel *et al.*, 2015). However, in longer events, due to the duration of the event (i.e., *Tor Des Glaciers*: 450 km, cut-off time 190 h), complete sleep deprivation

is not an option, particularly for an average runner. For races longer than 36 h, sleep is correlated with race duration

Several strategies to reduce the negative effects of sleep deprivation have been found in the literature: caffeine (McLellan, Bell and Kamimori, 2004; McLellan *et al.*, 2004, 2005, 2007; Khcharem *et al.*, 2022); modafinil (Wesensten *et al.*, 2002); naps (Waterhouse *et al.*, 2007; Blanchfield *et al.*, 2018; Martin *et al.*, 2018); and sleep extension (Martin *et al.*, 2018).

Perception of effort (RPE) is a key factor in endurance performance (Marcora, 2008; Oliver *et al.*, 2009; Marcora and Staiano, 2010; Temesi *et al.*, 2013). RPE and endurance performance are negatively affected by mental fatigue (Van Cutsem *et al.*, 2017). Brain endurance (BET) is a novel method, aiming to increase resistance to mental fatigue, based on a systematic repetition of cognitive demanding tasks, aiming to improve endurance performance by reducing RPE. BET as part of a traditional aerobic program reduced RPE and improved endurance performance (Marcora, Staiano and Merlini, 2015). Due to the aforementioned definition of sleep deprivation as a mental fatigue state, based on BET principles, a systematic repetition of physical exercise under sleep deprivation is proposed as an alternative strategy to reduce the negative effects of sleep deprivation on endurance performance, improving tolerance to sleep deprivation. At an anecdotal level, according to informal conversation with elderly former athletes, and alpine mountaineers, this type of training may have been already used as part of their preparation for an event, or summit. The therapeutic use of sleep deprivation has been reported in patients with depression (Giedke and Schwärzler, 2002). Kolb and Whishaw (1998) stated that structural and functional adaptations, as a response to a stimulus, can be observed in the brain (i.e., brain plasticity). Therefore, a training program combining a traditional physical training with some exercise bouts in a sleep deprived state might increase training load, inducing adaptations at a brain level that would reduce RPE and improve endurance performance.

The aim of the current study was to investigate the effects of certain bouts of exercise under sleep deprivation in a group of soldiers, as part of their preparation for a mountain ultra-marathon. We hypothesised that exercise under sleep deprivation would

improve tolerance to sleep deprivation, reducing RPE, and improving endurance running performance.

5.3 Methods

Participants

Twenty-one male members of the Italian Army (*5° Reggimento Alpini*) were recruited. Participants involved in this study were free of any known disease, injury, and medical treatment. Throughout the course of the experiment, eleven participants were excluded from the study (one was transferred to a different unit, five reported musculoskeletal injuries prior to the testing sessions, five reported sleep times > 1 h during the sleep deprivation visits). Therefore, data from ten participants were analysed. Prior to taking part in the experiment, all of them were informed about the experiment, and were asked to sign an informed consent form. Ethical approval was granted by the University of Kent, School of Sport and Exercises Sciences Research Ethics and Advisory Group. The study was conducted in accordance with the Declaration of Helsinki (2013).

Study design

As a result of the special characteristics and commitments of the population (active soldiers) recruited, we were not able to run a study with two groups (experimental, control). Therefore, all participants were asked to attend the laboratory (Pro Motus S.r.l / GmbH, Bolzano, Italy) six times in total: three visits prior to the training (pre-training) and another three immediately after (post-training). Testing periods were separated by twelve weeks. Each testing period consisted of one preliminary visit 48 h prior to the two experimental visits. Sleep deprivation nights during experimental visits took place in several rooms of the building where the laboratory is based. All experimental visits were conducted during the time frame (08:00 am - 11:00 am) when lack of sleep seems to have the most negative impact on cognitive function (Mollicone *et al.*, 2010). Participants were encouraged to keep their habitual routines during the whole duration of the experiment:

food and sleep were monitored using 3-day diaries prior to each experimental visit. Participants' physical activity behaviour throughout the study was monitored using a daily and physical activity diary, and the leisure and occupational physical activity level scales (Saltin and Grimby, 1968). An overview of the study protocol can be found in Figure 5.1.

Due to the intrinsic logistics involved in testing military personnel, for testing purposes, participants were split into two groups, according to their working schedule. All members of the group performed each visit on the same day, including the sleep deprivation nights. Testing periods between groups were separated by seven days. To optimise the TTE testing process, after the preliminary visit, participants were paired within their group according to their delta speed (Δ speed). Therefore, during the experimental visits two participants were tested at the same time. However, to prevent potential influences while being tested, they were kept separated from each other at different locations in the same laboratory space. During the time to exhaustion tests, treadmills were separated by hospital ward folder screens, so they could not see each other. Additionally, they were told to perform to their best, independently of the other person. To avoid variability, tests were always performed on the same day of the week, pairs were tested in the same time slot both during normal sleep and sleep deprivation.

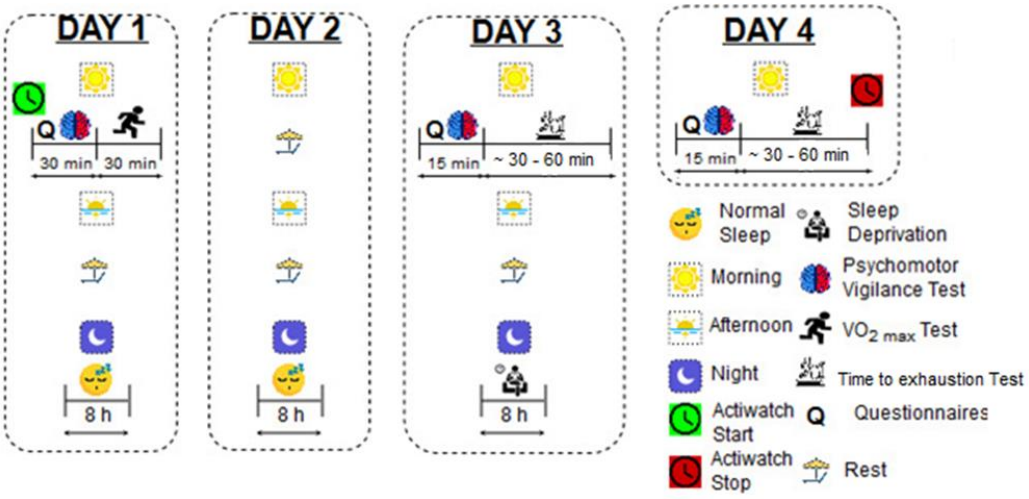


Figure 5.1 Overview of the study protocol

Testing procedures

Preliminary visit

Participants were informed about the testing procedures, and a PAR-Q questionnaire was used for health screening. They were asked to complete several questionnaires related to their chronotype (Horne and Ostberg, 1976); daytime sleepiness (Johns, 1991); mental and physical fatigue (Wewers and Lowe, 1990); mood (Terry, Lane and Fogarty, 2003); motivation (Matthews, Campbell and Falconer, 2001); physical activity (Jackson *et al.*, 1990); sleep quality (Buysse *et al.*, 1989); and competition history. Stature and body mass were measured to the nearest 0.1 cm and 0.1 kg using a stadiometer and a weight scale (Seca, Birmingham, UK) with participants in stocking feet. In order to determine maximal oxygen consumption ($\dot{V}O_{2\ max}$) participants performed a maximal incremental test on a motorised treadmill (Woodway Pro Series, Woodway USA, Waukesha, WI, USA) set at a fixed gradient of 1% to replicate the energetic cost of running outdoor in a flat surface (Jones and Doust, 1996). After a 5 min warm-up at 8 $km \cdot h^{-1}$, speed increased every minute by 1 $km \cdot h^{-1}$. Test was terminated when participants reached volitional exhaustion. Gas exchange measures were collected breath-by-breath throughout the entire test (MetaMax 3B-R2; Cortex Biophysik GmbH, Leipzig, Germany). Metabolic cart was calibrated before the tests, according to the manufacturer's specifications. Criteria for achieving $\dot{V}O_{2\ max}$ was accepted when two of the following were attained: heart rate within 10 $beats \cdot min^{-1}$ of age-predicted maximum; Respiratory exchange ratio (RER) $\geq$ 1.10; Rating of perceived exertion (RPE) $\geq$ 17 (American College of Sports Medicine, 2014).

Heart rate (HR) response was recorded continuously during the test using a chest band and a monitor (Polar V800, Polar Instruments, Kempele, Finland). RPE was collected every minute using the 15-point Borg RPE scale following standard instructions (Borg, 1998). Prior to the test, standard instructions about the scale were given to the participants. Furthermore, anchoring procedures to determine low and high anchor points in the scale were used (Noble and Robertson, 1996). Then, participants were familiarised with the Psychomotor Vigilance Test (PVT), administered using a PVT-192 device (PVT-192, Ambulatory Monitoring, Inc., Ardsley, New York, USA), and the questionnaires utilised during the experimental visits. Participants were asked to wear a wrist actigraphy

device (Actiwatch Spectrum, Philips Respironics, Murrysville, Pennsylvania, USA) for the entire duration of the experiment in order to monitor their sleep/wake activity.

Experimental visits

Normal sleep

Prior to the visit, participants were asked to rest as much as possible, refraining from any strenuous exercise, and alcohol consumption. In addition to that, to ensure proper nutritional and hydration status (Smith, Maben and Brockman, 1993), they were instructed to maintain their habitual diets and meal times, and to drink an amount of water corresponding to 35 $mL \cdot kg^{-1}$ of body mass during the day. Any source of caffeine was forbidden in the twelve hours preceding the visit. Participants were asked to sleep at least for seven hours, allowing them to go to bed from 09:00 pm onwards. Wake up time was set to 06:00 am. To prevent the risk of hypoglycaemia during exercise (Sedliak *et al.*, 2007), participants were asked to have a standardised breakfast prior to each visit, consisting of 200 mL of orange juice (52 kCal per 100 mL, Despar Italia S.C.a, r.l., Fruttagel S.C.p.A., Alfonsine, Ravenna, Italy) and two snack bars (108 kcal per bar, Corny Classic, Hero group, Schwartauer Werke, Bad Schwartau, Germany). Upon arrival in the laboratory at 07:00 am, participants completed a pre-experiment checklist to ensure adherence to the instructions previously given. After that, they completed five questionnaires regarding: expectations; mental and physical fatigue; mood; motivation; and sleepiness (See *Psychological questionnaires* section). Then, participants performed a 10-min PVT test.

After a 5 min warm up at 50% $\dot{V}O_{2\ max}$, participants performed a time to exhaustion test (TTE) at Δ speed. Heart rate was recorded continuously. RPE was asked at the first minute, and then every five minutes. Prior to the start, participants were instructed to perform their best until reaching exhaustion. No verbal encouragement was provided. Participants were blinded to any kind of feedback (i.e., distance, speed, time). Test terminated when the participants decided that they could not run anymore.

After the TTE, participants completed a questionnaire related to their perceived workload.

Since the sleep deprivation visit was scheduled in the evening of the same day as the normal sleep visit, participants were instructed to follow their usual routines. They were reminded to refrain from any source of caffeine and sleep.

Sleep deprivation

The same day of the normal sleep visit, at 08:00 pm, participants were asked to arrive at the laboratory, where they were strictly monitored by two members of the Italian Army to avoid any napping, food consumption and the use of caffeine. During the night, participants were allowed to spend their time in activities such as reading; chatting; listening to music; playing video games; reading; and watching series / movies (Plukaard, 2015). In order to control food intake during the night, at 02:00 am participants were allowed to eat two snack bars, as mentioned in a previous section. The following morning, the testing procedures described in the *Normal sleep* section were replicated.

Psychological questionnaires

Expectations. A categorical scale (Gift, 1989; Wewers and Lowe, 1990) was used to assess the participants' expectations in relation to running performance. They were asked to circle one of the twenty-line intervals for both scales according to how they expected to perform in relation to their best performance. The total range of scores is between 0 and 20. Descriptors of extreme expectations ("much better" and "much worse") were anchored to the first and last lines.

Mental and Physical Fatigue. Subjective mental and physical fatigue were assessed using two visual analogue scales. Descriptors of extreme feelings of fatigue ("no fatigued at all" and "extremely fatigued") were anchored at its end points.

Mood. To evaluate participants' mood, The Brunel Mood Scale (BRUMS) was used (Terry, Lane and Fogarty, 2003). This 24-item questionnaire is a shorter version of The Profile of Moods States. A 5-point Likert scale (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, 4 = extremely) is used as an answer for each item. Six specific subscales with four related items each, are defined: anger, confusion, depression, fatigue, tension, and vigour. Scores are between 0 and 16.

Motivation. As a way of evaluating motivation related to the running task, ISMS (Matthews, Campbell and Falconer, 2001) were used. Using a 5-point Likert scale (0 = not all; 1 = a little; 2 = somewhat; 3 = very much; and 4 = extremely), participants are asked to select the number that best describes their attitude to a task in relation to 14 items. Items are allocated in two scales: intrinsic motivation, and success on task. Each scale consists of seven items. The range of scores of each scale is from 0 to 28.

Sleepiness. In order to measure subjective levels of sleepiness, the Karolinska Sleepiness Scale, KSS (Akerstedt and Gillberg, 1990) was used before and after each experimental visit. Using a 9-point Likert scale (1 = extremely alert; 2 = very alert; 3 = alert; 4 = rather alert; 5 = neither alert nor sleepy; 6 = some signs of sleepiness; 7 = sleepy but no effort to keep awake; 8 = sleepy, some effort to keep awake; and 9 = very sleepy, great effort to keep awake, fighting sleep).

Workload. The multidimensional rating scale NASA-TLX (Hart and Staveland, 1988) was used to evaluate the subjective workload experienced during the running task. This scale has six subscales: Mental Demand; Physical Demand; Temporal Demand; Performance; Effort; and Frustration. For each subscale, two descriptors are anchored at its end points: "very low" on the left end, and "very high" on the right one, with the exemption of Performance, which has "perfect" on the left endpoint and "failure" on the right one. Participants were required to complete this at the end of each experimental visit.

Cognitive performance

The Psychomotor Vigilance Test (PVT) is an objective assessment of cognitive impairment (vigilance) produced by sleep loss (Balkin *et al.*, 2004; Basner and Dinges,

2011). PVT-192 device is considered the gold standard for simple visual reaction time testing (Dinges and Powell, 1985; Khitrov *et al.*, 2014). A four-digit millisecond counter shown in a LED display acts as both visual stimulus and performance feedback. Response to the stimulus is done by pressing a button. The time difference between the stimulus presentation and the response is defined as reaction time (RT). Inter-stimulus intervals between 2000 and 10,000 ms are randomised by the device before each stimulus presentation. Right button was set to register responses.

Tests were conducted in a quiet area, during the familiarisation visit, participants performed a trial, with the purpose of finding their most comfortable setup. Participants were asked to always use the finger from their dominant hand. They were instructed to press and release the right button as soon as they saw the red numbers in the display. They were instructed to do their best and get the lowest number possible. Three parameters were extracted from the tests: mean RT, number of lapses (RT > 500 ms); and number of errors (i.e., false starts).

Actigraphy

Actigraphy watches (Actiwatch Spectrum, Philips Respironics, Murrysville, Pennsylvania, USA) were used to monitor sleep. This device continuously records light exposure and quantity of body movement (30-second epochs for this study) with a piezo-electric accelerometer. Default sleep/wake threshold (activity counts above 40) was used. Using a proprietary algorithm and software (Philips Respironics Actiware version 6.0.9) sleep parameters are calculated. This device and method have been used to assess sleep/wake behaviour in athletes several occasions (Halson *et al*., 2014; Sargent *et al*., 2014; Lastella *et al*., 2015a; Lastella *et al*., 2015b). For the purpose of this study, only total sleep times were reported.

Training

As part of their preparation for a mountain-ultra marathon during the 12-week period between tests two sleep deprivation nights were planned on weeks four and six respectively in addition to their physical programme. The first one as part of a 36-h mountain march. The second one took place during a 105-km mountain ultra-marathon (Gran Trail Courmayeur). Considering the sleep deprivation protocol of the experimental visits, over a 14-week period, participants performed three sleep deprivation nights during weeks 1, 5, 7, and 14 respectively (days 3, 35, 48, and 94). The purpose of the training was to exercise in a sleep deprived state, a frequent experience for athletes competing in ultra-marathons (Martin *et al.*, 2018). For this reason, participants were asked to avoid sleep during the day when sleep deprivation night was planned. In order to quantify the load, participants were asked to complete a diary, including: distance; time; and session-RPE (Foster *et al.*, 2001). Results are presented in Table 5.1.

Table 5.1. Training parameters

Sessions per week (N)	Hours per week (h)	Distance per week (km)	Mean session-RPE (A.U.)
4 ± 0	16.7 ± 5.2	44.8 ± 14.7	6 ± 3

Data are presented as mean ± SD.

Mountain-ultramarathon race

Participants were registered to participate in the 2019 edition of *Tor des Géants*, a ~340 km mountain-ultra marathon taking part in Aosta Valley (Italy) where participants must complete the course in less than 150 h. With a cumulative elevation gain of 24,000 m and altitude ranges between 322 and 3,300 metres above the sea level and twenty mountain passes over 2,000 m, this race is often described as one of the world's most challenging mountain-ultra-marathon.

Data analysis

$\dot{V}O_{2\ max}$ was calculated as the highest 30-s average oxygen uptake. The first ventilatory threshold (VT1) was determined using the following criteria: the first unequal increase in $\dot{V}CO_2$ from a visual inspection of $\dot{V}CO_2$ vs $\dot{V}O_2$ single plots; an increase in $\dot{V}E/\dot{V}O_2$ with no changes in $\dot{V}E/\dot{V}CO_2$; and increase in $PETO_2$ with no fall in $PETCO_2$. The second ventilatory threshold (VT2) using the following criteria: the first unequal increase in $\dot{V}CO_2$ from a visual inspection of $\dot{V}E$ vs $\dot{V}CO_2$ plots; an increase in $\dot{V}E/\dot{V}CO_2$; and a decrease in $PETCO_2$. Moreover, speeds corresponding at VT1 and VT2 were calculated.

Peak Treadmill Speed (PTS) and Δ speed were calculated using the following formulas:

$$PTS\ (km \cdot h^{-1}) = speed\ in\ the\ last\ completed\ stage + [(seconds\ at\ last\ uncompleted\ stage / 60\ s)]\ (Kuipers\ et\ al., 2003)$$

$$\Delta\ speed\ (km \cdot h^{-1}) = Speed\ at\ VT1 + [(Speed\ at\ VT2 - Speed\ at\ VT1) \cdot 0.5]$$

To prevent potential data loss caused by differences in TTE, an individual iso-time method was applied to HR and RPE values during TTE (Blanchfield, Hardy and Marcora, 2014). For each participant, the worst TTE was taken into consideration, four time points were calculated corresponding to 25%, 50%, 75% and 100% of TTE. The same time points were considered for the best TTE. This method to analyse TTE data has been suggested in the literature due to its sensitivity to changes in performance (Nicolò *et al.*, 2019).

Statistical analysis

Data were checked for assumptions of all statistical tests. Two-tailed paired-samples t-tests were used to assess differences before and after the training on the following variables: body mass; chronotype; daily sleepiness; Δ speed; HR_{max}; number of sessions per week; peak treadmill speed; physical activity; sleep quality; sleep time before each experimental visit; hours per week; speed at VT1; speed at VT2; $\dot{V}O_{2\,max}$; VT1; and VT2. Two-way repeated measures ANOVAs were conducted to investigate interactions between training (before and after the training) and condition (normal sleep and sleep deprivation) for: expectations; mental and physical fatigue; mood; motivation; sleepiness; perceived workload; PVT mean reaction time; PVT number of lapses; PVT number of errors; and TTE. Three-way repeated measures ANOVAs were used to assess interactions between, sleep condition, and iso-time points for: HR, and RPE. When the assumption of sphericity was not met, Greenhouse-Geisser correction was used. When significant interactions were found. Post-hoc analyses using pairwise comparisons were performed using Bonferroni correction. For ANOVAs, partial eta squared (ηp^2) effect sizes were reported when relevant. Values greater than 0.01, 0.06 and 0.14, were anchored to small, moderate, and large effect sizes, respectively (Cohen, 1988). To identify outliers (i.e., ± 3 SD) or input errors, studentized residuals were checked, if confirmed, outliers were not excluded. A 1/x transformation was applied to TTE and PVT mean reaction time in order to meet the normality assumption. Sleep time prior to the normal sleep visit, sleep time prior to the sleep deprivation visit, PVT number of lapses, and PVT number of errors were not normally distributed. Therefore, a series of Wilcoxon signed-rank tests were used. Spearman's rank correlation coefficient (r_p) test was used to assess the strength of association between physiological variables investigated in the study and race time. The strength of the associations between variables are defined as low (> 0.3), moderate (> 0.5), high (> 0.7), and very high (> 0.9), respectively (Hinkle, Wiersma and Jurs, 2003). Data are presented as means ± SD unless otherwise stated. Statistical significance was accepted a*t* $p < 0.05$ (two-tailed). Statistical analyses were performed using SPSS Statistics 28.0 (IBM, Armonk, New York, USA).

5.4 Results

Participants' characteristics

Normative data and cardio-respiratory fitness

No differences were found before and after the training in body mass ($p = 0.975$). Compared to prior to the training, speed at VT2 was lower after the training ($p < 0.001$). No differences were found before and after the training for: peak treadmill speed ($p = 0.051$); $\dot{V}O_{2\ max}$ ($p = 0.555$); HR_{max} ($p = 0.130$); VT1 ($p = 0.535$); speed at VT1 ($p = 0.132$); VT1 as of %$\dot{V}O_{2\ max}$ ($p = 0.648$); VT2 ($p = 0.128$); and VT2 as % of $\dot{V}O_{2\ max}$ ($p = 0.148$). Values are reported in Table 5.2.

Table 5.2. Normative data and cardio-respiratory variables pre-training and post-training.

	PRE	POST
Age (years)	29 ± 4	29 ± 4
Stature (m)	1.76 ± 0.06	1.76 ± 0.06
Body mass (kg)	79.6 ± 9.89	79.6 ± 12.0
Peak treadmill speed ($km \cdot h^{-1}$)	16.8 ± 1.03	16.3 ± 1.0
$\dot{V}O_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	52.1 ± 5.4	51.4 ± 7.5
HR_{max} (bpm)	183 ± 10	189 ± 12
Speed at VT1 ($km \cdot h^{-1}$)	10.8 ± 4.1	10.4 ± 0.7
VT1 (% of $\dot{V}O_{2\ max}$)	74.8 ± 5.2	74.0 ± 5.1
Speed at VT2 ($km \cdot h^{-1}$)	13.1 ± 1.0	12.6 ± 0.8*
VT2 (% of $\dot{V}O_{2\ max}$)	88.5 ± 3.2	86.5 ± 4.0

Data are presented as mean ± SD. * denotes difference from pre-training ($p < 0.05$).

Sleep time, sleep quality, chronotype, and daytime sleepiness

No significant differences before and after the training for: sleep time before the preliminary visit ($p = 0.266$); sleep quality ($p = 0.343$); chronotype ($p = 0.341$); and daytime sleepiness ($p = 0.496$). Table 5.3.

Table 5.3. Sleep quality, chronotype, and daytime sleepiness pre-training and post-training.

	PRE	POST
Sleep time prior to the preliminary visit (h)	7.77 ± 0.92	8.15 ± 1.45
PSQI (A.U.)	4 ± 1	4 ± 1
MEQ (A.U.)	59 ± 5	57 ± 6
ESS (A.U.)	5 ± 2	5 ± 2

Pittsburgh Sleep Quality Index (PSQI), Morningness-eveningness Questionnaire (MEQ), Epworth Sleepiness Scale (ESS). Data are presented as mean ± SD.

Training characteristics

Significant differences before and after the training were found number of sessions per week ($p = 0.009$), and for number of hours per week ($p = 0.003$). Values are shown in Table 5.4.

Table 5.4. Training variables pre-training and post-training.

	PRE	POST
Training (sessions per week)	3 ± 1	4 ± 0*
Training (hours per week)	10.1 ± 4.0	16.7 ± 5.2*

Data are presented as mean ± SD. * denotes differences from pre-training ($p < 0.05$).

Manipulation checks

Expectations

No significant interaction was found between condition (normal sleep, sleep deprivation) and training (pre, post) for expectations ($p = 0.252$). A main effect of

condition was found (p = 0.022). Compared to normal sleep, participants expected to perform (out of 20) 1.4 points worse in sleep deprivation. Figure 5.2.A. No main effect of training was found (p = 0.638).

Mental and physical fatigue

No significant interaction was found between condition and training for mental fatigue (p = 0.965). Main effects of condition (p = 0.001) and training (p = 0.027) were found. Post-hoc analysis revealed that, compared to normal sleep, participants perceived mental fatigue (out of 20) 4.65 points higher in sleep deprivation. Follow-up analysis indicated that, compared to pre-training, participants perceived mental fatigue 2.25 points lower after the training. Figure 5.2.B.

No significant interaction was found between condition and training for physical fatigue (p = 0.678). A main effect of condition (p = 0.002) was found. Post-hoc analysis revealed that, compared to normal sleep, participants perceived physical fatigue (out of 20) 4 points higher in sleep deprivation. No main effect of training was found (p = 0.330). Figure 5.2.C.

Mood

No significant interaction was found between condition and training for total mood disturbance (p = 0.898). Neither a main effect of condition (p = 0.068), nor a main effect of training (p = 0.558) were found.

No significant interaction was found between condition and training for vigour (p = 0.461). A main effect of condition (p < 0.001) was found. Post-hoc analysis revealed that, compared to normal sleep, participants were feeling (out of 28) 3.55 points less vigorous in sleep deprivation. No main effect of training (p = 0.726) was found. Figure 5.2.D.

No significant interaction was found between condition and training for tension (p = 0.478). Neither a main effect of condition (p = 0.377), nor a main effect of training (p = 0.069) were found.

No significant interaction was found between condition and training for confusion (p = 1.000). Neither a main effect of condition (p = 0.713), nor a main effect of training (p = 0.085) were found.

No significant interaction was found between condition and training for depression (p = 0.749). Neither a main effect of condition (p = 0.120), nor a main effect of training (p = 0.087) were found.

No significant interaction was found between condition and training for anger (p = 0.753). Neither a main effect of condition (p = 0.075), nor a main effect of training (p = 0.110) were found.

No significant interaction was found between condition and training for fatigue (p = 0.075). Neither a main effect of condition (p = 0.318), nor a main effect of training (p = 0.896) were found.

Motivation

No significant interaction was found between condition and training for intrinsic motivation (p = 0.502). Neither a main effect of condition (p = 0.227) nor a main effect of training (p = 0.851) were found.

No significant interaction was found between condition and training for success in the task (p = 0.339). Neither a main effect of condition (p = 0.069) nor a main effect of training (p = 0.900) were found.

Sleepiness

No significant interaction was found between condition and training for subjective sleepiness (p = 0.168). A main effect of condition was found (p < 0.001). Post-hoc analysis revealed that, compared to normal sleep, participants' sleepiness was (out of 9) 3.3 points higher in sleep deprivation. No main effect of training was found (p = 0.147) Figure 5.2.E.

Sleep time

No significant interaction between condition and training were found for sleep time (p = 0.435). A main effect of condition was found (p < 0.001). Post-hoc analysis revealed that, in comparison to normal sleep, participants' sleep was 6.47 h lower in sleep deprivation. No main effect of training was found (p = 0.436). Figure 5.2.F.

Workload

No differences were found between pre-training and post-training for the following variables: effort (p = 0.095); frustration (p = 0.232); mental demand (p = 0.494); performance (p = 0.310); physical demand (p = 0.247); and temporal demand (p = 0.232).

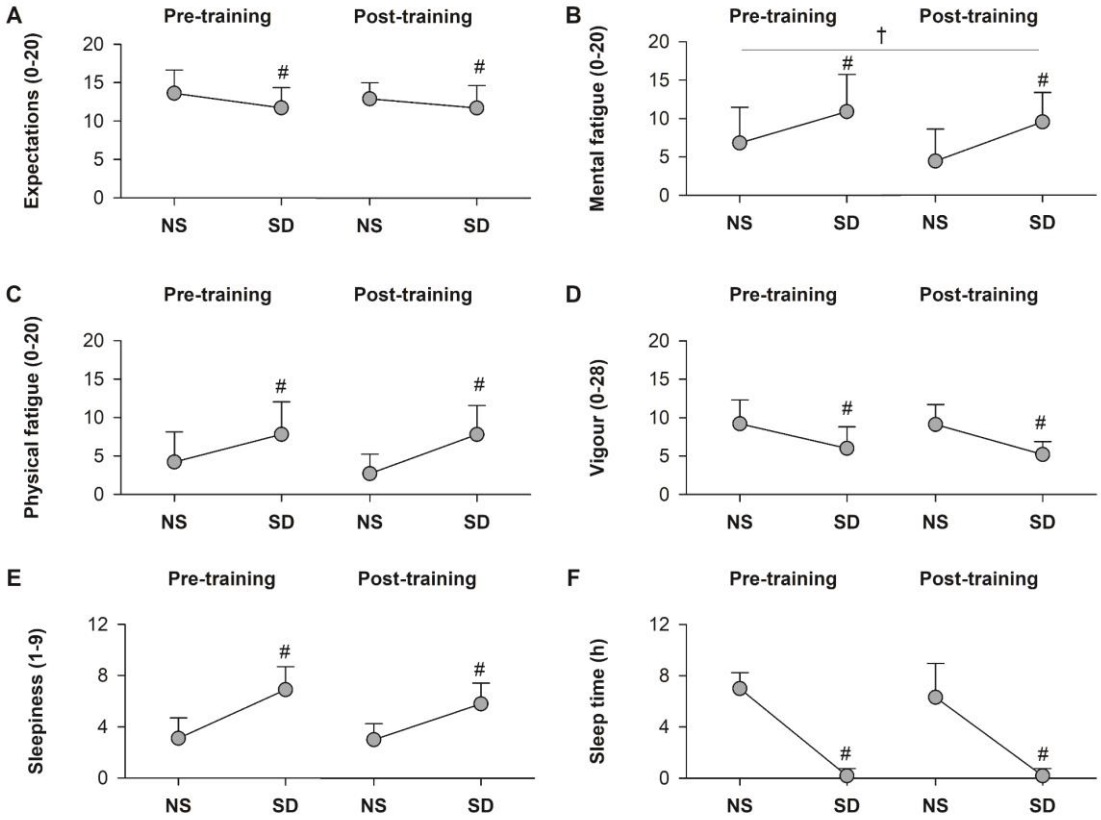


Figure 5.2 Performance expectations (A). Mental fatigue (B). Physical fatigue (C). Vigour (D). Sleepiness (E). Sleep time (F). NS = normal sleep. SD = sleep deprivation. Data are presented as mean ± SD. # denotes differences between conditions ($p < 0.05$). † denotes differences between training phase ($p < 0.05$).

Time to exhaustion

Performance

No differences were found before and after the training for Δ speed (PRE: 11.9 ± 0.7 km·h^{-1}; POST: 11.5 ± 0.8 km·h^{-1}, $p = 0.062$). Δ speed as % of PTS was not different before and after the training (PRE: 71.2 ± 3.9%; POST: 70.6 ± 4.0%, $p = 0.701$).

No interaction between condition (normal sleep, sleep deprivation) and training (pre-training, post-training) was found for TTE ($p = 0.832$). A main effect of condition was found ($F_{1,1} = 44.621$, $p < 0.001$, $\eta p^2 = 0.832$). TTE in normal sleep was longer than in

sleep deprivation. No main effect of training was found (p = 0.084). Values are shown in Table 5.5. and Figure 5.3.

Table 5.5. Time to exhaustion pre-training and post-training.

	PRE	POST
TTE NS (s)	1547 ± 421	1624 ± 477
TTE SD (s)	1132 ± 418#	1160 ± 572#
δ performance (s)	-415 ± 376	-463 ± 229
δ performance (%)	-25.8 ± 23.5	-30.8 ± 15.4

Data are presented as mean ± SD. TTE = time to exhaustion. NS = Normal sleep. SD = Sleep deprivation. # denotes differences from NS (p < 0.05).

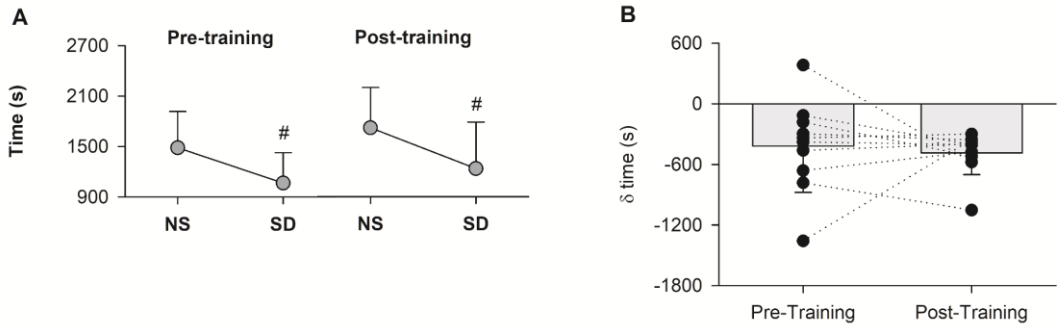


Figure 5.3 Time to exhaustion (A). Absolute change in time to exhaustion (B). Black circles depict individual performances. NS = normal sleep. SD = sleep deprivation. Data are presented as mean ± SD. # denotes differences between conditions (p < 0.05).

Heart rate

No interaction between condition (normal sleep, sleep deprivation), iso-time (25%, 75%, and 100%), and training (pre, post) was found for HR (p = 0.548). A two-way interaction between condition and iso-time was found ($F_{1.138, 10.240}$ = 6.228, p = 0.028,

$\eta p^2 = 0.409$. Neither a two-way interaction was found between condition and training ($p = 0.054$), nor between iso-time and training ($p = 0.056$). Main effects of condition ($F_{1, 9} = 21.198$, $p = 0.001$, $\eta p^2 = 0.702$) and iso-time ($F_{1.237, 11.130} = 36.512$, $p < 0.001$) were found. HR was lower in sleep deprivation than in normal sleep. HR increased over time. No main effect of training was found ($p = 0.543$). Post-hoc analysis revealed that, compared to normal sleep, HR was lower in sleep deprivation at 50% ($p < 0.001$), 75% ($p < 0.001$), and 100% ($p < 0.001$). No differences were found at 25% ($p = 0.973$) Figure 5.4.A.

RPE

No interaction between condition, training, and iso-time was found ($p = 0.958$). A two-way interaction between condition and iso-time was found ($F_{1.408, 12.674} = 5.745$, $p = 0.024$, $\eta p^2 = 0.390$). Neither a two-way interaction was found between condition and training ($p = 0.935$), nor between iso-time and training ($p = 0.443$). Main effects of condition ($F_{1, 9} = 12.525$, $p = 0.006$, $\eta p^2 = 0.582$) and iso-time ($F_{1.689, 15.201} = 139.421$, $p < 0.001$) were found. Compared to normal sleep, RPE was higher in sleep deprivation. RPE increased over time. Post-hoc analysis revealed that, compared to normal sleep, RPE was higher in sleep deprivation at 25% ($p = 0.007$), 50% ($p = 0.017$), and 75% ($p = 0.006$). No differences were found at 100% ($p = 0.591$) Figure 5.4.B.

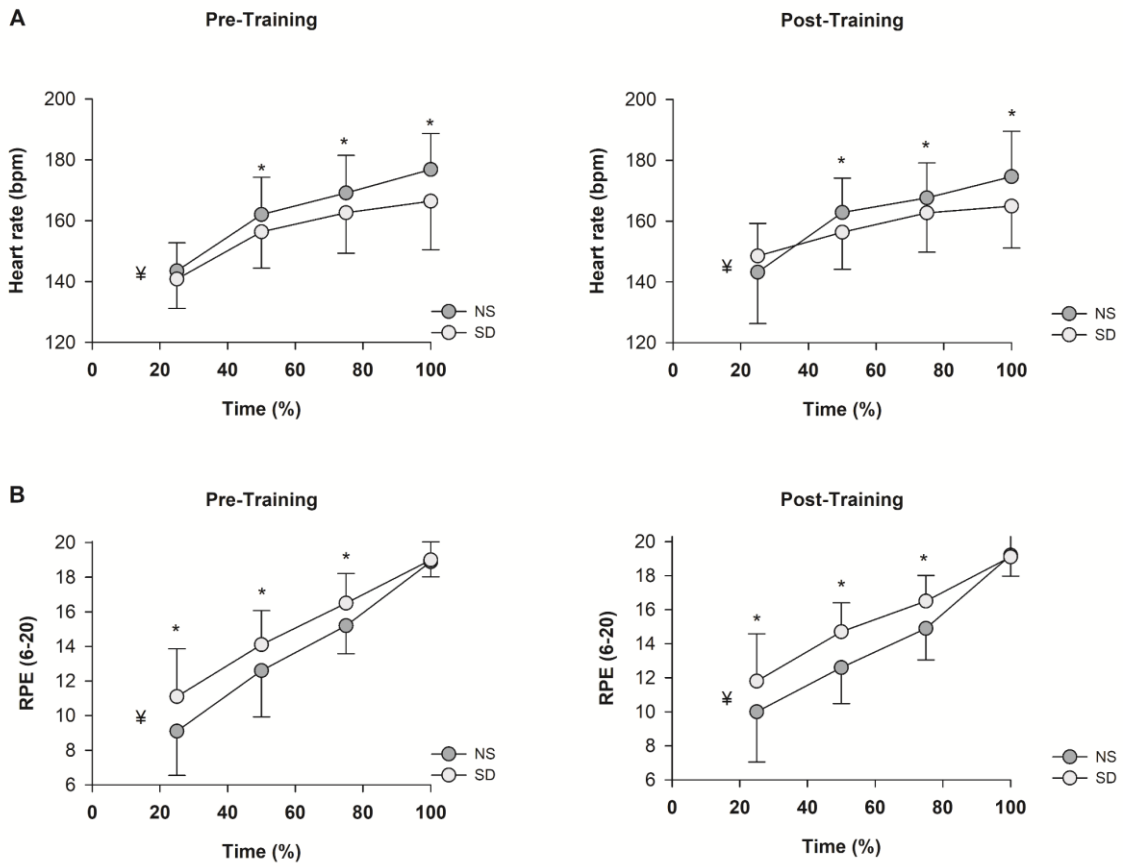


Figure 5.4 Time to exhaustion test: Heart rate (A). RPE (B). NS = normal sleep. SD = sleep deprivation. ¥ denotes interaction between condition and iso-time ($p < 0.05$). * denotes difference from normal sleep ($p < 0.05$). Data are presented as mean ± SD.

Cognitive performance

Reaction time

No interaction between condition (normal sleep, sleep deprivation) and training (pre, post) was found for mean reaction time ($p = 0.156$). A main effect of condition was found ($F_{1,9} = 12.590$, $p = 0.006$ $\eta p^2 = 0.583$). Mean reaction time was lower in normal sleep than in sleep deprivation. No main effect of training was found ($p = 0.838$). Values are shown in Table 5.6. Figure 5.5.A. and Figure 5.5.B.

Table 5.6. Mean reaction time pre-training and post-training.

	PRE	POST
Normal sleep (ms)	255 ± 30	261 ± 32
Sleep deprivation (ms)	319 ± 115#	293 ± 59#
δ performance (ms)	63.9 ± 94.2	32.2 ± 31.6
δ performance (%)	-23.1 ± 31.4	-11.6 ± 11.0

Data are presented as mean ± SD. TTE = time to exhaustion. NS = Normal sleep. SD = Sleep deprivation. # denotes differences from NS ($p < 0.05$).

Number of lapses

Before the training, the number of lapses was lower in normal sleep, compared to sleep deprivation (Z = -2.060, $p = 0.039$). After the training, no differences were found for the number of lapses between normal sleep and sleep deprivation ($p = 0.093$). Neither a difference was found in the number of lapses in normal sleep, pre-training and post-training ($p = 0.655$), nor in the number of lapses in sleep deprivation, pre-training and post-training ($p = 0.588$). Figure 5.5.C.

Number of errors

Before the training, no differences were found for the number of errors between normal sleep and sleep deprivation ($p = 0.655$). No differences were found after the training for the number of errors between normal sleep and sleep deprivation ($p = 0.564$). Neither a difference was found in the number of errors in normal sleep, pre-training and post-training ($p = 0.655$). Compared to pre-training, the number of errors in sleep deprivation was lower post-training (Z = -1.992 $p = 0.046$). Figure 5.5.D.

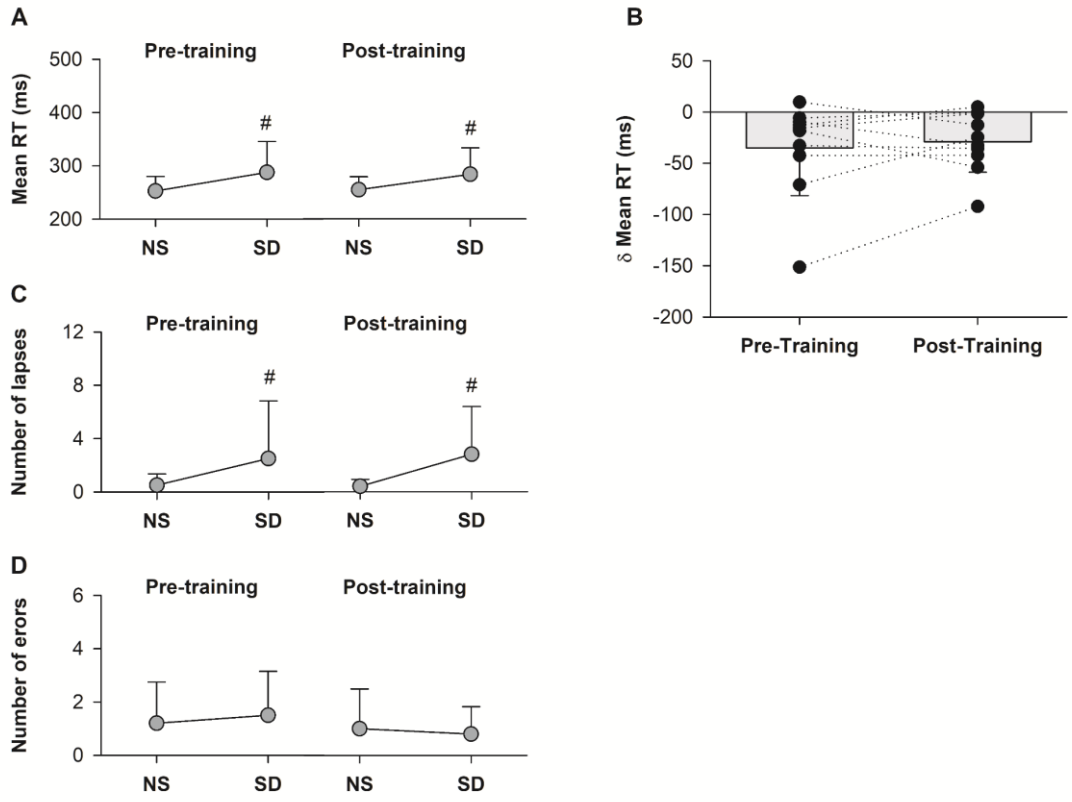


Figure 5.5 PVT performance: Mean RT (A). Number of lapses (B). Number of errors (C). Data presented as mean ± SD. Black circles depict individual performances. # denotes difference between conditions. a denotes difference from pre-training ($p < 0.05$).

Association between race performance and other variables

Race performance

As a consequence of the race strategy followed by the participants, aiming to complete the event running as a platoon, they did not take into consideration the amount of time lost when working as a group (i.e., refuelling). Due to the substantial risk of not finishing the event due to the cut-off time at some checkpoints, they were forced to change the plan. Some athletes had to retire due to physical issues (i.e., blisters), others voluntarily withdrew from the event due to fatigue. Therefore, only four out of fifteen participants finished the race (26.7%) in 148:59:00 ± 00:00:49 (HH:MM:SS). For comparison

purposes, total distance covered was considered as a performance parameter (the more distance you covered, the better your race performance was).

Participants' characteristics

No significant associations were found between distance covered and the following variables: age (p = 0.159), body mass (p = 0.438), session-RPE (p = 0.189), training distance per week (p = 0.132), training hours per week (p = 0.411), and training sessions per week (p = 0.253).

Sleep time, sleep quality, chronotype, and daytime sleepiness

A moderate positive association between distance covered and chronotype was found (r_p = 0.630, p = 0.012). Participants with higher MEQ score (the higher the MEQ the more "Morning type" chronotype) covered more distance during the race. Figure 5.6.A.

A moderate negative association between distance covered and weekly sleep time was found (r_p = -0.641, p = 0.010). Participants that reported less weekly sleep time during the training period covered more distance during the race. Figure 5.6.B.

No significant associations were found between distance covered and the following variables: daytime sleepiness (p = 0.683), and sleep quality (p = 0.830).

Cardio-respiratory fitness

A moderate positive association between distance covered and peak treadmill speed was found (r_p = 0.533, p = 0.041). Participants that reached a higher peak treadmill speed during the preliminary visit covered more distance during the race. Figure 5.6.C.

No significant associations with distance covered were found for the following variables: HR_{max} (p = 0.147), $\dot{V}O_{2\,max}$ (p = 0.160), VT1 (p = 0.215), and VT2 (p = 0.974).

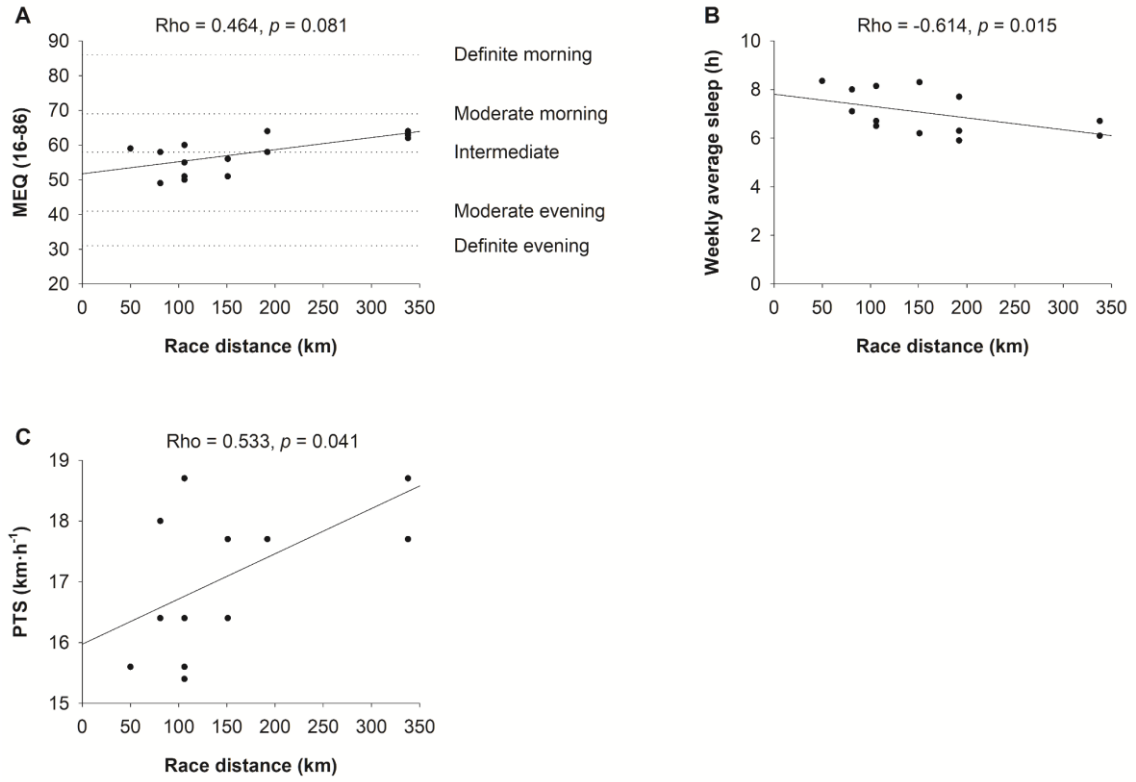


Figure 5.6 Associations with distance covered. Chronotype (A). Average sleep per week (B). Peak treadmill speed (C). MEQ = Morningness-eveningness questionnaire. PTS = Peak treadmill speed.

Time to exhaustion

A moderate positive association between distance covered and TTE in normal sleep was found (r_p = 0.569, p = 0.027). Participants with a longer TTE covered more distance during the race. Figure 5.7.A.

A high positive association between distance covered and TTE in a sleep deprived state was found (r_p = 0.771, p < 0.001). Participants with a longer TTE covered more distance during the race. Figure 5.7.B.

A moderate negative association between distance covered and TTE difference (as a %, TTE in sleep deprivation, compared to TTE in normal sleep) was found (r_p = 0.587, p =

0.021). Participants with a lower decrease in TTE covered more distance during the race. Figure 5.7.C.

A high positive association between distance covered and total TTE performance (the sum of TTE performance in both conditions, normal sleep, and sleep deprivation) was found ($r_p = 0.767$, $p < 0.001$). Participants with a higher total TTE covered more distance during the race. Figure 5.7.D.

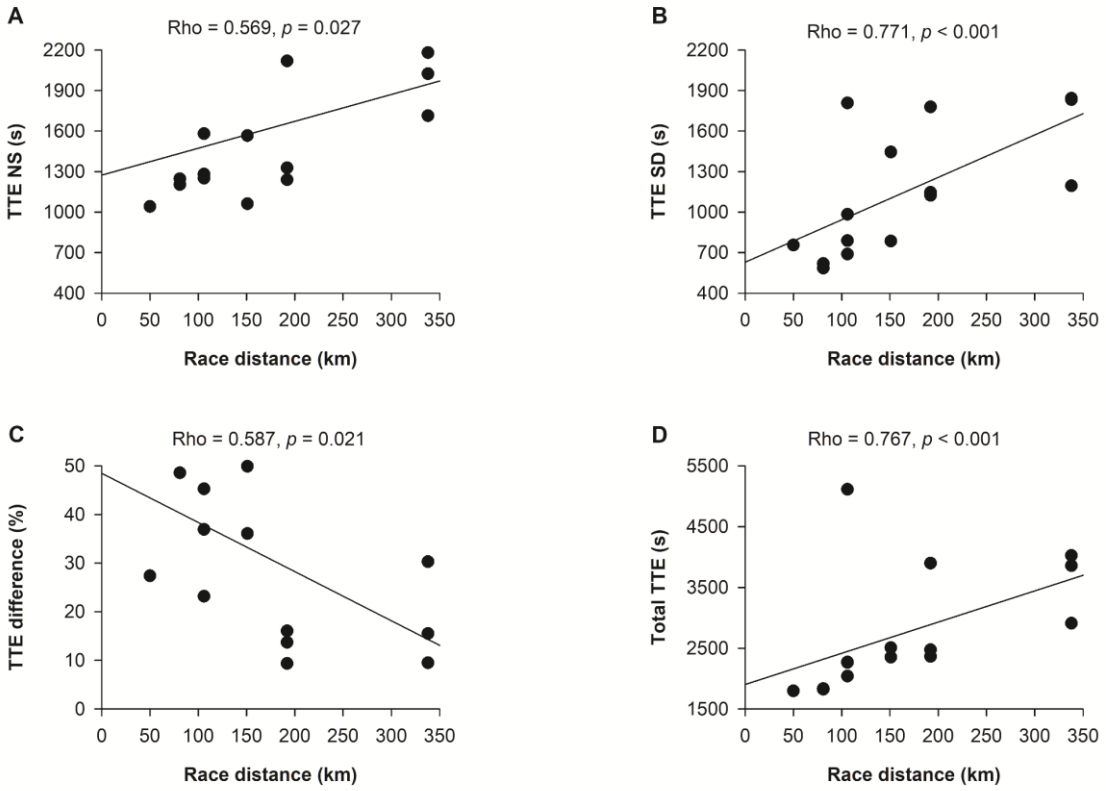


Figure 5.7 Associations with distance covered. TTE. NS = Time to exhaustion in normal sleep (A). TTE SD = Time to exhaustion in sleep deprivation (B). TTE difference (C). Total TTE (D).

Cognitive performance

No significant associations were found between distance covered and the following variables: PVT mean reaction time in normal sleep ($p = 0.274$), PVT mean reaction time in sleep deprivation ($p = 0.114$), PVT difference, as a %, mean RT in sleep deprivation

compared to mean RT in normal sleep (p = 0.205), and the sum of mean RT in both conditions (p = 0.290).

5.5 Discussion

The main findings of the study indicated that three bouts of exercise under sleep deprivation over a 14-week period training for a mountain ultra-marathon did not improve tolerance to sleep deprivation. Further research is required to investigate the number of sessions required to induce any potential adaptation. Furthermore, sleep deprivation had a negative effect on both endurance and cognitive performance. TTE was impaired by 28.3 ± 19.9% (439 ± 311 s). PVT mean reaction time was increased by 17.4 ± 23.5% (48.1 ± 70.3 ms). From a physical perspective, the increment in the number of sessions and hours did not improve cardio-respiratory fitness. Regarding cognitive performance, no differences in PVT mean reaction time, number of errors, and number of lapses were observed between pre- and post-training. Associations were found between race performance, established as distance covered during a ~340 km mountain-ultramarathon, chronotype, average sleep per week during training, peak treadmill speed, and time to exhaustion performance.

Endurance performance

TTE in normal sleep was longer than in sleep deprivation. Our results are in line with previous studies: a 20.1% reduction in TTE running at 80% of $\dot{V}O_{2\,max}$ after 27 h of sleep deprivation (McLellan *et al.*, 2005). A 9.5% reduction in TTE walking at 80% of $\dot{V}O_{2\,max}$ after 36 h of sleep deprivation (Martin, 1981). A 21.9% reduction in TTE walking at 5.6 $km \cdot h^{-1}$ after 50 h of sleep deprivation (Martin and Chen, 1984). Using other protocols, similar performance impairments have been reported: a 7.94% decrease in a 8-km TT run after 26 h of sleep deprivation (Khcharem *et al.*, 2022). A 3% decrease in a 30-min TT run after 30 h of sleep deprivation (Oliver *et al.*, 2009).

This reduction in TTE performance might be explained by an increased RPE in sleep deprivation, in agreement with the literature across different exercise modalities and sleep deprivation durations: running after 28 h (McLellan, Bell and Kamimori, 2004). Walking after 36 h (Martin, 1981); after 60 h (Myles, 1985); and after 64 h (Plyley *et al.*, 1987). Cycling after 1 night (Temesi *et al.*, 2013); and after 30 h (Chen, 1991).

Cognitive performance

Compared to normal sleep, mean reaction time was worsened in sleep deprivation. Our results are supported by the evidence reported in the literature. The effects of sleep deprivation on cognitive performance are quite clear in relation to exercise: several cognitive performance markers are impaired as a result of sleep loss (Martin, Bender and Chen, 1986; Fullagar *et al.*, 2015). In the context of reaction time during ultra-endurance events, only a few studies have been published: solo sailing races (Hurdiel *et al.*, 2014), and a 168-km mountain ultra-marathon (Hurdiel *et al.*, 2015). However, inter-individual variability has been described in various studies: some subjects might be particularly vulnerable to sleep deprivation, while other seemed to be more resilient to the negative effects of sleep deprivation on cognitive performance (Van Dongen *et al.*, 2004; Van Dongen, Maislin and Dinges, 2004; Lim, Choo and Chee, 2007; Rupp, Wesensten and Balkin, 2012). Several factors can influence vulnerability to sleep deprivation: sleep prior to the sleep deprivation caused by family responsibilities, social activities, or work commitments will reduce sleep deprivation tolerance; young medical doctors were more vulnerable to acute sleep deprivation than their older colleagues (Czeisler, 2009); potential genetic factors, such as *PER3* gene polymorphism *PER3(3/5)*, present in 10-15% of the population, responsible for decrements in cognitive performance in response to sleep loss (Viola *et al.*, 2007). A genetic variation of the *ADORA2A* gene may be associated with tolerance to the negative effects of sleep deprivation on cognitive performance (Bodenmann *et al.*, 2012).

Behavioural responses

Participants expected to perform worse in sleep deprivation. However, no changes were observed in motivation and success. Motivation to perform a task might be reduced by lack of sleep, due to a reduction in effort willingness (Lim and Dinges, 2008; Massar *et al.,* 2019). No differences were found in total mood disturbance, whereas vigour was lower in sleep deprivation than in normal sleep. A lower level of vigour has been related to sleep deprivation (Martin, Bender and Chen, 1986; Meney *et al.*, 1998). Subjective sleepiness, physical and mental fatigue were higher in sleep deprivation than in normal sleep. Our results are in line with the literature, where increased levels of sleepiness and subjective fatigue were associated with sleep deprivation (Dinges *et al.*, 1997). No changes in perceived workload were found.

Training

A 12-week plan, consisting of four training sessions per week, averaging 16.7 ± 5.2 hours and 44.8 ± 14.7 km per week, did not significantly change any physiological parameters measured. Actually, speed at VT2 was 0.5 ± 0.91 $km \cdot h^{-1}$ lower after the training. A potential explanation for this is the fact that the last two weeks of the training plan corresponded with a two-week break. During this period soldiers were on holidays, and therefore, away from the barracks. Despite being instructed to continue with their training plan during the holidays, the vast majority of the group did not train as usual. Participants were tested immediately after the training period. A partial loss of training-induced adaptations might have occurred due to insufficient training stimulus during the last two weeks. In 6 well-trained endurance runners, $\dot{V}O_{2\ max}$ was reduced by 4% after fifteen days of inactivity (Houston, Bentzen and Larsen, 1979). More recently, in a group of endurance-trained runners, fourteen days of inactivity led to a 4.7% reduction in $\dot{V}O_{2\ max}$ (Houmard *et al.*, 1992). On the other hand, $\dot{V}O_{2\ max}$ did not decrease in a group of distance runners after ten days of inactivity (Cullinane *et al.*, 1986), recent endurance performance improvements can be maintained for at least two weeks without training. The amount of physical activity performed by the athletes during the period of training cessation could partly explain these contradictory results found in the literature.

Reductions in $\dot{V}O_{2\,max}$ due to training stoppage seems to be dependent on time and initial fitness level. (Mujika and Padilla, 2001).

Contrary to our hypothesis, apart from perceived mental fatigue, no differences were found pre- and post-training in variables that could have possibly indicated an improvement in sleep deprivation tolerance, such as TTE performance, RPE, sleepiness, perceived workload, vigour, etc. A potential explanation could be that three bouts of exercise under sleep deprivation, over a 14-week period, is not enough to induce any adaptation in the resilience to the negative effects of sleep deprivation in psychophysiological parameters. In a recent case study published, a Sleep Deprivation Training, consisting of 6 nights of sleep deprivation over a 6-week period, had a beneficial effect on sleepiness and perceived mental effort in a multi-day testing setting (Gattoni *et al.*, 2022). In the light of the results of that case study and our results, further research is needed, such as a randomised controlled trial experiment, to confirm whether Sleep Deprivation Training could improve sleep deprivation tolerance, and consequently, improve endurance performance.

Associations between race performance and other variables

Several associations were found between variables investigated and race performance (distance covered during a ~340 km mountain ultra-marathon). Participants with a higher MEQ score (the higher the score, the more "morning-type" chronotype) covered more distance during the race, supporting the results reported in a systematic review, showing that morning-types perceived less effort when performing a sub-maximal task in the morning, when compared to neutral and evening types Therefore, morning-type athletes perform better in morning events (Vitale and Weydahl, 2017). It has been proposed that recreational endurance sport individuals have an earlier chronotype than non-competitive males, finding associations between half-marathon and marathon performances and morning types (Henst *et al.*, 2015). Additionally, morning-type chronotypes are overrepresented in several sport disciplines: solo sailing, (Filardi, Morini and Plazzi, 2020), a group of cyclists, runners, and Ironman triathletes from South Africa (Kunorozva *et al.*, 2012), South African and Dutch half-marathon and marathon

runners (Henst *et al.*, 2015), and ultra-marathon runners (Martin *et al.*, 2018). Authors hypothesised that chronotype may be influenced by a commonly early start times of diverse endurance sports competitions (Henst *et al.*, 2015; Roden, Rudner and Rae, 2017). Further However, results are not always consistent, this could be partially explained by heterogeneous samples and different levels of fitness (Vitale and Weydahl, 2017).

Participants with a lower weekly sleep duration covered more distance during the event. Our results are in contrast to the sleep habits of ultra-marathon runners, a population with a high percentage of nappers and similar sleep durations as the general population and other athletic populations (Martin *et al.*, 2018). Our results might be partially explained by a lower sample size (N = 15), compared to the 636 responses from participants in the study from Martin *et al.* (2018).

Participants with a higher peak treadmill speed covered more distance in the mountain ultra-marathon event investigated in this study. According to a review of the limiting factors during an ultra-marathon, higher levels of performance in long-distance running are reliant of high $\dot{V}O_{2\,max}$, high fraction of $\dot{V}O_{2\,max}$ utilisation, and cost of oxygen transport (Garbisu-Hualde and Santos-Concejero, 2020). On the other hand, other researchers have reported that the main limiting factor is $v\dot{V}O_{2\,max}$ (Balducci *et al.*, 2017; Fornasiero *et al.*, 2018).

Participants with a better sleep deprivation tolerance (defined as a lower difference in TTE between normal sleep and sleep deprivation, or as the sum of TTE in normal sleep and sleep deprivation conditions, or as a longer TTE in a sleep deprived state) covered more distance during the ultra-marathon event. To the best of our knowledge, this concept has not been widely explored. Further research may explore the assessment of reliable and valid tests to evaluate sleep deprivation tolerance. Additionally, following studies may investigate sleep deprivation tolerance as a limiting factor in ultra-endurance performance.

Limitations

The present study has several limitations that may have affected the final findings: 1) participants were not strictly monitored during the training phase. Consequently, they may have failed to properly control the training load. 2) Two of the three exercise bouts under sleep deprivation were done in the field, one in a 36-h military march, the other during an ultra-endurance event, both were part of the training plan. However, participants may have fallen asleep during these trials. 3) During TTE participants were tested in pairs. Despite being instructed to do their best, and not being influenced by the other person. This setup may have affected their engagement during the test. Future research should be conducted using an individual setting. 4) Sleep and training were monitored weekly during the training phase via questionnaires. In order to obtain more accurate data, the use of actiwatches and a heart rate monitor is recommended for future studies. 5) During the last two weeks of the training phase, participants were on holidays, therefore, adherence to the training plan was reduced. Participants were immediately tested after that period. Therefore, any potential adaptation may have been affected because of this factor. Future studies may need to develop a more effective training plan. 6) Training intervention and number of exercise bouts under sleep deprivation may not have been enough to induce any potential effect. Further research needs to explore a longer intervention with more frequent sleep deprivation bouts. 7) Several participants raced together during the mountain ultra-marathon event. Despite being a common behaviour of these types of events, where participants with a similar level tend to race together for the whole duration of the event, or at least a substantial portion of the event, this may have influenced our results. Future studies may want to explore larger sample sizes of unrelated participants. Furthermore, we based our analysis in the distance covered as a marker of performance. Moreover, further research needs to aim for an analysis of race time in finishers only to better address race performance.

It is important to emphasise that several limitations were inherent to the fact that this research was conducted in military personnel. Therefore, future research needs to be conducted in a better controlled environment, with a different population, and using a longer intervention with more frequent exercise bouts under sleep deprivation.

5.6 Conclusions

In conclusion, the results of this study provide novel information about the negative effects of sleep deprivation on cognitive and physical performance, addressing the importance of counteracting the effects of sleep deprivation in order to improve performance. Furthermore, the protocol used, including one night of sleep deprivation, seems a suitable way to assess cognitive and physical performance in a sleep deprived state, partially simulating an ultra-endurance event. The inclusion of exercise bouts after one night of sleep deprivation in a training plan (Sleep Deprivation Training) as part of the preparation for a mountain ultra-marathon event may improve the finish rate in an ultra-endurance race. Our results failed to show any effect of these innovative type training. Several limitations may have affected our results. However, further research needs to be carried out to investigate the length and number of bouts per week required to potentially reduce the negative effects of sleep deprivation. A case study providing some initial information about the effects of Sleep Deprivation Training on endurance performance have been published recently. Additionally, the combination of Sleep Deprivation Training with other strategies to counteract the effects of sleep deprivation, such as caffeine, may be explored in future investigations.

Moreover, the results of our study have shown moderate and high associations between sleep deprivation tolerance (i.e., the sum of TTE in normal sleep and sleep deprivation) and performance during a ~340 km mountain ultra-marathon. Sleep deprivation tolerance may be further explored as a potential factor of ultra-endurance performance. Methods to improve sleep deprivation tolerance, and therefore, ultra-endurance performance, as well as valid and reliable protocols to assess sleep deprivation tolerance may be studied in future investigations.

Chapter 6: General Discussion

6.1 Main findings

The present PhD thesis aimed to investigate sleep deprivation in ultra-endurance performance. Therefore, two main categories have been distinguished in order to discuss the main findings: assessment and countermeasures. The main findings have been discussed primarily in the context of endurance exercise, and its associated perceptual and physiological responses. The main effects of sleep deprivation on cognitive performance have not been included in this chapter as they are not the central findings of this PhD thesis. Furthermore, they have been already widely investigated and reported in the literature.

Sleep assessment during mountain ultra-marathon events

To the best of our knowledge, the first sleep study in a mountain ultra-marathon event longer than 330 km has been conducted during this doctoral research program (Chapter 3), providing a new insight, and filling that gap in the literature. The aim of the study was to investigate sleep-related parameters during a ~431 km ultra-marathon.

The main findings of this descriptive field study have shown that there is a moderate positive association between sleep-related parameters and race time, suggesting that faster runners sleep less and spend less time in activities (i.e., restoring food/drinks at the checkpoints, resting, sleeping) other than moving towards the finish line. These results are in accordance with studies done in shorter events, such as *Ultra-Trail du Mont Blanc* - 168 km (Hurdiel *et al.*, 2015, 2018; Poussel *et al.*, 2015), and self-reported sleep duration in races longer than 60 h (Martin *et al.*, 2018). Before the first sleep bout during the race, participants were awake for more than 24 hours. A potential explanation for this

is that participants, as part of their sleep strategy, deliberately decided not to sleep during the first night, in order to minimise the impact of sleep time on finish time (Martin *et al.*, 2018). Total sleep times reported in this study are within the range of similar field studies (Saugy *et al.*, 2013; Hurdiel *et al.*, 2015, 2018; Poussel *et al.*, 2015; Savoldelli *et al.*, 2017).

Assessment of the negative effects of sleep deprivation on endurance performance

The effects of sleep loss have been well reported in the context of exercise performance, for a review see Fullagar *et al.* (2015). Two experiments of this doctoral research program have explored the negative effects of sleep deprivation in different scenarios (Chapters 3 and 5). In the descriptive field study investigating sleep during mountain ultra-marathons (Chapter 3), during the course of the race, sleep episodes, rest and sleep times increased, whereas speed decreased. This could be explained by the fact that small amounts of sleep (naps), which is one of the main sleep strategies used by ultra-marathon runners (Poussel *et al.*, 2015; Martin *et al.*, 2018), may not be sufficient to compensate for the sleep debt accumulated during the race. Consequently, the number of sleep episodes may increase later in the race as a result of cumulative fatigue (Hurdiel *et al.*, 2018), and psychophysiological demands of ultra-endurance exercise (Millet, 2011). Furthermore, a low positive association ($r_p = 0.409$) between RPE and distance, and a low negative association ($r_p = -0.436$) between RPE and split speed were found, in line with several results reported in the literature (Utter *et al.*, 2003; Chapman and Mickleborough, 2009; Oliver *et al.*, 2009; Martin *et al.*, 2010; Mann *et al.*, 2015; Howe *et al.*, 2019). A moderate positive association was found between sleepiness and distance run ($r_p = 0.513$). This is in line with the results reported by Hurdiel *et al.* (2018).

In the context of fatigue during prolonged exercise, categorised by two main factors: a decline in muscular force, and an increased perception of effort (Mosso, 1891; Enoka and Stuart, 1992; Marino, Gard and Drinkwater, 2011), the results of this study are encompassed in the second category, aligned with the model designed to explain the role of fatigue on ultra-endurance performance (Millet, 2011), where sleep deprivation increases RPE. Therefore, as one of the factors involved in pace regulation, according to

the psychobiological model of endurance performance (Marcora, 2008; Marcora and Staiano, 2010; Pageaux, 2014), the negative association between RPE and split speed may suggest that athletes compensate the increment in perceived effort, resulting from the demands of ultra-endurance exercise and sleep deprivation, by reducing their running speed.

As part of the preparation for a mountain ultra-marathon (Chapter 5), a novel testing protocol was designed, in order to test the resistance to the negative effects of sleep deprivation on endurance performance in a more ecologically valid setup, partially simulating an ultra-endurance event. Participants were required to perform two time to exhaustion tests (TTE), running at ~71% of their peak treadmill speed, on consecutive days, one after a night of normal sleep, followed by the other one after a day of total sleep deprivation. TTE decreased by ~28% after one night of total sleep deprivation. Results are within the range of performance decrements reported in TTE studies (Martin, 1981; Martin and Chen, 1984; McLellan *et al.*, 2005; Azboy and Kaygisiz, 2009), and TT protocols (Oliver *et al.*, 2009; Khcharem *et al.*, 2022). A potential explanation for a reduction in TTE performance is due to an increase in RPE after sleep deprivation, as reported in similar studies performed at a constant intensity (Martin, 1981; Martin and Gaddis, 1981; Martin and Haney, 1982; Mougin *et al.*, 1991; Marcora, 2009; Pageaux, Marcora and Lepers, 2013). In agreement with the psychobiological model of endurance performance (Marcora, 2008, 2009; Pageaux, 2014), where perception of effort is postulated as one of the main limiting factors. In sleep-deprived individuals, perception of effort seems to be a key factor on pacing, and therefore, endurance performance. Central processing sensory inputs related to perception of effort during exercise, might be affected by an acute mentally-fatiguing state (Marcora, 2009), such as sleep deprivation, as well as changes in activity at specific areas of the brain associated with RPE (Williamson *et al.*, 2001, 2002; Williamson, Fadel and Mitchell, 2006), such as ACC (Williamson, 2015). Subjective sleepiness was higher in the sleep deprivation condition, in agreement with a previous study showing an association between increased levels of sleepiness and subjective fatigue and sleep deprivation (Dinges *et al.*, 1997).

HR response in sleep deprivation was lower, when compared to normal sleep. These results are in accordance with previous studies found (Holland, 1968; Martin and

Gaddis, 1981), suggesting that lack of sleep may affect the activity of sympathetic and parasympathetic nervous systems during exercise.

In addition to that, the concept of sleep deprivation tolerance, defined as the resistance to the negative effects of sleep deprivation on endurance performance, has also been explored. A lower decrease in TTE performance in a sleep deprived condition, compared to TTE performance after normal sleep, may be further investigated as a valid marker of sleep deprivation tolerance. Moreover, individuals with a better sleep deprivation tolerance covered more distance in a ~340 km mountain ultra-marathon (r_p = 0.767).

The use of caffeine to counteract the negative effects of sleep deprivation on endurance performance

A randomised, double-blind, placebo-controlled, counterbalanced, crossover experimental study design was used to investigate the effects of caffeine on running performance after one night of sleep deprivation (Chapter 4).

The main findings revealed that 6 $mg \cdot kg^{-1}$ of caffeine after one night of sleep deprivation, improves 30-min running time trial performance by ~6%. Our results are in agreement with similar running time trial studies reported in both sleep deprived subjects (McLellan *et al.*, 2005; Khcharem *et al.*, 2022) and non-sleep-deprived individuals (Cohen *et al.*, 1996; O'Rourke *et al.*, 2008; Church *et al.*, 2015; Khcharem *et al.*, 2021, 2022). The effect size of our study (Cohen's d = 0.24) is within the effect size ranges reported in a recent review of 21 published meta-analyses of caffeine supplementation and aerobic endurance (Grgic *et al.*, 2020). Compared to placebo, RPE values were lower in the caffeine trial. This could potentially explain the observed improvement in performance. Several studies have reported an increase in RPE during exercise in a sleep deprived state (Martin, 1981; Myles, 1985; Symons, VanHelder and Myles, 1988; Rodgers *et al.*, 1995; McLellan, Bell and Kamimori, 2004; Oliver *et al.*, 2009). Unfortunately, in the context of sleep deprivation and caffeine research, only a study has reported RPE values, revealing that caffeine decreases RPE during a run to exhaustion test after 24 h of sleep deprivation, compared to placebo (McLellan, Bell and Kamimori,

2004). Central processing sensory inputs, responsible for RPE production during exercise, might be negatively affected by a mental fatigued state, such as sleep deprivation (Marcora, 2009). However, caffeine seems to reduce RPE due to the decrease in the activity of the cortical premotor and motor areas required to produce a particular force (de Morree, Klein and Marcora, 2014).

Furthermore, as part of the same study (Chapter 4), the effects of caffeine after sleep deprivation on endurance exercise at a constant workload were also investigated. In comparison to placebo, caffeine reduces RPE values during submaximal exercise.

From a sleepiness perspective, compared to placebo, caffeine reduces sleepiness following a night of sleep deprivation. Our results are in line with what has been published in both, sleep and non-sleep deprived individuals (Biggs *et al.*, 2007; Beaven and Ekström, 2013; Hansen *et al.*, 2019).

The measure of cardiopulmonary parameters during both, constant workload and time trial tests aimed to provide novel insight into the field of caffeine and exercise in a sleep deprived state. The effects of sleep deprivation on cardiopulmonary variables during exercise is negligible (Fullagar *et al.*, 2015). In comparison to placebo, all these parameters, and HR response, were higher in the caffeine trial. Results are complicated to compare due to the lack of studies in the context of sleep deprivation, caffeine supplementation and cardiopulmonary response. However, several interpretations can be found in relation to caffeine and ventilation (Chapman, Wilhite and Mickleborough, 2012). It has been argued that, in conditions where pulmonary gas exchange is jeopardised, caffeine increases oxygen saturation and alveolar partial pressure, when performing submaximal exercise. On the other hand, it has been also proposed that any potential benefit of enhanced alveolar oxygenation might be compromised by the effects of caffeine on the central nervous system. Increased ventilation might decrease blood flow of locomotor muscles as a result of an increase in the work done by breathing muscles.

Exercise in a sleep deprived state as part of a training protocol to improve tolerance to the negative effects of sleep deprivation.

An innovative approach, inspired in the Brain Endurance Training (BET) proposed by Marcora, Staiano and Merlini (2015), was investigated as part of the training for a mountain ultra-marathon (Chapter 5). Opposed to BET, which is a systematic repetition of demanding cognitive tasks over prolonged periods of time, aiming to increase resistance to mental fatigue, and therefore, improve endurance performance, three bouts of exercise after a night of sleep deprivation were proposed during a 14-week period. The purpose was to have a preliminary observation about its potential effects on sleep deprivation tolerance. Main findings have shown that sleep deprivation tolerance was not improved. A potential explanation for these results could be that the number of bouts proposed were not enough to induce significant adaptations. A similar method based on the same BET principles, called Sleep Deprivation Training (SDT) has been used in a case study (Gattoni *et al.*, 2022). Over a 6-week program prior to a 6-day race, an ultra-endurance runner performed one night of sleep deprivation per week, in addition to his regular physical training. SDT had a beneficial effect in perceived mental effort and sleepiness. Interestingly, we found a training effect on mental fatigue perceived by the individuals. But no training effects were observed in any other physiological or perceptual parameter measured. In the light of these optimistic preliminary results, a randomised control trial is needed to support SDT as a potential way to counteract the negative effects of sleep deprivation. In order to induce sufficient structural and functional adaptations in brain areas, such as ACC (Shenhav *et al.*, 2017), physical and cognitive training should be combined, as reported in previous research (Fabre *et al.*, 2002; Holzschneider *et al.*, 2012).

Summarising, the main findings presented above propose perception of effort as a critical factor in several aspects related to sleep deprivation and ultra-endurance performance. In mountain ultra-marathon events, runners try to sleep as little as possible in order to reduce the impact of sleep time on finish time. Over the course of the race, reductions in speed are observed resulting from an increased perception of effort due to the combination of accumulated sleep debt and fatigue. Similar to mental fatigue, RPE increases when performing endurance exercise in a sleep deprived state. Caffeine has

been proposed as a countermeasure to reduce the negative effects of sleep deprivation on endurance performance due to the reported reduction in RPE during exercise at submaximal and maximal intensities after one night of sleep deprivation. Further investigations may explore a systematic repetition of exercise in sleep deprived conditions as a training method to improve sleep deprivation tolerance, and therefore, improve endurance performance, due to structural as functional adaptations at a brain level, aiming to reduce RPE.

6.2 Limitations and weaknesses of the research project

Various limitations might have impacted the final results of the experiments conducted during this doctoral research program.

In Chapter 3, inherent to the nature of field studies, we found diverse limitations. In relation to the sample size: small number of subjects in comparison to the number of participants in ultra-marathon events, and the dropout rate in this type of competitions. The first one was predictable, due to logistics and equipment available. We tried to compensate for this by collecting data during several editions, a widespread practice found in several studies investigating ultra-marathon performance. Therefore, we managed to collect data from twenty-seven participants out of the 284 competitors (9.5%). Regarding dropouts, the average in the events investigated was ~43%, whereas in our sample we experienced a ~40% dropout rate. Another limiting factor is the fact that during one of the events studied the race was stopped for several hours due to adverse weather conditions. Participants were instructed to remain at the closest checkpoint. This might have influenced their pacing strategy. Because the race was stopped during the night, most of the runners decided to sleep until the following morning, when the racing was permitted again. Other limitations found in this experiment were related to sleep: the devices used to monitor sleep, and the lack of data about sleep strategies adopted prior to the race. In order to obtain objective sleep data accurately, actiwatches (a wrist-worn actigraphy device) were used to collect data in the field. However, this technology cannot record the sleep periods shorter than 30 s (microsleep). It has been suggested that athletes may experience several microsleep episodes over the course of an ultra-marathon

(Hurdiel *et al.*, 2015). 55% of ultra-marathon runners have described sleep extension, an increase in sleep time using daytime naps, or more sleep at night, as their main sleep strategy before a competition (Martin *et al.*, 2018). Sleep extension can protect against the cognitive impaired induced by sleep deprivation, and reduce RPE during exercise (Arnal *et al.*, 2015, 2016). Another limitation revealed in this study was related to the lack of data collected about caffeine intake during the race. 13% of ultra-marathon runners have reported the use of stimulants to resist the sleep pressure (Martin *et al.*, 2018). Furthermore, caffeine reduces RPE during exercise (Doherty and Smith, 2005), and promotes wakefulness (McLellan, Caldwell and Lieberman, 2016).

In Chapter 4 we found a limitation related to the experimental design used. Despite being originally conceptualised as a 2 x 2 factorial design (caffeine / placebo, normal sleep / sleep deprivation) as used in related studies (McLellan, Bell and Kamimori, 2004; McLellan *et al.*, 2005; Donald *et al.*, 2017; Khcharem *et al.*, 2022). For practical reasons, we removed the normal sleep condition. Therefore, we lacked data from a non-sleep deprived state. Consequently, we could not test the negative effects of sleep deprivation on running performance after one night of sleep deprivation. Additionally, in this chapter, in relation to caffeine, plasma concentrations of caffeine were not measured due to technical reasons. Therefore, we lacked reliable data of participants' adherence to the pre-experiment instructions, consisting of a pre-visit checklist regarding any source of caffeine in the 12 h prior to each visit. Another limitation regarding caffeine was in relation to the influence of genetic polymorphisms in the individual's response to caffeine (Grgic *et al.*, 2021). Two genes have been proposed: *ADORA2A*, related to individual caffeine sensitivity, and *CYP1A2*, mainly responsible for the speed of caffeine metabolism. For technical reasons, data from participants' regarding *ADORA2A* and *CYP1A2* were not available.

In Chapter 5 several methodological limitations were revealed. Due to logistical reasons, participants were not strictly monitored during the training phase. A mandatory two-week holiday period occurred during the last two weeks of the training phase. Therefore, training might be reduced because of this factor. Participants were tested immediately after the training phase. Any potential training adaptation might have been lost. Furthermore, the training intervention and the number of exercise bouts under sleep

deprivation may not have been sufficient to induce any potential effect. Sleep data during this period was collected using a weekly sleep diary. Only during the testing period sleep was monitored using actigraphy. The two sleep bouts in a sleep deprived state performed during the training phase were not properly standardised: One was performed during a 36-h military march. The other one was during a 105-km mountain ultra-marathon. Therefore, despite being instructed to not sleep during these events, individuals may have fallen asleep. During the TTE, for logistical reasons, participants were tested in pairs. This could have affected their performance. In order to control this factor, participants were separated and instructed to perform their best, irrespective of the performance of the other participant. However, this setup may have affected their motivation during the test. It is important to underline that various limitations of this study described above were an inevitable consequence of conducting research in military personnel.

Gender bias was present in Chapters 4 and 5. Only males were recruited for the studies conducted, in order to avoid hormonal variations related to the menstrual cycle and oral contraceptive measures, which have been shown to have an effect in cognitive and physical performance (Kimura and Hampson, 1994; Wright and Badia, 1999; Constantini, Dubnov and Lebrun, 2005). Therefore, the results cannot be extrapolated to women.

Lastly, as a measure to remove any potential belief bias from the main researcher, no verbal encouragement was used during the experimental trials in all the experiments conducted as part of this doctoral research program. It is well established in various endurance tasks, verbal encouragement improves performance (McCormick, Meijen and Marcora, 2015).

6.3 Conclusions and future research

Overall, this doctoral research program has investigated several research questions that were not answered in the current literature. New understanding of knowledge in the field of sleep deprivation and ultra-endurance performance have been proposed, not only to fill a gap in the research literature, but also to provide athletes and

coaches with some evidence-based guidance about sleep during ultra-marathon events, and the use of caffeine to counteract the negative effects of sleep deprivation. Furthermore, more research is needed to explore future measures that could improve sleep deprivation tolerance, and therefore, improve endurance performance.

The first study (Chapter 3) was conducted in the field, aiming to better understand the sleep management of athletes during a mountain ultra-marathon longer than 330 km. It can be argued that this is a challenging scenario, where there are many variables that cannot be as controlled as in a more standardised environment (i.e., a laboratory). Nevertheless, a field study is a highly ecologically valid scenario, providing real insights during a real competition. The results of this observational study have described how athletes deal with sleep during a mountain ultra-marathon, using actigraphy, a reliable and valid tool to collect objective sleep data. It has been confirmed that there is a positive association between sleep duration and race time, as reported in the literature during shorter events, or using a less reliable method (sleep diary). It has been observed that participants did not sleep for almost 24 h before their first sleep episode during the race. However, sleep parameters increased over the course of the competition. Future studies should explore the effects of diverse sleep strategies, aiming to investigate whether there is an optimal amount of sleep that could minimise the negative effects of sleep deprivation, and therefore, allowing a better recovery that could be translated into a lower decline in performance. A better understanding of sleep management during mountain ultra-marathon events may help athletes to choose a sleep strategy aiming to optimise their performance during the race.

The negative effects of sleep deprivation on endurance performance have been described in Chapter 5. In agreement with previous studies found, a decrease in time to exhaustion running at ~71% of peak treadmill speed after one night of sleep deprivation was reported. However, several methodological limitations, such as the fact that participants were tested in pairs, may require additional studies to provide more precise data. Future research may explore different sleep loss, exercise protocols, intensities, and/or sport disciplines. Nevertheless, the effects of sleep loss in endurance performance are well documented in the literature. A novel protocol to test the negative effects of sleep deprivation has been used, emerging as a direction for further investigation about the

validity and reliability of this testing protocol. Furthermore, in Chapter 5 the concept of sleep deprivation tolerance has been proposed as the difference in performance between normal sleep and sleep deprivation. Actually, the results of the study have reported a positive association between sleep deprivation tolerance and distance covered in a ~340 mountain ultra-marathon. However, several aspects must be taken into consideration regarding this association: dropout rate during the race (73%), finishing time variability (± 49 s). A larger sample size needs to be considered in future experiments.

Two countermeasures to reduce the negative effects of sleep deprivation have been investigated: caffeine (Chapter 4), and as part of the training for a mountain ultra-marathon (Chapter 5), punctual exercise bouts in a sleep deprived state aiming to experience that scenario before the competition. In Chapter 4, after one night of sleep deprivation, 6 $mg \cdot kg^{-1}$ of caffeine, improved running performance by 5.54%. Further research is required to explore various aspects, such as the timing and dosage of several caffeine doses during the day; repeated doses of caffeine over several days of competition; the combination of various sources of caffeine; the influence of caffeine-related genetic polymorphisms; the relationship between caffeine intake and sleep time. There are only a few studies that have investigated the effects of caffeine in sleep deprivation. Therefore, additional research may explore other types of exercise; testing protocols; sport disciplines; and sleep loss induced. Additionally, future research may aim to investigate and report the use of caffeine during a mountain ultra-marathon. In Chapter 5, proposed as part of the preparation for a mountain ultra-marathon, three bouts of exercise in a sleep deprived state over a 14-week period were not effective reducing the negative effects of sleep deprivation. It may have not been sufficient stimulus to induce any potential adaptation at the brain level. However, several limitations may have affected the results. Interestingly, a case study using a more systematic approach to this concept, called Sleep Deprivation Training, has been published recently (Gattoni *et al.*, 2022), reporting that, exercising once a week in a sleep deprived state for six weeks, in addition to a regular physical training program, improved perceived sleepiness and mental effort. Further research should aim to study the effects of this innovative training method on perception of effort and endurance performance, using a randomised controlled trial. Additionally, future studies may explore the combination of this new method with other countermeasures to reduce the negative effects of sleep deprivation, such as caffeine.

In general, future work in the areas of directions mentioned above need to be investigated in other populations, such as females. Lastly, in the context of sleep deprivation and endurance performance, further studies using EEG and fMRI are critical to identify structural and functional adaptation in certain brain areas.

To conclude, the findings of this doctoral research program provide an assessment of the sleep during a ~431 km mountain ultra-marathon. Sleep duration is positively associated with race duration. Participants aimed to stay awake almost 24 h before the first sleep episode. However, throughout the race, sleep increases whereas running speed decreases. A combination of accumulated sleep debt and fatigue as a consequence of the demands of ultra-endurance performance seems to be a plausible explanation for an increase in RPE, and therefore, a reduction in performance. The negative effects of sleep deprivation on endurance performance have been assessed using a novel testing protocol: a time to exhaustion run after a night of normal sleep, and the following day another time to exhaustion run after one night of sleep deprivation. Therefore, the difference in performance between normal sleep and sleep deprivation has been proposed as sleep deprivation tolerance. A positive association has been found between sleep deprivation tolerance and distance covered in a ~340 mountain ultra-marathon. 6 $mg \cdot kg^{-1}$ of caffeine has been suggested as a countermeasure for the negative effects of sleep deprivation. Caffeine reduces RPE, and therefore, running performance was improved by ~6% in a 30-min time trial after one night of sleep deprivation. Despite not having shown an effect reducing the negative effects of sleep deprivation as part of the preparation for a mountain ultra-marathon. In the light of recent preliminary results from a case study, a novel method, consisting of a systematic repetition of exercise bouts in a sleep deprived state (Sleep Deprivation Training), in combination with regular physical training, might be explored in a randomised control trial, in order to further investigate its potential as a countermeasure for the negative effects of sleep deprivation. Athletes aiming to improve their performance in ultra-endurance events might benefit from the countermeasures aimed to reduce the negative effects of sleep deprivation.

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