PERIOD3 Variable Number Tandem Repeat Genotype Associations with Performance, Injury, Illness and Re-entrainment

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Submitted to the University of Cape Town in fulfilment of requirements for the degree: Doctor of Philosophy in Science

Faculty of Science

University of Cape Town

To be submitted: 22 February 2016

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PLAGIARISM DECLARATION

I, **Lovemore Kunorozva**, hereby declare that the work on which this Dissertation/Thesis is based on is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

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ACKNOWLEDGEMENTS

My academic and research career is the product of inspiration, guidance and assistance from multiple individuals without which this thesis would not have been possible.

To start with, I wish to acknowledge the direct or indirect contributions of Professor Timothy Noakes, team physicians from the Sharks, Stormers, Free State Cheetahs, Blue Bulls and the Lions. Specifically, the physicians helped with the drawing of blood and capturing of injury and illness data.

I thank Mr Willy Merwe for assistance with the Super Rugby footage and Mrs Marisce Blackaller-Smal for assistance with managing the injury and illness data. Members of the Molecular & Cell Biology and Exercise Science & Sports Medicine Departments for being supportive and providing me with a home to carry out my experiments. Members of the Rhythms and Blooms Laboratory both past and present, Dr Eric Banda, Nyambura Shawa, Rageema Joseph, Chenjerai Muchapireyi, Denford Banga, Kim Stephenson and Rob Henst for their forbearance, understanding, advice and support in recruiting the control population.

More specifically, I want to thank Dr Sharief Hendricks and Mr Ryan Williams who helped me with the post-play game analysis aspect of my study. Furthermore, I want to thank Mrs Hendriena Victor for the help with meal planning for the sleep intervention trial. My greatest debt however, is to my supervisors Associate Professor Laura Roden and Dr Dale Rae. As the project supervisors, they instilled in me both an interest in the project and a questioning attitude. I am forever grateful for the opportunities you have given me and the knowledge you have shared. Working with both my supervisors has been a great learning experience, and one that I hope can continue in the future. I would like to thank the volunteers, without whose participation this study would have been impossible.

To my friends, family including SOS Children's Villages, and more importantly Chumisa Palesa Qhumza, special thanks for your continued encouragement, it has been a good four years and I have really valued your support.

This work was supported by research bursaries to Mr Lovemore Kunorozva from the University of Cape Town's International/Refugee Scholarship and the Molecular & Cell Biology's Equity Development Programme (EDP) fund as well as UCT Research Associate grant.

TERMS AND DEFINITIONS

ANOVA	Analysis of variance
ASPS	Advanced Sleep Phase Syndrome
AUC	Area under the curve
AUS	Australia
bHLH	Basic helix loop-helix
BMAL1	Brain and muscle aryl hydrocarbon receptor nuclear translocator like 1
BMI	Body mass index
Bp	Base pair
CBS	Chronobiology and Sleep Laboratory
CBT	Core body temperature
cDNA	Complementary deoxyribonucleic acid
CHO	Carbohydrate
CK1ε	Casein kinase 1ε
CLOCK	Circadian locomotor output cycles kaput
CON	Control group
CR	Constant routine
CRY	Cryptochrome
CSM	Composite scale of morningness
DET	Definite evening type
DLMO	Dim light melatonin onset
DMT	Definite morning type
DNA	Deoxyribonucleic acid
dNTPS	Deoxyribonucleotide triphosphates
DSPS	Delayed Sleep Phase Syndrome
EDTA	Ethylenediaminetetraacetic acid
EE	Energy expenditure
EEG	Electroencephalogram/Electroencephalography

EtBr	Ethidium bromide
E-W	Westward travel
GA	Game activity
gDNA	Genomic deoxyribonucleic acid
h	Hour
HÖ	Horne Östberg
$h \cdot wk^{-1}$	Hours per week
HREC	Human Research Ethics Committee
iPRGC	Intrinsically photosensitive retinal ganglion cells
KPI	Key performance indicator
LAI	Low physical activity individuals
LB	Luria broth
MCTQ	Munich chronotype questionnaire
MET	Moderate evening type
min	Minute
MMT	Moderate morning type
MAPK	Mitogen activated protein kinase
mRNA	Messenger ribonucleic acid
MSF	Midpoints of sleep on free days
MW	Molecular weight
NREM	Non-rapid eye movement
NT	Neither type
NTZD	No time zone difference
NZ	New Zealand
PAS	Per-arnt-sim protein signal sensor
PCR	Polymerase chain reaction
PER	PERIOD
PSQI	Pittsburgh Sleep Quality Index
REM	Rapid eye movement

RER	Respiratory exchange ratio
RHT	Retinohypothalamic tract
RMR	Resting metabolic rate
ROR	Retinoic acid-related orphan receptor
RRE	Retinoic acid-related orphan receptor response elements
RUG	Super 15 rugby group
s	Seconds
SA	South Africa
SARU	South African Rugby Union
SCN	Suprachiasmatic nuclei
SDS	Sodium dodecyl sulphate
SNP	Single nucleotide polymorphism
SO	Sleep opportunity
SSS	Stanford Sleepiness Scale
SWS	Slow wave sleep
TAE	Tris-acetate-EDTA
TE	Tris-EDTA
TSD	Total sleep deprivation
TZ	Time zone
TZD	Time zone difference
UCT	University of Cape Town
UV	Ultra violet
VNTR	Variable number tandem repeat polymorphism
WASO	Wake after sleep onset
W-E	Eastward travel

GENERALLY USED TERMINOLOGY AND NOMENCLATURE

Generally accepted gene names, symbols and genetic terminology were used in this thesis. Gene names are given in upper case letters and italicised e.g. *PERIOD* abbreviated to *PER*. The abbreviations are defined in text upon first use in each chapter.

When referring to protein products, names are given in upper case letters but not italicised e.g. PERIOD/PER.

ABSTRACT

Background: Circadian rhythms are internally driven biological variations that fluctuate with a period of approximately 24 hours, even in the absence of external environmental time cues. These rhythms enable organisms to synchronise their internal clock time with external environmental time. This ensures appropriate timing of biological and metabolic processes, and allows anticipation of daily changes in the environment. Circadian rhythms also play an important role in sports in terms of optimising performance time-of-day and aiding adjustment to global time zone changes. Thus, performance, which is under the control of the athlete, may be impacted by event time-of-day scheduling in the new time zone. It has previously been shown that individual sport athletes in South Africa tend to be morning-types and carry the *PERIOD3* (*PER3*) Variable Number Tandem Repeat (VNTR) 5-repeat allele, which has been associated with a preference for mornings. The distribution of the *PER3* VNTR polymorphism in combination with an individual's preference for mornings or evenings has not yet been described in team sports. Differences in the *PER3* VNTR genotype between team and individual sport athletes are expected, given that individual sport athletes may be free to choose the time-of-day at which they train. In contrast, team sport athletes usually train in groups, thus these individuals may not have the flexibility to choose their preferred training times.

There are notable inter-individual differences in adjustment to jet-lag after time zone changes. A possible genetic candidate that may be responsible for some of this variation is the *PER3* VNTR gene. This gene consist of two alleles corresponding in size to 4-repeats ($PER3^{4}$) or 5-repeats ($PER3^{5}$). Individuals are either homozygous for the 4-repeat allele ($PER3^{4/4}$) or the 5-repeat allele ($PER3^{5/5}$), while others are heterozygous for the *PER3* gene ($PER3^{4/5}$). The *PER3* VNTR genotype might explain individual sensitivity to bright light and has been reported to be associated with sleep pressure- an increase in the brain's pressure and need for sleep, following an extended period of awakening. Individuals homozygous for the longer variant of the gene *(*i.e. $PER3^{5/5}$) experience a higher sleep pressure during extended wakefulness. The $PER3^{5/5}$ genotype has been

reported to be more sensitive to the alerting and melatonin suppression effects of blue enriched light than the *PER3*$^{4/4}$ genotype.

Aims: Therefore, the aim of Study 1 was to compare the chronotype and *PER3* VNTR genotype distribution of South African Super Rugby players to individuals of low physical activity (i.e. those who are physically active ≤2 times per week). The aim of Study 2 was to determine whether *PER3* VNTR genotype might contribute to inter-individual variation in the extent to which game involvement and quality of play are affected following trans-meridian travel. Further, the aim of Study 3 was to compare the impact of time zone travel during the 2012 Super Rugby competition in South African players genotyped as *PER3*$^{4/4}$, *PER3*$^{4/5}$ and *PER3*$^{5/5}$ on the incidence of illnesses and injuries. Lastly, the aim of Study 4 was to compare the extent to which individuals genotyped as *PER3*$^{4/4}$ or *PER3*$^{5/5}$ respond to appropriately-timed blue light exposure in order to resynchronise their circadian rhythm, following simulated eastward travel, based on changes in dim-light melatonin onset and cortisol circadian phases.

Methods: The cohort for Study 1 consisted of male, South African rugby players (n=205) and a healthy, active, but non-competitive control population (n=191). Firstly, the distribution of morning-, neither- or evening-preferring individuals was measured using the Horne-Östberg morningness-eveningness personality questionnaire. The genotype and allele frequency of the *PER3* VNTR polymorphism were determined from genomic DNA products extracted from human blood/buccal cell samples amplified and digested with *Nco*I.

In Studies 2 and 3, the impact of time zone travel during the 2011 and 2012 Super 15 Rugby competitions on South African players categorised as *PER3*$^{4/4}$, *PER3*$^{4/5}$ and *PER3*$^{5/5}$ was compared. Specifically, effects on game activity rate and quality of play in players categorised as *PER3*$^{4/4}$ (n=20), *PER3*$^{4/5}$ (n=20) and *PER3*$^{5/5}$ (n=20) and the incidence of illness and injury in the three *PER3* VNTR genotypes were measured.

Lastly, in Study 4, the extent to which untrained males genotyped as *PER3*$^{4/4}$ (n=8) or *PER3*$^{5/5}$ (n=8) responded to appropriately-timed blue light exposure in order to resynchronise their circadian rhythm was examined. Jet-lag was artificially induced in the laboratory to simulate eastward travel crossing six time zones, and circadian resynchronisation was assessed by measuring changes in DLMO and cortisol circadian phases.

Results and Discussion: Results from Study 1 showed that the *PER3* VNTR genotype distribution was similar in the rugby (*PER3*$^{4/4}$: 41.5%, *PER3*$^{4/5}$: 45.4%, *PER3*$^{5/5}$: 13.1%) and control (*PER3*$^{4/4}$: 36.6%, *PER3*$^{4/5}$: 51.7%, *PER3*$^{5/5}$: 11.7%, p=0.738) groups. The frequencies of the 4- and 5-repeat alleles in the rugby (*PER3*^{4}: 64.1%; *PER3*^{5}: 35.9%) and control (*PER3*^{4}: 62.5%; *PER3*^{5}: 37.2%, p=0.598) groups were similar to those previously reported in other populations. However, they were different from findings by Kunorozva et al. (2012) in individual sports' athletes (*PER3*^{4}: 41.7%, *PER3*^{5}: 58.3%). The chronotype distribution was significantly different between the rugby (MT: 48.9%, NT: 48.8%, ET: 3.3%) and control (MT: 24.6%, NT: 56.5%, ET: 18.9%, p<0.001) groups. An association between *PER3* VNTR genotype and chronotype was only observed in the control group (r=0.293, p=0.008).

In study 2, game involvement was similar in all players regardless of direction travelled (No time zone travel: 0.24 (0.78 events·min^{-1}), Westward travel: 0.00 (0.39 events·min^{-1}), Eastward travel: 0.18 (0.45 events·min^{-1}), p=0.072) or number of time zones crossed (No time zone travel: 0.24 (0.77 events·min^{-1}), 6-8 Time zones: 0.08 (0.49 events·min^{-1}), >10 Time zones: 0.13 (0.45 events·min^{-1}), p=0.351). Similarly, no significant differences in game involvement were noted when the *PER3* VNTR genotype was taken into account (*PER3*$^{5/5}$: 0.18 (1.08 events·min^{-1}), *PER3*$^{4/5}$: 0.13 (0.41 events·min^{-1}), *PER3*$^{4/4}$: 0.26 (0.57 events·min^{-1}), p=0.216). In contrast, quality of play was significantly higher after westward (90.63 (12.4%)) compared to eastward (88.8 (12.3%), p=0.031) travel. Further, quality of play was significantly higher following westward travel (89.2 (14.7%)) compared to eastward travel (86.9 (14.0%), p=0.040) only when more than ten time zones were crossed. Lastly, play quality was worse in the *PER3*$^{4/4}$ (85.1 (13.2%)) compared to the

PER3$^{4/5}$ (93.2 (16.5%), p=0.041) and *PER3*$^{5/5}$ (92.1 (13.1%), p=0.026) groups following westward travel across ten or more time zones.

Results from study 3 indicated that significantly more of the players became ill after both eastward and westward travel compared to when there was no time zone travel (No time zone travel: 16.5/1000 player days, Eastward travel: 42.6/1000 player days, Westward travel: 30.4/1000 player days, p<0.001). Likewise, significantly more players sustained injuries following time zone travel (No time zone travel: 36.3/1000 exposure hours, Westward travel: 48.1/1000 exposure hours, Eastward travel: 66.4/1000 exposure hours, p=0.022). Specifically, high injury incidence rates were noted following eastward travel (66.4/1000 exposure hours) compared to no time zone travel (36.3/1000 exposure hours, p=0.010).

Results from Study 4 indicated that, regardless of genotype, a phase advance was achieved for all individuals, suggesting that the protocol was effective. In the simulated jet-lag travel study, mean DLMO time for the *PER3*$^{5/5}$ group (17h31±0h21) occurred earlier than that of the *PER3*$^{4/4}$ group (17h58±0h24) following appropriately-timed blue-enriched light exposure (p=0.032).

Conclusions: Chronotype distribution in rugby players may have been affected by the conditioning effect of morning training sessions. The *PER3* VNTR genotype may explain some of the variations in an individual's match quality of play, injury and illness incidence rates in South African Super Rugby players, as well as re-entrainment in healthy, active, but non-competitive individuals following simulated jet-lag. These findings may further the understanding of possible cause/s of illnesses and injuries, and improve health and performance in rugby players who travel to participate in international competitions. The *PER3* VNTR genotype results have improved our understanding of inter-individual differences in response to jet-lag. This has the potential to transform the way team physicians manage their players in order to maintain proper circadian alignment with the external 24h day in the new environment.

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CHAPTER ONE: INTRODUCTION AND BACKGROUND

1.1 Introduction

Air travel across multiple meridians prior to or between competitions is a common feature of the lifestyle of many elite athletes, not only for international events such as the Olympic Games, but also in places such as the USA and Australia, where even domestic competitions can involve significant travel across time zones. Such travel often results in "jet-lag", the misalignment between the internal body clock, also known as the circadian clock, and the external environmental time in the new time zone (Reilly et al., 2007). The impact that circadian disruption as a consequent of trans-meridian travel can have on injury and illness is of fundamental importance for regular travellers (Waterhouse and Reilly, 2009, Baron and Reid, 2014). More importantly, in professional athletes whose livelihoods depend on their ability to train and compete.

In addition, different systems (e.g. psychological) resynchronise into the new time zone at different rates, which might affect the rate at which optimal performance is restored. Specifically, the effects of psychological risk factors (e.g. mood) on injury are mediated by the cumulative effect of acute and/or chronic physiological responses (Vandekerckhove and Cluydts, 2010). Cumulative influences of both physical and psychological factors deplete perceptual and sensor motor reserves potentially exacerbating the risk of injury (Vandekerckhove and Cluydts, 2010, Evans et al., 2007). Thus, athletes who undergo time zone travel are more likely to have a higher compound allostatic load (i.e. stressor) and an even more diminished threshold for mounting situational appropriate responses. Likewise, disruption of a system physiologically affects its function, for example, disruption of the respiratory and cardiovascular systems may limit endurance performance (Walsh, 2000, Kottke, 1966).

Under synchronised conditions, aspects of performance such as reaction time (Reilly et al., 2007), leg strength (Coldwells et al., 1994) and neuromuscular coordination (Ilmarinen et al., 1980) all peak in the late afternoon to early evening. The variation in the peak of the above-mentioned

variables may also be related to changes in the environment (i.e. light-dark cycles, ambient temperature), body temperature, time since awake, chronotype and sleep-wake cycles, just to mention a few (Lee and Galvez, 2012, Youngstedt and O'Connor, 1999).

Super Rugby athletes engage in frequent trans-meridian travel prior to and between matches played in South Africa, Australia and New Zealand. This results in recurrent desynchronisation of circadian rhythms in the new time zone which leads to the undesirable symptoms of jet-lag including, altered sleep patterns, changes in mood state, impaired reaction time and gastrointestinal disturbances (Reilly et al., 2005, Waterhouse and Reilly, 2009). The extent to which an individual is affected by jet-lag symptoms varies and depends on several factors chief among them being number of time zones crossed, direction of travel, age, genetics and chronotype (Choy et al., 2011, Reilly et al., 2007, Wesentsten et al., 2003). The key to alleviating jet-lag symptoms in the new time zone lies in proper resynchronisation strategies. This is important particularly in the Super Rugby competition considering that athletes may cross up to ten time zones with very little time to recover before a match. Ideally a recovery time of one day per time zone crossed would be allowed (Manfredini et al., 1998), highlighting the importance of the amount of lead time desirable for body clock adjustment when scheduling international travel itineraries. However, because of budget and time constraints this is not usually the case in many sports including the Super Rugby competition.

Strategies such as appropriately-timed light exposure (Forbes-Robertson et al., 2012) and external melatonin administration (Burke et al., 2013) may be used to aid resynchronisation into the new time zone. However, their effectiveness is complicated by the inter-individual variability in response to the same entraining stimuli. Therefore, effective jet-lag alleviation strategies following time zone travel will have to understand the basis for the inter-individual variabilities in addition to number of time zones crossed and direction of travel in order to be useful and reduce the impact of time zone travel on health and performance.

1.2 The circadian system

Living organisms possess an intricately precise, internal molecular circadian system that times daily events ranging from sleep and wakefulness in humans to photosynthesis in plants (Shea et al., 2011, Morris et al., 2012, Takahashi et al., 2008, Crowley et al., 2014). This system represents an evolutionarily conserved adaptation to the temporal environment that dates back to the earliest life forms (Kleitman, 1949). The circadian clock generates rhythms, which are endogenously (internally) driven fluctuations in biological, physiological and behavioural variables that have a period of about 24h (Ebisawa, 2013, Mohawk et al., 2012). These rhythms often take the form of sinusoidal waves (Figure 1.1) that can be described in terms of period, phase and amplitude. The circadian phase is the time at which the circadian rhythm of a variable reaches a particular state relative to a marker (Roenneberg et al., 2003a, Korczak et al., 2008). An individual's free-running (non-entrained) period determines the phase angle between endogenous and exogenous phase and subsequently this has an impact on the timing of entrainment to a *zeitgeber* (Aschoff, 1979, Roenneberg and Merrow, 2000).

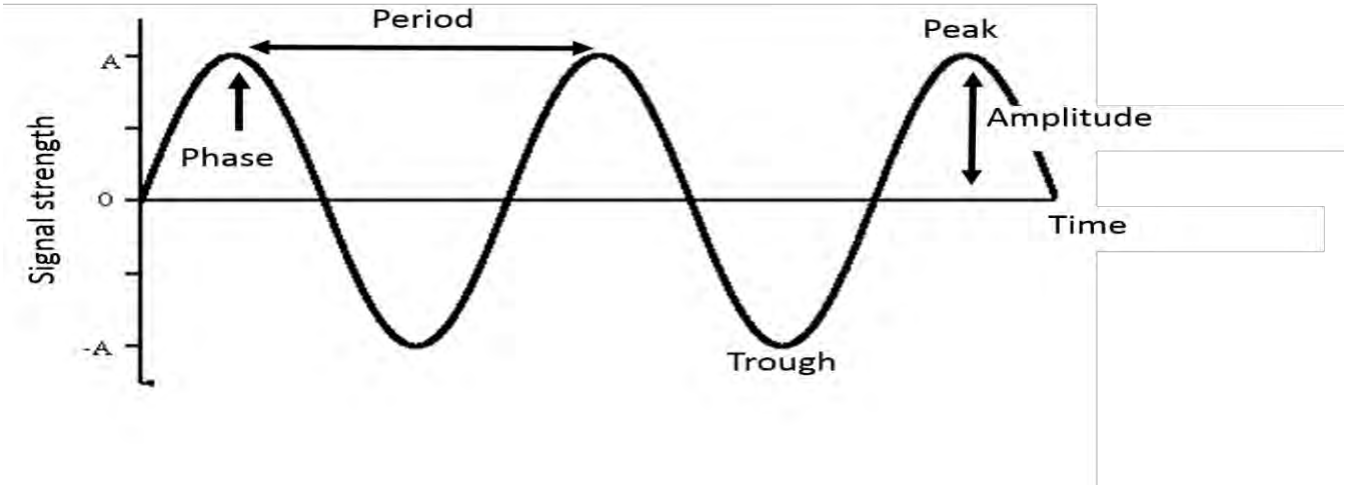


Figure 1.1: Sinusoidal waves showing key terms used to describe circadian rhythms. Period: the time during which a physiological process completes one circadian cycle, Phase: a stage (time-of-day) in the circadian cycle relative to a marker, Amplitude: one-half the distances from peak to trough during one period of an oscillation, Peak: the maximum point reached, Trough: the lowest point reached. Modified from Hsuchou (2013).

The circadian system consists of three parts: (1) the input pathways, which transmit environmental signals to entrain or synchronise (2) the "clocks", which in turn generate rhythms that are relayed as (3) outputs (Figure 1.2). The outputs can also feedback to the clocks via fluctuations in endocrine rhythms or through changes in body temperature. Each cell in the body possesses the molecular mechanisms to generate rhythms, but mammals possess a central pacemaker or master clock in the suprachiasmatic nuclei (SCN) in the brain, which express clock genes.

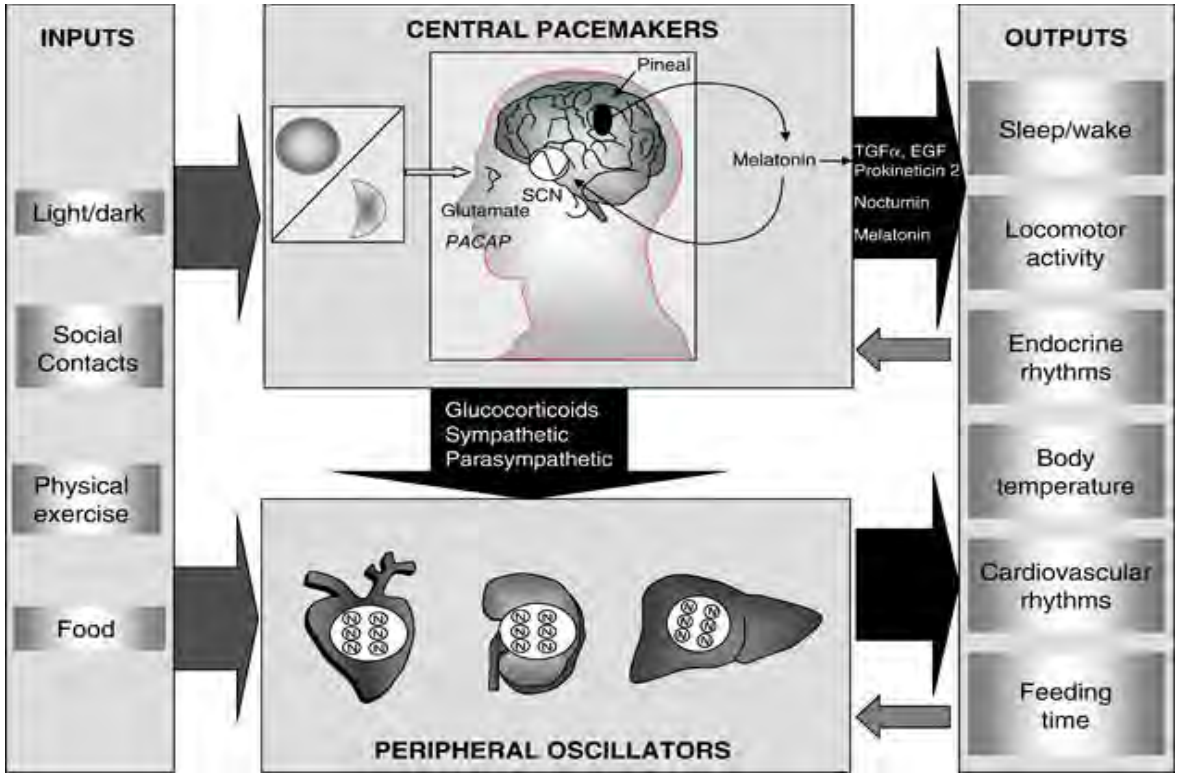


Figure 1.2: A simplified conceptual framework of the mammalian circadian system.

The central clock is entrained mainly through light signals. The peripheral clocks (indicated by ~) can be affected by food, feeding time and temperature signals independent of the light entrainment pathway of the SCN. Peripheral clocks may receive direct signals from the SCN via the sympathetic and parasympathetic nervous systems. Sleep-wake cycle, locomotor activity and endocrine output rhythms feedback to the master clock and peripheral clocks regulating their function. In the diagram SCN: Suprachiasmatic nuclei, PACAP: Pituitary adenylate cyclase-activating peptide; TGFα: Tumour growth factor alpha; EGF: Epidermal growth factor. Reproduced from Garaulet et al. (2010).

Peripheral clocks are time keeping systems found in tissues which rhythmically express clock genes, are entrained by daily feeding and may receive input from the SCN (Giebultowicz, 2001). For example, cellular peripheral clocks in the heart, kidney, pancreas and the liver are entrained (synchronised) by signals from the SCN as well as food, social contact, physical exercise and temperature cues (Garaulet et al., 2010, Ebisawa, 2007, Schibler et al., 2015). Likewise, Schibler and co-workers (2003) observed that when nocturnal rodents were fed during daytime for one week, the phase of circadian gene expression in the liver, heart, kidney and pancreas were all inverted. This was not seen in the SCN. In addition, Hara et al. (2001) reported that restricted feeding was able to reactivate and phase entrain circadian liver gene expression in SCN-lesioned mice which were completely arrhythmic.

In mammals, the circadian clock exerts its effects throughout the body by producing daily rhythms in all aspects of physiology such as core body temperature (Dijk et al., 2012), metabolism (Huang et al., 2011), DNA synthesis (Champier et al., 2012), brain wave activity (Dijk and Czeisler, 1995, Marshall et al., 2011) and the regulation of plasma hormone levels such as melatonin (Dijk et al., 2012, Pevet and Challet, 2011), adrenalin (Vieira et al., 2013) and cortisol (Kudielka et al., 2006). The rhythmic fluctuations of the aforementioned variables govern several of our habits and influence the activities that we perform during the 24h day (Roenneberg et al., 2013, Takahashi, 2015, Albrecht, 2012). Furthermore, these rhythms are self-sustaining, persisting in conditions where there are no external time cues, with an intrinsic period length close to 24h, indicating the presence of an internal time keeping mechanism (Hirota and Fukada, 2004, Saini et al., 2011).

Aschoff's work in the 1960's was pioneering in elucidating the endogenous nature of mammalian circadian rhythms. In his experiments, one person was kept in a self-contained underground chamber (i.e. living room with a bed, kitchen, and bathroom) for several weeks. Day length was altered to be either longer than 24h, shorter than 24h or exactly 24h; while body temperature, urinary cortisol, wake-up and bed-times were measured throughout the experiment (Aschoff, 1965). A normal 24h rhythm was maintained for the first seven days of the experiment when the

day length was exactly 24h, as evidenced by the rhythms in wakefulness and sleep, rectal temperature and urinary cortisol excretion. However, when external time cues were removed the rhythm was reported to free-run, with the circadian period measured between successive awakenings lengthening to 26.1h. When the day was shorter than 24h the circadian period measured between successive awakenings was shortened to 22.7h. The circadian period can adjust by lengthening or shortening to reset to the correct phase. In Aschoff's experiment, the mean rhythmicity for the individual under free running conditions was 25.5h, implying that circadian rhythmicity persists, but with a period deviating slightly from 24h.

The endogenous nature of mammalian circadian rhythms has also been demonstrated *in-vitro*, using cultured rat SCN cells which continued to generate 24h rhythms seven weeks post removal from the rat's brain (Welsh et al., 1995). In a separate experiment using circadian gene reporter methods, it was demonstrated that nearly all peripheral organs and tissues can express circadian oscillations in isolation yet still receive, and may require, input from the SCN *in-vivo* (Yoo et al., 2004). Furthermore, Akhtar et al. (2002) reported loss of circadian rhythms in the liver following SCN ablation in mice.

The function of the SCN in setting time was demonstrated in an experiment were neural grafts from the SCN region were used to restore circadian rhythmicity to arrhythmic mutant hamsters whose own nucleus had been ablated (Ralph et al., 1990, Lehman et al., 1987). Furthermore, recent experiments in mammals have indicated that genetic mutations or knockout of essential clock genes result in either the loss of circadian rhythmicity or gross deficits in circadian timing of the SCN (Partch et al., 2014, Hastings et al., 2014). This indicates that the SCN is the endogenous timekeeper, with an important role in circadian timing.

1.2.1 Molecular components of the circadian clock

The basis of the circadian system is the presence of a molecular mechanism in each cell of the body. This mechanism consists of a network of transcriptional and translational feedback loops, which are similar at the molecular level in both the SCN and peripheral clocks (Balsalobre, 2002,

Yoo et al., 2004, Schibler and Sassone-Corsi, 2002). Protein products from core clock genes are important for generating and regulating circadian rhythms within each cell throughout an organism (Lowrey and Takahashi, 2004). The underlying molecular mechanisms of the circadian clock exhibit an exceptional amount of evolutionary conservation in diverse organisms ranging from cyanobacteria, fungi and plants to mammals (Muller et al., 2013, Tarrant and Reitzel, 2013, Chang and Guarente, 2013). However, the individual clock components for these organisms vary between species. In mammals, the molecular clockwork of rhythm generation consists of a network of interacting positive and negative auto-regulatory transcription-translation feedback loops involving central clock genes and proteins (Figure 1.3) (Partch et al., 2014, Robinson and Reddy, 2014, Beckwith and Yanovsky, 2014). The mammalian circadian behaviour can be viewed as an integrated system, beginning with transcription of clock genes and leading ultimately to behavioural outputs. Furthermore, the mammalian circadian clock model is based mostly on what has been discovered in mice.

At the core of the circadian clock network are transcriptional activators, circadian locomotor output cycles kaput (CLOCK) and brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT)-like 1 (BMAL1) which, as heterodimers, positively regulate the expression of a number of genes, including the *PERIOD* (*PER*), *CRYPTOCHROME* (*CRY*) and Rev-erb alpha/beta (*REV-ERBα/β*) genes at the start of each cycle. *PER* and *CRY* gene products accumulate, dimerise, and form a multimeric complex that translocates into the nucleus where they interact with CLOCK and BMAL1, inhibiting their own transcription (Kucera et al., 2012, Yoo et al., 2013, Okamura et al., 2010). The enzymes casein kinase 1ε (CK1ε) and the mitogen activated protein kinase (MAPK) phosphorylate the PER, CRY, CLOCK and BMAL1 proteins regulating their stability and/or function, nuclear localisation, and degradation (Yoo et al., 2013, Lefta et al., 2011).

An abundance of BMAL1 protein promotes CLOCK-BMAL1 heterodimerisation necessary to restart the *PER/CRY* transcriptional cycles. The positive-feedback loop in this manner augments regulation of the negative-feedback loop, perpetuating the clock cycle. The negative feedback loop includes the regulation of three *PER* genes (in humans, designated *hPER1-3*) and two CRY

genes (*hCRY1-2*). The rhythmic transcription of the *PER* and *CRY* genes is driven by the basic helix loop-helix (bHLH)-PER-ARNT-SIM (PAS) domain, consisting of CLOCK and BMAL1 transcription factors (Partch et al., 2014, Kucera et al., 2012). It takes approximately 24h for a single cycle to be completed; however, the exact stoichiometry and kinetics involved in this transcription-translation auto-regulatory feedback loop are quite complex and not clearly understood at present.

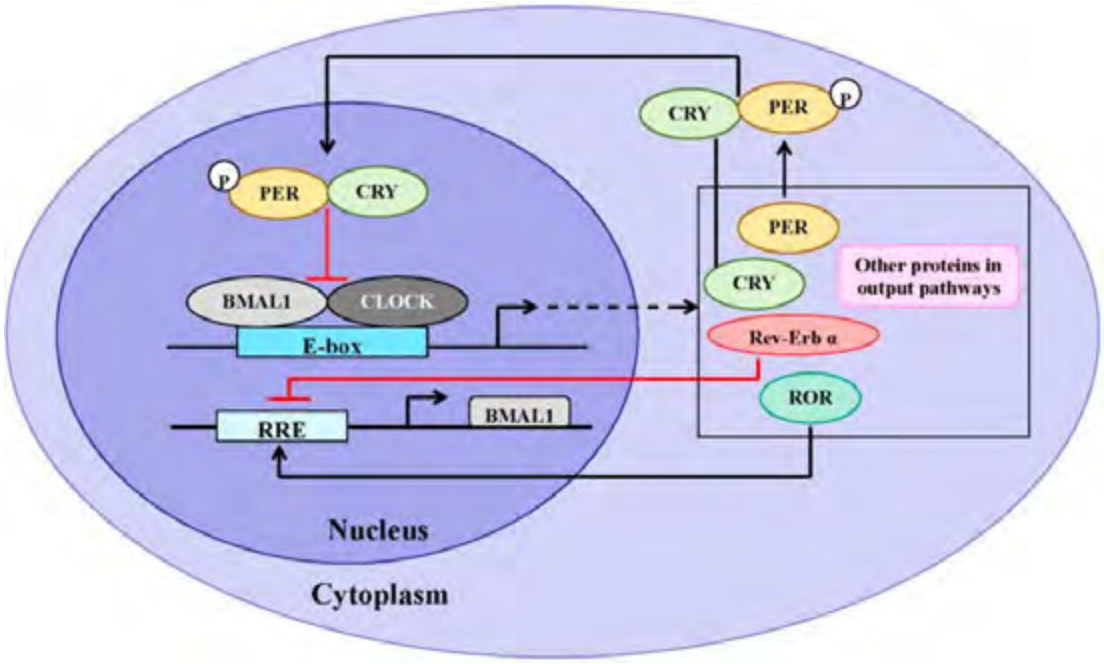


Figure 1.3: A schematic outline of the transcription/translation feedback loops modulating circadian rhythms in mammals. CLOCK and BMAL1 heterodimerise and bind to the E-box promoter elements to activate the transcription of PER1-3, CRY1-2, ROR, REV-ERBα and other genes in output pathways, forming the positive loop of the molecular clockwork of rhythm generation. Phosphorylated PER and CRY proteins heterodimerise and negatively regulate their own transcription and that of REV-ERBα. ROR and REV-ERBα also regulate the activity of the loops by increasing and repressing the transcription of BMAL1 expression, respectively. Post-translational modification of clock factors, for example, phosphorylation, sequestration and sub-cellular localisation influence the regulation of the feedback loop. Reproduced from Wang et al. (2014).

There are numerous other feedback loops interlocked with the core CLOCK-BMAL/PER-CRY loop, including the *REV-ERBα* (Vieira et al., 2013, Kojetin and Burris, 2014) and the retinoic acid-related orphan receptor (ROR) loops (Ko and Takahashi, 2006). REV-ERBα is a component in the secondary auto-regulatory feedback loop that represses *BMAL1* transcription and competes with a ROR to bind ROR response elements (RREs) in the *BMAL1* promoter (Kojetin and Burris, 2014).

1.2.2 Entrainment of the circadian clock

Entrainment is the process by which the circadian clock synchronises to a time cue (Roenneberg et al., 2013, Buhr and Van Gelder, 2014, Husse et al., 2015). This happens to ensure that physiological and behavioural rhythmic events match the period and phase of the immediate external environmental oscillation. This is achieved by altering the concentration or activity of clock components through altered gene expression and protein stability (Sotak et al., 2013, McClung, 2013) in response to an entraining agent. The circadian clock keeps time accurately and adjusts to environmental time cues in order to ensure the efficacy of entrainment. Circadian rhythms are synchronised to the 24h day and exhibit specific phase relationships with the external light-dark cycles and internally with each other. For example, melatonin peaks during the dark period, in the middle of the habitual sleep episode, approximately 2h before the nadir of core body temperature rhythm and approximately 5h before the peak of the cortisol rhythm (Dijk et al., 2012, Morris et al., 2012).

Several endogenous and exogenous time cues that entrain the circadian system in mammals exist. Chief amongst them are the light and temperature cues (Forbes-Robertson et al., 2012), with light being the primary entrainer of the SCN. Other factors also play a role in entrainment. For example, sound and feeding time (Ware et al., 2012, Escobar et al., 2011, Carneiro and Araujo, 2012), hormone levels (Bookout et al., 2013, Morris et al., 2012) and physical activity (Schroder and Esser, 2013). The rate and extent to which the circadian clock entrains to any *zeitgeber* depends on: (1) the free-running period of the clock (Tau or τ); (2) photoperiod; (3) the strength of the *zeitgeber*; (4) the amplitude of the *zeitgeber*, and (5) the clock's sensitivity and responsiveness to a *zeitgeber* at the time of application/perception (Roenneberg et al., 2013,

Golombek and Rosenstein, 2010). The SCN is only found in mammals and is involved in the light entrainment pathway. Output rhythms from the SCN are fundamental to the signalling of other molecules and hormones. As the site of light input in mammals, the retina is vital for image-forming and non-image-forming tasks (Dijk and Archer, 2009). It is the non-image forming tasks of the retina, which are important for circadian entrainment. The SCN receives light input from both classical photoreceptors (rods and cones), as well as from intrinsically-photosensitive retinal ganglion cells (LeGates et al., 2014). Melanopsin is found in intrinsically photosensitive retinal ganglion cells (iPRGC) (Schmidt et al., 2011). These cells play a pivotal role in relaying light information in the blue-light wavelength spectrum (450-495 nm) to the SCN via the retina (LeGates et al., 2014, Chellappa et al., 2014). The SCN uses information from the retino-hypothalamic pathway and is able to coordinate daily 24h rhythms including hormonal secretion, temperature fluctuation and neural activation in line with solar time and the sleep-wake cycles (Buijs et al., 2003, Waterhouse et al., 2005a).

The visual system for entrainment in humans functions optimally under light in the blue portion of the spectrum (Duffy and Wright, 2005, Wright et al., 2013, Lockley et al., 2003). The pathway relevant to light-entrainment relies mainly on information regarding the intensity and length of exposure of the light signal. Thus, a weak light signal leads to a smaller change in phase positioning than a stronger light signal; and an extended light exposure leads to a stronger change than a short exposure. Depending on when during the 24h cycle the light signal is presented to the eye, it will phase advance, phase delay or result in no shift at all in circadian rhythms (Figure 1.4). For example, light exposure during late subjective night accelerates (phase advances) the clock into the next day and exposure to light during early subjective night decelerates (phase delays) the clock (Vosko et al., 2010, Kaur et al., 2009, Schwartz et al., 2011). Exposure during the middle of the subjective day causes no change in phase. Processes such as chromatin remodelling (Nakahata et al., 2008, Bunney and Potkin, 2008) are responsible for re-entrainment, which result in the repression or transcription of central clock genes. Since the same clock genes are expressed in the SCN and retina as in the cells of the rest of the body, it is thus possible to use other cells (e.g. hair follicle, cheek cells) to investigate the change in, for example, chromatin methylation.

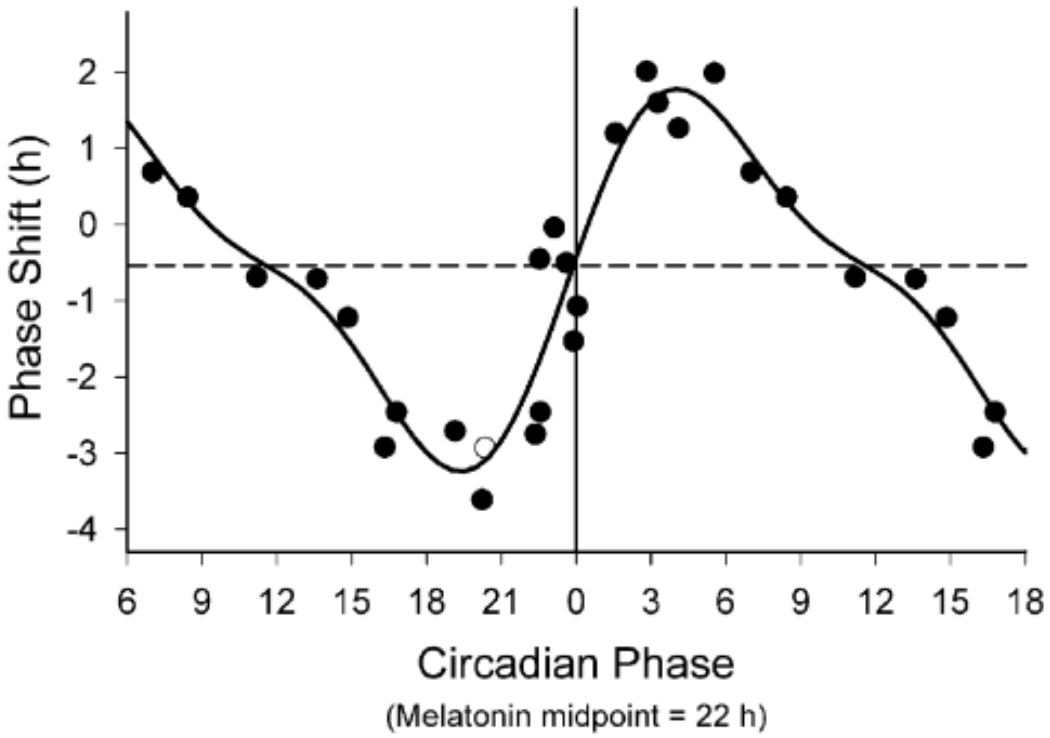


Figure 1.4: Shift (change) in melatonin midpoint as the circadian phase marker with time of day of bright light stimulus. Phase advances (positive values) and delays (negative values) are plotted against the timing of the centre of the light exposure (alternating 6 min fixed gaze: ~10 000 lux; free gaze: ~5000-9000 lux for 6.7h) relative to the melatonin midpoint on the pre-stimulus constant routine in 21 healthy, entrained individuals. The filled circles represent data from plasma melatonin of 20 healthy, entrained individuals, and the open circle represents data from salivary melatonin in one individual from whom blood samples were not collected. The horizontal dashed line represents the 0.54h average delay drift of the body clock between the pre- and post-stimulus phase assessments. Reproduced from (Khalsa et al., 2003)

1.3 Diurnal preference or chronotype

The phase relationship between the external *zeitgeber* and the internal body clock varies between individuals resulting in circadian phenotypes that reflect one's subjective preference for morning or evening activity (also known as 'chronotype'). Such a concept has long been recognised with Kleitman (1949) documenting that some individuals have a consistent preference for daytime activities whilst others have a preference for evening- or nighttime

activities. Specifically, individuals are characterised along a continuum of preference for mornings or evenings. On one end are extreme morning-types (also called 'larks'), who wake up early and are most alert in the first part of the day (Taillard et al., 2004). On the other end are extreme evening-types (also called 'owls'), who wake up later and are most alert in the second part of the day (Zencirci and Arslan, 2011, Loureiro and Garcia-Marques, 2015). In the middle are neither-types, who have no particular preference for mornings or evenings, but can function optimally either in the mornings or evenings. The majority of people fall into this category.

Chronotype differences may arise as a result of (1) tau length differences and (2) timing of the circadian rhythms of particular aspects of physiology such as sleep timing, body temperature, and hormone release (Archer et al., 2008, Baehr et al., 2000, Groeger et al., 2008). For example, the daily rhythms of core body temperature and melatonin secretion have been used to distinguish the timing of circadian rhythms between individuals preferring mornings compared to those preferring evenings (Scheer and Czeisler, 2005, Weinert and Waterhouse, 2007). Specifically, the rise in core body temperature in morning-types takes place at about ±04h00 which is typically two hours ahead of evening-types as illustrated in Figure 1.5 (Baehr et al., 2000, Horne and Ostberg, 1976, Kerkhof and Van Dongen, 1996).

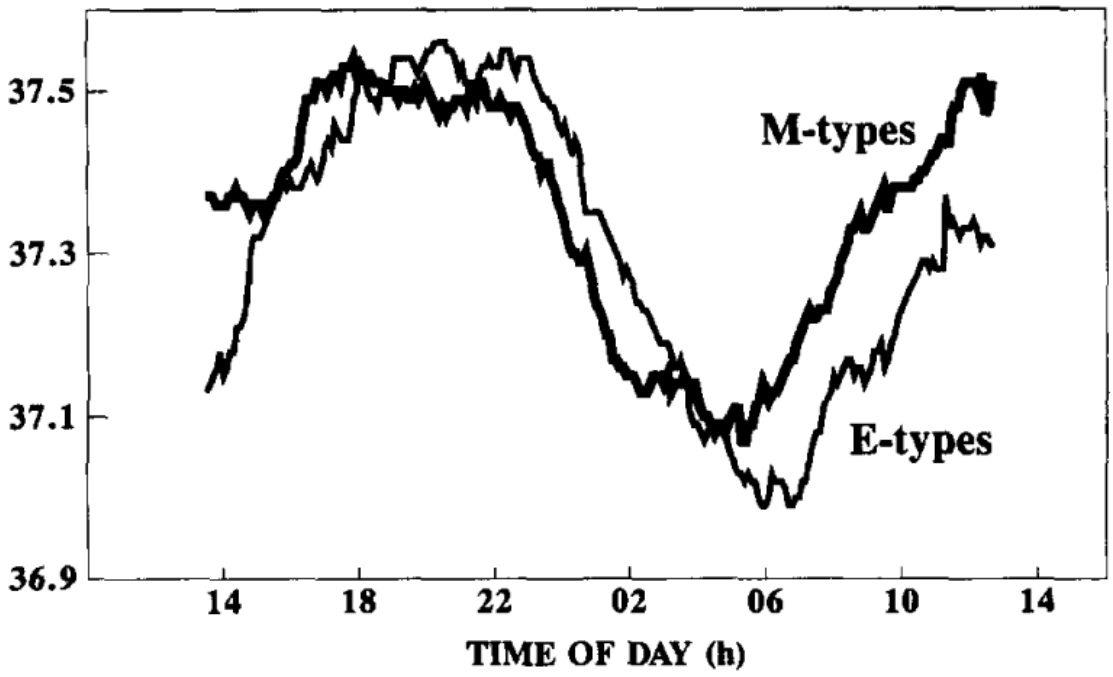


Figure 1.5: Change in rectal body temperature (°C) as a function of time of day in seven morning- and seven evening-types during a constant routine laboratory experiment. E-types: evening-types (bold line), M-types: morning-types (thin line). Reproduced from (Kerkhof and Van Dongen, 1996).

It has been illustrated that while the peak in urinary adrenalin occurs earlier in evening-types, the amplitude is higher in morning-types (Kerkhof, 1985). In addition, subjective alertness, fatigue, heart rate and vigilance performance have been shown to occur earlier in morning-types than in evening-types. While there were no amplitude differences, the onset, peak, and offset of melatonin were reported to occur about 3h earlier in morning-types than in evening-types (Mongrain et al., 2005). These combined data indicate that chronotype may be seen as a psychological preference for early or late rising, but it is influenced by the internal time keeping mechanism.

Both genetics and the environment play an important role in determining chronotype, with estimates of heritability of about 50% (Barclay et al., 2010). In addition to polymorphisms in the circadian clock genes playing a part in the genetic determination, differences in resetting of the body clock, brought about by differences in conditioning or differences in exposure to phase-

resetting stimuli, such as the light-dark cycle also play a role (Archer et al., 2008). For example, chronotype studies from various researchers tend to report more morning-types in temperate/warmer climates than in cooler ones (BaHammam et al., 2011, Henst et al., 2015, Kunorozva et al., 2012, Paine et al., 2006, Rae et al., 2015).

1.3.1 Measuring chronotype

While there are numerous validated questionnaires available to determine chronotype, including the Munich Chronotype Questionnaire (Roenneberg et al., 2003b), Composite Scale of Morningness (Smith et al., 1989) and the Circadian Energy Scale (Ottoni et al., 2011), the Horne-Östberg (HÖ) questionnaire remains the most commonly used subjective tool (Adan and Natale, 2002, Zavada et al., 2005, Randler, 2008, Punduk et al., 2005). Moreover, besides being translated into different languages, psychometric evaluation of this questionnaire confirms that it is made up of a fairly homogenous set of items with reputable internal consistency (Taillard et al., 2004, Randler, 2008, Urban et al., 2011, Rhee et al., 2012). In addition to being validated in a number of settings, the HÖ-questionnaire has also been shown to correlate well with several mechanisms involved in the regulation and timing of the natural sleep-wake cycle (Randler, 2008, Horne and Ostberg, 1976, Lehnkering and Siegmund, 2007) and the secretion of melatonin (Taillard et al., 2003), providing further validation of its use.

The HÖ-questionnaire is a 19-item questionnaire used to categorise individuals into one of five different chronotype groups (Horne and Ostberg, 1976). The scoring scale ranges from 16-86 with lower scores indicating a greater preference for evenings, and higher scores indicating a greater preference for mornings. Scores in the intermediate area indicate neither morning- nor evening-type preference. The HÖ-questionnaire evaluates behavioural aspirations rather than actual behavioural patterns subject to circadian differences, based primarily on preferences for morning- and evening-orientation of activity (Juda et al., 2013, Roenneberg, 2012, Zavada et al., 2005). Questions about the timing of actual sleep-wake behaviour on the HÖ-questionnaire are ideally fit for quantification of the circadian phase, as sleep-wake behaviour is easily observable and can be reported with no difficulties. The proportion of morning-, neither- and evening-type

individuals within various populations around the world has been reported in the literature, and is shown to vary considerably. The bulk of studies conducted thus far report more individuals in the neither- and evening-type categories and very few in the morning-type category in American, European, and Asian male and female general and student populations (Tonetti et al., 2010, Zavada et al., 2005, Osland et al., 2011, Chelminski et al., 1997). However, Paine et al. (2006) and Kunorozva et al. (2012) reported low proportions of evening-types (6% and 7%, respectively), and roughly equal proportions of neither-types (45% and 49%), and morning-types (49% and 41%) in Caucasian populations in temperate climates.

1.3.2 Factors influencing chronotype

Chronotype is influenced by developmental changes and ageing (Adan and Natale, 2002, Bliwise et al., 2005, Edelstein et al., 2012). For example, Horne and Östberg (1976) reported that a bed time of 23h30 may be indicative of a morning-type individual within a student population, but might be more related to an evening-type in the 40-60 years age group. Several other studies have reported age-related chronotype differences between young, middle-aged and older adults (Adan, 1992, Paine et al., 2006, Tonetti et al., 2010, Borisenkov et al., 2010, Reilly et al., 1997b, Kerkhof, 1988, Monk, 1989, Reilly et al., 1993, Waterhouse and Minors, 1995, Koskenvuo et al., 2007b). For example, Tonetti et al. (2010) reported a chronotype distribution of morning (11.3%) and evening (23.4%) types in 1 041 young adults aged between 18-30 years. In another study, Koskenvuo and colleagues (2007a) reported that evening-type individuals were more prevalent in adolescents and young adults, while morning-types were more common in adolescents and older adults. Reilly et al. (1997b) illustrated that chronotype changes with age in 31 participants between the ages of 25-74. Specifically, they reported a reduced circadian amplitude and phase relation between systolic blood pressure and heart rate in older adults, implying an internal dissociation of rhythms with aging. This suggests an increase towards morningness with aging.

In contrast, a study which assessed chronotype based on midpoints of sleep on free days (MSF) in people aged 8-to-94 years old indicated that males between 8-20 years show a later MSF, which is indicative of an evening chronotype (Roenneberg et al., 2004). In the same study, it was

noted that MSF displayed a reversed pattern in individuals above 21 years, changing to morning chronotype. The change to morning preference in the older generation becomes more apparent around the age of 50 years (Bliwise et al., 2005, Taillard et al., 2004). However, confounding this association is the strong synchronising effect of societal demands, particularly work habits, academic and family commitments in altering the natural chronotype of adolescents and young adults (Adan and Natale, 2002, Roepke and Duffy, 2010, Harb et al., 2012). In addition, when older people retire they do not have to wake early for work, thus they may become more evening-types through conditioning. Work-type is another factor, which may influence chronotype, for example, nighttime shift work may perturb the normal sleep-wake cycle, potentially altering an individual's chronotype. This is conceivable given that in one study, only 32% of healthy daytime shift workers were categorised as evening-types, while 75% of nighttime shift workers were categorised as evening-types (Burch et al., 2009).

An individual's sex may influence chronotype; however, there is no consensus as to which gender is more prone to being more morning- or evening-oriented. For example, Paine et al. (2006) reported no sex effect in 2 526 New Zealand participants between the ages of 30-49 years. Similarly, Lazar et al. (2012) illustrated no sex effect in 413 males and 262 females aged between 20-35 years. In another study sex was not associated with chronotype using the reduced version of the HÖ-questionnaire in 330 males and 578 females (Adan, 1992). However, Adan and Natale (2002) reported that females scored highly on the HÖ-questionnaire compared to males in a 2 135 University student population. In another study, Cavalera et al. (2011) reported that males were more evening-oriented compared to females.

1.4 Relationship between clock genes and chronotype

There are differences in circadian rhythm generation mainly due to genetic polymorphisms in clock genes, which lead to phenotypic differences in individuals. Several studies have demonstrated that mutations and polymorphisms in clock genes are associated with particular circadian abnormalities in humans, as well as with more subtle non-pathological phenotypes and chronotype. For instance, polymorphisms in a number of clock genes are associated with

increased or decreased risk of cancer (Kelleher et al., 2014, Gery et al., 2006, Winter et al., 2007) and sleep disorders, such as familial advanced sleep phase syndrome (ASPS) and delayed sleep phase syndrome (DSPS) (Reid et al., 2012, Archer et al., 2003, Ebisawa, 2007, Jones et al., 1999, Pereira et al., 2005), while others are associated with chronotype (Archer et al., 2003, Carpen et al., 2006, Katzenberg et al., 1998, Pedrazzoli et al., 2007).

For the purpose of this thesis, the focus is on polymorphisms in clock genes associated with chronotype, focusing specifically on the *PER3* gene. *PER3* is a core clock gene, which is one of the transcriptional repressors that form the negative limb of the transcriptional-translational feedback loop. It has a redundant role with other PER proteins (PER1 and PER2) and is not essential for circadian rhythm maintenance. However, it plays an important role in the sleep-wake timing and sleep homeostasis, without influencing circadian parameters (Dijk and Archer, 2010, Viola et al., 2007, Maire et al., 2014, Hasan et al., 2014). It is highly expressed on the skin, in blood, epithelium of mammary- and salivary secreting-glands, just to mention a few. Several methods such as affymetrics, RNA sequencing and in-situ hybridisation can be used to measure *PER3* expression levels (Hamada et al., 2001, Akashi et al., 2010, Li et al., 2013).

Variations in *PER3* including variable number tandem repeat (VNTR) and promoter have been associated with chronotype. The *PER3* gene consist of two alleles, the longer version of the allele (*PER3*^{5}) is associated with morningness while the shorter variant (*PER3*^{4}, GeneBank AB047536) is associated with eveningness (Archer et al., 2003, Ebisawa et al., 2001). The functional roles of *PER1* and *PER2* the other two *PER* genes are well documented and have been shown to directly influence circadian rhythm maintenance (Jenkins et al., 2005, Zheng et al., 2001). Polymorphisms in these two genes have also been correlated with morning-evening preference (Carpen et al., 2005, Lee et al., 2011, Toh et al., 2001, Carpen et al., 2006, Matsuo et al., 2007). For example, Lee et al. (2011) demonstrated an association between the *PER2* 3853G allele with morningness in 299 Korean college students (108 females and 191 males; mean age 22.1±2.1).

In another study, Carpen et al. (2005) illustrated an association between *PER2* and diurnal preference in 105 British individuals (mean age: 40.1±14.1). Specifically, they reported a higher percentage of the *PER2* 111G in individuals with extreme morning preference. In addition, *PER2* 2114A allele was shown to be associated with eveningness in 71 healthy Japanese individuals (50 females and 21 males; mean age 23.5±7.6; Matsuo et al., 2007). There have been a few studies, which found no association in the other *PER2* polymorphisms and chronotype (Carpen et al., 2005, Lee et al., 2011, Choub et al., 2011). In two of these studies, no association was found between chronotype and either the C or G alleles of the *PER2* gene in 156 healthy volunteers of Italian origin (104 females and 52 males; mean age 33.1±12.0; Choub et al., 2011) and 299 Korean college students (108 females and 191 males; mean age 22.1±2.1; Lee et al., 2011).

The *CLOCK* 3111C (rs1080126) polymorphism has been associated with evening preference (Katzenberg et al., 1998, Mishima et al., 2005), while the *PER2* 3853G (rs934945) and 111G (rs2304672) polymorphisms have been associated with morning preference (Lee et al., 2011, Choub et al., 2011). Robillard et al. (2002) and Pedrazzoli et al. (2007) failed to reproduce the CLOCK 3111C in British and Brazilian samples, respectively, and Rae et al. (2015) and Henst et al. (2015) have found no association between the *PER3* VNTR and chronotype in South African and Dutch marathon and professional swimming populations. Possible reasons for the contradictory findings in these studies vary and range from, the use of different ethnicities, differences in sample sizes and differences in latitude.

1.4.1 The *PER3* variable number tandem repeat (VNTR) and chronotype

To date, there have been conflicting findings with respect to whether the VNTR polymorphism within the coding region of the *PER3* gene is associated with diurnal preference or not. Specifically, just as many studies have reported an association between *PER3* and diurnal preference (Henst et al., 2015, Archer et al., 2003, Groeger et al., 2008, Pereira et al., 2005, Ellis et al., 2009, Jones et al., 2007, Archer et al., 2010, Kunorozva et al., 2012) compared to those studies which have failed to find an association (Henst et al., 2015, Rae et al., 2015, Osland et al., 2011, Voinescu and Coogan, 2012). This polymorphism comprises a 54 base pair repeat sequence

on exon 18 of the *PER3* gene encoding eighteen amino acids in positions 966-1055, which is repeated four (*PER3*^{4}) or five (*PER3*^{5}) times (Figure 1.6), resulting in two forms of the protein (Ebisawa et al., 2001). A minority (10%) of people in most populations are homozygous for the *PER3*^{5} allele, while approximately 50% are homozygous for the *PER3*^{4} allele (Ciarleglio et al., 2008, Henst et al., 2015, Archer et al., 2003). In some populations, for example, the Papua New Guinea, the prevalence of these alleles is reversed (Ciarleglio et al., 2008).

The 5-repeat allele has more serine or threonine residue phosphorylation sites; therefore it's believed to be turned-over more rapidly than the 4-repeat allele which has fewer phosphorylation residues (Archer et al., 2003). The *PER3*^{4} allele was reported to be associated with the evening chronotype in a British population (Archer et al., 2003), a finding that was reproduced in a Caucasian South African population (Kunorozva et al., 2012). Kunorozva et al. (2012) investigated the distribution of chronotype and *PER3* VNTR genotype in South African, male, individual sport athletes and a control population of active, but non-competitive individuals (exercised more than three times per week). They reported a higher proportion of individuals carrying the *PER3* 5-repeat allele in the athletic population compared to the control group.

One of the studies that found no association between chronotype and *PER3* genotypes cite level of physical activity as a confounding factor (Henst et al., 2015). Specifically, they reported a higher percentage of morning-types in marathon runners regardless of *PER3* VNTR genotype. The mismatch between *PER3* genotype and chronotype in the Henst et al. (2015) study may also be attributed to the time of competition event. Specifically, that taking part in an endurance event with an earlier start time (i.e. between 06h00-08h00 for South African runners and 10h00-12h00 for the Dutch runners) may affect chronotype. This is plausible given that while the majority of the runners trained in the evening, they were more morning-oriented than the control population. Therefore, biological effects (e.g. *PER3* genotype) may be masked by environmental and social pressures subsequently affecting the chronotype and genotype association. Increased exercise frequency *per se* may influence the distribution of chronotype, but has no influence on

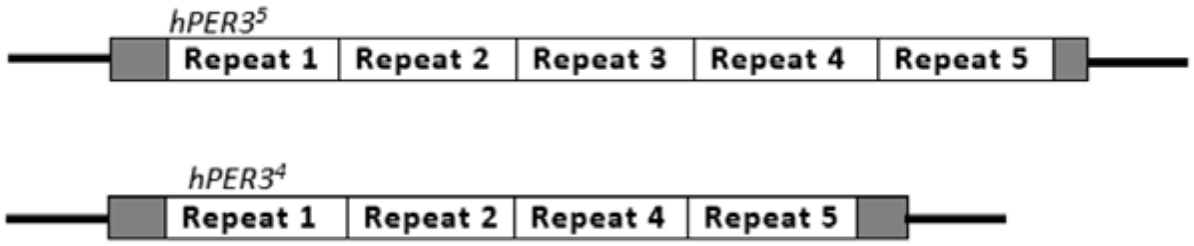


Figure 1.6: A graphic representation of the *hPER3* VNTR polymorphism located on exon 18. Grey boxes indicate the flanking regions. White boxes indicate the 54 base pair (bp) repeats, which are repeated four (*PER3*^{4}) or five (*PER3*^{5}) times, encoding proteins of different sizes. VNTR: Variable number tandem repeat polymorphism and *PER3*: *PERIOD3*.

the *PER3* gene expression, thus this may only affect the association between chronotype and genotype. There are noticeable differences in the distribution of the *PER3* VNTR polymorphism worldwide, as demonstrated by the varying allele frequency in the African American, European American, Han Chinese, Ghanaians and Papua New Guinea populations (Ciarleglio et al., 2008, Nadkarni et al., 2005). In particular, the 4-repeat allele frequency in the European American (59%), Han Chinese (81%) and Papua New Guinea (41%) populations differed immensely (Ciarleglio et al., 2008). This is interesting because unlike the other clock genes, the *PER3* VNTR allele frequency was reported not to be affected by environmental parameters such as seasonal fluctuations, photoperiod and temperature, which vary as a function of latitude (Ciarleglio et al., 2008, Nadkarni et al., 2005).

1.5 Sleep and circadian rhythms

The sleep-wake cycle is the most obvious circadian output of the circadian system. Sleep is part of a daily biological rhythm that is necessary for survival, enhancing well-being, energy conservation and optimal bodily function (Siegel, 2005, Leproult and Van Cauter, 2010, Wang et al., 2011). Its widespread presence in nature highly suggests that it somehow serves an important evolutionary function. During the sleep period, humans are less aware of their surroundings and less able to respond immediately to danger, a situation that one would normally consider as being

highly disadvantageous to an organism's chances of survival, yet sleep remains. Sleep is influenced by both the homeostatic (need based) and circadian (i.e. time-of-day) processes (Dijk and Archer, 2010, Caldwell and Redeker, 2005, Van Dongen et al., 2012, Daan et al., 1984, Borbely, 1982). The homeostatic process relates to the sleep-wake history of an individual, with a drive for sleep (sleep pressure) mounting during episodes of wakefulness (Dijk and Czeisler, 1994, Kerkhof and Van Dongen, 2010, Cohen et al., 2010). The circadian process relates to the variation in the timing of preferred sleep and wake-up times over the course of 24 hours (Van Dongen et al., 2012, Wulff et al., 2010). These two processes combined influence sleep propensity and duration as well as the quality of wakefulness including cognitive function and physical performance (Morris et al., 2012, Dijk, 2010, Banks et al., 2010).

Under suitable entraining conditions, the homeostatic and circadian systems interact to consolidate wakefulness during the biological day and sleep during the biological night. Importantly, sleep is not a single homogenous state; it has been reported to consist of four different sleep stages (Chellappa et al., 2012b, Viola et al., 2007, Dijk and Czeisler, 1995)**.** It is broadly divided into two states namely; rapid eye movement (REM) sleep and non-rapid eye movement sleep (n-REM). REM sleep is mostly characterised by higher levels of electroencephalogram (EEG) activation, and episodic bursts of rapid eye movement under closed eye lids (Viola et al., 2007, Horovitz et al., 2008). In contrast, n-REM sleep is characterised by a reduction in electrical output from the brain cortex relative to waking as well as a reduction in muscle tone in the voluntary muscles.

In addition, n-REM is sub-divided into four stages, with stage 1 sleep being the closest to wakefulness and having a much lower threshold for arousal. In contrast, stage 4 sleep is marked by a reduction in cortical activity along with a high arousal threshold. Being awoken from this stage of sleep is often accompanied by feelings of sleepiness. More often than not, sleep stages 3 and 4 are combined to form the deep or slow wave sleep (SWS). This is because they are both characterised by a marked decrease in the frequency of electrical activity in the cortex (Diekelmann and Born, 2010, Crunelli and Hughes, 2010). REM sleep is believed to play a

fundamental role in sleep consolidation, perhaps due in some part to the fact that the brain is usually active during this phase of sleep (Goldstein and Walker, 2014). Sleep timing varies greatly in individuals and has been recognised as an interesting aspect of chronotype (Randler, 2014, Adan et al., 2012). Furthermore, it is a variable trait in humans, with some individuals retiring to bed at a time when others are waking up. This variability allows one to distinguish individuals that have a great sleep need and retire to bed earlier (morning-types) from those that retire to bed later (evening-types) (Roenneberg et al., 2004).

Higher sleep need has also been reported in individuals homozygous for the *PER3* 5-repeat allele during sleep restriction/deprivation studies (Viola et al., 2007, Chellappa et al., 2014). Specifically, the $PER3^{5/5}$ genotype has been shown to be associated with a shorter sleep latency, more theta and alpha activity in wake and rapid eye movement (REM) sleep, and more slow wave activity in non-REM sleep compared to the $PER3^{4/4}$ genotype; which is indicative of an increased sleep pressure in the $PER3^{5/5}$ genotype (Chellappa et al., 2012b, Goel et al., 2009, Maire et al., 2014). Therefore, it is likely that $PER3^{5/5}$ individuals or those individuals with earlier sleep timing will be greatly affected by extended wakefulness or disruption of the sleep-wake cycle compared to the $PER3^{4/4}$ individuals or those with later sleep timing.

1.5.1 Sleep and its effects on individual and team sport athletes

Sleep is fundamental for athlete recovery due to its physiological and restorative effects (Lastella et al., 2014a). At present, a number of studies have explored the effects of sleep extension, deprivation and restriction on the sleep-wake behaviour of elite athletes (Lastella et al., 2015, Mah et al., 2011, Halson, 2014, Juliff et al., 2015, Robey et al., 2014, Richmond et al., 2007, Kline et al., 2007, Oliver et al., 2009, Mougin et al., 1991, Leeder et al., 2012). Several of these studies have reported sleep effects under partial or total sleep deprivation conditions (Blumert et al., 2007, Oliver et al., 2009, Kline et al., 2007, Mougin et al., 1991), which makes their application on elite athletes during competition difficult. In addition, the amount of sleep athletes obtain varies and may depend on the type of sport (Lastella et al., 2015). For example, current evidence in the literature indicate that individual sport athletes may obtain less sleep at night than team

sport athletes (Leeder et al., 2012, Richmond et al., 2007, Sargent et al., 2014a). These differences may be attributed to training time-of-day, which has been reported to influence total sleep time (Sargent et al., 2014b). Sargent et al. (2014b) suggest that individual sport athletes may sleep less at night because they are able to nap during the afternoon to compensate for shorter nighttime sleep, given that they are flexible to plan their days.

It has been reported that individual sport athletes from swimming, rowing and canoeing obtain between 5-7h of sleep per night (Sargent et al., 2014a, Leeder et al., 2012). In contrast, team sport athletes, for example, the Australian Rules Football athletes have been reported to obtain between 8-9h nighttime sleep (Richmond et al., 2004, Richmond et al., 2007). This is higher than the 5-7h obtained by individual sport athletes; suggesting that any sleep deficit may alter the rhythm of performance, particularly in team sport athletes, consequently reducing both the average level of performance and peak-to-peak amplitude (Souissi et al., 2003, Bougard et al., 2008). Caution must however be exercised when interpreting total sleep time findings between individual and team sport athletes, given that there may be methodological and cultural differences between studies (Lastella et al., 2015). Small decrements in performance due to sleep deficit might separate a winning team or individual from another, whose performance is only slightly below peak values (Leger et al., 2005).

In summary, it is difficult to apply findings on effects of sleep following partial and total sleep deprivation in elite athletes, given that the extent of disturbed sleep before and during a competition or after time zone travel is different to total or partial sleep deprivation.

Table 1.1: Sleep amount and its effects on various parameters in athletes

Hours of sleep	Population	Parameters	References
≥10h	Basketball players	↓Sprint time, ↑Shoot accuracy	(Mah et al., 2011)
10h	Active individuals	↑ Leucocyte production	(Faraut et al., 2011)
10h	Swimmers	↓15m sprint time, reaction time, turn time, improved mood	(Mah et al., 2008)
9h	Speed skaters	↓Sleep efficiency	(Leeder et al., 2012)
<8h	Adolescent athletes	↑1.7 increased risk of injury than athletes sleeping >8h	(Milewski et al., 2014)
8h	Olympic athletes	↓Sleep quality	(Leeder et al., 2012)
<7h	Healthy individuals	↑T-cell function ↓Natural killer cell activity	(Fondell et al., 2011)
5h	Endurance athletes	↑Heart rate and lactate accumulation	(Mougin et al., 1991)
3h	Swimmers	↓Swim performance	(Kline et al., 2007)
3h	Weightlifters	↓Maximal bench press, leg press and deadlifts	(Reilly and Piercy, 1994)
2.5h	Swimmers	↓ Mood state	(Sinnerton and Reilly, 1992)
2.5h	Endurance athletes	↓Psychomotor performance	(Reilly and Deykin, 1983)
24h SD	Weightlifters	↓Mood state	(Blumert et al., 2007)
24h SD	Marathon runners	↓Running performance	(Oliver et al., 2009)
30 SD	Elite male	↓ Mean and total sleep time, ↓ Muscle glycogen content	(Skein et al., 2011)
36 SD	Cyclists	↓anaerobic power	(Souissi et al., 2003)

↑↓ represent an increase and decrease respectively, SD- sleep deprivation

1.6 Disruption of the circadian clock

1.6.1 Circadian disruption

One of the primary ways in which the circadian clock can be disrupted is through circadian misalignment, which may be transient or chronic. Both transient and chronic rhythm disruption lead to the misalignment of the sleep-wake cycle and the internal body clock with geophysical time resulting in physiological activation and hormone secretion at unusual times during the 24h day (Morris et al., 2012, Turek and Gillette, 2004, Muhlbauer et al., 2009, Scheer and Czeisler, 2005). This upsets the normal functioning of various bodily functions. Some symptoms of

circadian disruption include fragmented sleep, changes in mood and gastrointestinal disturbances (Waterhouse and Reilly, 2009, Samel et al., 1995, Eastman and Burgess, 2009, Vosko et al., 2010). Transient rhythm disruption has been noted in numerous settings including after trans-meridian travel, while chronic rhythm disruption has been noted in nighttime shift workers. Transient circadian disruption occurs because of the disparity between the internal circadian rhythm and the external day-night environment (Lee et al., 2009, Haus and Smolensky, 2006). In contrast, chronic disruption is noted pathologically in sleep disorders such as ASPS and DSPS, which occur as a result of a body clock advance or delay relative to external time, consequently causing the sleep episode to occur at socially abnormal times during the day or night (Boulos et al., 1995, Pandi-Perumal et al., 2008, Lack and Wright, 2007).

Shift work involves working outside the normal daylight hours (Loudoun and Bohle, 1997, Nachreiner, 1998). The temporal structure of shift work (nighttime vs daytime) interferes with both the prevailing time of society and internal body rhythms. In particular, the frequent changes in shift schedule and circadian disruption that ensues following nighttime shift work has been linked with increased risk of obesity (Buxton et al., 2012), metabolic disorders (Knutson et al., 2007); cardiovascular diseases (Stevens, 2009) and cancer (Davis and Mirick, 2006). For example, significantly more digestive problems have been reported in individuals working nighttime shifts compared to those working daytime shifts (Nagaya et al., 2002, Costa, 1996). This suggests that a chronically misaligned circadian system may increase the risk of illness. While, nighttime shift work results in circadian desynchronisation, its further discussion is beyond the scope of this thesis.

Circadian disruption similar to that which occurs following time zone travel result in acute changes in the feeding system and hormonal secretion, which is detrimental to metabolism (Baron and Reid, 2014, Mullington et al., 2003). This is conceivable given that dysregulation of feeding behaviour, changes in appetite stimulating hormones and glucose metabolism have been reported in nighttime shift workers (Spiegel et al., 2004, Scheer et al., 2009, Gonnissen et al., 2012). For example, Scheer et al. (2009) reported a decrease in leptin levels and an increase in

glucose levels in eight adults that ate and slept 12h out of phase from their habitual time. Another study reported a decrease in leptin levels and elevated ghrelin in a group of sleep deprived volunteers from the Wisconsin sleep cohort study (Taheri et al., 2004). Circulating leptin levels are responsive to acute changes in energy balance resulting from increased or decreased caloric intake. Decreased leptin levels stimulate appetite and decrease intake. While these studies were performed in controlled laboratory conditions, circadian disruption following time zone travel may lead to changes in hormonal levels affecting metabolism. This may contribute to energy imbalances by altering energy balance via changes in the source of fuel being oxidised.

In summary, while recovering from chronic circadian disruption may take months, recovery from transient circadian disruption may take a few weeks or days. There is inter-individual variation in response to and recovery from transient circadian disruption, for example, following time zone travel. However, no one has tried to explain this inter-individual variation in relation to clock gene polymorphisms.

1.6.2 Physiological variables measured to assess circadian rhythm disruption

Several physiological variables including core body temperature, melatonin and cortisol can be measured to investigate the normal functioning of the circadian clock following circadian disruption activities such as nighttime shift work, frequent time zone travel, space flights and submarine missions. The decision about which physiological variable to use in order to measure circadian phase is vital as the above-mentioned phase makers are affected differently by confounders. For example, body temperature has the advantage of being able to be collected continuously using either an ingested thermometric pill (Byrne and Lim, 2007) or wrist-worn temperature data loggers (Sarabia et al., 2008). However, variations in body temperature across the day are influenced not only by circadian rhythms, but also by factors such as posture, sleep-wake state and activity level (Sarabia et al., 2008, Donaldson et al., 1996). Furthermore, the influence of these behavioural confounders on body temperature is phase dependent, such that the change in temperature produced by the behaviour is different depending on when during the

24h day the behaviour occurs. As such, diurnal variations in temperature may not reflect the underlying circadian variation.

Melatonin levels on the other hand are quite robust to confounders such as posture, activity and arousal state compared to temperature (Zeitzer et al., 2007, Deacon and Arendt, 1996). However, there is some evidence, suggesting that periodic changes in behaviour (e.g. exercise, posture) can influence melatonin levels (Deacon and Arendt, 1994, Monteleone et al., 1990). One disadvantage of using melatonin as a circadian phase maker is that collection of samples (i.e. salivary) during sleep may require interruption, although specialised blood collection systems used in many laboratories do not require interruption (Keijzer et al., 2011). For this reason, studies that investigate both sleep patterns and salivary melatonin levels have measured only the onset of melatonin secretion as a phase marker, rather than collection over the entire 24h period. While the onset of melatonin secretion is sufficient to determine melatonin rhythm timing, it misses out any changes in rhythm amplitude, duration or offset timing. The endogenous increase of melatonin levels is suppressed by ambient lighting; as such, samples have to be taken in dim light throughout all sampling segments.

While cortisol (Figure 1.7) and temperature can be used as circadian phase markers of circadian disruption, the onset of endogenous melatonin production is thought to be a more reliable marker of the circadian phase (Griefahn, 2002, Benloucif et al., 2005). Furthermore, melatonin has been shown to free run in blind animals and humans in line with core body temperature (Czeisler et al., 1995, Nakagawa et al., 1992) and following extended wake periods under constant routine, dim light conditions (Archer and Mench, 2014, Boivin and James, 2002, Weibel and Brandenberger, 1998).

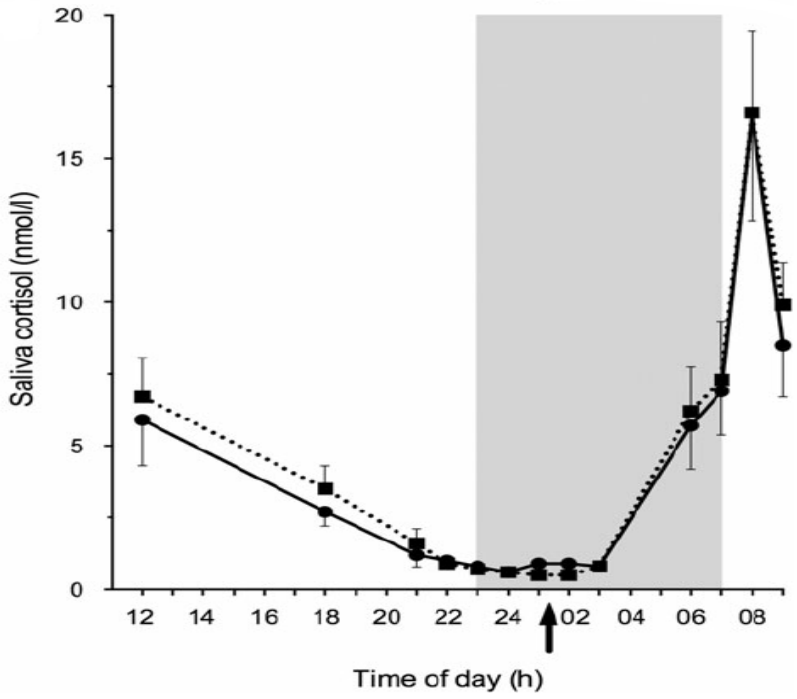


Figure 1.7: Circadian rhythm of saliva cortisol of eight young healthy volunteers (solid line). The dotted line indicates saliva cortisol levels when light was shined in the volunteers' ears. Data are presented as mean ± SEM and the arrow indicates time of ear light exposure, 24 min (01h10-01h34). The grey area shows lights off (23h00-07h00) (Jurvelin et al., 2014).

1.6.2.1 Melatonin "a marker of innate" circadian rhythm and disruption

Melatonin is a ubiquitous molecule synthesised from serotonin in the pineal gland and secreted at night where it is centrally involved in sleep regulation (Pandi-Perumal et al., 2007). It is also an important molecule in terms of spreading the "time-of-day" information to peripheral clocks (Pevet and Challet, 2011, Underwood et al., 1984, Gern et al., 1986). Specifically ablation of the mammalian pineal gland suppresses melatonin production affecting synchronisation of peripheral clocks and regulation of other hormones (Chakir et al., 2015, Bell-Pedersen et al., 2005). Melatonin secretion happens under dim-light conditions, and is supressed by exposure to bright light (Figure 1.8). Its secretion regulates the entire thermoregulatory cascade (i.e. decrease in heat production and increase in heat loss) which starts with the rise in endogenous melatonin levels in the evening (Cajochen et al., 2003). Onset of melatonin secretion acts as a hormonal signal timing the rise in blood flow in distal skin regions, which results in heat loss, the degree of

which is the best physiological predictor for the rapid onset of sleep (Krauchi et al., 1999). The correlation between sleep and the melatonin rhythm has been reported in blind individuals in whom the circadian clock is not entrained (Lockley et al., 1997, Nakagawa et al., 1992) and in sighted individuals with non-24h sleep-wake syndrome (Uchiyama et al., 2000, McArthur et al., 1996).

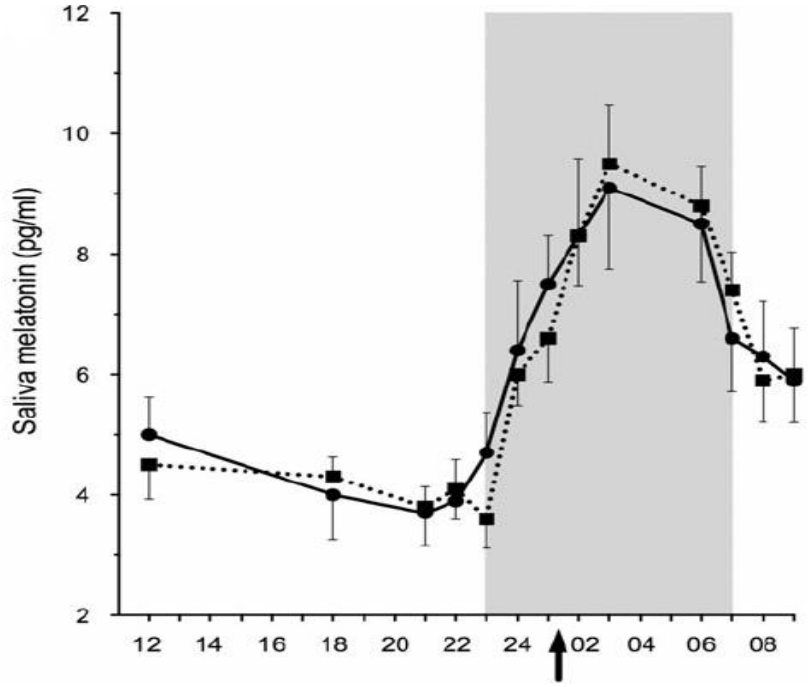


Figure 1.8. Circadian rhythm of saliva melatonin of eight young healthy volunteers (solid line). The dotted line indicates saliva melatonin levels when light was shined in the volunteers' ears. Data are presented as mean ± SEM and the arrow indicates time of ear light exposure, 24 min (01h10-01h34). The grey area shows lights off (23h00-07h00) (Jurvelin et al., 2014).

The daily circadian onset of melatonin secretion coincides with dim-light or sunset starting about ±2-3h before bedtime (Figure 1.8), which correlates with an increase in sleep propensity (Dijk and Cajochen, 1997). This natural phenomenon is known as dim-light melatonin onset (DLMO) (Gooley et al., 2011, Hofstra and de Weerd, 2008, Lewy et al., 1980, Weitzman et al., 1981) and it is the most reliable marker of the timing of the central clock in humans (Danilenko et al., 2014, Klerman et al., 2012). Under normal conditions salivary or plasma melatonin concentration increase during the evening, levels continue to increase during the sleep period, and gradually

deteriorates during the second half of the night (Arendt, 2005, Pandi-Perumal et al., 2007, Hilaire et al., 2007). EEG activation during wakefulness is timed at a specific time phase relative to the circadian cycle (Cajochen et al., 2002). EEG experiments indicate that the circadian clock drives the rhythms of melatonin synthesis, thermoregulation, sleep consolidation and activation during wakefulness among other things (Cajochen et al., 2003, Dijk et al., 1999). While there may be feedback from the pineal gland to the circadian clock and thermoregulatory centres in the hypothalamus, the consensus is that melatonin weakens the circadian signal from the SCN promoting heat loss. This induces sleepiness via the preoptic area of the anterior hypothalamus.

It is possible that the effect of melatonin on sleepiness and sleep is relative rather than absolute, since individuals who do not secrete melatonin seem to sleep normally (Wehr et al., 2001). Therefore, the function of melatonin appears to be the mediation of dark signals and providing night information, rather than be the hormone of sleep (Wright et al., 2013). It is also thought to be an internal synchroniser that stabilises and reinforces various circadian rhythms in the body. During the day, melatonin is not produced in measurable quantities (Gooley et al., 2011, Keijzer et al., 2014, Zeitzer et al., 2007). In healthy individuals who have been maintaining a stable sleep-wake schedule with sleep at night, DLMO can be estimated with reasonable accuracy as it is related to environmental light exposure (Keijzer et al., 2011, Martin and Eastman, 2002, Burgess and Eastman, 2005, Wright et al., 2013).

1.6.3 Phase shifting of circadian rhythms

A phase shift is a change in the timing of a rhythm in relation to an environmental time cue (Eastman et al., 1995, Minors et al., 1991). This may shift bedtime or wake-up time to either earlier or later than usual during the day similar to that which happens following time zone travel. Minor phase shifts occur on a daily basis to synchronise the circadian rhythm with the external environment (Beaumont et al., 2004, Burgess et al., 2002, Edwards et al., 2000, Gronfier et al., 2007, Wright et al., 2013). More major adjustments are required to resynchronise one's internal body clock with the temporal environment following trans-meridian travel. For example, following long-haul time zone travel, normal sleep-wake patterns may be disrupted and this is

likely to persist for a few days. The rhythm of the master circadian clock in the SCN is entrained by the rhythmic change of light and dark (Lewy et al., 1998, Warman et al., 2003). The SCN subsequently synchronises peripheral clocks in the liver, kidney and pancreas for example, with each other and thus aligns the entire circadian system to the external light. These peripheral clocks take longer to be resynchronised compared to the SCN. Peripheral clocks can potentially be entrained directly by other signals such as rhythmically timed food intake without affecting rhythms in the SCN (Buijs et al., 2013, Damiola et al., 2000). A more federated organisation of independent clocks offers the opportunity of different signals acting independently on different peripheral clocks. In mice, light and feeding rhythms may be uncoupled when sleep rhythms are disrupted (e.g. jet-lag), alignment by different zeitgeber signals is impaired, which results in the uncoupling of the SCN and peripheral clocks (Barclay et al., 2012). In the event that rhythms from the SCN are disrupted, a great number of peripheral clocks would produce disharmony of rhythms that would make coordination of circadian behaviour impossible.

Two features of the mammalian circadian system provide flexibility in circadian programming to exploit temporal regularities of social stimuli or food availability (Mistlberger and Antle, 2011). Sensitivity of the SCN to behavioural arousal stimulated during the sleep period is one of the features, which can reset its phase and modulate its response to the light-dark stimuli. For example, the cortisol rhythm phase adjusts more slowly compared to other rhythms following a circadian phase shift (Sack et al., 2007). As a result, its rhythm will be dissociated in timing for a few days relative to rhythms that have synchronised faster. However, the apparent difference in the rate of phase shifting may be due to different masking influences of external factors on simultaneous entrainment of internal rhythms. For example, differences in the rate at which the SCN and peripheral clocks readjust was demonstrated following a simulated phase shift in a transgenic rat model in which the mouse *PER1* gene promoter was linked to a luciferase reporter (Yamazaki et al., 2000). The rats were housed in 12h:12h light-dark cycles, sacrificed 30-60 min before lights off and the SCN, liver, lung and skeletal muscle explants cultured under static conditions in constant darkness and temperature. Light output was measured continuously from individual cultures with a Hamamatsu photomultiplier tube detector assembly (Geusz et al.,

1997). Light emission from the SCN was robustly rhythmic, implying that the engineered mouse *PER1*-luc transgene was rhythmically transcribed under the regulation of normal circadian mechanisms. In contrast, circadian rhythms of light output in the liver, lung and skeletal muscle phase lagged the SCN rhythm by 7-11h. This suggests that the SCN may be able to adjust quickly compared to peripheral clocks following a phase shift similar to that, which occurs following time zone travel.

Several behavioural and pharmacological methods such as timed light exposure or avoidance, napping, nutrition, caffeine containing products, exogenous melatonin and physical exercise can be employed to accelerate adaptation of physiological and neuroendocrine systems following desynchronisation (Forbes-Robertson et al., 2012, Wright et al., 2013, LeGates et al., 2014, Carneiro and Araujo, 2012, Edwards et al., 2000, Schroder and Esser, 2013, Beaumont et al., 2004). Important to note is that there is considerable inter- and intra-individual variation in the way individuals respond to these interventions making it difficult to provide general therapeutic advice (Waterhouse and Reilly, 2009). For the purposes of this thesis, I will focus on light exposure or avoidance, exogenous melatonin and exercise strategies, for interventions that can be employed to accelerate adaptation to the new environment.

1.6.3.1 Light exposure and melatonin

Ocular light exposure at an appropriate time-of-day has been shown to be the most important entraining stimulus of the circadian system (Boivin and James, 2002, Gronfier et al., 2007, Forbes-Robertson et al., 2012). The reason for light exposure is to help not only the SCN resynchronisation, but also melatonin levels, which in turn can help peripheral clocks to reset. Appropriately-timed light exposure can therefore be used to accelerate resynchronisation of the melatonin rhythm so that sleep can be initiated more easily. Light exposure induces a range of circadian, neuroendocrine and neurobehavioural responses (Burgess et al., 2002, Lockley et al., 2006). Timed exposure, dosing pattern, duration and wavelength are all important in determining the extent of the response and should be considered when phase shifting one's circadian rhythms (section 1.2.3). For example, in one study, participants were exposed to either 3h of bright light

(3 000 lux) or red dim light (10 lux) at 19h00 local time for two consecutive evenings following westward travel across six time zones (Boulos et al., 2002). A 1h phase delay in salivary DLMO - a marker of innate circadian rhythm was noted in the bright light treated group of participants. In another study, three groups of participants underwent a 1h phase advance for three days in a simulated jet-lag experiment (Burgess et al., 2003). Specifically, group one was exposed to 3.5h of bright light (>3 000 lux), group two to intermittent bright light (alternating 0.5h on and off, >3 000 lux) and a control group which was exposed to ordinary dim indoor light (about 60 lux). Following light treatment, DLMO was phase advanced by about 2.1h in the group treated with continuous bright light, 1.5h in the group with intermittent light treatment and 0.6h in the control group.

Light avoidance techniques can also be used to effect the phase of adjustment required (Crowley et al., 2003, Eastman et al., 1994). Following eastward travel a circadian phase advance is required, while a phase delay is required following westward travel, as such time of light avoidance may vary depending on the direction of travel (Duffy et al., 1998, Waterhouse et al., 2007). For example, following westward travel circadian rhythms require a phase delay, as such light exposure may be avoided between 02h00-08h00 departure time within the first 24h upon arrival in the new time zone in order to realise a phase delay. Daan and Lewy (1984) suggested avoiding bright light in the early hours of the morning following long westward travel, and in the first few hours of the evening following eastward travel. They suggested wearing low transmittance sunglasses or goggles if going out during these hours to reduce exposure. For example, Eastman et al. (1994) demonstrated circadian re-alignment in nighttime shift workers who wore dark goggles when going home in the morning. Wearing goggles at appropriate times during a long-haul flight across time zones is a useful strategy to avoid light at the new “end of day” in order to promote melatonin onset.

Exogenous melatonin administration can have both a hypnotic effect as well as a chronobiotic effect if administered at the appropriate time-of-day (Turek and Gillette, 2004, Pandi-Perumal et al., 2008). Administration in the early evening before sunset produces a phase advance, while

administration in the early hours of the morning produces a phase delay effect (Beaumont et al., 2004). Furthermore, Manfredini et al. (2000) reported that melatonin administration in the afternoon resulted in a phase advance following eastward travel across eight time zones in eight males who had a delayed core body temperature acrophase. The differences in timing of melatonin administration as well as differences in an individual's endocrinological patterns complicate the use of external melatonin in travelling people. This complicates the use of melatonin as a strategy for re-entrainment hence; its use should be treated with caution. While external melatonin has been shown to be useful in mild jet-lag under laboratory conditions (Paul et al., 2011, Manfredini et al., 2000), other studies have failed to show its efficacy following actual travel across twelve time zones (Richmond et al., 2004, Edwards et al., 2000).

1.6.3.2 Exercise

While some studies have reported no effect of phase shift following exercise (Moog and Hildebrant, 1987), there is consensus that appropriately timed physical activity can effect a phase shift (Brand et al., 2010, Youngstedt et al., 2006, Yamanaka et al., 2010). Appropriately-timed exercise may be performed to obtain the desired direction of circadian phase shift; the timing depends on whether a circadian phase advance or delay is required. Specifically exercising in the morning in the new time zone produces a phase advance and at night a phase delay (Buxton et al., 2003, Yamanaka et al., 2010). Exercise at night would be beneficial following westward travel in order to phase delay the circadian clock, while exercise in the morning would be beneficial after eastward travel where a phase advance would be required.

Timed-exercise has been used as a stimulus to accelerate entrainment to the new sleep-wake cycle of healthy young adults in simulated nighttime shift work and constant routine studies (Eastman et al., 1995, Miyazaki et al., 2001, Yamanaka et al., 2010, Vanreeth et al., 1994). Specifically, in two of these studies exercise at night accelerated melatonin phase delays (Eastman et al., 1995, Vanreeth et al., 1994), while daytime exercise accelerated melatonin phase advances (Miyazaki et al., 2001). Klein and Wegmann (1974) reported an accelerated resynchronisation of the rectal temperature rhythm and psychomotor performance in a group of

eight students who were allowed to exercise compared to the group that was not allowed to exercise following westward travel across six time zones. In another study, Shiota et al. (1996) reported that outdoor exercise increased resynchronisation of the 17-hydroxy-corticosteriods rhythm to the new time zone. While the above-mentioned studies indicate that exercise was effective in facilitating readjustment into the new environment, it must be acknowledged that a great number of time cues (e.g. light) from the environment may have played a role since exercise took place outside the experimental facilities.

The intervening effect of light exposure and social cues might explain the accelerated rectal temperature phase delay following westward travel in the exercise group only. In addition, the people who exercised reported higher levels of fatigue (Klein and Wegmann, 1974), a finding which the authors did not address. The obvious importance of controlling for light exposure, in addition to accounting for an individual's prior fitness and level of habitual physical activity (Eastman et al., 1995) must be accounted for when using exercise to phase shift circadian rhythms. It is however difficult to control activity and other confounding time cues in the new time zone in an effort to accelerate adaptation, which then complicates the use of exercise as a strategy to facilitate re-entrainment.

1.6.4 Circadian disruption and trans-meridian travel

Trans-meridian travel is travel east-to-west or west-to-east, i.e. across meridians and not in the north-to-south or south-to-north direction. This travel is more common in today's society where air travel is accessible to large portions of the population. The misalignment that occurs following trans-meridian travel arises as a function of the disparity between timing of one's internal body rhythms and external environmental time in the new time zone, subsequently leading to symptoms of jet-lag (Atkinson et al., 2014, Samuels, 2012, Petit et al., 2014, Sack, 2009). In particular, jet-lag symptoms are thought to result from various physiological and psychological systems' inability to resynchronise swiftly to sudden changes of time in the immediate environment. These symptoms include impaired concentration, decreased vigour and energy, difficulties initiating and maintaining sleep at night, elevated daytime sleepiness, lack of

situational awareness, distorted estimation of time, space and distance and impairment of the immune system (Leatherwood and Dragoo, 2013, Waterhouse and Reilly, 2009, Samuels, 2012, Manfredini et al., 1998, Youngstedt and O'Connor, 1999, Eastman and Burgess, 2009, Reilly et al., 2005). These symptoms are temporary and improve after a few days in the new time zone.

Jet-lag symptoms are generally experienced after the first 24h in the new time zone until entrainment is realised (Atkinson et al., 2014). During the first 24h, it is difficult to separate effects of jet-lag from fatigue resulting from the long-haul flights. Jet-lag symptoms make it difficult to sustain a normal daily routine and are exacerbated by crossing time zones in quick succession (Sasaki et al., 1993). For example, deterioration of leg and grip strength as well as reaction time rhythms were reported in 17 individuals following a 9h intercontinental flight between the United Kingdom and Florida (Reilly et al., 2001). The abovementioned variables were in phase with the circadian rhythm of oral temperature and gradually improved such that performance of these variables had stabilised 5-7 days post flight. This indicates that trans-meridian travel disrupts circadian rhythms, which in turn influence performance variables. Circadian disruption also influences an individual's daily sleep.

It has been reported that prolonged wakefulness by as little as three hours in a single night similar to that which may occur following trans-meridian travel can produce performance decrements (Arnedt et al., 2001). Likewise, the effects of one to two hours of nightly sleep loss over a week have been reported to result in decrements in daytime function which lead to human error and accidents (Powell et al., 2001, Mitler et al., 1988).

1.6.4.1 Circadian disruption and the sleep-wake cycle

The sleep-wake cycle is disrupted because of the desynchronisation between internal and external rhythms following a change in time zones. For example, eastward travel from South Africa to New Zealand crossing eleven time zones may result in difficulties initiating and maintaining sleep for sufficient periods because the circadian rhythm is phase advanced. Thus, visitors are likely to experience a lower homeostatic drive for sleep at night as the sleep episode

occurs in their biological afternoon (home time); they are required to shorten their innate circadian rhythm to match the new environmental time zone time. Figure 1.9 illustrates how sleep is affected by circadian misalignment following trans-meridian travel.

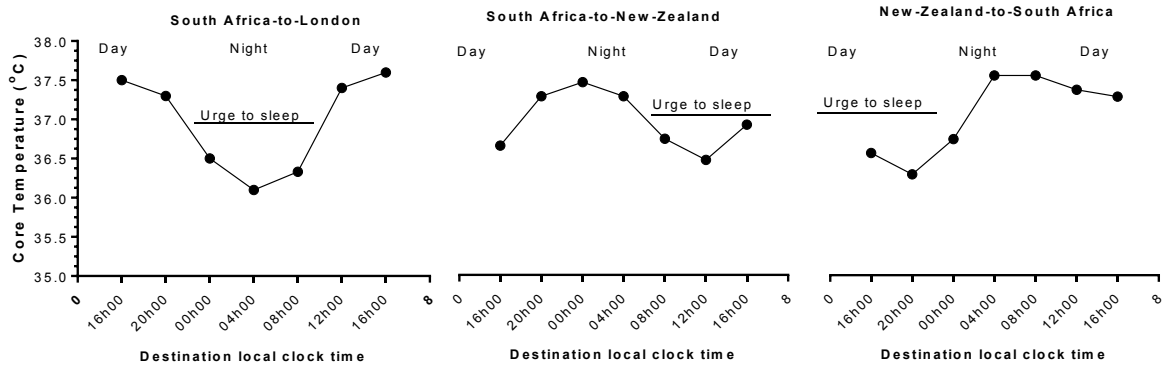


Figure 1.9: Diagrammatic representation of the 24h rhythm of core (rectal) temperature in an individual who normally sleeps from 23h00-07h00. The "urge to sleep" represents bedtime for the individual, coinciding with a drop in core body temperature. In the left hand panel, the urge to sleep coincides with local nighttime at the destination, whereas in the central and right-hand panels, the urge to sleep coincides with daylight hours at the destinations. Adapted from Waterhouse et al. (1997).

Furthermore, following trans-meridian travel the melatonin rhythm is disrupted subsequently affecting melatonin levels, as such individuals may find it difficult to initiate sleep in the new time zone. Poor sleep quality would therefore exacerbate the effects of jet-lag and negatively affect an individual's performance. In one study, sleep was assessed using polysomnography for 9 nights in 27 healthy volunteers following eastward travel across seven time zones (Beaumont et al., 2004). A longer sleep onset latency was noted in the first three nights in the new time zone, while less REM sleep than normal was noted only for the first four nights. This only corrected to normal proportions after five nights. Another study reported altered sleep architecture in 15 participants following both simulated eastward and westward travel across eight time zones after a week in the new environment (Hume, 1980). Specifically, Hume (1980) reported that participants spent more time in REM sleep following simulated westward compared to eastward

travel. Carrier et al. (1996) observed a reduction in sleep efficiency, duration, total sleep time, sleep onset latency and an increase in the number of awakenings in the first part of the sleep episode in 25 healthy individuals after a simulated 6h phase advance.

The majority of research performed using subjective measures of sleep patterns has been focused on sleep disruption and/or recovery following trans-meridian travel in business people (Edwards et al., 2000), the military (Beaumont et al., 2004), sedentary individuals (Jamieson et al., 2001), academics (Takahashi et al., 2002, Edwards et al., 2000), airline employees (Lowden and Akerstedt, 1999, Suvanto et al., 1993) and athletes (Lemmer et al., 2002, Hill et al., 1993, O'Connor et al., 1991). The consensus from all these studies is that sleep disruption as a consequent of trans-meridian travel is a concern to all travelling individuals. However there is great inter-individual variability in sleep response and thus there is a need to understand what makes an individual's response worse/better.

1.6.4.2 Circadian disruption and the immune system

Most immune cells express circadian clock genes, which exhibit a 24h rhythm. This has an effect on the 24h rhythm of immune functions including phagocytosis, synthesis and release of cytokines and chemokines as well as trafficking to inflamed or infected tissues (Labrecque and Cermakian, 2015). Therefore, circadian disruption either due to inconsistent sleep-wake schedules, nighttime shift work or trans-meridian travel has significant consequences for human health (Labrecque and Cermakian, 2015, Knutson et al., 2007, Scheiermann et al., 2013). This is conceivable given that many functions and parameters key to immune functioning such as natural killer cell activity (Arjona and Sarkar, 2005), humoral immune response (Keller et al., 2009), cytokine levels (Keller et al., 2009), serum cortisol (Fatima et al., 2013) and rhythms of absolute and relative numbers of circulating white blood cells (Bridges et al., 1992) are time-of-day dependent.

Desynchrony of internal body rhythms and external time has been reported to result in several behavioural, circuitry, endocrine/metabolic and immune disorders under laboratory conditions

(Lechner et al., 2000, Shearer et al., 2001, Dinges et al., 1994). For example, Spiegel et al. (2002) reported a 50% decrease in antibody titter 10 days after immunisation with the influenza vaccine in individuals who had been subjected to 4h nightly sleep restriction for 6 days prior to immunisation compared to individuals who followed usual sleep durations. However, antibody levels were shown to be similar between the two groups 3-4 weeks post-immunisation, when synchronisation to the local environment had occurred. This indicates that sleep restriction and circadian disruption such as that which occurs after time zone transitions alters the immune response. Balachandran et al. (2002) reported an attenuation of the febrile response to *E. Coli* endotoxin challenge in individuals who had 4h nightly sleep for 10 days compared to individuals who had 8h nightly sleep for the same period. This suggests that misalignment between the internal body clock and external time may reduce an individual's immune response to antigens presented in the new time zone.

Under normal sleep-wake cycles immune cells belonging to the innate (specific e.g. natural killer cells) or adaptive (antigen-specific e.g. B-lymphocytes) immune systems show robust peaks at different times of the day, with some (e.g. naive Th-cells) peaking at night. Therefore, circadian disruption that ensues following trans-meridian travel, for example, may negatively affect cells responsible for antigen recognition leaving an individual vulnerable to pathogen attack in the new time zone. The extent to which each individual's circadian rhythms are disrupted after time zone travel determines the degree to which the immune system is compromised. Therefore, it will be important to find out which individuals are more prone to circadian disruption, and thus more susceptible to illnesses.

1.6.4.3 Circadian disruption and injury

Intercontinental travel can lead to a disrupted circadian rhythm, whose consequences may result in impaired cognitive function, mood and loss of motivation; subsequently increasing the risk of injury (Samuels, 2012). A recent study, reported that baseball teams whose circadian rhythms were synchronised to optimal performance times stood a better chance of succeeding in a competition, suggesting detrimental effects of intercontinental travel and/or desynchronised

circadian rhythm (Winter et al., 2009). To date several studies exist on the epidemiology of professional rugby injuries in which matches are played when circadian rhythms are synchronised to optimal performance times (Jakoet and Noakes, 1998, Fuller et al., 2010, Brooks et al., 2005, Targett, 1998, Quarrie and Hopkins, 2008). While these studies are important for educating coaches and athletes, they do not attempt to describe the effect of intercontinental travel on the risk of injury in rugby union players. Only one group- The Clinical Sports Medicine Research group at the University of Cape Town has conducted a study investigating the 2012 competition injuries in Super Rugby athletes (Schwellnus et al., 2014). Of concern was that while New-Zealand and Australian teams were able to field a full strength squad in weeks 13-18 of the 2012 Super Rugby tournament, most South African teams were hit by injuries and could not field a full strength squad. As a result, teams had to make do with players at their disposals at the time.

It is possible that circadian disruption following time zone travel (Forbes-Robertson et al., 2012, Youngstedt and O'Connor, 1999, Leatherwood and Dragoo, 2013), which places a high physical and psychological demand on the travelling athlete, may be one the reasons why there are more injuries in the SA Super Rugby athletes. This is conceivable given that match injuries in the Super Rugby tournament where players have to contend with demanding travelling schedules is higher (Schwellnus et al., 2012) than in other tournaments that are played in one geographical zone (Jakoet and Noakes, 1998, Hagglund et al., 2009, Parkkari et al., 2004, Vanmechelen, 1992). Much of what is known about the effect of circadian disruption on injuries has been extrapolated from studies in occupational settings and indicates an increased risk of accidents or errors following both total and partial sleep deprivation (Smith et al., 1994, Akerstedt, 1998, Folkard et al., 2005). Other inferences on the subject of injury risk have been made using known knowledge regarding circadian disruption of muscle activity, core temperature and cognition (Shrier and Gossal, 2000, Capezuti et al., 1998, Howland et al., 2007).

Circadian disruption under competition conditions may have a more profound effect on functioning than circadian disruption under simulated jet-lag conditions in a laboratory. Factors such as mood, psychomotor and cognitive function that influence performance have been

reported to deteriorate much faster than physical capabilities following circadian disruption in military personnel and volunteers in laboratory studies (Dinges, 1995, Rosa, 1995). Such deterioration can adversely influence the interaction between a player's individual motivation and emotional response to a situation. For example, changes in mood state were shown to result in a significant proportion of injuries in an American College basketball team (Lavallee and Flint, 1996). Cumulative influences of physical and psychological stressors, which occur following circadian disruption (Dijk and Czeisler, 1994, Buxton et al., 2012, Burch et al., 2009) deplete perceptual and sensor motor reserves potentially exacerbating the risk of injury (Vandekerckhove and Cluydts, 2010). This is conceivable given that the capacity to respond appropriately to acute superimposed physical and psychological stressors is limited when allostatic load (stressor) is high. Allostatic load is the deviation from homeostatic levels between the sympathoadrenal, hypothalamic-pituitary adrenal mediator and hormone secretion (Evans et al., 2007, Vandekerckhove and Cluydts, 2010).

This is supported by findings from Illhan et al. (2006), who reported a higher (75.8%) incidence of injuries in nurses working rotating nighttime and daytime shifts compared to nurses working either daytime- or nighttime-shifts (50%). In this study, rotating shift work was associated with a 1.7 fold increased risk of sustaining a needle stick injury and a 2.7 fold increased risk that an injury was from a contaminated device. Another study reported significantly more injuries (60 injuries per hour per 1000 individuals) in a group of 296 personnel working nighttime shifts compared to a group of 1 102 personnel (40 injuries per hour per 1000 individuals) working daytime shifts (Park et al., 2001). In the same study, a 1.5 fold increased risk of injury was noted in the group working nighttime shifts (18h00-05h59) compared to the group working daytime shifts (06h00-17h59). A decrease in vigilance and performance as well as an increase in the frequency of injuries has been reported when exercise was performed at night or in the early morning (Winget et al., 1985, Horne and Reyner, 1999). As such, there is a high possibility that circadian disruption- the mismatch between internal body time and external time in the new environment may increase the risk of injury in players.

It is plausible to assume that athletes who undergo frequent time zone travel will have a higher compound allostatic load and even more diminished threshold for mounting situational appropriate responses as a consequent of circadian disruption. This is conceivable given that athletes in a competition such as the Super Rugby do not have adequate lead-time to recover completely from jet-lag effects before match kick-off. As a result, failure to recover from jet-lag effects may decrease the reserves for emergence response to situations during training sessions or matches.

1.6.5 Severity of circadian disruption following trans-meridian travel

The severity of circadian disruption following trans-meridian travel is influenced by several factors chief among them being age, sex, chronotype, individual variability, direction of travel, number of time zones crossed, rhythm amplitude, strength of local zeitgeber upon arrival and physiological systems being measured (Waterhouse and Reilly, 2009, Vosko et al., 2010, Waterhouse et al., 2007, Moline et al., 1992).

1.6.5.1 Chronotype

With respect to chronotype differences, individuals preferring evenings have been shown to be less vulnerable to the effects of jet-lag compared to those preferring mornings for both directions of travel (Manfredini et al., 1998, Winget et al., 1985). In particular, individuals with low amplitudes (ETs) in core body temperature appear to be less affected by a phase shift than those with large circadian amplitudes (MTs) (Winget et al., 1984). For example, morning-type individuals were reported to have longer resynchronisation periods and rigid sleep-wake cycles in comparison to their evening-type counterparts following simulated eastward travel (Suvanto et al., 1993). The circadian rhythm of evening-types was reported to take a shorter resynchronisation period following a simulated phase delay, further suggesting that evening-type individuals may find it easier to adapt following trans-meridian travel, particularly after westward travel (Winget et al., 1985).

1.6.5.2 Light exposure

An individual's ability to synchronise in to the new time zone also depends on the strength of *zeitgeber* upon arrival, for example, light exposure. The amount of daylight in the natural environment is a strong predictor of how fast adaptation to the new environment will take following trans-meridian travel (Suvanto et al., 1993, Duffy et al., 1996). It has been reported that re-adjustment to the new time zone is faster during summer than during winter, as a consequent of varying exposure to natural day light (Harma et al., 1994). Specifically, Harma et al. (1994) measured salivary melatonin and cortisol levels five days prior to, during and after a four day round-trip from Helsinki-to-Los Angeles-to-Seattle-to-Helsinki during summer and winter.

Melatonin and cortisol circadian rhythms adaption was faster after both eastward and westward travel in the group that travelled in summer compared to the group that travelled in winter. In summer, there is more environmental light during both morning and evenings. Therefore, late sleeping times increased exposure to phase delaying evening light; following westward travel, while early morning light exposure resulted in a phase advance following eastward travel. In another study, physiological processes were reported to resynchronise at a slower rate in participants who had limited exposure to outdoor light compared to those who were exposed to natural light following eastward travel across six time zones between Germany and the United States (Klein et al., 1972). Bright light exposure has also been shown to successfully speed up resynchronisation during recovery days following nighttime shift work (Ahasan et al., 2001). The above-mentioned studies indicate that the degree of phase shifting will differ depending on light intensity, duration and exposure time of day, the degree of phase shifting may vary from one individual to the other depending on chronotype.

1.6.5.3 Number of time zones crossed and direction of travel

The rate of synchronisation to the new time zone following trans-meridian travel is associated with the number of time zones crossed (Reilly et al., 2005, Haimov and Arendt, 1999). Alterations in physiological function become more evident following three or more time zone crosses (Reilly and Edwards, 2007). For example, Suvanto and Ilmarinen (1989) reported a worse response in

terms of sleep quality, adaptation and days required for recovery following travel across ten time zones compared to travel across seven time zones. In addition to the number of time zones crossed, direction of travel also influences the extent of desynchronisation and severity of jet-lag symptoms. In general, adaptation following trans-meridian travel follows an orthodromic response, whereby physiological systems adapt in the same direction as the phase of adjustment (Edwards et al., 2000). Thus, adaptation to the new time zone following eastward or westward travel requires a phase advance or phase delay, respectively (Fowler et al., 2015b, Waterhouse et al., 2007, Drust et al., 2005). However, when the number of time zones crossed approach near the limit of entrainment (10-12 time zones); physiological systems synchronise in the opposite direction of the phase shift (Takahashi et al., 2002, Reilly et al., 2005). As a general rule, the rate of re-adjustment is faster following westward travel irrespective of time-of-day of departure and whether it is an outbound or return flight (Takahashi et al., 2002, Chapman et al., 2012, Sack, 2010, Reilly et al., 2005).

Klein and Wegmann (1980) reported that the re-entrainment rate is slower when travelling eastward (56min/day) than when travelling westward (88min/day). This difference in re-adjustment shift rate between eastward and westward travel is influenced by the internal body clock (O'Connor and Morgan, 1990, Winget et al., 1984, Waterhouse et al., 2007, Klein and Wegmann, 1980). For example, Lemmer et al. (2002) reported that rhythmic patterns in body temperature and grip strength were greatly disturbed on day one in 15 elite athletes following eastward travel compared to westward travel across eight time zones. In the same study, jet-lag symptoms were shown to persist between 1-2 days longer in eastbound than westbound athletes.

Adaptation following eastward travel requires a phase advance, resulting in a circadian period length shorter than 24h (Waterhouse et al., 2007, Burgess et al., 2003, Eastman and Burgess, 2009). After eastward travel, the environmental clock is effectively brought forward, resulting in a shorter day such that the homeostatic drive for sleep at socially acceptable bedtime in the new time zone is not high enough yet to initiate sleep (Chapman et al., 2012, Meir, 2002, Eastman et

al., 2005). On the other hand, the day is lengthened following westward travel. Bedtime in the new time zone is latter than usual such that the homeostatic sleep drive is very high and thus promoting sleep. Resynchronisation requires a phase delay, resulting in a period length longer than 24h.

Since circadian rhythms in humans tend to have a natural period length slightly longer than 24h under free-running conditions (Czeisler et al., 1999, Aschoff, 1965, Roenneberg and Merrow, 2007), it is thought that adaptation of rhythms is better suited to a phase delay than a phase advance. Compounding the direction of travel effect is the day and night relationships. In particular, most westward flights are usually scheduled during daytime, while eastward flights are scheduled during nighttime (Samel et al., 1995). Travelling at night has been reported to result in significant sleep loss and disruption compared to travelling during the day (Samel et al., 1997, Gander et al., 1998). For example, Preston and colleagues (1973) illustrated that sleep loss was associated with number of night flights rather than number of time zones crossed in twenty-four flight attendants following trans-meridian travel.

In addition to the above-mentioned variables, the intensity and extent of jet-lag symptoms exhibit a relationship with other variables such as time of arrival, activity in the first 24h upon arrival, the ability to nap while travelling and the availability of external time cues upon arrival (Reilly and Edwards, 2007). In practice, more than one of the abovementioned variables can act as a significant predictor of jet-lag. There is a huge inter-individual variability and different predictors may be more important at different stages of the process of adjustment. Effects related to the flights themselves such as fatigue would be most pronounced in the first 24h in the new environment, while those associated with the direction of phase of adjustment may become more apparent after a few days.

Differences in the way individuals adapt to a phase shift has been previously demonstrated in simulated jet-lag and nighttime shift work studies (Hida et al., 2014, Maire et al., 2014, Chellappa et al., 2012b, Wirth et al., 2013, Gamble et al., 2011). The above-mentioned studies indicate that

there is great inter-individual variability in adaptation response after time zone travel, however, none of these studies investigated an individual's adaptation response based on his/her clock gene genotype. Thus, the severity of jet-lag following time zone travel is not just a combination of the number of time zones crossed and/or direction of travel, but an individual's genetics, for example, may also warrant consideration.

1.6.5.4 Effect of trans-meridian travel on different physiological systems

Following trans-meridian travel, the circadian rhythm of various systems has been reported to become desynchronised. Complicating this is the fact that various physiological systems including sleep-wake patterns, body temperature and the cognitive system re-adjust at a different pace following perturbation**,** for example, after time zone travel (Beaumont et al., 2004, Buxton et al., 2003). The circadian rhythm of core body temperature (Edwards et al., 2000, Beaumont et al., 2004), hormones (Lemmer et al., 2002, Bullock et al., 2007), gastro-intestinal function (Waterhouse et al., 2005a) and sleep-wake patterns (Edwards et al., 2000, Beaumont et al., 2004, Takahashi et al., 2002) have all been shown to desynchronise.

The rate of resynchronisation varies between physiological systems, with some systems synchronising more rapidly than others (Winget et al., 1984). For example, following a 9h simulated phase advance, core body temperature rhythm was reported to take only two days to return to baseline, while sleep patterns, mood and performance took five days (Deacon and Arendt, 1996). Similarly, core body temperature and urinary excretions (i.e. sodium and chloride) were reported to re-adjust more rapidly than plasma steroids and urinary potassium in 23 participants following a 6-8h simulated phase advance (Elliott et al., 1972). In another study, salivary cortisol and melatonin were shown to resynchronise faster after westward than eastward travel across ten time zones in 35 female flight attendants (Harma et al., 1994). Furthermore, Klein and colleagues (1972) demonstrated that resynchronisation of the core body temperature rhythm occurred after eight days following eastward travel and only after five days following westward travel. The abovementioned studies demonstrated a group effect (i.e. overall outcome in a cohort) with respect to physiology and jet-lag symptoms; however, accounting for

each individual's response and susceptibility to trans-meridian travel will add more value to jet-lag studies following time zone transitions.

1.6.6 Trans-meridian travel and sports performance

The influence of circadian rhythms in athletic performance has long been acknowledged in sport (Youngstedt and O'Connor, 1999, Atkinson and Reilly, 1996, Winget et al., 1985). Disruption of these rhythms would undoubtedly influence athletic performance negatively. It is very difficult to measure individual performance, therefore studies investigating the effects of jet-lag on performance have used proxies "for performance", such as heart rate, aerobic endurance and capacity, muscle strength and flexibility or team match outcome (Hill et al., 1993, Atkinson and Reilly, 1996, Drust et al., 2005). Circadian misalignment from trans-meridian travel may influence circadian variation and peak time for optimal efficiency of several markers of physical performance. All the studies that have investigated the impact of trans-meridian travel on both specific and non-specific sport indicators of performance are listed in Table 1.2.

While some studies have reported a decrease in markers of performance (Wright et al., 1983, O'Connor and Morgan, 1990, Hill et al., 1993, Recht et al., 1995, Jehue et al., 1993), others have found no effect (Hill et al., 1993, Bullock et al., 2007, Beaumont et al., 2004, Fowler et al., 2015b, Bishop, 2004, Chapman et al., 2012). The available literature on athletic performance following trans-meridian travel is confounding and inconclusive. More importantly, most of these studies investigated non-sport specific indicators of athletic performance following time zone travel. Possible reasons for the lack of consensus include the lack of performance assessment prior to trans-meridian travel in some of the studies (O'Connor and Morgan, 1990). Furthermore, the disparity in the results may have arisen as a consequent of different participant types, aspect of performance being investigated, sex, number of time zones crossed and direction of travel. Likewise, the time-of-day that these aspects were investigated varies in different studies, with some studies conducted in the morning while others were conducted in the evening (Smith et al., 1997).

Table 1.2: Effects of time zone travel on markers of physical performance and match outcomes

Direction of travel	TZ crossed	Performance measure	Outcome	Participant group	Reference
Westward	11	Strength	↔muscle strength ↔motion	Rugby players	(Fowler et al., 2015a)
Westward	8	Squat jump	↓squat jump ↔Counter movement jump	Elite athletes	(Chapman et al., 2012)
Eastward	8	Sprint, jump	↔30 m sprint ↓Jump performance	Elite athletes	(Bullock et al., 2007)
Eastward	7	Grip strength	↓Grip strength	US air force	(Lagarde et al., 2001)
Eastward	6	Grip strength, aerobic power	↔Grip strength, ↓Anaerobic power	Students	(Hill et al., 1993)
Eastward	6	Sprint, muscular strength	↓Distance running and sprinting, muscular strength	Untrained individuals	(O'Connor and Morgan, 1990)
Eastward	6	Arm strength, sprint, perceived exertion	↓Dynamic arm strength, 270 m sprint, 2.8 km run and RPE	Military	(Wright et al., 1983)
Westward	5	Grip strength, reaction time	↓ grip strength, simple choice reaction time	Elite athletes	(Reilly et al., 2001)
Eastward/ Westward	4	Heart rate, muscle soreness	W-E travel ↑Heart rate E-W & W-E travel ↓Muscle soreness	Elite swimmers	O'Connor et al., 1991
Eastward/ Westward	2	Match win or loss	W-E travel ↓Performance	NFL athletes	(Jehue et al., 1993)
Eastward/ Westward	2	Match win or loss	E-W travel ↔Performance	ANNC athletes	(Bishop, 2004)
Eastward/ Westward	1-3	Match win or loss	W-E travel↑Performance	MLB athletes	(Winter et al., 2009)
Eastward/ Westward	1-2	Match win or loss	W-E travel ↑Performance E-W travel ↓Performance	NBA athletes	(Steenland and Deddens, 1997)
Eastward/ Westward	1-2	Match win or loss	W-E travel ↑Performance E-W travel ↓Performance	NFL athletes	(Smith et al., 1997)
Eastward/ Westward	1-2	Match win or loss	W-E travel ↓Performance	American baseball	(Recht et al., 1995)
Eastward/ Westward	1	Match win or loss	W-E travel ↓Performance	NCAA athletes	(Worthen and Wade, 1999)

↓, ↔ ↑ represent a decrease, no change or increase respectively in measured variables. HR- heart rate; VO_2max- maximum oxygen uptake; RPE- rate of perceived exertion; ANNC- Australian National Netball Competition; MLB- Major League Baseball; NBA- National Basketball Association; NCAA- National Collegiate Athletic Association; NFL- National Football League; E-W travel- Westward travel; W-E travel- Eastward travel and TZ- Time zones.

In addition, some studies, which investigated the impact of time zone travel on performance, used untrained individuals and student populations (Hill et al., 1993, O'Connor and Morgan, 1990). Physical performance and physiological systems of non-athlete populations differ to those of athletes, given that athletes are trained and thus their physiological systems have adapted and may therefore respond differently after trans-meridian travel. For example, repetitive testing rather than trans-meridian travel may have decreased performance in a healthy non-athletic population where increased fatigue and muscle soreness were noted after trans-meridian travel (O'Connor and Morgan, 1990). Studies on non-athletes measuring performance following trans-meridian travel are flawed for this reason. Therefore, while findings from non-athletic populations (O'Connor and Morgan, 1990, Hill et al., 1993) shed some light on the impact of trans-meridian travel on performance, it is important not to generalise these findings as they may differ in trained populations during actual competitions.

With regards to direction of travel, Jehue et al. (1993) and Steenlands & Deddens (1997) noted significant reductions in points scored in professional baseball and Australian teams after eastward compared to westward travel. In contrast, Smith et al. (1997) reported that West Coast American National Football League teams undergoing eastward travel were more likely to win a game than East Coast teams undergoing westward travel. In this study, West Coast teams won 63.5% of their matches following eastward travel compared to the 36.5% of East Coast teams following westward travel. Smith and colleagues (1997) suggested that the 21h00 match kick-off time, which was closer to the time that West Coast teams trained may have enhanced their athletic performance. Likewise, the night matches possibly eliminated the beneficial effects of home-field advantage for East Coast teams. This complicates the interpretation of direction of travel effect (i.e. extent of circadian rhythm disruption) on both an individual's performance and the overall outcome of the competition.

The above-mentioned studies do not however indicate direct evidence that the reduction in the performance indicators was due to individual differences in coping with the effects of jet-lag, since team measures (win/loss) of performance were used. Of the studies that have actually

measured match outcome performance, the problems encountered include individual *vs* team performance, strength of the opposition, match location, environmental conditions as well as the time-of-day the match took place (Taylor et al., 2008, Atkinson and Reilly, 1996, Thomas et al., 2008, Smith et al., 1997). Overall, these confounders make it difficult to know what the true effect of trans-meridian travel on performance at an individual level is.

1. Team versus individual performance- Team performance does not reflect each individual's contribution on the pitch. Some individuals may adapt faster and perform better; while others may take a bit longer to adapt resulting in performance decrements, however, this is not reflected in the global match outcome.
2. Opponents- Opposing team's performance is not accounted for. For example, the visiting team may encounter a stronger and better skilled opposition. Thus, poor team performance may be attributed to a superior opponent; rather than effects of jet-lag from trans-meridian travel.
3. Number of time zones crossed- The effects of jet-lag become obvious following three or more time zone crosses (Waterhouse et al., 2007). This partly explains the discrepancies in athletic performance following a single time zone transition (Steenland and Deddens, 1997) compared to two time zones (Jehue et al., 1993, Recht et al., 1995), specifically failure to show the linkage between poor performance and jet-lag.
4. Match status- Tactics employed and physical aspects of performance have been extensively explored (Bloomfield et al., 2005, Taylor et al., 2010). A team is likely to register an improved match performance in, for example, the semi-final match compared to a mid-season match after travelling across the same number of time zones and in the same direction.
5. Match location- Factors contributing to match location have been well documented in literature and include crowd behaviour, venue familiarity, travel fatigue and referee bias (Tenga et al., 2010, Taylor et al., 2008). For example, Recht et al. (1995) reported that teams had more victories when playing at home (54%) compared to away matches (46%) in 19 North American NFL teams.

While the information gathered on team performance is helpful, it is highly confounded and thus difficult to interpret the effects of trans-meridian travel on performance. Therefore, studies are needed in which individual performance is assessed instead.

1.7 Summary and conclusions

Trans-meridian travel across multiple time zones results in the disruption of the normal 24h rhythms and social schedules (Waterhouse et al., 2007, Roenneberg et al., 2013). In the context of sports performance, circadian rhythm disruption may influence an individual's immune function, physical performance, and injury risk (Waterhouse and Reilly, 2009, Samuels, 2012). This has potential implications on both an individual's training efficacy, and competition performance. Furthermore, circadian disruption is a major concern not only for the travelling athlete, but also for team coaches who have to make decisions on player combinations and replacements when certain players are not fit to play or become ill following trans-meridian travel.

In addition, the circadian clock adapts slowly to new time cues following trans-meridian travel. Specifically the phase relationship between circadian rhythms and external time cues is out of synchronisation for a number of days (LeGates et al., 2014, Waterhouse et al., 2005b). Equally important, adaptation following time zone travel varies from 4-12 days in those individuals who resynchronise with difficulty as well as for various physiological variables (Desir et al., 1981, Harma et al., 1994, Klein and Wegman, 1980). This suggests that while the circadian clock is slow to adapt to time cues in the new environment, different rates of resynchronisation for a host of physiological and behavioural measures persist until the correct phase relationship between internal body rhythms and external time cues is realised (Deacon and Arendt, 1996, Waterhouse et al., 2002).

Resynchronisation to a new time zone depends on several factors chief among them being an individual's chronotype, number of time zones crossed as well as the direction of travel. This affects the duration of circadian disruption potentially exacerbating jet-lag symptoms (Winget et

al., 1985, Eastman and Burgess, 2009, Vosko et al., 2010). These symptoms are a significant factor for nearly two thirds of athletes and negatively influence physical performance in matches played before circadian rhythms have resynchronised in to the new time zone. While, no individual is immune to the effects of jet-lag, the extent to which each individual responds to circadian disruption determines how quickly they will be able to resynchronise into the new environment.

Rhythm disruption of cognitive and neuromuscular functions including, reaction time, decision making and alertness may result in impaired physical performance and subsequently injuries. For example, lack of situational awareness and decision-making may result in a rugby player holding onto the ball longer than necessary, which increases the chances of him being tackled. Specifically, a significant number of injuries in rugby have been shown to occur as a result of tackles (Fuller et al., 2010, King and Gabbett, 2008). An injury to a single player could affect a team's game plan, motivation and/or match outcome on a given day.

In addition, the degree to which one's sleep and circadian rhythms are disrupted determines the extent to which their immune function is compromised given that different cells key to immune functioning peak at different times during the 24h day. Therefore, identifying which players are more prone to illnesses and injuries following time zone travel is key to improving performance in sporting activities. Identifying a player's chronotype may play a fundamental role in assessing their susceptibility to illnesses and/or injuries.

Chronotype has been reported to vary greatly in various general and athletic populations around the world (Lastella et al., 2010, Henst et al., 2015, Zavada et al., 2005, Kunorozva et al., 2012, BaHammam et al., 2011). A recent chronotype finding from UCT's circadian rhythms research group (Kunorozva et al., 2012) has shown that South African individual sport athletes tend to be more morning-oriented than the control population. This led to the question that is the chronotype distribution in team sport athletes similar or different to individual sport athletes. It was therefore, hypothesised that team sport athletes may have different proportions of morning- and evening-types compared to individual sport athletes as a consequence of time of

competition of the sport. However, one needs to consider chronotype in association with external factors. The interaction between an individual's genetics and external factors play an important role in shaping chronotype, which may subsequently influence performance. This highlights the need to study the extent to which different chronotypes desynchronise to a new time zone following trans-meridian travel in team sport athletes, since chronotype may strongly influence the efficacy of training and individual match performance. This is particularly important today when athletes are required to travel across multiple time zones (up to 12 time zones) for matches and competitions with little lead-time for synchronisation to the new time zone.

At present, nothing is known about the inter-individual effects of trans-meridian travel on individuals with various *PER3* VNTR genotypes with respect to injuries, illnesses and performance. Since there are physiological and psychological differences in the three *PER3* VNTR groups noted in sleep deprivation and nighttime shift work studies (Wirth et al., 2013, Chellappa et al., 2012b, Maire et al., 2014), it seems logical that these individuals may be affected differently by time zone travel. Specifically, understanding how each *PER3* VNTR genotype desynchronises and resynchronises into a new time zone could help improve the care of athletes. Furthermore, it could facilitate implementation of preventative measures especially for those *PER3* VNTR groups more susceptible to circadian disruption.

Since the day is shortened following eastward travel and the $PER3^{5/5}$ genotype has been shown to have a greater sleep propensity following sleep loss and a shorter circadian period compared to the $PER3^{4/4}$ genotype (Goel et al., 2009, Viola et al., 2007), it is possible that $PER3^{5/5}$ individuals may find it easier to initiate and maintain sleep in the new time zone. Likewise, the $PER3^{5/5}$ genotype has been shown to be more sensitive to blue-light exposure during a sleep deprivation study (Chellappa et al., 2012b). It was therefore hypothesised that $PER3^{5/5}$ Super Rugby players would adapt better (i.e. more advanced DLMO phase) to eastward travel compared to their $PER3^{4/4}$ counterparts.

Therefore, the aims of this thesis were:

(1) To compare the chronotype and *PER3* VNTR genotype distribution of Super Rugby players to individuals of low physical activity (i.e. those who train ≤2 times per week).

(2) To determine whether *PER3* VNTR genotype can explain inter-individual variation in the extent to which physical performance is affected following trans-meridian travel.

(3) To compare the impact of time zone travel during the 2012 Super Rugby competition in South African players genotyped as $PER3^{4/4}$, $PER3^{4/5}$ and $PER3^{5/5}$ on the incidence of injuries and illnesses.

(4) To compare the extent to which individuals genotyped as $PER3^{4/4}$ or $PER3^{5/5}$ respond to appropriately-timed blue light exposure in order to resynchronise their circadian rhythm, following simulated eastward travel, based on changes in dim-light melatonin onset and cortisol circadian phases.

CHAPTER 2: *PERIOD3* VARIABLE NUMBER TANDEM REPEAT POLYMORPHISM AND CHRONOTYPE DISTRIBUTION IN THE SOUTH AFRICAN SUPER RUGBY AND CONTROL POPULATIONS.

2.1 Introduction

A previous study carried out by Kunorozva et al. (2012), showed a very good correlation between chronotype and *PER3* VNTR genotype in Caucasian male athletes participating in individual endurance sports in South Africa. There were more individuals preferring mornings compared to those preferring evenings in the athletic populations compared to a control population of recreationally active, but non-competitive individuals. More of the individual sport athletes carried the *PER3* VNTR 5-repeat allele, which has been shown to be associated with morningness (Archer et al., 2003), compared to the control population. Furthermore, there was a positive association between chronotype and genotype such that individuals carrying the 5-repeat allele were more likely to be morning-types and those carrying the 4-repeat allele were more likely to be evening-types (Kunorozva et al., 2012).

Of interest is the fact that these distributions are not reflective of what other studies around the world have reported in the general population. Typically the evening-type chronotype is more common than the morning-type chronotype (Kabrita et al., 2014, Tonetti et al., 2010, Zavada et al., 2005), and the *PER3* 4-repeat allele is more common than the 5-repeat allele (Ciarleglio et al., 2008, Pereira et al., 2005, Nadkarni et al., 2005). This led to questions: Were the chronotype and genotype distributions observed by Kunorozva et al. (2012) specific to individual sport endurance athletes, who typically train and race in the early morning? Are the same distributions of chronotype and *PER3* VNTR genotype observed in team sport athletes who have more varied training session times and match schedules? These questions were necessary, given that chronotype and *PER3* VNTR genotype distributions of active controls (i.e. train ≥3 times per week) have already been described in South African populations (Rae et al., 2015, Kunorozva et al., 2012, Henst et al., 2015). These studies reported a high prevalence of morning-types compared to evening-types in active South African populations.

Team sport players may be less morning-oriented than individual sport athletes may, but more morning-oriented than the general population. This may be explained in part by timing differences in the training, and match or race schedules between individual and team sport athletes. Individual sport athletes tend to be free to choose their preferred training times unlike their team sport counterparts who typically train according to a set team schedule. The aim of this study was to compare the chronotype and *PER3* VNTR genotype distribution of Super Rugby players to individuals of low physical activity (i.e. those who are physically active ≤2 times per week).

Therefore, the objectives of this study were:

1. To describe and compare the chronotype distribution of South African Super Rugby players (RUG) and a control population of individuals who have low levels of physical activity (CON).
2. To genotype the same two populations for the VNTR polymorphism within the *PER3* gene.
3. To determine whether there is an association between chronotype and *PER3* VNTR genotype in these two groups.

It was hypothesised that individual sport athletes will have more morning-types than team sport athletes. Further, it was hypothesised that the rugby group would have a higher frequency of the $PER3^4$ allele compared to the low physical activity control group. Lastly, it was hypothesised that there would be an association between chronotype and *PER3* VNTR genotype in both the rugby and control groups. Specifically, that the $PER3^4$ allele would be associated with lower HÖ-scores and a preference for evenings, and the $PER3^5$ allele with higher HÖ-scores and a preference for mornings.

2.2 Methods

2.2.1 Participants

Professional male rugby players (RUG, n=205) from five South African Super Rugby teams participated in this study. Players were recruited with the help of each of the respective South

African provincial Super 15 team physicians, who were given detailed information about the nature of the research. Each team physician was able to explain the details of the study to the players, and all players received a participant information sheet (Appendix 1A), detailing the study purpose, outline, requirements, as well as all the potential risks and benefits. Players from both the 2011 and 2012 Super Rugby tournaments, who had played at least half of the Super Rugby tournament matches and had travelled with the team to play Super Rugby matches in Australia/New Zealand, were included in this study. The control group (CON, n=191) comprised males with habitually low levels of physical activity. This was assessed based on a questionnaire on which participants indicated the type of physical activity they usually undertook, for example, running, going to the gym, soccer, tennis, swimming and the frequency. Low levels of physical activity were defined as exercise no more than twice a week. CON participants were recruited at the Bayside and Cascades shopping malls, Cape Town Gardens Park and at the University of Cape Town and the University of Kwazulu Natal campuses at all times of the day to avoid bias in sampling that could arise by recruiting only in the mornings or evenings. The control group exercised no more than twice a week in the past year.

To be included in this study, all participants (RUG and CON) were required to be (i) between the ages of 18-40 years, (ii) in self-reported good mental and physical health, and (iii) free from any chronic medical condition. Participants who had taken any of the following substances: amphetamines, modafinil, soporific drugs, hypnotics or melatonin in the past three months prior to participation were excluded from the study as these drugs affect the sleep-wake cycle and/or circadian system. The descriptive characteristics of both groups are presented in Table 2.1.

2.2.2 Study design

This is a cross-sectional genetic association study comparing the *PER3* variable number tandem repeat polymorphism and chronotype distributions between the RUG and the CON populations. All volunteers were informed of the purpose of the study and gave written informed consent to participate (Appendix 1B). Participants completed a brief personal, health, training and competition-history questionnaire to determine their eligibility for the study and to document

their general characteristics (Appendix 1C). They also completed the Horne-Östberg (HÖ) morningness-eveningness personality questionnaire to determine their chronotype (Appendix 1D). Buccal cell samples or blood samples were obtained from participants for DNA extraction. Genomic DNA was used to genotype each individual for the VNTR polymorphism within the *PER3* gene (Archer et al., 2003). The study was approved by the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee (HREC Ref No: 412/2009), and performed in accordance with the principles of the Declaration of Helsinki (Fortaleza, Brazil 2013), the International Conference on Harmonisation and South African Good Clinical Practice guidelines.

2.2.3 Detailed testing procedures

2.2.3.1 Horne-Östberg Morningness-Eveningness Personality Questionnaire

A sub-group of the RUG (n=120) and all CON (n=191) participants completed the HÖ-questionnaire. This is the most widely used questionnaire that describes chronotype, and is highly correlated to the timing of melatonin release and core body temperature changes over a 24h period, the two most important markers of innate circadian phase (Horne and Ostberg, 1976). The questionnaire consists of 19 items relating to sleep-wake behaviour, and schedules and yields overall scores ranging from 16-86. Based on their score, individuals were placed into one of three main chronotype categories: evening-type (score: 16-41), neither-type (score: 42-58) and morning-type (score: 59-86). The internal consistency of the HÖ-questionnaire for this study was "good" with a Cronbach's α score of 0.82 (George and Mallery, 2003).

2.2.3.2 Genomic DNA extraction

2.2.3.2.1 Buccal cell samples

Buccal cell samples were obtained by scraping the inside of both cheeks using Epicentre Catch-All™ Sample Swabs (Epicentre Biotechnologies, Madison, USA). Following collection, the swabs were stored at room temperature, for no more than seven days. A simple salting out protocol was used to extract total human gDNA from the buccal cell swabs (Aljanabi and Martinez, 1997), with some modifications. After 1h incubation at room temperature in lysis buffer (0.4 M NaCl, 10 mM Tris-HCl (pH 8.0), 2 mM EDTA and 50 µl of 10% sodium dodecyl sulphate (SDS)), 10 µl of

proteinase-K was added to tubes containing the swabs. The tubes were incubated for 3h in a water bath at 55°C to allow the cells to lyse. Two hundred and twenty five microliters of 6 M NaCl was added to each tube. Samples were centrifuged for 10 min at 9200 *g* at room temperature using a bench centrifuge (Labnet International, Edison, USA) to remove NaCl and lysed cell debris. The supernatant was transferred to a clean microfuge tube and the centrifugation process repeated. After extraction and purification, samples were either left to air dry at room temperature for 1h or dried for 10 min at 37^{o}C. The pellet was re-suspended in 25 µl of 1 X sterile 1 mM Tris 1 mM EDTA (TE) buffer and DNA quantified using a NanoDrop® ND-1000 spectrophotometer (NanoDrop® Technologies, Wilmington, Delaware, USA). The quality was assessed after electrophoresis through a 1% (w/v) agarose gel in 1 X Tris Acetate EDTA (TAE; 40 mM Tris, 1 mM EDTA and 0.11% (v/v) glacial acetic acid) buffer. DNA samples were stored at -20°C until further analysis.

2.2.3.2.2 Blood samples

Blood samples were collected from the antecubital vein by a qualified phlebotomist into 5 ml EDTA vacutainer tubes (Becton Dickinson, New Jersey, USA). The vacutainer tubes were stored at -20^{o}C until further analysis. Total gDNA was extracted from the 5 ml blood samples according to the procedure described by Lahiri (1991), with slight modifications (Collins et al., 2004). The blood samples were transferred to sterile 15 ml polypropylene tubes, to which 10 ml of TKM1 buffer (10 mM Tris-HCl pH 7.6, 10 mM KCl, 10 mM $MgCl_2$ and 2 mM EDTA) containing 2.5% Nonidet P-40 was added in order to lyse the blood cells. Centrifugation of samples was performed at 3000 *g* for 10 min at room temperature in a Beckman TJ-6 Tabletop Centrifuge (Beckman Coulter, Minnesota, USA). Fifty microliters of 10% SDS was added to 800 µl of TKM2 buffer (10 mM Tris-HCl pH-7.6, 10 mM KCl, 10 mM $MgCl_2$) suspension, mixed thoroughly by pipetting back and forth several times and incubated for 1h in a water bath at 55°C. Extracted DNA was suspended in 50 µl of TE buffer and stored at -20^{o}C until further analysis.

2.2.3.2.3 *PER3* VNTR amplification and confirmation

In order to genotype individuals for the *PER3* VNTR polymorphism, exon 18 of *PER3* and its adjoining regions were amplified by PCR from 80-100 ng of gDNA using standard methods described by Ebisawa et al. (2001) and Archer et al. (2003). Polymerase chain reaction products were digested with *Ncol* restriction enzyme, and products assessed after electrophoresis through a 2% (w/v) agarose gel in 1 X Tris Acetate EDTA (TAE; 40 mM Tris, 1 mM EDTA and 0.11% (v/v) glacial acetic acid) buffer and viewed under ultra-violet (UV) light using ethidium bromide staining. Those individuals homozygous for the 5-repeat allele were shown by a banding pattern of 420 bp and 200 bp, respectively. While those homozygous for the 4-repeat allele had a banding pattern of either 600 bp only or 600 bp, 380 bp and 200 bp, or 380 bp and 200 bp. The individuals that were heterozygous for the *PER3* gene had a combination of either 600 bp, 420 bp and 200 bp or 420 bp, 380 bp and 200 bp. The 4- and 5-repeat alleles of the *PER3* VNTR polymorphism were genotyped as previously described by Kunorozva et al. (2012). Each *PER3* VNTR genotype was checked and confirmed by an independent person other than the candidate.

2.2.4 Data and statistical analyses

Data are reported as mean ± standard deviation (SD, for parametric data) and median with interquartile range (IR, for non-parametric data) or frequency (%). A Shapiro-Wilks test was performed to check for normality of the data. Hardy Weinberg (http://genepop.curtin.edu.au/genepop_op1.html) exact tests were performed to determine if the percentage frequencies of the 4- and 5-repeat alleles were normally distributed. Group characteristics were compared using an independent t-test and Mann-Whitney U test. A Chi-squared or Fisher's Exact test were performed to compare both the chronotype and *PER3* VNTR polymorphism frequency distributions between the two groups. Correlations were performed using multinomial logistic regression. Data were analysed using Statistica version 11 (StatSoft Inc., Tulsa, Oklahoma, USA). Significance was assumed for $p<0.05$.

2.3 Results

2.3.1 Participant characteristics

Characteristics of the RUG and CON groups are presented in Table 2.1. There were differences in all the variables except for age between the RUG and CON groups. The RUG group was taller ($p<0.001$), heavier ($p<0.001$) and had a higher body mass index ($p<0.001$). The RUG group trained on more days each week compared to the CON group ($p<0.001$). The RUG group consisted of 171 (83.4%) Caucasians, 20 (9.7%) mixed ancestry and 14 (6.8%) black African individuals, while the CON group consisted of 71 (37.2%) Caucasians, 56 (29.3%) mixed ancestry, 63 (32.9%) black Africans and 1 (0.5%) Indian. There were significant differences in ethnicity distribution between the RUG and CON groups ($p<0.001$).

Table 2.1: General characteristics of the Rugby and Control groups.

	RUG (n=205)	CON (n=191)	p-value
Age (y)	25.0 (5.0)	25.0 (8.0)	0.835
Weight (kg)	102.0 (20.0)	74.0 (20.0)	<0.001
Height (cm)	186.4±7.4	175.5±8.7	<0.001
BMI (kg·m^{-2})	28.7 (3.5)	23.8 (4.4)	<0.001
Training (days·week^{-1})	4.3±0.9	1.7±0.4	<0.001

Data are presented as the median with IR and mean ± SD. BMI: Body mass index, CON: Low physical activity control group and RUG: Super Rugby group. Training data for the RUG group are for the two weeks prior to the tournament starting. The p-values represent significance as determined by either a Mann-Whitney U test or an independent t-test.

2.3.2 Chronotype distribution

The scores for all participants ranged from 26 to 77, but the HÖ-score data were significantly skewed towards the higher scores ($p<0.001$) in the RUG group. The RUG group had a higher mean HÖ-score compared to the CON group (RUG: 57.4±8.4 and CON: 50.6±10.4, $p<0.001$). Figure 2.1 depicts the frequencies of morning-types (MT), neither-types (NT), and evening-types (ET) in both groups. A significant difference in the chronotype distribution between the two groups was found ($p<0.001$). A *post-hoc* analysis indicated that there were more MT individuals in the RUG group

than in the CON (p<0.001) group. In contrast, there were more ET individuals in the CON group than in the RUG (p=0.003) group.

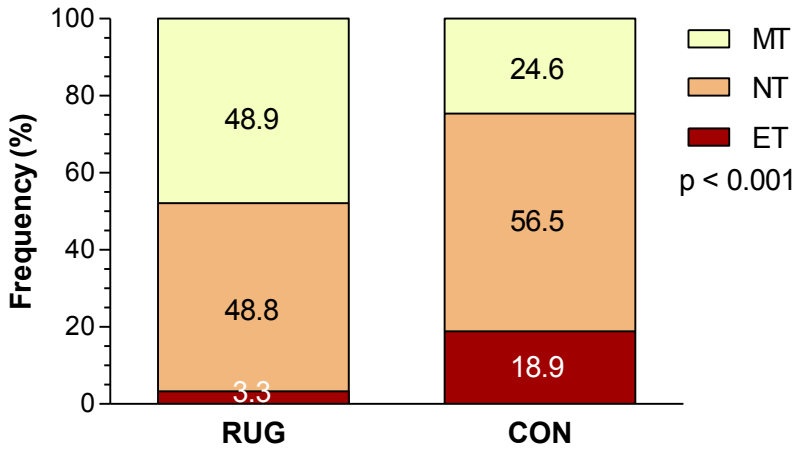


Figure 2.1: Proportion of chronotype categories in the RUG (n=120) and CON (n=191) groups. CON: Low physical activity control group, ET: evening-type, MT: morning-type, NT: neither-type, and RUG: Super Rugby group. The p-value represents significance determined using the Fisher's exact test.

2.3.3 *PER3* VNTR polymorphism frequency distribution

All of the RUG group, but only 120 individuals in the CON group were genotyped successfully. Of the 191 CON participants, gDNA from 48 CON individuals was non-amplifiable, and 23 of the CON participants were lost to follow-up for the DNA sample collection. The genotype and allele frequencies were not different between the two groups (Figure 2.2). In both groups, approximately one third of the individuals carried the 5-repeat allele while two thirds carried the 4-repeat allele.

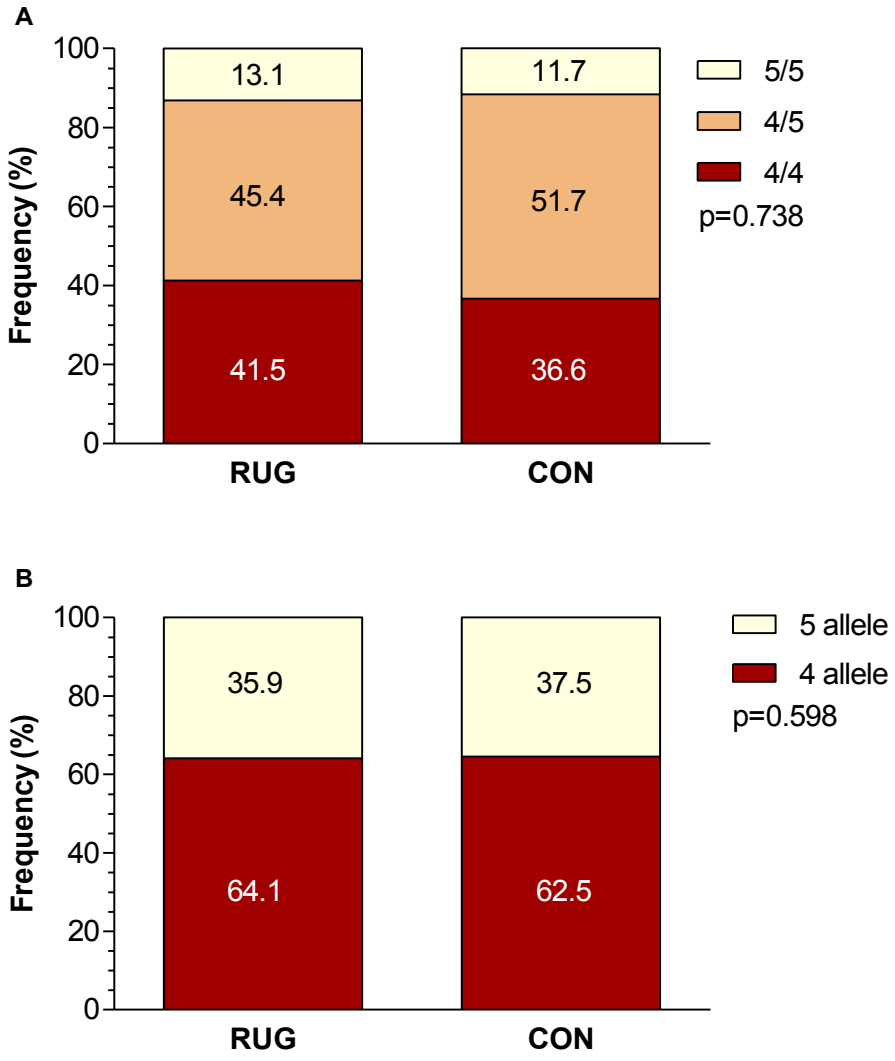


Figure 2.2: Frequency distributions of the *PER3* VNTR genotypes (A) and alleles (B) in the RUG (n=205) and CON (n=120) groups. 4/4: $PER3^{4/4}$, 4/5: $PER3^{4/5}$, 5/5: $PER3^{5/5}$, CON: Low physical activity control group and RUG: Super Rugby group. The p-values represents significance determined using a Chi-squared test.

2.3.4 Relationship between chronotype and the *PER3* VNTR genotype

The association between the HÖ-questionnaire score for each individual in relation to his *PER3* VNTR genotype is presented in Figure 2.3. There was no significant correlation between the HÖ-score and an individual's genotype when data from both the RUG and CON groups were pooled for analyses. When analysed separately, the CON group showed a low, but significant association

between HÖ-score and the *PER3* VNTR polymorphism (p=0.008, r=0.293); but the RUG group data showed no association (p=0.574, r=0.009).

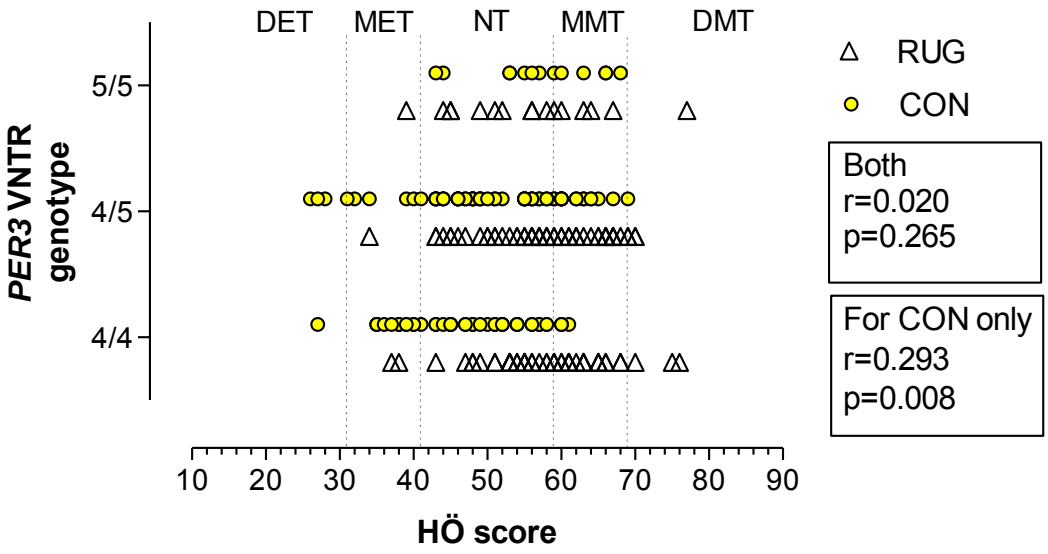


Figure 2.3: Relationship between *PER3* VNTR genotype and the HÖ-score in the RUG (n=120) and CON (n=120) groups. 4/4: $PER3^{4/4}$, 4/5: $PER3^{4/5}$, 5/5: $PER3^{5/5}$, CON: Low physical activity control group, DET: definite evening-type, DMT: definite morning-type, HÖ: Horne-Östberg questionnaire, MET: moderate evening-type, MMT: moderate morning-type, NT: neither-type, RUG: Super Rugby group and VNTR: variable number tandem repeat. The p-value represents significance determined using multinomial logistic regression.

The chronotype distributions within each of the three genotype groups for both the RUG and CON groups are presented in Figure 2.4. Chronotype distribution was significantly different between the three *PER3* VNTR genotype groups in the CON group (p=0.011, Figure 2.4A). A *post-hoc* analysis indicated that there were more MTs in the $PER3^{5/5}$ group than the other two groups (p=0.009). Furthermore, there were more ETs in the $PER3^{4/4}$ group compared to the other two groups (p=0.030). The number of NTs in each of the three groups was similar. The same pattern was not observed in the RUG group (Figure 2.4B).

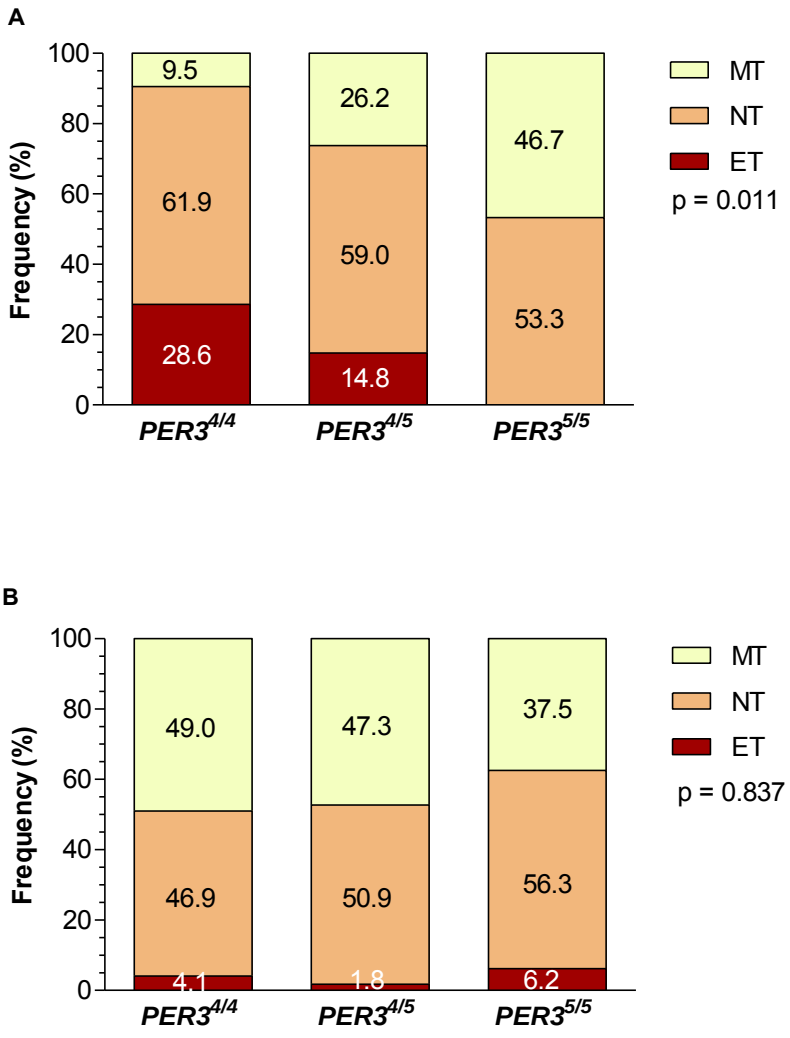


Figure 2.4: Chronotype distribution in the three *PER3* VNTR genotype groups in the CON (A, n=120) and RUG (B, n=120) groups. ET: evening-type, MT: morning-type and NT: neither-type. The p-value represents significance determined by Chi-squared (CON) and Fisher's exact (RUG) tests, respectively.

2.4 Discussion

The main aim of this study was to describe the genotype and chronotype distribution in the South African, Super Rugby population and an age- and gender-matched control population of individuals with low levels of physical activity. The main findings were that while the two groups had similar *PER3* VNTR genotype distributions (Figure 2.2), the chronotype distribution in the Super Rugby population was significantly different to that of the control group (Figure 2.1). More importantly, an association between genotype and chronotype was only observed in the control group. The chronotype and genotype results of the control group in this study are similar to a control group of individuals with low levels of physical activity of previous study (unpublished data).

Regarding the genotype data, the Super Rugby and control populations in the present study had similar *PER3* VNTR genotype and allele frequencies. Specifically, these groups had high frequencies of individuals who were homozygous for the *PER3* 4-repeat allele compared to those individuals who were homozygous for the *PER3* 5-repeat allele (Figure 2.2A). Furthermore, the majority (two thirds) of participants in both the RUG and CON groups carried the *PER3* 4-repeat allele, while only one third carried the *PER3* 5-repeat allele (Figure 2.2B). This result is congruent with findings from other studies around the world (Henst et al., 2015, Rae et al., 2015, Archer et al., 2003, Nadkarni et al., 2005, Ciarleglio et al., 2008), in which the *PER3* 4-repeat allele has also been predominant in the general population and marathon runners. For example, Ciarleglio et al. (2008) reported a higher frequency of the *PER3* 4-repeat allele in African American (59%), Ghanaian African (64%) and European American (65%) populations.

The *PER3* 5-repeat allele has only been found to be the predominant allele in a few studies investigating Papua New Guinea (81%), South African Caucasian (male athletes) (60%) and Yemenis (52%) populations (Ciarleglio et al., 2008, Nadkarni et al., 2005, Lazar et al., 2012, Kunorozva et al., 2012). The similarity between the *PER3* VNTR genotype distribution observed in the present study and other populations (Nadkarni et al., 2005, Ciarleglio et al., 2008, Archer et al., 2003, Pereira et al., 2005), suggests that the observed differences are not due to random

selection or differences in geographical location, but rather due to random selection through sexual selection.

The chronotype spread in the CON group was normally distributed with the majority of participants being characterised as neither-types (Figure 2.1). This finding concurs with results from Li et al. (2011), who reported ratios of 18:47:35 of ET:NT:MT in a Chinese population from the Beijing district consisting of 188 males and females in a ratio of 1:1 and aged between 19-51 years (mean age: 30.8±7.8). Of interest however is the fact that neither the CON group nor RUG chronotype findings in the present study are reflective of what other studies around the world have reported in the general population, where typically the evening chronotype is more prevalent than the morning chronotype (Kabrita et al., 2014, Zavada et al., 2005, Tonetti et al., 2010). These researchers reported ET:NT:MT ratios of 25:68:7 in a Lebanese population (mean age: 19.8±1.5, 1:1 males to females, Kabrita et al., 2014), 24:65:11 in a Spanish population (mean age: 22.8±2.9, 1:1 males to females, Tonetti et al., 2010) and 33:55:14 in a Dutch population (age range: 10-80 years, Zavada et al., 2005).

Taking a closer look at the chronotype data from this study, one observes that the RUG group contained twice as many morning-types (48.8%) compared to the CON group (24.6%). In contrast, very few RUG players were characterised as evening-types (<5%), while a notable proportion of individuals in the CON group were categorised as evening-types (18.9%). In general, there was a higher proportion of morning-types compared to evening-types in both the RUG and CON populations compared to previous studies (Adan and Natale, 2002, Kabrita et al., 2014, Osland et al., 2011, Kudielka et al., 2007). This is interesting because this finding is similar to results from other age-matched male SA individual endurance sport and recreationally active populations (Henst et al., 2015, Kunorozva et al., 2012, Rae et al., 2015). A higher proportion of morning-types in this study may have been a result of habitual early morning training times, also referred to as “conditioning” in the rugby group. This means that habitual early morning training times might be driving the morning-chronotype. In response to this problem “conditioning”, an argument can be made for the inclusion of sleep diaries in future. The sleep diaries will give

information pertaining to the athletes' bedtime and wake-up time, which might facilitate interpretation of the HÖ-score better.

The chronotype distribution in the RUG group was however skewed towards morning-orientation, with a high proportion of participants being characterised as morning- or neither-types (Figure 2.1). This finding is in line with previous studies (Rae et al., 2015, Kunorozva et al., 2012, Lastella et al., 2010)**,** which investigated chronotype distribution in trained athletes. For example, these researchers reported ET:NT:MT ratios of 0:48:52 in an Australian population (mean age: 20.9±2.9, 2:1 males to females, Lastella et al., 2010), 0:42:58 (mean age: 32.6±5.7, 2:1 males to females, Rae et al., 2015) and 8:28:64 (mean age: 36.5±6.8, males only, Kunorozva et al., 2012) in Caucasian South African populations. The RUG chronotype distribution is however different to numerous other studies around the world just like the CON group in this study (Tonetti et al., 2010, Zavada et al., 2005, BaHammam et al., 2011).

It is interesting to note that the rugby and control populations had more morning-types than evening-types compared to other populations. A possibility for having a high proportion of morning- compared to evening-chronotypes in both the SA rugby and control populations in this study could be societal/cultural differences between SA and other countries. For example, the average school start time in SA is 07h45 vs 09h00 for most parts of Europe and the United States of America. Studies by Hidalgo et al. (2009) and Pedrazzoli et al. (2007) reported that most of the Brazilian population, for example, tend to be evening-types. In Brazil, individuals have been shown to start their personal activities in the evening and thus may be more active and alert in the late afternoon into the early evening. Therefore, perhaps one is conditioned to being more morning- or evening-type in nature by the society in which he/she resides in. This is plausible since the HÖ-questionnaire scoring is based on answers where precise times are given, thus societal norms may influence the questionnaire scoring in different countries (Taillard et al., 2004, Li et al., 2011, Smith et al., 2002).

Another potential reason for this finding may be geographical differences between cities or countries. Specifically, differences in latitude may contribute to the differences observed as latitude influences photoperiod experienced in a given city or country during a particular season (Borisenkov et al., 2010, Randler, 2008, Miguel et al., 2014, Masal et al., 2015). For example, Borisenkov (2010) observed a higher frequency of evening-types in the Republic of Komi in Russia. He reported differences in chronotype between towns that were 4.5^{o} apart in latitude. Likewise, he compared chronotype distribution between high latitude lying towns in the Republic of Komi and lower latitude European cities. He observed a significantly higher proportion of late chronotypes in those towns in high latitude areas, suggesting that photoperiod may contribute to differences in chronotype distribution. South Africa has a latitude of 30^{o} S compared to the lower latitude of 19^{o} S in Turkey (Masal et al., 2015), 22^{o} S of Paraiba do Sul (Hidalgo et al., 2009) and 23^{o} S of Sao Paulo (Rique et al., 2014) where higher percentages of evening-types were found. The latitude of Cape Town (34^{o} S) is similar to 34^{o} S of Adelaide, South Australia where a high percentage of morning-types were observed in athletes (Lastella et al., 2010). Therefore, latitude may play a role in influencing chronotype.

In addition to latitude, ambient temperature in a given society may also influence chronotype. Specifically, climate zones differ significantly in ambient temperature (e.g. mean annual temperature- tropics 25^{o}C, subtropics 18^{o}C and temperate zones 10^{o}C) (Randler, 2008). The tropics have similar daylength nearly all year round, while daylength near the poles increases with increasing latitude in the summer and decreases with latitude in winter. Prolonged periods of ambient lighting (e.g. during summer) might extend the time individuals retire to bed by an hour in a given society. For example, an eight year longitudinal study (Lehnkering and Siegmund, 2007) indicate a significantly longer actual sleep time in autumn than in spring in 2 697 participants. Another study using the Composite Scale of Morningness reported that late chronotypes were more prevalent in subtropics than tropic regions in a group of adolescent participants (Randler, 2008). Based on the abovementioned evidence, it can be said with relative certainty that ambient temperature may affect chronotype distribution in a given region.

Chronotype has also been shown to vary considerably with age in many populations (Horne and Ostberg, 1976, Taillard et al., 1999, Randler et al., 2012, Roenneberg et al., 2004). Specifically, adolescent individuals tend to have a propensity for delayed sleep periods, which are associated with eveningness; while young adults and the elderly have a tendency for advanced sleep periods associated with morningness. Therefore, it is possible that the differences observed between this study and the above-mentioned studies could be due to age differences. For example, the Kabrita et al. (2014) and Tonetti et al. (2010) studies which had high prevalence of evening-types used young student (17-25 years) and university (18-30 years) populations, respectively, an age group known to be biased towards evening behaviour.

An important finding of the current study was that there was no association between chronotype and *PER3* VNTR genotype when the two groups were analysed together (Figure 2.3). However, there was a positive association between chronotype and genotype in the CON group when data were assessed separately, such that individuals homozygous for the 5-repeat allele were more likely to be categorised as morning-types; while those homozygous for the 4-repeat allele were more likely to be scored as evening- or neither-types. This is in line with previously published studies that reported an association between the *PER3* VNTR genotype and chronotype (Kunorozva et al., 2012, Archer et al., 2003, Pereira et al., 2005, Archer et al., 2010, Jones et al., 2007). Based on the high proportion of the *PER3* 4-repeat allele result in the CON group, a high percentage of evening- and neither-types was expected.

In contrast to the CON group, there was no association between chronotype and genotype in the rugby population. Based on the players' genotype, a higher proportion of evening-types was expected; instead, a high proportion of morning-types was noted. This is different to most studies that have reported an association (Archer et al., 2003, Archer et al., 2010, Ebisawa et al., 2001, Pereira et al., 2005, Kunorozva et al., 2012), but similar to a few other studies (Henst et al., 2015, Goel et al., 2009, Barclay et al., 2011), which reported no association between genotype and chronotype. A few studies have reported that repeated training at a particular time-of-day may influence chronotype (Chtourou and Souissi, 2012, Chtourou et al., 2012, Hill and Smith, 1991).

However, other studies in literature suggest that highly active individuals tend towards morning orientation (Antunez et al., 2013, Urban et al., 2011, Shechter and St-Onge, 2014). This is further supported by previous findings at the University of Cape Town in Associate Professor Roden and Dr Rae's research group (unpublished data) that individuals with higher levels of physical activity tend towards morning orientation.

What is potentially interesting is the finding of evening-types in the rugby's *PER3*$^{5/5}$ group which have not been observed before. Perhaps conditioning could explain the lack of association between chronotype and *PER3* VNTR genotype in the RUG group. South African Super Rugby teams perform most of their training sessions in the morning between 08h00-11h00 (training time information obtained from the participant questionnaires). Thus, RUG players may have become used to the repeated early morning training times, such that their chronotype shifted to a more morning-orientation, despite their *PER3* VNTR genotype being associated with the evening-chronotype. Accordingly, this conditioning may have resulted in the mismatch of the players' chronotype with their *PER3*-based genetic predisposition weakening any association between chronotype and *PER3* genotype.

Another aspect is that morning-type individuals typically have earlier sleep schedules compared to their evening-type counterparts and therefore differ in their bed and wake-up times (Lo et al., 2014, Mongrain et al., 2006). It is possible that heavy training loads in the morning, such as is the case in most Super Rugby teams, may lead to players being more tired at the end of the day, and thus players may sleep earlier than they usually would on days without training. Perhaps some *PER3*$^{5/5}$ individuals nap, leading to them staying up later and considering themselves as evening-types. In addition, research on sleep habits in elite athletes indicate that attaining adequate sleep prior to training or competition is important for achieving optimum performance and satisfaction (Mah et al., 2011, Juliff et al., 2015). For example, Mah et al. (2011) demonstrated a faster sprint time as well as an improved shooting accuracy in the Stanford University men' s basketball players following sleep extension (≥10h) compared to baseline (6-9h). In this study, basketball players also reported improved overall ratings of physical and cognitive function during training

and matches, suggesting that adequate sleep is beneficial for optimal performance. While sleep timing was not measured, it is possible that South African Super Rugby players retire to bed earlier on the majority of training days. Either early morning training times or heavy training load appear to be driving the morning-type phenotype in the rugby population in this study as players are tired at the end of the day.

2.4.1 Limitations

Data collection spanned over 18 months, from March 2012-September 2013. Therefore, seasonal variations in photoperiod, known to affect chronotype, may have influenced the results. The use of a subjective tool to measure chronotype was a limitation in this study. Specifically, the questions on the HÖ-questionnaire are fixed and do not allow for flexibility when there is no suitable option for an individual's preferred answer. This likely affected those individuals, whose preferred answer was not on the multiple choice options list. For this reason, the questionnaire does not reflect an individual's true chronotype, but a close approximation of an individual's chronotype. Likewise, the HÖ-questionnaire has not been validated for use in a team sport structure, where individuals do not have the flexibility to choose their preferred time for physical performance. Thus, individuals were likely to choose team-training times as the times they prefer to train, and not their actual preferred training time-of-day. Use of sleep diaries (at least two month prior to tournament kick-off) would have assisted in determining prior sleep history for the Super Rugby population since it is unlikely that they chose their own sleep time during training days and days prior to a match. This would have helped explain some of the mismatch between chronotype and genotype in this study.

2.5 Conclusion

The *PER3* VNTR genotype and chronotype distributions were different between the SA Super Rugby and control populations. There was no chronotype and *PER3* VNTR genotype association in the RUG group. Perhaps repeated exposure to morning training sessions or heavy training load lead to early wake times or increased fatigue both of which may shift chronotype. This is conceivable, because the two groups had similar *PER3* allele frequency distributions, but the lack

of association was only observed in the RUG group. The only aspect that was different between the two populations was the level of physical activity. It appears that with a combination of repeated morning training times and a higher level of physical activity, individuals tend towards more morning orientation (Henst et al., 2015, Rae et al., 2015, Lastella et al., 2010). While the CON group in this study extends the work of previous studies (Kunorozva et al., 2012, Pereira et al., 2005, Archer et al., 2003, Ebisawa et al., 2001); the RUG group raises interesting questions with regards to the relationship between chronotype and *PER3* VNTR genotype in active individuals. Therefore, future studies in other team sport athletes that will make use of a sleep diary or actigraphy to measure sleep habits may be warranted to explore this hypothesis.

CHAPTER 3: THE IMPACT OF TRANS-MERIDIAN TRAVEL DURING COMPETITION ON INDIVIDUAL MATCH PERFORMANCE IN RUGBY PLAYERS GROUPED BY *PER3* VNTR GENOTYPE.

3.1 Introduction

Travelling across multiple time zones leads to a mismatch between the internal body clock and the external environment at the destination venue, resulting in an assortment of symptoms known as jet-lag (Waterhouse et al., 2007, Sack, 2010). These symptoms include impaired judgement, reaction time, alertness, daytime fatigue and disruption of the sleep-wake cycle (Edwards et al., 2000, Manfredini et al., 1998, Reilly et al., 2005). Physical performance following time zone transitions in particular is greatly affected by jet-lag and circadian rhythm disruption. Jet-lag and circadian rhythm disruption have been shown to influence not only cognitive function (e.g. decision making, alertness), but also neuromuscular and physiological parameters that are crucial for achieving optimum physical performance (Reilly et al., 2005, Leatherwood and Dragoo, 2013). For instance, time zone transition disrupts circadian rhythms in muscle flexibility (Reilly et al., 2007), muscle strength (Jasper et al., 2009) as well as reaction time (Reilly et al., 1997a), all of which are fundamental for an individual's match performance.

Research has shown that the extent to which individuals are affected by jet-lag and circadian rhythm misalignment varies following trans-meridian travel (Winget et al., 1984, Eastman and Burgess, 2009, Chapman et al., 2012). One possible explanation may be that polymorphisms in clock genes, such as those found in the *PERIOD3* gene, may influence individual tolerance to circadian rhythm disruption caused by time zone transitions. For example, individuals with various *PER3* VNTR genotypes have been shown to respond differently to sleep deprivation and disruption (Dijk and Archer, 2010, Chellappa et al., 2012b), such as that which occurs following time zone transitions. Specifically, the $PER3^{5/5}$ individuals are more susceptible to sleep deprivation than the $PER3^{4/4}$ individuals.

These differences in susceptibility may arise due to differences that have been observed in sleep pressure and cognitive performance following sleep deprivation between *PER3*$^{4/4}$ and *PER3*$^{5/5}$ individuals (Maire et al., 2014, Viola et al., 2007). The genotype groups may also entrain to new light/dark cycles differently due to the differences in sensitivity to blue light suppression of melatonin in *PER3*$^{4/4}$ and *PER3*$^{5/5}$ genotypes (Chellappa et al., 2012a). The aim of this study was to determine whether *PER3* VNTR genotype might contribute to inter-individual variation in the extent to which game involvement and quality of play are affected following trans-meridian travel. To do this, game activity and play quality were analysed in the South African Super Rugby players competing in the 2011 and 2012 Super Rugby tournaments. The Super Rugby tournament involves teams from three Southern hemisphere countries (South Africa (SA), Australia (AUS) and New Zealand (NZ)) and is a unique competition in that teams are required to undergo repeated trans-meridian travel, crossing up to 12 time zones between matches. This provides a good platform to study the effects of trans-meridian travel on subsequent match involvement and quality of play.

Therefore, the objectives of the study were:

(1) To compare players genotyped as *PER3*$^{5/5}$, *PER3*$^{4/5}$ and *PER3*$^{4/4}$ with respect to game activity rate and quality of play in games preceded either by no time zone travel, east-to-west or west-to-east travel.
(2) To determine whether the number of time zones crossed prior to a match influences individual game activity rate and quality of play in these three groups of players.

It was hypothesised that individuals carrying the *PER3*$^{5/5}$ genotype would have a higher game activity rate and quality of play than the other two *PER3* VNTR groups following time zone travel. Further, it was hypothesised that game activity rate and quality of play would decrease as the number of time zones crossed prior to a match increases in the three *PER3* genotypes. Lastly, it was hypothesised that game activity rate and quality of play would be higher following westward travel compared to eastward travel.

3.2 Methods

3.2.1 Setting

Each country has five teams participating in the tournament. Teams play matches weekly for the duration of the 21-week tournament period, with the exception of two byes, one of which is when teams travel between countries for subsequent matches. The competition involves both home and away matches as well as frequent trans-meridian travel to play at Australian (AUS) and New Zealand (NZ) match venues. While the gruelling travel schedule during the competition places exceptional physiological and psychological stresses on players, it also provides an opportunity to study the effects of jet-lag adaptation in athletes. Each team participates in a round robin over 16 weeks beginning towards the end of February or early March each year until August, with a one-month break from the tournament during June allowing selected players to represent their respective countries in international matches. After the round-robin stage, four teams (viz. teams in position 3-6 on the combined Super Rugby log) participate in the two play-offs of the tournament with winners proceeding to join the first and second ranked teams on the combined Super Rugby log in the two semi-final matches. Lastly, the two teams that win the semi-final games proceed to play in the Super Rugby final match of the tournament.

3.2.2 Participants

Sixty Super Rugby players from all five SA teams were included in this study based on the genotype results from Chapter 2. Players genotyped as *PER3*$^{5/5}$ (n=20), *PER3*$^{4/5}$ (n=20) and *PER3*$^{4/4}$ (n=20) who had played a minimum of 70% of their 2011 or 2012 Super Rugby matches were randomly selected for this study. However, if a player played ≥70% of his matches in both the 2011 and 2012 tournaments, the 2012 data were used. The HÖ-questionnaire data were disregarded when randomly selecting players for this study, because no association was found between the HÖ-score and genotype in the rugby group analysed in Chapter 2. The characteristics of the three groups of players (*PER3*$^{5/5}$, *PER3*$^{4/5}$ and *PER3*$^{4/4}$) are presented in Table 3.1.

3.2.3 Study design

In this retrospective observational study, individual quality of play and involvement of players grouped by *PER3* VNTR genotype was compared when players travelled east-to-west (E-W), west-to-east (W-E) and when there was no time zone difference (NTZD) between match venues. Given the nature of a team game like rugby and the differences in play between positions (i.e. lock vs full back), it was not possible to directly measure and compare involvement or play quality of players to each other in each match. Using post-match video analysis, the extent to which an individual was involved in a match was determined, and a "game activity (GA) rate" score was calculated. Change in GA rate score for the W-E travel or E-W travel was calculated as the difference in GA rate for the match after trans-meridian travel and the last match played in the NTZD environment. Change in GA rate score between matches when no time zones were crossed was calculated as the difference between GA rate score of the latest match minus that of the previous match.

All Super Rugby matches in which South African teams played during the 2011 and 2012 competitions were recorded digitally, edited to remove adverts and half time commentary, and stored on the Human Biology server at the University of Cape Town. The Human Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town approved this study (HREC Ref No: 008/2011). Prior to commencement of both the 2011 and 2012 Super Rugby tournaments, all participants gave written informed consent agreeing to participate in this study (Appendix 1B, Chapter 2). The study was performed in accordance with the principles of the Declaration of Helsinki (Fortaleza, Brazil, 2013), the International Conference on Harmonisation and South African Good Clinical Practice guidelines.

3.2.4 Detailed testing procedures

3.2.4.1 *PER3* VNTR genotype and descriptive characteristics of players

The genotype and other descriptive data presented in this chapter were obtained as described in section 2.2.3 of Chapter 2.

3.2.4.2 Super Rugby tournament statistics, travel and match schedules

Collectively, all five SA teams played 59 games in SA (i.e. no time zone difference) during weeks 1-12 of the 2011 and 2012 tournaments. They then travelled to AUS and NZ for five weeks to play a further four matches each (i.e. 40 matches for all five SA teams in the 2011 and 2012 tournaments). Then teams travelled back to SA for a further 1-10 weeks of play (63 matches), depending on progression through the tournament. Furthermore, two SA teams travelled back to AUS/NZ during the knockout stages of the tournament in three different weeks to play three more games.

Match outcome data were obtained from the South African Rugby Union (SARU) official website (www.sarugby.net). In addition, each SA team provided their travel itinerary schedule for the whole tournament each year, so that precise departure and arrival dates and times for both local and international travel could be used for analysis. A combination of information from the travel itinerary schedules and SARU website allowed the construction of a table with match outcomes for matches played at home and away as well as following E-W or W-E travel.

Matches were grouped into those played after crossing 6-8 time zones, >10 time zones and when no time zone crosses took place. Specifically, matches played after crossing 6-8 time zones were combined to boost the sample size. Matches played after crossing two time zones were not considered in these analyses. A match was defined as being preceded by “W-E travel” if a team travelled in an easterly direction and played within a number of days after arrival that would not have allowed complete re-entrainment. To calculate this, the concept that for every time zone crossed a player needs approximately a day to resynchronise to the new time-zone environment was employed (Manfredini et al., 1998, Waterhouse and Reilly, 2009). For example, if a team travelled from SA to Perth, AUS crossing six time zones and played within six days of arrival, the match was defined as being preceded by W-E travel. The same concept was used to categorise “E-W travel” when teams travelled in the opposite direction. Matches were categorised as being preceded by “no time zone change (NTZD)” when (i) it was a home game for the player of interest

not preceded by E-W or W-E travel, or (ii) the match took place after "domestic" travel (i.e. travel between cities within SA, AUS or NZ during which no time zones were crossed).

3.2.4.3 Player game activity rate and quality of play determination

A total of 166 matches were analysed for both the 2011 and 2012 Super Rugby tournaments. During these matches, the 60 players of interest in this study played an average of 15 games each during the tournament period. The GA rate score for each player in each match was determined objectively using post-play video coding analysis with specifically designed sports coding software (SportsCode Elite, version 8, Sportstec, England). The video data were used retrospectively to analyse the game statistics for each of the 60 players in each match throughout the two tournaments, from which GA rate was calculated and used as the outcome of interest. The GA rate score is thought to be a reasonable marker of an individual's involvement during a particular match (Hughes and Bartlett, 2002, Ortega et al., 2009). The first step for GA rate analysis in this study was to identify and measure a range of 'Key Performance Indicators (KPIs)', that have a real bearing on the outcome of a game, for each player during each match (Glazier, 2010, Hughes and Bartlett, 2002). A performance indicator is an action variable or selection of action variables that defines an aspect of an individual's involvement during a match. The methods to define KPIs of play in rugby have been described previously (James et al., 2005). Play instances were labelled using the standard KPIs that were divided into five possible play scenarios: tackles, lineouts, handling, breakdowns, and infringements (as defined by World Rugby).

Thus, using this method, a GA score was calculated for each player for each match he played throughout the tournament. This score reflected the number of events counted for each KPI for the player for that particular game. This score was then converted to a GA rate by dividing it by the number of minutes that the player was on the field for during a particular match (events/min). Finally, each player's GA rate was normalised to his first match of the tournament. This approach was necessary to account for differences in GA levels that could arise due to an individual's playing position. Changes in the GA rate of each player were then correlated with

aspects of trans-meridian travel, such as number of time zones crossed, and direction of travel as the tournament progressed.

A player's quality of a play in a given match was calculated by summing all scored KPIs (with those considered to be a positive quality of play being preceded by a + sign and negative aspects of play were allocated a score of zero), and expressing this number as a percentage of the total KPIs counted for that player in that match. For example, a good pass, a poor pass and a broken tackle, would add up to three events (say Score A). The quality of those events (say Score B) was calculated as good pass (+1), poor pass (0) and broken tackle (+1), which is 2. Thus, the overall quality of play was obtained by dividing Score B by Score A and multiplying by one hundred (Score B/Score A X 100) to obtain a percentage quality of play score, which in the example above (2/3 X 100) would be 67%.

3.2.5 Data and statistical analyses

Descriptive data are reported as mean ± standard deviation (SD, for normally distributed data) or median with interquartile range (IR, when data are not normally distributed), counts or frequency (%). A Shapiro-Wilks test was used to test for normality of the data. Chi-squared or Fisher's exact tests were used to determine differences between counts. Three group comparisons were made with one-way ANOVA or Kruskall-Wallis ANOVA tests. Repeated variables were assessed using either dependent t-tests, Wilcoxon matched pair's tests, ANOVA with repeated measures or Friedman's ANOVA tests. *Post-hoc* analyses were performed using Tukey, Fishers LSD or Dunnett multiple comparison tests. Data were analysed in Statistica (version 11, StatSoft Inc., Tulsa, Oklahoma, USA) and Stata (version 14, StataCorp, Texas, USA). Significance was assumed for $p<0.05$.

3.3 Results

3.3.1 Participant characteristics

Participant characteristics of the Super Rugby players for this study are presented in Table 3.1. There were no significant differences between the three genotypes for any of the variables.

Table 3.1: General characteristics of the three *PER3* VNTR groups of rugby players.

	PER3$^{4/4}$ (n=20)	*PER3*$^{4/5}$ (n=20)	*PER3*$^{5/5}$ (n=20)	p-value
Age (y)	25.4±3.1	26.4±2.9	25.7±2.9	0.489
Weight (kg)	100.1±11.7	105.8±10.0	103.8±10.1	0.459
Height (cm)	187.3±6.6	188.0±7.6	187.0±5.4	0.950
BMI (kg·m^{-2})	29.2±2.8	30.0±2.5	29.8±3.2	0.321

The data are presented as the mean ± standard deviation. BMI: Body mass index. The p-value represents significance as determined by a one-way ANOVA test.

3.3.2 Tournament related data

Match outcomes for the 2011 and 2012 tournaments, as well as those for both tournaments combined, without taking into account home ground advantage, are presented in Table 3.2. The data represent that of all five SA teams combined. There were no significant differences in the number of matches won or lost in the 2011 or 2012 Super Rugby tournaments when analysed independently. Similarly, there were no significant differences in the number of matches won or lost in the combined 2011 and 2012 Super Rugby tournaments.

Table 3.2: Success of South African teams in the 2011 and 2012 Super Rugby tournaments.

Tournament	Won	Lost	p-value
2011	41 (50.6)	40 (49.4)	0.652
2012	45 (52.3)	41 (47.7)	0.792
Both	86 (51.5)	81 (48.5)	0.378

Data are presented as counts, with the percentages in parentheses. Significance was determined using a Chi-squared test.

3.3.2.1 Match outcomes: Effect of home ground advantage

Match outcomes for the 2011 and 2012 tournaments, as well as those for both tournaments combined, taking into account home ground advantage, are presented in Table 3.3. The data represent that of all five SA teams combined. No significant differences were observed in the number of matches won compared to matches lost by SA teams either when playing at home or when playing away in the 2011 and 2012 tournaments, or when data for both tournaments were combined (Table 3.3).

Similarly, the ratio of matches won: lost was similar for home vs away matches during the 2011 tournament. In contrast, significantly more matches were won compared to matches lost in the 2012 tournament for home versus away matches. Specifically, more matches were won when playing at home (p=0.018) compared to matches played away. However, the ratio of matches won: lost was similar for home versus away matches when the data for the 2011 and 2012 tournaments were combined.

Table 3.3: Effect of SA teams' home ground advantage in the 2011 and 2012 Super Rugby tournaments.

	Location	Won	Lost	p_1-value	p_2-value
2011	**Home**	17 (47.2)	19 (52.8)	0.542	0.584
	Away	24 (53.3)	21 (46.7)	0.674	
2012	**Home**	28 (65.1)	15 (34.9)	0.133	0.018
	Away	17 (39.5)	26 (60.5)	0.349	
Both	**Home**	45 (57.0)	34 (43.0)	0.476	0.181
	Away	41 (46.6)	47 (53.4)	0.562	

Data are presented as counts, with the percentages in parentheses. p_1: significance between matches won or lost when either playing at home alone or away alone, p_2: significance for matches won or lost between home and away. Significance was determined using a Chi-squared test.

3.3.2.2 Match outcomes: Effect of prior trans-meridian travel

Match outcomes of the 2011 and 2012 tournaments taking into account pre-match trans-meridian travel are presented in Table 3.4. The data represent that of all five SA teams combined. There were no significant differences in the number of matches won compared to matches lost by the SA teams following W-E travel, E-W travel or NTZD, in the 2011 tournament alone. Similarly, no significant differences were observed in the number of matches won compared to matches lost by the SA teams in the 2012 tournament, or when data for both tournaments were combined.

Table 3.4: Match outcomes of all SA teams during the 2011 and 2012 Super rugby tournaments - the effect of prior trans-meridian travel.

	W-E (n=19)		E-W (n=18)		NTZD (n=126)		p-value
	Won	Lost	Won	Lost	Won	Lost	
2011	5 (62.5)	3 (37.5)	1 (33.3)	2 (66.7)	14 (50.0)	14 (50.0)	0.875
2012	3 (27.3)	8 (72.7)	3 (60.0)	2 (40.0)	12 (41.4)	17 (58.6)	0.284
Both	8 (42.1)	11 (57.9)	4 (50.0)	4 (50.0)	26 (45.6)	31 (54.4)	0.937

Data are presented as counts with the percentages in parentheses. W-E: west-to-east travel, E-W: east-to-west travel, NTZD: no time zone difference. Significance was determined using a Fisher's exact test.

3.3.3. Game activity rate

3.3.3.1 *PER3* genotype and change in game activity (GA) rate with tournament progression

All GA rate data presented in this section represents the 2011 and 2012 tournaments combined. Figure 3.1 illustrates how GA rate changes with tournament progression. There was a significant change in GA rate over time during the combined 2011 and 2012 Super Rugby tournaments (Figure 3.1A). However, no significant differences were noted in the GA rate between the $PER3^{5/5}$ and $PER3^{4/4}$ VNTR groups as the tournament progressed (Figure 3.1B). Similarly, no significant differences were observed between the three *PER3* VNTR groups (Figure 3.1C).

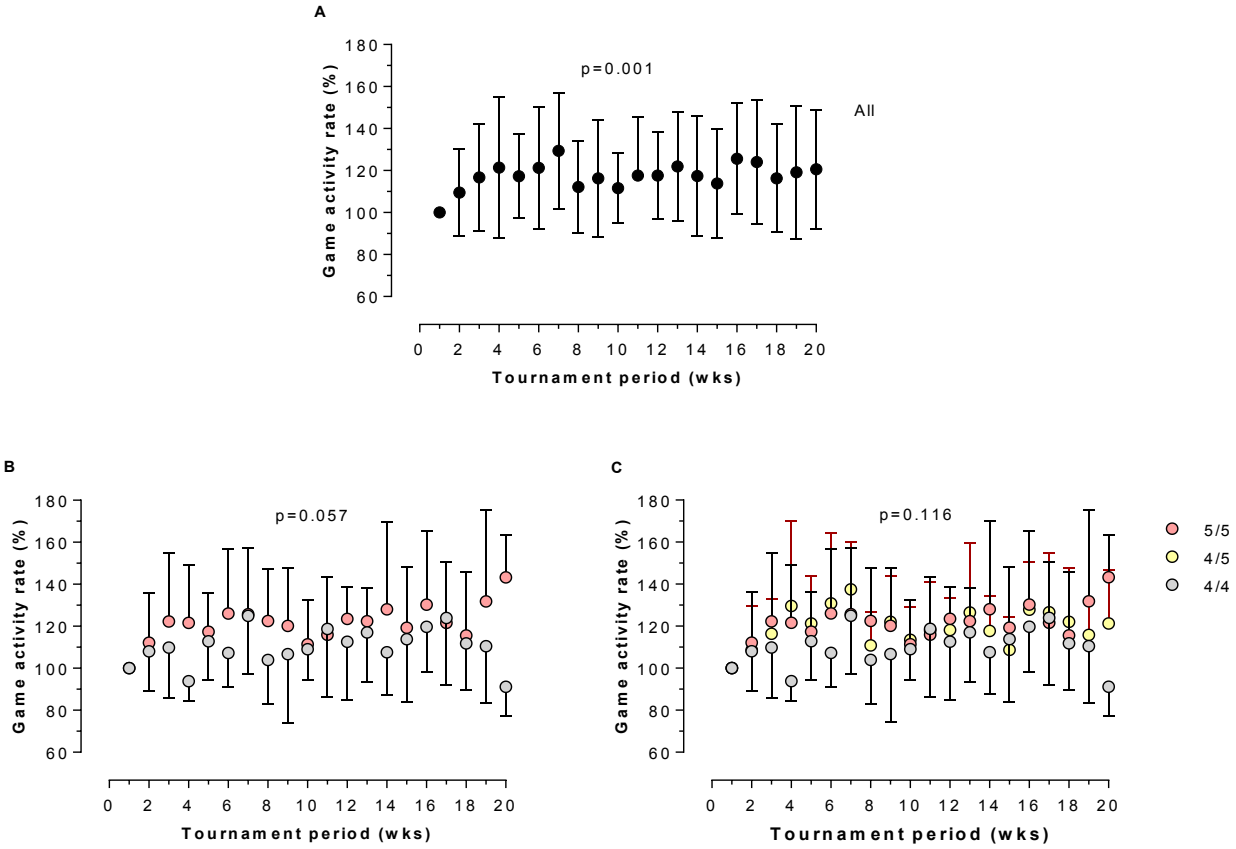


Figure 3.1: Change in player game activity rate with tournament progression. (A) All players (n=60), (B) *PER3*$^{5/5}$ (n=20) and *PER3*$^{4/4}$ (n=20) players and (C) all three genotype groups, *PER3*$^{4/4}$ (n=20), *PER3*$^{4/5}$ (n=20) and *PER3*$^{5/5}$ (n=20). Data are presented as mean ± SD. 4/4: *PER3*$^{4/4}$, 4/5: *PER3*$^{4/5}$ and 5/5: *PER3*$^{5/5}$. Significance was determined using Friedman's ANOVA test (A), an independent t-test (B) and one-way ANOVA test (C) to compare each group's area under the curve (AUC) values.

3.3.3.2 Direction of travel and change in player game activity rate

Changes in player GA rates grouped by direction of travel prior to the match are presented in Figure 3. 2. There were no significant differences between the three groups.

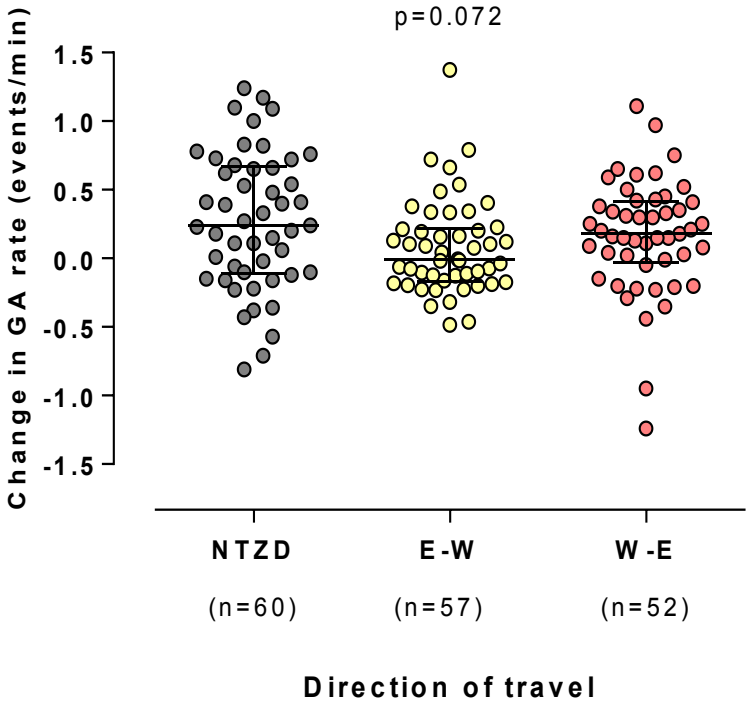


Figure 3.2: Change in game activity rate in players grouped by direction of travel prior to the match. Data are presented as median with IR and individual data points are plotted. NTZD: No time zone difference, E-W: Westward travel, W-E: Eastward travel. Significance was determined using Friedman's ANOVA test.

3.3.3.3 Number of time zones crossed and change in player game activity rate

Figure 3.3 shows change in GA rate in players grouped by number of time zones crossed (i.e. NTZD, 6-8 TZD and >10 TZD) immediately preceding a match. There were no significant differences between the three groups.

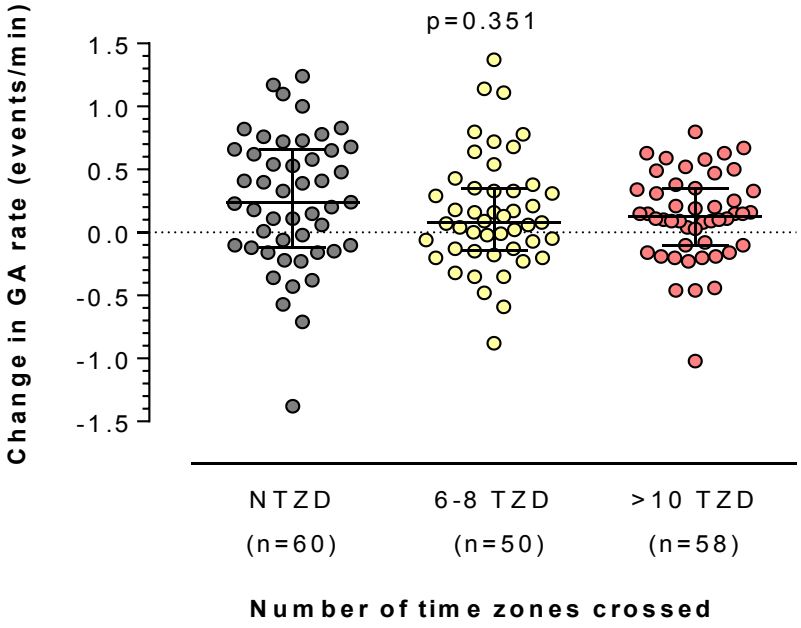


Figure 3.3: **Change in game activity rate in players grouped by number of time zones crossed prior to a match.** Data are presented as median with IR and individual data points are plotted. NTZD: No time zone difference, 6-8 TZD: Between six and eight time zone difference, >10 TZD: More than ten time zone difference. Significance was determined using Friedman's ANOVA test.

3.3.3.4 Direction of travel, number of time zones crossed and change in player GA rate

Change in GA rate grouped by direction of travel and number of time zones crossed prior to a match are presented in Figure 3.4. There were no significant differences between the two directions of travel (i.e. eastward or westward) when data from the 6-8 TZD and >10 TZD were combined for analysis (Figure 3.4A). Furthermore, no significant differences were noted between the two directions of travel following either 6-8 TZD (Figure 3.4B) or >10 TZD (Figure 3.4C) travel.

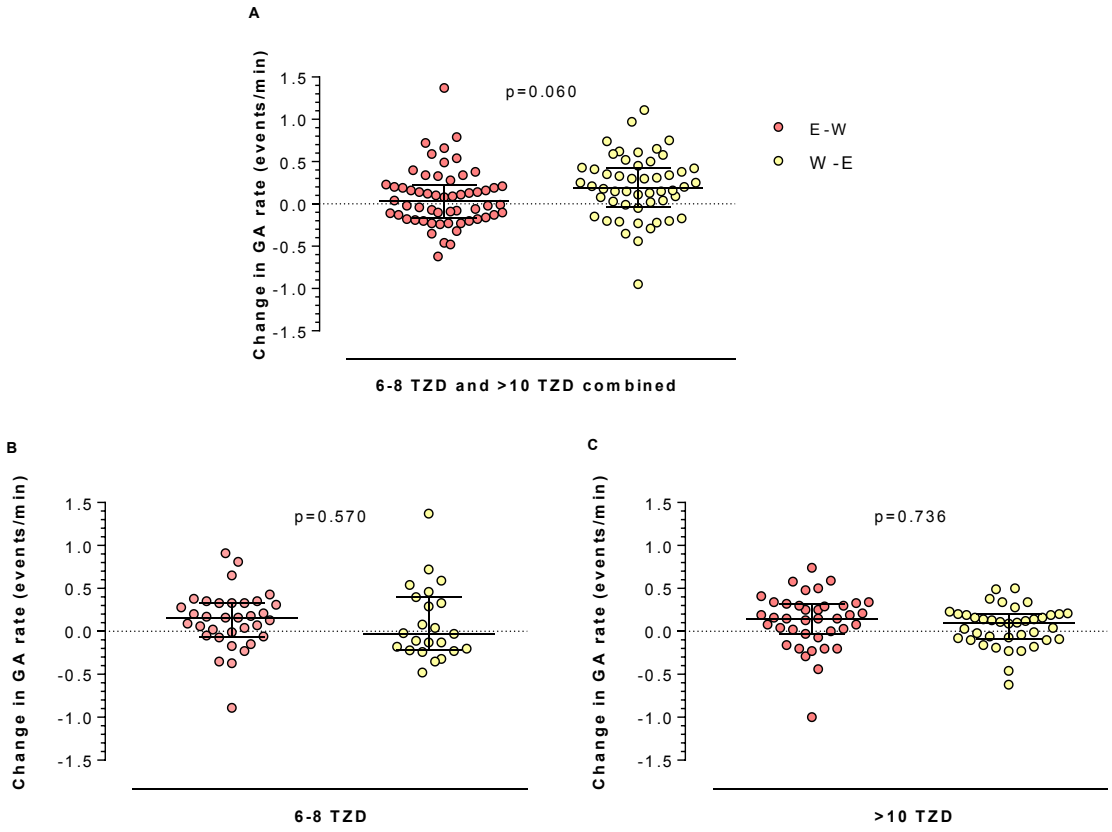


Figure 3.4: Change in player game activity rate grouped by direction of travel immediately prior to a match taking into account number of time zones crossed. Data are presented as median with IR and individual data points are plotted. (A) 6-8 and >10 TZD (time zone differences) combined, E-W (Westward travel): n=57, W-E (Eastward travel): n=52; (B) 6-8 TZD, E-W: n=24, W-E: n=35; (C) >10 TZD, E-W: n=39, W-E: n=39. Significance was determined using a Wilcoxon matched pairs test.

3.3.3.5 *PER3* VNTR genotype, direction of travel and player game activity rate

Change in player GA rate scores from one match to the next grouped by *PER3* VNTR genotype taking into account direction of travel are presented in Figure 3.5. There were no significant differences in the three genotype groups when the E-W or W-E travel data were combined for analysis (Figure 3.5A). Likewise, no significant differences were noted between the three genotype groups following either E-W or W-E travel (Figure 3.5B and Figure 3.5C, respectively).

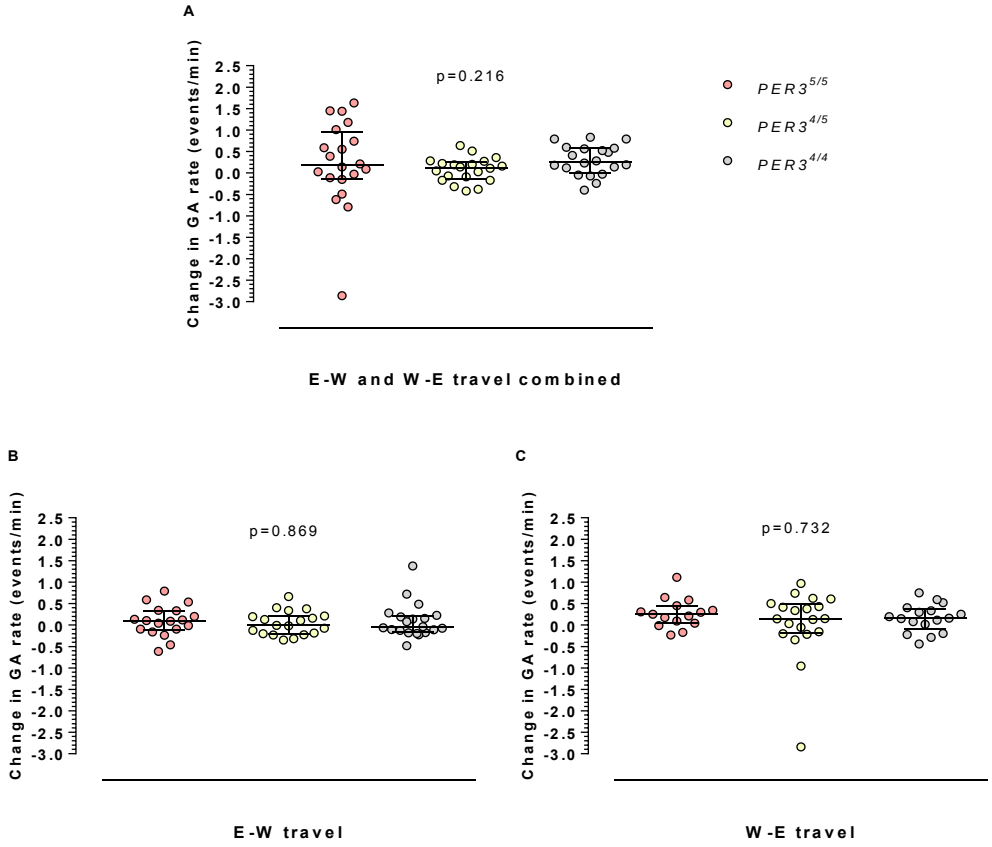


Figure 3.5: Change in game activity rate in players grouped by *PER3* VNTR genotype taking into account direction of travel prior to the match. Data are presented as median with IR and individual data points are plotted. (A) E-W travel and W-E travel combined: ($PER3^{5/5}$: n=20, $PER3^{4/5}$: n=20 and $PER3^{4/4}$: n=20); (B) E-W travel: ($PER3^{5/5}$: n=18, $PER3^{4/5}$: n=19 and $PER3^{4/4}$: n=20); (C) W-E travel: ($PER3^{5/5}$: n=15, $PER3^{4/5}$: n=17 and $PER3^{4/4}$: n=20). Significance was determined using the Kruskal-Wallis ANOVA test.

3.3.3.6 *PER3* VNTR genotype, number of time zones crossed and change in player GA rate.

Change in player GA rates from one match to the next grouped by *PER3* VNTR genotype following either NTZD, 6-8 TZD or >10 TZD travel are presented in Figure 3.6. There were no significant differences between the three *PER3* VNTR groups when the groups were combined for analyses (Figure 3.6A). Furthermore, no significant differences were noted between the three genotype groups after either travel across 6-8 TZD or >10 TZD travel (Figure 3.6B and Figure 3.6C, respectively).

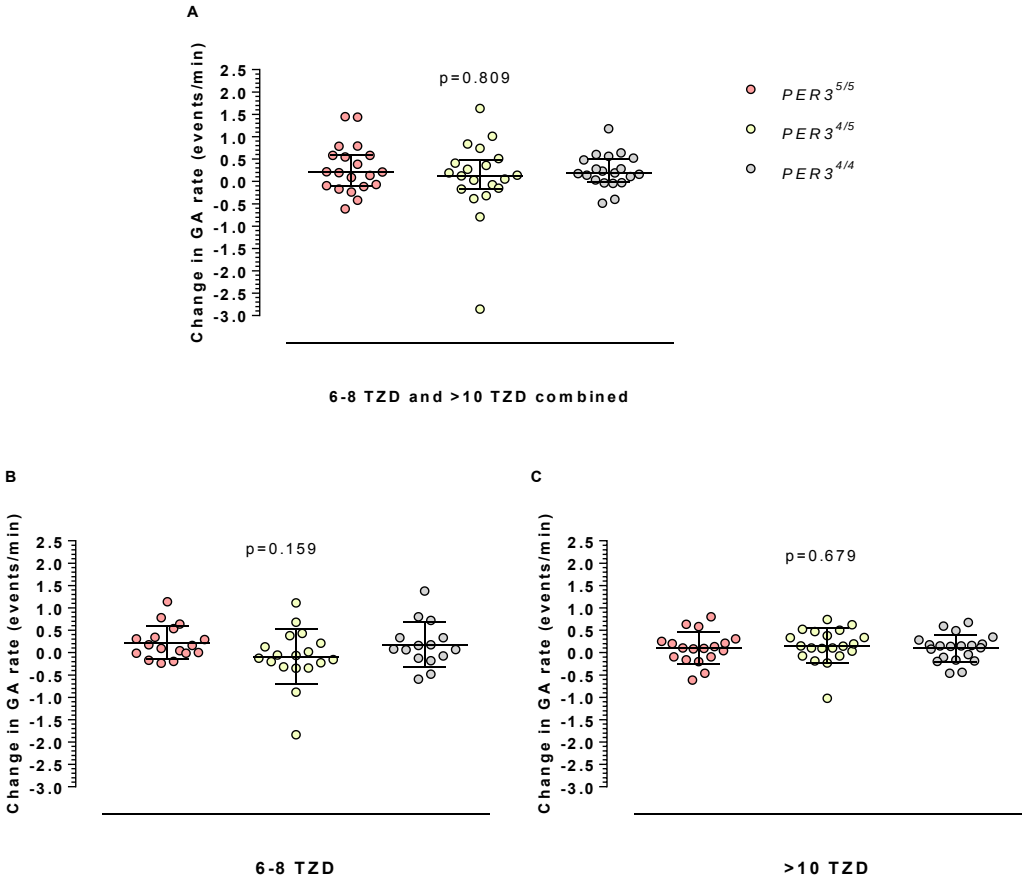


Figure 3.6: Change in game activity rate in players grouped by *PER3* VNTR genotype taking into account number of time zones crossed prior to the match. Data are presented as median with IR (A, C) or mean ± SD (B) and individual data points are plotted. (A) 6-8 and >10 time zone differences (TZD) combined: (*PER3*$^{5/5}$: n=20, *PER3*$^{4/5}$: n=20 and *PER3*$^{4/4}$: n=20); (B) 6-8 TZD: (*PER3*$^{5/5}$: n=17, *PER3*$^{4/5}$: n=18 and *PER3*$^{4/4}$: n=15); (C) >10 TZD: (*PER3*$^{5/5}$: n=18, *PER3*$^{4/5}$: n=20 and *PER3*$^{4/4}$: n=20). Significance was determined using one-way ANOVA and Kruskal-Wallis ANOVA tests.

3.3.3.7 *PER3* VNTR genotype, direction of travel, number of time zones crossed and change in player GA rate

Change in GA rate from one match to the next in players grouped by *PER3* VNTR genotype taking into account both direction of travel and number of time zones crossed is presented in Figure 3.7. There were no significant differences between the three *PER3* VNTR groups after 6-8 TZD travel in either westward or eastward travel (Figure 3.7A, Figure 3.7B). Furthermore, no

significant differences were noted between the three *PER3* VNTR groups for >10 TZD travel after E-W and W-E travel (Figure 3.7C and Figure 3.7D).

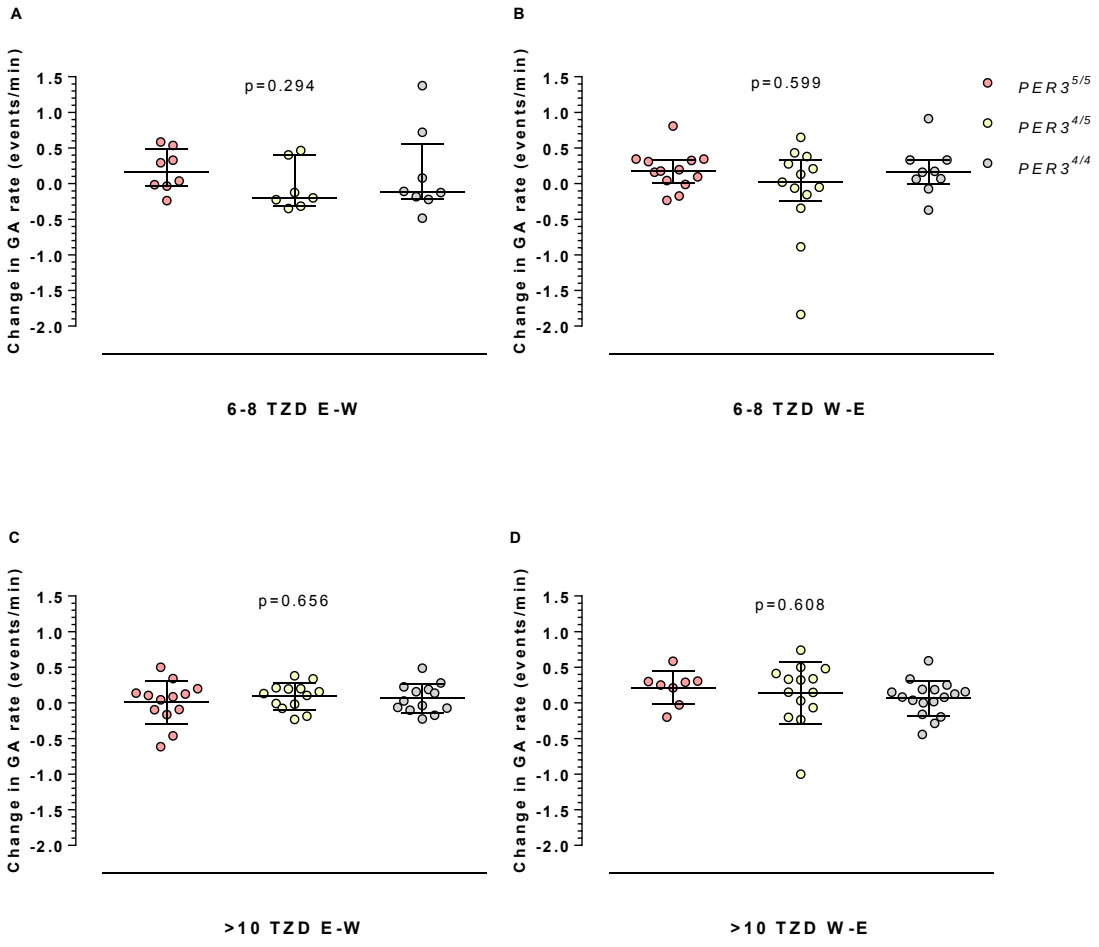


Figure 3.7: Change in game activity rate in players grouped by *PER3* VNTR genotype taking into account direction of travel and number of time zones crossed prior to the match. Data are presented as mean ± SD (C, D), median with IR (A, B) and individual data points are plotted. 6-8 TZD in (A) E-W travel: (*PER3*$^{5/5}$: n=8, *PER3*$^{4/5}$: n=7 and *PER3*$^{4/4}$: n=8) and (B) W-E travel: (*PER3*$^{5/5}$: n=13, *PER3*$^{4/5}$: n=13 and *PER3*$^{4/4}$: n=9); (C) More than ten time zone differences, E-W travel: (*PER3*$^{5/5}$: n=13, *PER3*$^{4/5}$: n=13 and *PER3*$^{4/4}$: n=13) and (D) W-E travel: (*PER3*$^{5/5}$: n=8, *PER3*$^{4/5}$: n=14 and *PER3*$^{4/4}$: n=17). Significance was determined using one-way ANOVA and Kruskal-Wallis ANOVA tests.

3.3.4 Quality of play data

3.3.4.1 *PER3* VNTR genotype and quality of play

All quality of play data presented in this section represents the 2011 and 2012 tournaments combined. Quality of play data in the three *PER3* VNTR groups of players regardless of direction of travel or number of time zones crossed are presented in Figure 3.8. There were no significant differences in the quality of play between the three *PER3* VNTR groups.

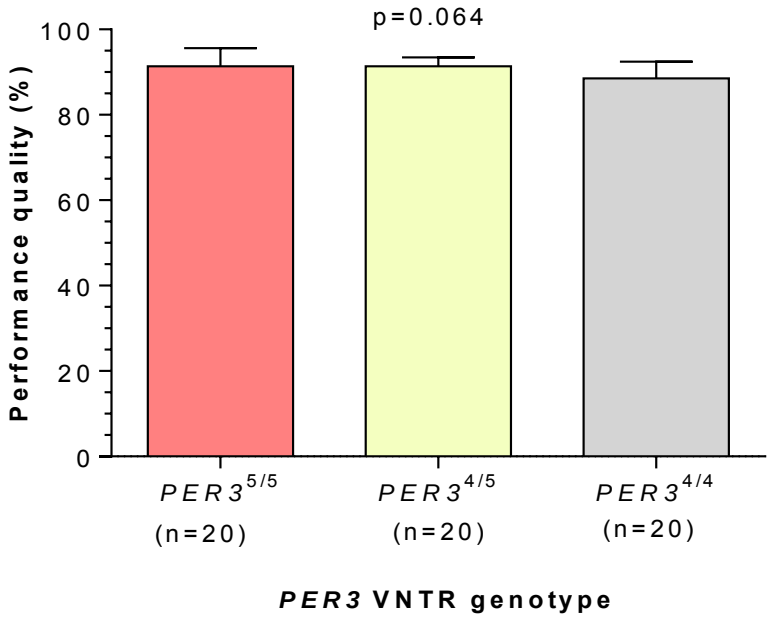


Figure 3.8: Quality of play in players grouped by *PER3* VNTR genotype. Data are presented as median with IR. Significance was determined using the Kruskal-Wallis ANOVA test.

3.3.4.2 Direction of travel and quality of play

Quality of play data in players grouped by direction of travel between matches preceded by either no time zone travel, eastward or westward travel are presented in Figure 3.9. Quality of play was significantly different between the three directions of travel (p=0.020). *Post-hoc* analysis indicated a lower quality of play following eastward travel compared to no time zone travel (p=0.024).

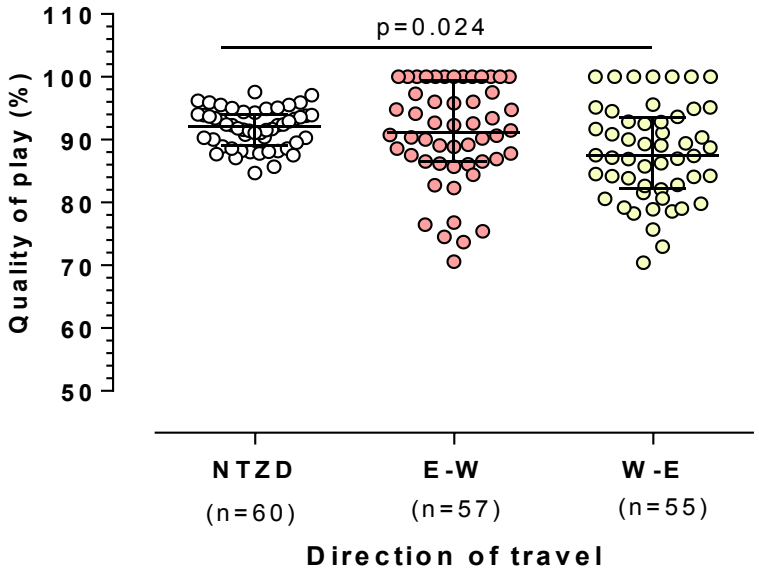


Figure 3.9: Player quality of play following either NTZD, E-W travel or W-E travel during the combined 2011 and 2012 Super Rugby tournaments. Data are presented as median with IR and individual data points are plotted. NTZD: No time zone difference, E-W: Westward travel, W-E: Eastward travel. Lines above the graph represent significant differences between groups at either end of the line. Significance was determined using Friedman's ANOVA test.

3.3.4.3 Number of time zones crossed and quality of play

Quality of play data in players grouped by number of time zones crossed prior to a match are presented in Figure 3.10. There were significant differences in the quality of play between the three groups (p=0.033). *Post-hoc* analysis indicated that quality of play was higher in the NTZD environment compared to >10 TZD travel (p=0.043).

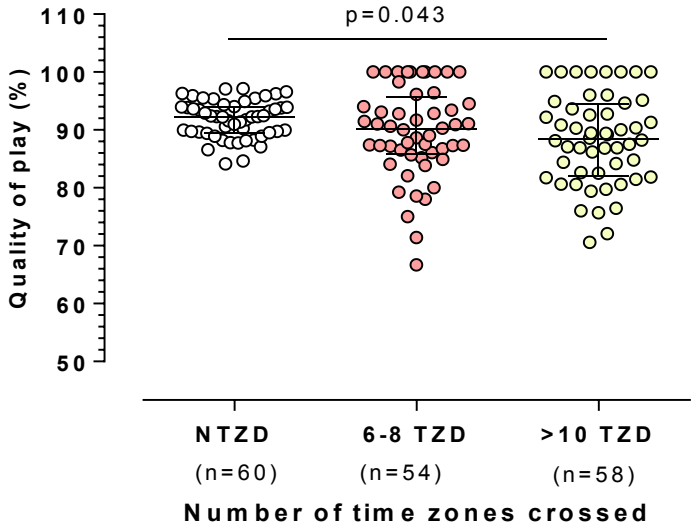


Figure 3.10: Quality of play in players grouped by the number of time zones crossed prior to the match. Data are presented as median with IR and individual data points are plotted. NTZD: No time zone difference, 6-8 TZD: Between six and eight time zone difference, >10 TZD: More than ten time zone difference. Lines above the graph represent significant differences between groups at either end of the line. Significance was determined using Friedman's ANOVA test.

3.3.4.4 Number of time zones crossed, direction of travel and quality of play

Quality of play data in players grouped by direction of travel taking into account number of time zones crossed prior to the match are presented in Figure 3.11. Quality of play was higher when players travelled west over more than six time zones compared to when they travelled east across the same number of time zones (p=0.031, Figure 3.11A). When analysed separately, there were no significant differences between E-W and W-E travel when only 6-8 time zones were crossed prior to the match (Figure 3.11B). However, when the players crossed >10 time zones quality of play was higher following E-W travel compared to W-E travel (p=0.040, Figure 3.11C).

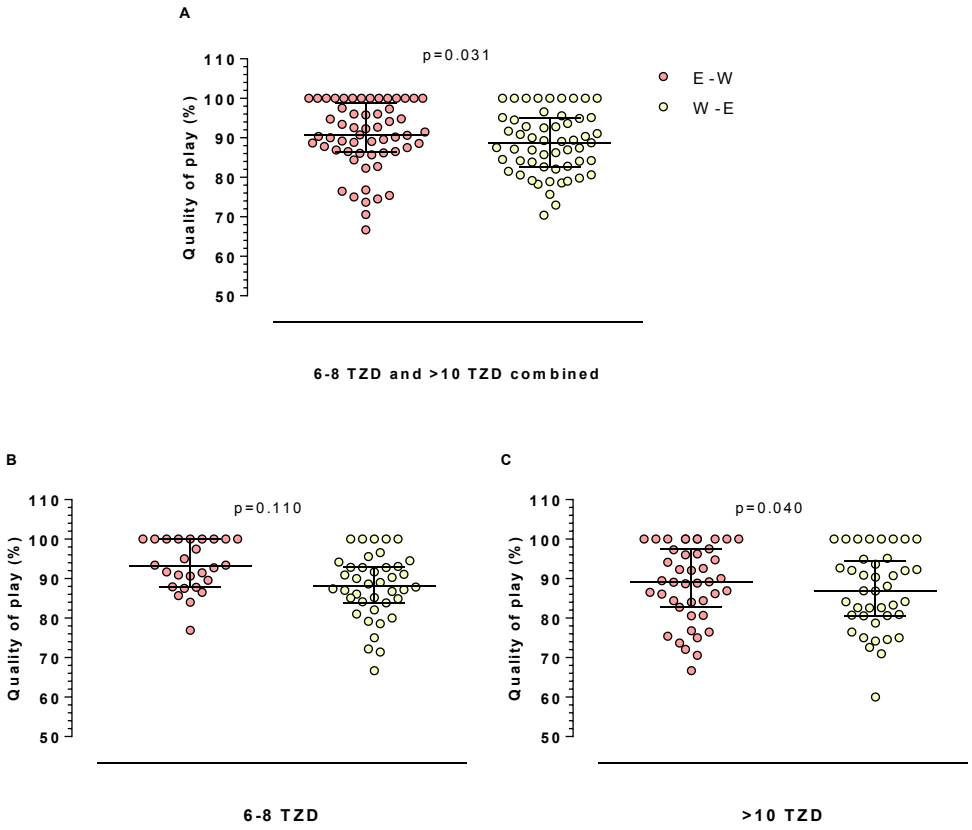


Figure 3.11: Quality of play in players grouped by direction of travel taking into account number of time zones crossed prior to the match. Data are presented as median with IR, and individual data points are plotted. (A) E-W travel: n=58, W-E travel: n=55; (B) E-W travel: n=40, W-E travel: n=26; (C) E-W travel: n=44, W-E travel: n=40. Significance was determined using a Wilcoxon matched pairs test.

3.3.4.5 *PER3* VNTR genotype, direction of travel and quality of play

Quality of play data in players grouped by *PER3* VNTR genotype taking into account direction of travel prior to the match are presented in Figure 3.12. Quality of play was significantly different between the three *PER3* VNTR groups when E-W and W-E travel were combined for analysis (p=0.035, Figure 3.12A). *Post-hoc* analysis indicated that quality of play was higher in the $PER3^{5/5}$ group compared to the $PER3^{4/4}$ group (p=0.033). However, no significant differences were noted following E-W travel (Figure 3.12B) and W-E travel (Figure 3.12C), respectively.

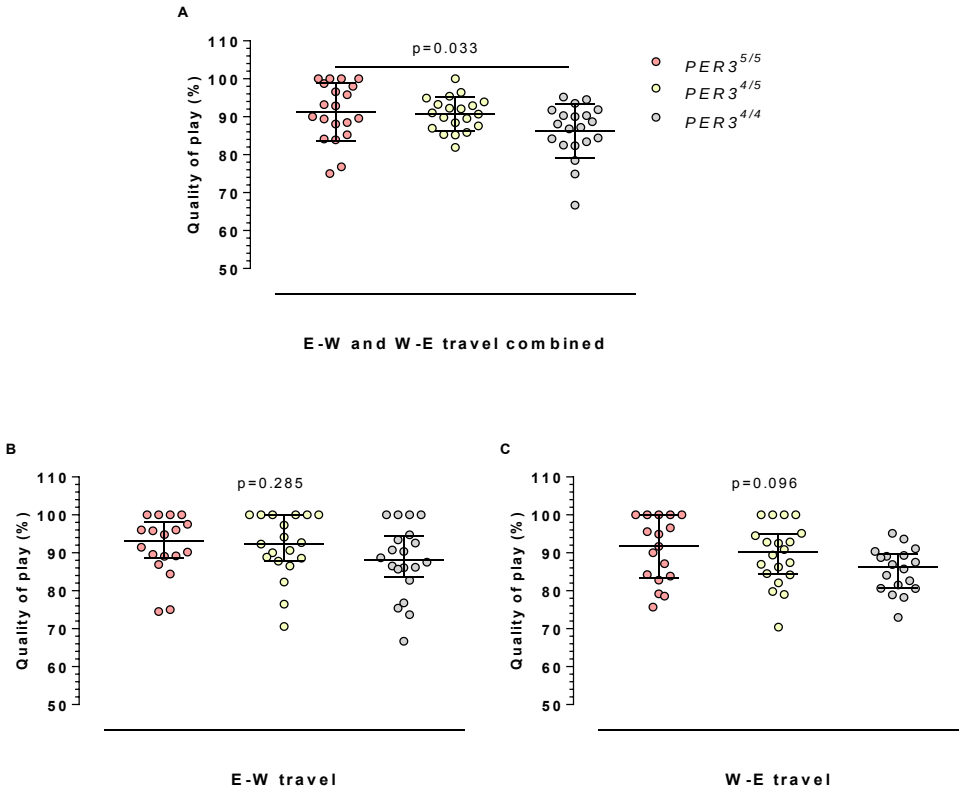


Figure 3.12: Quality of play in players grouped by *PER3* VNTR genotype taking into account direction of travel prior to the match. Data are presented as mean ± SD (A) and median with IR (B, C), and individual data points are plotted. (A) E-W and W-E travel combined: (*PER3*$^{5/5}$: n=20, *PER3*$^{4/5}$: n=20 and *PER3*$^{4/4}$: n=20); (B) E-W travel: (*PER3*$^{5/5}$: n=19, *PER3*$^{4/5}$: n=19 and *PER3*$^{4/4}$: n=20) and (C) W-E travel: (*PER3*$^{5/5}$: n=17, *PER3*$^{4/5}$: n=20 and *PER3*$^{4/4}$: n=18). Lines above the graph represent significant differences between groups at either end of the line. Significance was determined using one-way ANOVA and Kruskal-Wallis ANOVA tests.

3.3.4.6 *PER3* VNTR genotype, number of time zones crossed and quality of play

Quality of play data grouped by *PER3* VNTR genotype taking into account number of time zones crossed prior to the match are presented in Figure 3.13. There were no significant differences in the three *PER3* VNTR groups when data were combined for analysis (Figure 3.13A). While there were no significant differences in the quality of play between the three genotype groups for 6-8 TZs crossed (Figure 3.13B), significant differences were noted for >10 TZs crossed (p=0.039,

Figure 3.13C). *Post-hoc* analysis indicated a higher quality of play in the *PER3*$^{5/5}$ (p=0.030) and *PER3*$^{4/5}$ (p=0.026) groups compared to the *PER3*$^{4/4}$ group.

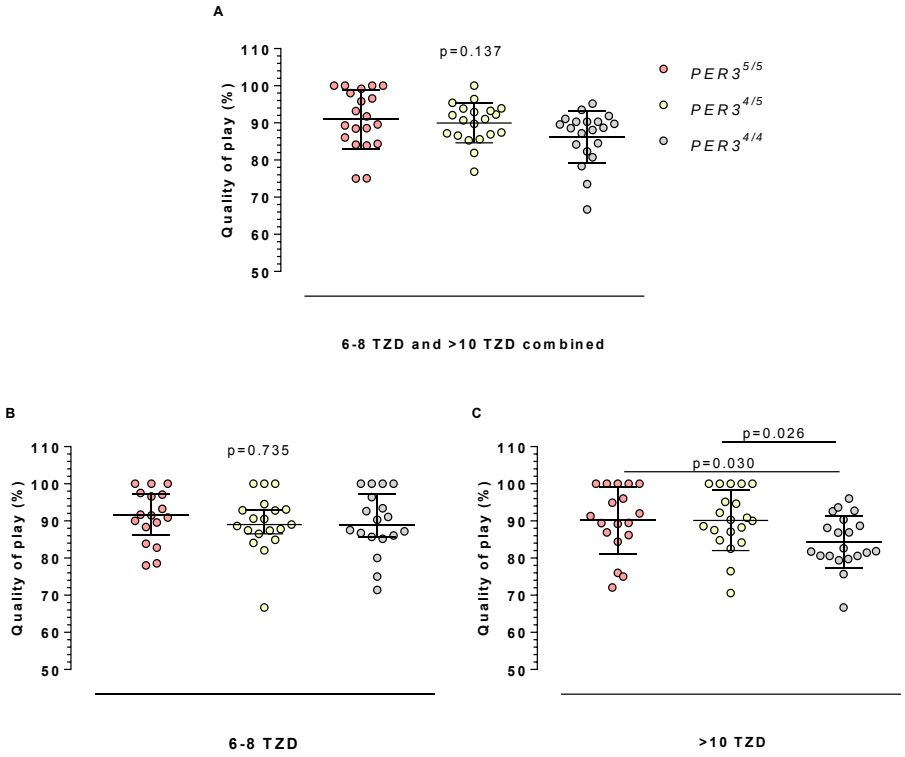


Figure 3.13: **Quality of play in players grouped by *PER3* VNTR genotype taking into account number of time zones crossed prior to the match.** Data are presented as mean ± SD (A, C), median with IR (B) and individual data points plotted. (A) 6-8 and >10 time zone differences (TZD) combined: (*PER3*$^{5/5}$: n=18, *PER3*$^{4/5}$: n=20 and *PER3*$^{4/4}$: n=20); (B) 6-8 TZD: (*PER3*$^{5/5}$: n=17, *PER3*$^{4/5}$: n=19 and *PER3*$^{4/4}$: n=18); (C) >10 TZD: (*PER3*$^{5/5}$: n=18, *PER3*$^{4/5}$: n=20 and *PER3*$^{4/4}$: n=20). Lines above the graph represent significant differences between groups at either end of the line. Significance was determined using one-way ANOVA and Kruskal-Wallis ANOVA tests.

3.3.4.7 *PER3* genotype, number of time zones crossed, direction of travel and quality of play

Quality of play data in players grouped by *PER3* VNTR genotype taking into account both direction of travel and number of time zones crossed prior to the match are presented in Figure 3.14. There were no significant differences between the three genotype groups when players crossed 6-8 time zones and direction of travel prior to the match was taken into account (Figure 3.14A and

Figure 3.14B). However, there were significant differences between the three groups after E-W travel across more than ten time zones (p=0.047, Figure 3.14C). *Post-hoc* test indicated a higher quality of play in the *PER3*$^{5/5}$ (p=0.026) and *PER3*$^{4/5}$ (p=0.041) groups compared to the *PER3*$^{4/4}$ group. No significant differences were noted after W-E travel across >10 time zones.

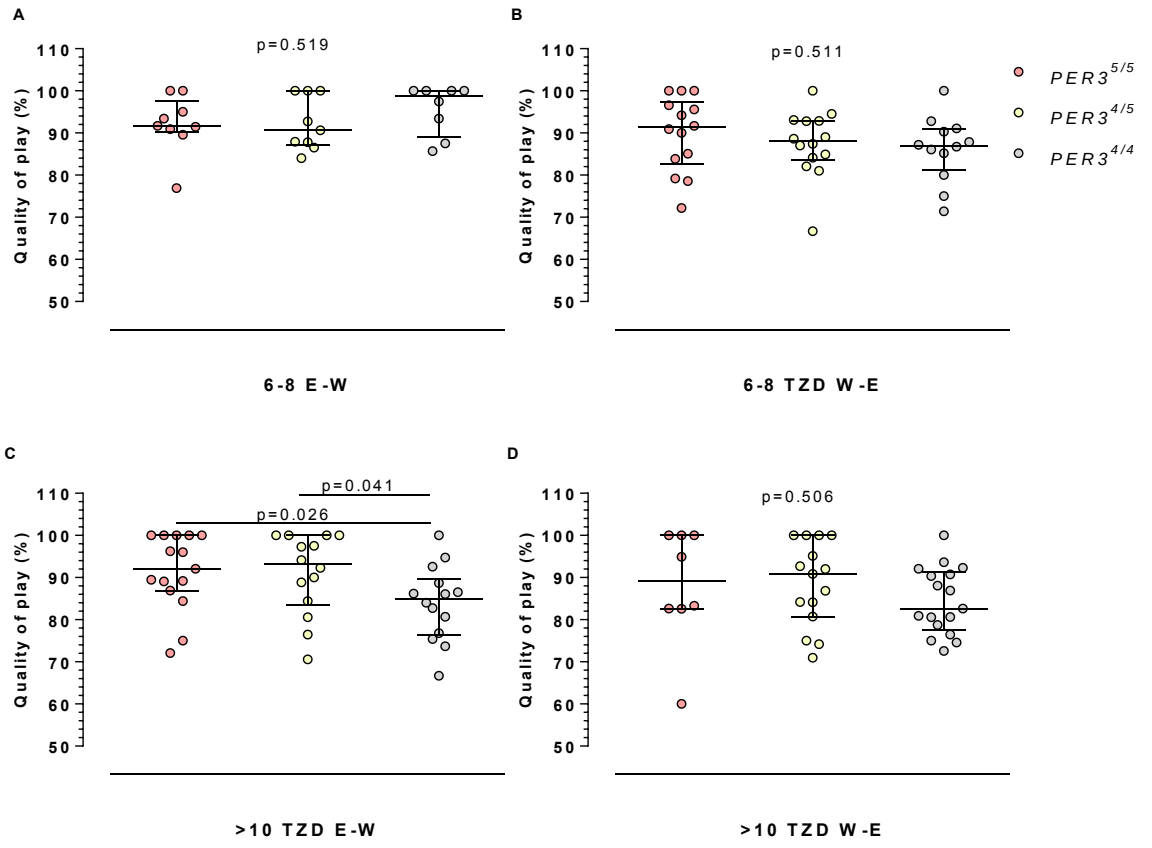


Figure 3.14: Quality of play in players grouped by *PER3* VNTR genotype taking into account direction of travel and number of time zones crossed prior to the match. Data are presented as median with IR and individual data points are plotted. (A) 6-8 time zone differences (TZD), E-W travel: (*PER3*$^{5/5}$: n=9, *PER3*$^{4/5}$: n=9 and *PER3*$^{4/4}$: n=8); (B) W-E travel: (*PER3*$^{5/5}$: n=14, *PER3*$^{4/5}$: n=14 and *PER3*$^{4/4}$: n=12); (C) **>**10 TZD, E-W travel: (*PER3*$^{5/5}$: n=15, *PER3*$^{4/5}$: n=14 and *PER3*$^{4/4}$: n=14); (D) W-E travel: (*PER3*$^{5/5}$: n=7, *PER3*$^{4/5}$: n=15 and *PER3*$^{4/4}$: n=17). Lines above the graph represent significant differences between groups at either end of the line. Significance was determined using a Kruskal-Wallis ANOVA test.

3.4 Discussion

The purpose of the present study was to determine whether *PER3* VNTR genotype moderates the effects of trans-meridian travel on player game involvement and quality of play in Super Rugby players. Specifically, the change in game activity (GA- a marker of involvement) rate and quality of play in players grouped by *PER3* VNTR genotype taking into account direction of travel and number of time zones crossed prior to a match were investigated. The extent to which players were involved in a game did not change when grouped by number of time zones crossed or direction of travel. This could have been because physically active people were used, and GA rate only measures the extent of involvement/participation rather than the quality of play, which is more likely to be indicative of any detrimental effects of time zone travel.

3.4.2 Changes in player quality of play taking into account number of time zones crossed and direction of travel prior to the match.

An important finding was that irrespective of *PER3* VNTR genotype quality of play was worse following both eastward travel and travel across >10 time zones compared to no time zone travel (Figure 3.9 & Figure 3.10). This indicates that the magnitude and direction of travel were important, with quality of play deteriorating as the number of time zones crossed increased, particularly in an eastward direction. Resynchronisation into the new environment was not measured in this study. In spite of this players were likely to have been jet-lagged, thus they may have suffered reductions in physical and mental capacity. It may be that jet-lag effects manifested themselves through a worsening quality of play. This is conceivable given that mood, cognitive and neuromuscular functions are understood to deteriorate significantly following sleep and circadian disruption (Golombek et al., 2013, Reilly et al., 2005, Waterhouse et al., 2005b, Davenne, 2009), similar to that which may occur following trans-meridian travel. However, factors other than circadian disruption and jet-lag may have changed quality of play such as opponent quality, playing out of position, tournament fatigue and homesickness.

While sleep was not measured in this study, speculation on its role in jet-lag adaptation will be made. The extent to which sleep is disrupted following time zone travel differs depending on the

direction of travel. Specifically, sleep episodes shift to an earlier time following eastward travel and to a later time following westward travel. Sleep quality may deteriorate following eastward travel because the sleep episode is phase advanced, which makes it difficult to initiate and maintain sleep at night in the new environment (Waterhouse and Reilly, 2009). Perhaps team physicians may want to schedule travel to AUS/NZ at night so that arrival in the new time zone is in the afternoon (preferably after 3pm), which could make a phase advance easier making use of exposure to outdoor light for a few hours before nighttime approaches. This may make it easier for individuals to sleep, as they may be fatigued from the journey. As a result, circadian rhythms in grip strength (Hill et al., 1993, Reilly et al., 2001), leg power (Chapman et al., 2012), body temperature (Miyazaki et al., 2002), muscle strength and flexibility (Sedliak et al., 2008, Drust et al., 2005), sprint and anaerobic efforts (Chtourou et al., 2012, Giacomoni et al., 2006), which influence performance may be less compromised compared to when travel is scheduled at other times of the day.

The above-mentioned physiological measures may however, be compromised following trans-meridian travel because biological timing for peak performance is out of synchronisation with local time at destination venue. Numerous physiological measures of performance peak when core body temperature is at its peak, which is usually in the late afternoon to early evening (Reilly et al., 2007, Reilly and Down, 1992, Hill and Smith, 1991). A significant proportion of Super Rugby matches were played between 14h00-17h00 local time. Between 14h00-17h00 New Zealand local time is equivalent to 04h00-06h00 in South Africa if resynchronisation has not yet occurred, which corresponds to the core body temperature nadir. It is therefore possible that incomplete resynchronisation could have influenced quality of play by reducing aspects of performance such as grip strength, reaction time and flexibility, due to differences in core body temperature.

Quality of play was lower for all players irrespective of *PER3* VNTR genotype after eastward compared to westward travel, only for travel across >10 time zones (Figure 3.11), however, there is great inter-individual variation. This further supports the notion that eastward travel is deleterious to the quality of play when the magnitude of time zone travel approaches the limit

of entrainment (10-12 time zones). This implies that players had not synchronised to the new time zone following eastward travel to the extent they had when playing after westward travel. This is conceivable given that individuals undergoing eastward travel tend to experience worse symptoms of jet-lag compared to westward travel including failure to maintain sleep at night and poor neuromuscular performance (Sack, 2010, Reilly and Edwards, 2007, Eastman and Burgess, 2009). Likewise, symptoms of jet-lag persist for longer and require a lengthier time for synchronisation after eastward compared to westward travel (Winget et al., 1984, Lemmer et al., 2002, Meir, 2002), due to the circadian clock's inability to quickly adjust by phase advance.

Findings in this study support that direction of travel and magnitude of time zone travel influence quality of play in the Super Rugby tournament. This poses a challenge to teams in the Super Rugby tournament who may not always have enough time to resynchronise into the new environment before match kick-off. It would have been ideal to analyse GA rate and quality of play data following W-E travel or E-W travel taking into account whether a team travelled to play a home or away match. However, this was not possible as there were no home matches for SA teams following W-E travel. Likewise, the grouping by W-E travel or E-W travel taking into account home or away matches would have reduced the sample size in each group. This would have subsequently made it difficult to interpret the findings due to small sample sizes potentially reducing the statistical power and leading to a type 1 statistical error.

3.4.3 Changes in quality of play in players grouped by genotype taking into account number of time zones crossed and direction of travel.

The three genotype groups had similar quality of play irrespective of direction of travel or number of time zones crossed (Figure 3.8), indicating that there were no starting differences in quality of play. However, differences in play quality were evident in the three genotypes following W-E travel or E-W travel (Figure 3.12A) regardless of number of time zones crossed, with quality of play lower in the *PER3*$^{4/4}$ group compared to the *PER3*$^{5/5}$ group. In particular, *PER3*$^{5/5}$ individuals have been reported to have better cognitive throughput (measured using a digit substitution test) and subjective sleepiness responses compared to the other two *PER3* genotypes, across five

nights during a partial sleep deprivation study (Goel et al., 2009). This may indicate that *PER3*$^{5/5}$ individuals are less vulnerable to jet-lag and thus quality of play was not as badly affected in this group.

While quality of play was not different between the three genotype groups after W-E or E-W travel when fewer than six time zones were crossed, it was different between the three genotype groups when more than ten time zones were crossed. Specifically, the *PER3*$^{4/4}$ group had a lower quality of play compared to the *PER3*$^{4/5}$ and *PER3*$^{5/5}$ groups when more than ten time zones were crossed (Figure 3.13C). Similarly, quality of play was lower in the *PER3*$^{4/4}$ group compared to the *PER3*$^{4/5}$ and *PER3*$^{5/5}$ groups following westward travel across more than ten time zones (Figure 3.14C). This suggests that *PER3*$^{4/5}$ and *PER3*$^{5/5}$ groups might have found it easier to adapt to the new environment or it could be that the *PER3*$^{4/4}$ group may have remained jet-lagged during matches reducing their capacity to perform well.

The exact mechanism by which *PER3*$^{4/5}$ and *PER3*$^{5/5}$ genotypes may use to adapt to the new environment is not known. However, it's possible that individuals carrying a single *PER3* 5-repeat allele may be able to utilise external time cues, for example, outdoor light in the new time zone faster, given that the *PER3*$^{5/5}$ genotype was reported to be more sensitive to blue-light (Chellappa et al., 2012). Super Rugby matches were not played immediately upon arrival in the new time zone. As such, high quality of play in the *PER3*$^{4/5}$ and *PER3*$^{5/5}$ groups compared to the *PER3*$^{4/4}$ group following time zone travel further suggests that carrying a single 5-repeat allele might have facilitated faster adaptation to the new time zone. There are however, no similar studies in literature with which to directly compare these findings. Therefore, further research in a competition environment, with larger sample sizes, across seasons and in multiple sports should be performed, to confirm this finding.

3.4.4 Match outcomes - effect of home ground advantage

A minor finding was that home ground advantage was evident for matches in the 2012 Super Rugby tournament, but not in the 2011 or the combined 2011 and 2012 Super Rugby tournaments. In the 2012 tournament, teams won 65% of their home matches, which is similar to the 73% found in the tri-nations Rugby Union and the 63% in the 2010 Super 12 Rugby tournament (Morton, 2006). This confirms that home ground advantage exists in the Super Rugby tournament, which is in line with previous studies (Du Preez and Walpole, 2004, Du Preez and Lambert, 2007). Home ground advantage has been noted in other sports such as soccer (Pollard, 2006), American Football (Pollard and Pollard, 2005, Watson and Krantz, 2003), baseball (Bray et al., 2005) and field hockey (Carre et al., 2006). One explanation for the lack of evidence for home ground advantage during the 2011 tournament might be that SA Super Rugby teams rested their key national team players. This might have been done to keep these players fresh for the Rugby World Cup tournament played later that year, which means that teams may not have had depth in their squads, thereby losing home ground advantage.

3.4.5 Match outcomes - effect of prior trans-meridian travel

Match outcomes were similar for matches played after eastward or westward travel and matches played after no time zone travel. This suggests that although players may have been at a disadvantage when undergoing eastward or westward travel due to the effects of jet-lag, match outcomes were not affected by eastward or westward travel. This result is in line with previous studies which reported that direction of travel accounted for less than 1% of the variance in match outcomes (Du Preez and Lambert, 2007, Goumas, 2014, Nevill et al., 1996). Some studies have however demonstrated a direction of travel effect on match outcomes, although these findings are contrasting (Jehue et al., 1993, Smith et al., 1997, Steenland and Deddens, 1997, Nutting, 2010, Winter et al., 2007, Nichols, 2012). Nichols (2012) for example, found that West Coast teams won 17% of their games after travelling to the East Coast, while East Coast teams won 53% of their matches after travelling to the West Coast during the 2008 NFL tournament. In contrast, Smith et al. (1997) reported that travelling westward is worse than travelling eastwards for NFL athletes. The variances in these findings are likely due to match kick off times. For

example, studies which indicated a lower percentage of matches won following westward travel, was typically matches which took place at night, and thus close to the visiting team's home bed-time.

Those studies, which reported lower percentages of matches won after eastward travel, were matches played in the early afternoon, which is the visiting team's home morning time. While these studies demonstrated a direction of travel effect, data from this study exhibit no measurable effect of eastward or westward travel in comparison to no time zone travel in terms of match outcomes. In a very long tournament such as the Super Rugby competition, extrinsic factors may have masked any effect of direction of travel on match outcome. These may include unequal number of matches played in a given travel direction, changes in squad size composition due to injury, stage of tournament (e.g. knock-out or round robin) and strength of opposition. Other factors that may influence a team performance on a particular day include tactical strategies of play, weather conditions (e.g. humidity and temperature), jet-lag, motivation, will to win, pressures from family, friends and media as well as an individual's personality, just to mention a few.

3.4.6 Limitations and future studies

A limitation of this study was that situational (e.g. opponents, weather conditions) or behavioural variables (e.g. mood, anxiety, arousal level) were not taken into account when analysing GA rate and quality of play data. These are difficult to account for since they are constantly changing, however, it will be interesting if a future study could consider this. Likewise, the strength of the opposition and position on the combined Super Rugby log was not taken into account. This would have provided a full account of the opponents as this is one of the factors that influence GA. Players in this study did not play in all matches following trans-meridian travel. This limited the sample sizes when GA rate and quality of play data were explored by number of time zones crossed or direction of travel.

The effect of the *PER3* VNTR genotype on trans-meridian travel and performance was indirectly determined. Perhaps in future a study with greater temporal resolution or planned times of sampling for example, 24h, 48h and 72h post travel or over the course of the new day in the new time zone for the first week would be warranted so that entrainment can be assessed more dynamically.

3.5 Conclusion

An individual's *PER3* VNTR genotype may explain some of the differences in quality of play in Super Rugby players after eastward and westward travel. The quality of play was lower in the $PER3^{4/4}$ group compared to the $PER3^{4/5}$ and $PER3^{5/5}$ groups following W-E travel or E-W travel, especially when the magnitude of travel was more than ten time zone crosse, suggesting that the *PER3* genotype may have had an impact on quality of play. It also suggests that the *PER3* VNTR genotype may play a role in entrainment through differential susceptibility to jet-lag effects. Perhaps the *PER3* genotype may mediate changes in the quality of play, which varied between the three genotypes through differential ability to resynchronise into the new environment. However, clock genes other than *PER3* or non-clock genes may confer susceptibility or create tolerance to the effects of jet-lag, subsequently influencing game activity rate.

The quality of play was worse after eastward compared to westward travel, suggesting that players had not synchronised to the new time zone after eastward travel to the same extent they had following westward travel. This implies that the magnitude and direction of travel are both important and should be considered when preparing for matches in the Super Rugby tournament. It is possible that implementing appropriate jet-lag alleviation strategies, such as timed exposure to light (Vosko et al., 2010, Fowler et al., 2015b), and use of external melatonin (Paul et al., 2011, Edwards et al., 2000) to contain the effects of jet-lag may enhance performance following time zone travel. This is the first study to investigate the *PER3* VNTR genotype with respect to adaptation to new time zones in a team sport of this magnitude and its effects on performance. Further research with larger sample sizes (especially during periods of international travel), ideally over multiple seasons and in other sports is warranted to confirm these findings.

CHAPTER 4: A COMPARISON OF THE EFFECT OF TRANS-MERIDIAN TRAVEL ON THE INCIDENCE OF ILLNESS AND INJURY IN SOUTH AFRICAN SUPER RUGBY PLAYERS GENOTYPED AS *PER3*$^{4/4}$, *PER3*$^{4/5}$ AND *PER3*$^{5/5}$.

4.1 Introduction

Trans-meridian travel is a challenge for athletes since it results in circadian disruption imparting a physical and emotional burden in the form of jet-lag. Therefore, team physicians try to ensure that such travel occurs with minimal disruption to health. As part of protecting an athlete's health, prevention of illnesses and injuries are key mandates for World Rugby. Appreciation of the significance of illness and injury surveillance studies is growing, with many governing bodies such as the International Association of Athletics Fédération and the Fédération Internationale de Football Association also regularly carrying out illness and injury epidemiological projects (Dvorak et al., 2011, Fuller et al., 2013, Schwellnus et al., 2013). Understanding factors that may increase or decrease a player's risk for illness or injury in the context of trans-meridian travel may be fundamental to team physicians for future planning during competitions such as the Super Rugby tournament. Specifically, knowledge from this research may provide data that will facilitate prevention initiatives to be properly arranged.

If it is possible to reduce the number and impact of illnesses and injuries experienced by players undergoing trans-meridian travel, some of the harmful effects to player health, performance limitations and time loss to training and competition may be reduced. This in turn could affect player performance, not just in the rugby population, but in other sports as well. Previous studies have reported that trans-meridian travel across multiple time zones negatively influences the incidence of illnesses and injuries in athletes with athletes who travelled across ≥5 time zones having a greater risk of getting ill and/or injured compared to those competing domestically (Schwellnus et al., 2012, Fuller et al., 2015). While individuals appear to cope differently, no individual is immune to the physiological and cognitive effects of jet-lag (Sack, 2010, Eastman and Burgess, 2009). Perhaps some aspect of an individual's circadian system might be key to understanding individual differences in susceptibility or tolerance to jet-lag.

The differences in susceptibility or tolerance to jet-lag may arise due to differences that have been reported in sleep pressure and cognitive performance between *PER3*$^{4/4}$ and *PER3*$^{5/5}$ individuals following sleep deprivation (Maire et al., 2014, Viola et al., 2007). Likewise, differences in the extent of sleep disruption may lead to altered hormonal levels and immune functions between the two *PER3* genotypes. The two genotype groups may also entrain to new light/dark cycles differently due to the differences in sensitivity to blue light suppression of melatonin in *PER3*$^{4/4}$ and *PER3*$^{5/5}$ genotypes (Chellappa et al., 2012a). Players genotyped as *PER3*$^{5/5}$ were compared to those genotyped as *PER3*$^{4/4}$ and to those heterozygous for the 4- and 5-repeat alleles. The aim of this study, therefore, was to compare the impact of time zone travel during the 2012 Super Rugby competition in South African players genotyped as *PER3*$^{4/4}$, *PER3*$^{4/5}$ and *PER3*$^{5/5}$ on the incidence of illnesses and injuries.

The objectives were to:
(1) Describe the incidence of illnesses and injuries in the three *PER3* VNTR genotype groups, and
(2) Compare these incidences taking into account direction of travel (i.e. west-to-east or east-to-west) and the number of time zones crossed (i.e. two or less and six or more time zones).

It was hypothesised that the risk of illness or injury would be higher in the *PER3*$^{4/4}$ genotype than the other two *PER3* genotype groups following time zone travel. Furthermore, it was hypothesised that illness and injury incidence rates would increase as the magnitude of time zone travel increases, and following west-to-east travel compared east-to-west travel.

4.2 Methods

4.2.1 Participants

Participants for this study comprised players from the five SA teams who participated in the 2012 Super Rugby tournament. The players (n=145) descriptive characteristics are presented in Table 4.1.

4.2.2 Study design

In this observational study, the illness and injury rates of three groups of players (*PER3*$^{4/4}$, *PER3*$^{4/5}$ and *PER3*$^{5/5}$) were compared when players travelled Westwards (E-W), Eastwards (W-E), and when there was no travel across time zones (NTZ) between matches. Team physicians for the five SA Super Rugby teams recorded illness and injury data for all players in their respective squads on a daily basis throughout the 2012 tournament. The tournament period included the week prior to the start of the first game and the day after the last match for each team. Each team physician collected this data using standardised daily medical illness and injury report forms, which were in electronic format (Appendices 4C and 4D). The team physician acquired the data through clinical assessment, kept the data secure and only submitted the completed report forms to the Division of Exercise Science and Sports Medicine, Department of Human Biology, University of Cape Town at the end of the tournament. There was full compliance on the return of completed data capture forms. Prior to commencement of the 2012 Super Rugby tournament, all participants gave written informed consent (Appendix 4B). The study was performed in accordance with the principles of the Declaration of Helsinki (Fortaleza, Brazil 2013), the International Conference on Harmonisation and South African Good Clinical Practice guidelines. Only the illnesses and injuries of individuals who gave written informed consent to participate were monitored (145 of 200 players). The study was approved by the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee (HREC Ref No: 008/2011).

4.2.3 Detailed testing procedures

4.2.3.1 Chronotype, genotype, match and travel schedules

Chronotype and genotype methods were described in Chapter 2, sections 2.2.3.1, 2.2.3.2 and 2.2.3.3. Match and travel schedules are described in Chapter 3, section 3.2.4.2.

4.2.3.2 Illnesses and injuries

For the purposes of this study, a medical illness was defined as any non-trauma related symptom or sign presenting in an individual that required medical attention from the team physician on a particular day (Schwellnus et al., 2012). Likewise, a medical injury was defined as any physical

complaint, ensuing from the transfer of energy that exceeded the body's ability to uphold its structural and/or functional integrity that was sustained by an individual during a match, training or gym session and required medical attention from the team physician on a particular day (Fuller et al., 2007b, King et al., 2006). Illnesses and injuries were defined as new (pre-existing and not fully rehabilitated were not logged) or recurring (individuals having resumed full participation after a previous condition). In cases where an illness was observed within seven days of the initial diagnosis it was recorded once only during the first diagnosis. Furthermore, in cases where a single event caused several injury types or affected various body parts, only the most severe diagnosis was recorded as determined by the team physicians. Severe illnesses and injuries were defined as illnesses or injuries requiring an estimated absence from training or competition of more than one week.

Each team's physician recorded the following information for illnesses experienced by an individual: venue, date of onset and frequency of symptom/s, description of symptom/s, suspected cause, final diagnosis and treatment as well as prescribed time off from training and/or match. Likewise, the following information was recorded for injuries: venue, date of injury, type of injury, mechanism of injury, final injury diagnosis, decision to continue or discontinue play, predicted number of days lost from training and/or matches and the severity of the injury. Furthermore, the stage of the match, facet of play, activity engaged in at time of injury (i.e. match, training, or gym) as well as the position the player was fielded were recorded.

4.2.3.3 Calculation of player days

The number of player days, necessary to determine illness incidence rates, was calculated separately for each of the five SA teams. While the study took place over a period of 21 weeks, the start and end dates of the competition were different for each team as these were dependent on the dates of the first and last matches played. For example, some teams commenced their tournament matches a few days later than other teams; and others were knocked out of the tournament before the quarterfinals, semi-finals or the final. Therefore, the total tournament days for each team and the daily squad size varied between teams. The team physician reported

the ‘daily squad size’ on each day, which varied from 5-18 players per team. This variation was in part due to squad sizes often being reduced during international travel times relative to matches played domestically. Moreover, since only illnesses for the individuals who gave written informed consent to participate was followed, some of the participating players may not have been part of the first team squad on a particular day. As such the total player days for each team was calculated as follows: total team tournament days × average daily squad size. The player days for all five SA teams combined were as follows: total player days for entire tournament: n=5 928; no time zone difference (NTZD) player days: n=4 656; Westward travel (E-W) player days: n=680; Eastward travel (W-E) player days: n=592; two time zone difference (2 TZD) player days: n=136 and six or more time zone difference (>6 TZD) player days: n=1 132.

4.2.3.4 Calculation of the illness incidence rate

Illness incidence data were calculated as the number of illness events expressed per 1 000 player days. During the competition, illness data were logged for a total of 5 928 player days for all five teams combined.

4.2.3.5 Calculation of exposure hours

Exposure hours were used to determine injury incidence rate. Total exposure hours were calculated as follows: total number of tournament matches/training sessions (for each team) X length of match/training session (hours) X total number of players in the study on the pitch in each match/training session (for each team). The exposure hours for all five SA teams combined were as follows: total exposure hours for entire tournament: n=3 957; NTZD exposure hours: n=3 082; W-E travel exposure hours: n=376; E-W travel exposure hours: n=498; 2 TZD exposure hours: n=142 and >6 TZD exposure hours: n=538, respectively.

4.2.3.6 Calculation of the injury incidence rate

Injury incidence data were calculated as the number of injuries per 1 000 exposure hours for each team for both training and match injuries. During the competition, injuries were logged for a total of 3 957 exposure hours for all five teams combined.

4.2.4 Data and statistical analyses

Data are reported as mean ± standard deviation (SD) for parametric data and median with interquartile range (IR, for non-parametric data), incidence rate (per 1 000 player days for illnesses and per 1 000 exposure hours for injuries), counts or frequency (%). A Shapiro-Wilks test was performed to check for normality of the data. A one-way ANOVA and Kruskal-Wallis ANOVA tests were performed to compare descriptive characteristics between the three *PER3* VNTR groups. Fisher's exact and Chi-squared tests were used to compare frequency count differences between groups for both illness and injury. *Post-hoc* analyses were performed using a Fisher's exact test. The data were analysed in Statistica version 11 (StatSoft Inc., Tulsa, Oklahoma, USA) and Stata MP/14, (StataCorp, Texas, USA). Significance was assumed when $p<0.05$.

4.3 Results

4.3.1 Participant characteristics

Participant characteristics of the Super Rugby players for this study are presented in Table 4.1. There were no significant differences between the three *PER3* genotype groups for any of the variables.

Table 4.1: General characteristics of the three *PER3* VNTR groups of rugby players.

	***PER3*$^{4/4}$ (n=59)**	***PER3*$^{4/5}$ (n=68)**	***PER3*$^{5/5}$ (n=18)**	**p-value**
Age (y)	24.6±3.6	25.2±3.2	24.5±2.7	0.547
Weight (kg)	100.7±11.6	103.4±12.3	100.2±12.7	0.360
Height (cm)	187.0±6.5	187.0±7.2	186±7.2	0.972
BMI (kg·m^{-2})	28.7 (3.7)	29.0 (3.3)	28.4 (3.1)	0.385

The data are presented as the mean ± SD and median with IR for BMI. BMI: Body mass index. The p-values represent significance as determined by a one-way ANOVA and Kruskal-Wallis (BMI) tests.

4.3.2 Illness during the 2012 tournament

A total of 124 illnesses were reported in 74 (51%) of the 145 players analysed. Of these, 119 (96%) were new illnesses. The overall incidence of illness was 20.9 per 1 000 player days. The illness incidence rate for each system is presented in Table 4.2. The respiratory and digestive systems were most affected by illness.

Table 4.2: Illness incidences for each system for all participating players during the tournament.

System	n	Incidence rate (per 1 000 player days)	Total (%)
Respiratory	88	15.0 (11.8-8.1)	71.0
Digestive	25	4.2 (2.6-5.9)	20.2
Skin	5	0.8 (0.1-1.6)	4.0
Nervous	3	0.5 (-0.1-1.1)	2.4
Ears and mastoid	1	0.2 (-0.2-0.5)	0.8
All other systems	2	0.3 (-0.1-0.8)	1.6

Data are presented as incidence rates (with 95% confidence intervals in parentheses) and percentages.

Table 4.3 shows the documented cause of each illness, as diagnosed by the team doctors. Infections were the predominant cause of illness, which together with environmental and pre-existing causes, accounted for nearly all reported illnesses.

Table 4.3: Causes of illnesses reported in the 2012 tournament.

Cause of illness	n	Incidence rate (per 1 000 player days)	Total (%)
Infection	81	13.8 (10.8-16.8)	65.4
Environmental	32	5.4 (3.6-7.3)	25.8
Pre-existing (e.g. asthma, allergy)	5	0.8 (0.1-1.6)	4.0
Diarrhoea	1	0.2 (-0.2-0.5)	0.8
Other	5	0.8 (0.1-1.6)	4.0

Data are presented as incidence rates (with 95% confidence intervals in parentheses) and percentages.

4.3.2.1 Direction of travel and illness

Seventy-seven illnesses were recorded in players in the NTZD environment, 18 following E-W travel and 29 following W-E travel during the tournament. Figure 4.1 shows the illness incidence rates when illnesses were grouped by direction of travel immediately prior to the onset of symptoms. The incidence rates were significantly different between the three directions of travel ($p<0.001$). Specifically, a *post-hoc* analysis indicated that the illness incidence rates were higher following E-W ($p=0.028$) and W-E ($p<0.001$) travel compared to NTZD.

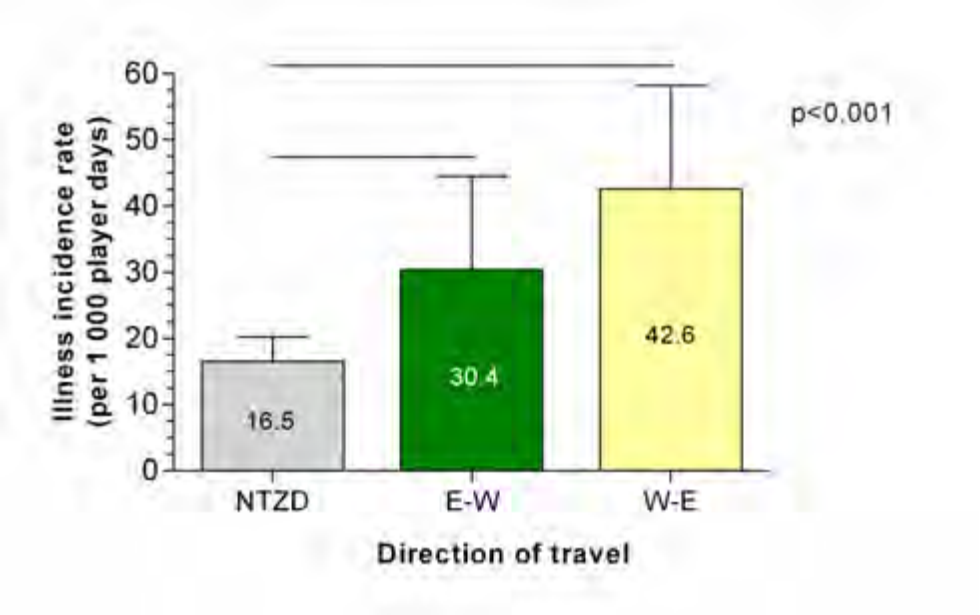


Figure 4.1: Illness incidence rates in players grouped by direction of travel immediately prior to the onset of symptoms. Data are presented as incidence rates with 95% confidence intervals. Error bars represent the 95% confidence intervals. NTZD: No time zone difference, E-W: Westward travel, W-E: Eastward travel. Significance was determined using a Chi-squared test. Lines above the graph represent significant differences between groups at either end of the line as determined by *post-hoc* analysis.

4.3.2.2 Number of time zones crossed and illness

Seventy-seven illnesses were recorded in players in the NTZD environment, 8 following travel across two time zones (2 TZD) and 39 following travel across six or more time zones (>6 TZD) during the tournament. Figure 4.2 shows the illness incidence rates in rugby players when illnesses were grouped by the number of time zones crossed immediately before appearance of symptoms. The incidence rates were significantly different between the three groups ($p<0.001$). *Post-hoc* analysis indicates that the incidence rates were higher following 2 TZD ($p=0.004$) and >6 TZD ($p<0.001$) compared to NTZD.

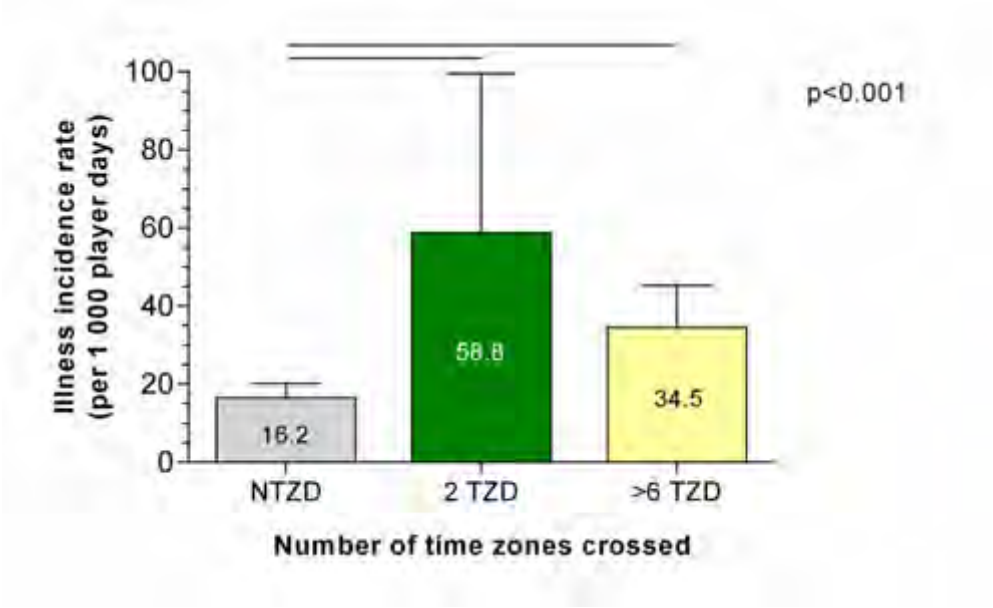


Figure 4.2: Illness incidence rates in players grouped by number of time zones crossed immediately prior to the onset of symptoms. Data are presented as incidence rates with 95% confidence intervals. Error bars represent the 95% confidence intervals. NTZD: No time zone difference, 2 TZD: Two time zone difference, >6 TZD: Six or more time zone difference. Significance was determined using a Chi-squared test. Lines above the graph represent significant differences between groups at either end of the line as determined by *post-hoc* analysis.

4.3.2.3 *PER3* VNTR genotype, direction of travel and illness

Figure 4.3 shows the illness incidence rates in players grouped by *PER3* VNTR genotype when direction of travel was taken into account. The incidence rate distributions were similar in the three genotype groups following NTZD, W-E and E-W travel. However, the incidence rate was higher in the $PER3^{5/5}$ ($p<0.001$) and $PER3^{4/5}$ ($p=0.018$) groups following W-E travel compared to NTZD when each *PER3* VNTR group was analysed independently. Furthermore, within the $PER3^{4/5}$ group the incidence rate was higher following E-W travel compared to NTZD ($p=0.076$).

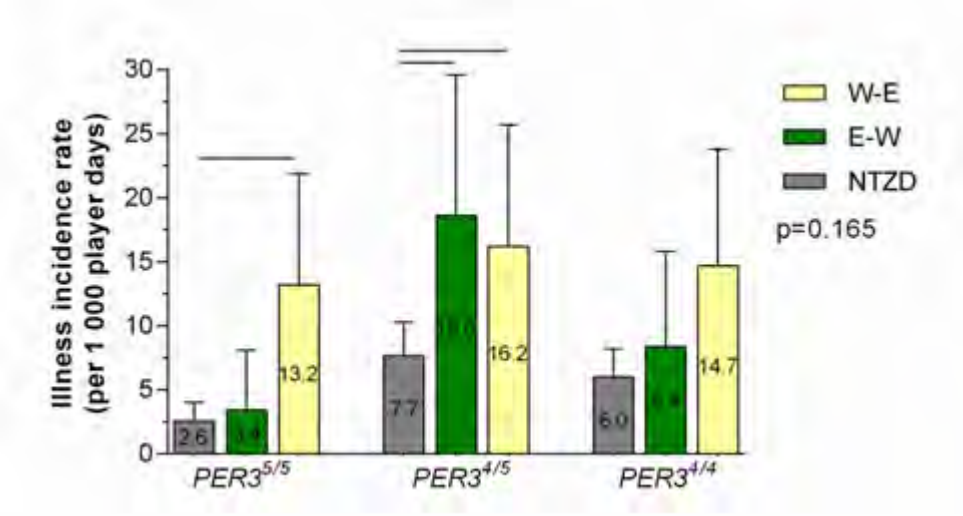


Figure 4.3: Illness incidence rates in players grouped by *PER3* VNTR genotype taking into account direction of travel immediately prior to the onset of symptoms. Data are presented as incidence rates with 95% confidence intervals. Error bars represent the 95% confidence intervals. NTZD: No time zone difference, E-W: Westward travel, W-E: Eastward travel. $PER3^{5/5}$: NTZD: n=12, E-W: n=2, W-E: n=9; $PER3^{4/5}$: NTZD: n=36, E-W: n=10, W-E: n=11; $PER3^{4/4}$: NTZD: n=28, E-W: n=5, W-E: n=11. Significance was determined using a Fisher's exact test. The p-value on the graph represents the direction of travel-by-*PER3* VNTR group comparison. Lines above the graph represent significant differences between groups at either end of the line as determined by *post-hoc* analysis.

4.3.2.4 *PER3* VNTR genotype, number of time zones crossed and illness

Figure 4.4 shows the illness incidence rates in players grouped by *PER3* VNTR genotype when the number of time zones crossed immediately before appearance of symptoms, was taken into account. The illness incidence rate was significantly different between the three genotype groups ($p<0.001$), with incidence rate higher in the $PER3^{4/5}$ group following >6 TZD compared to the other two groups ($p=0.044$). Furthermore, the illness incidence rates were higher in the $PER3^{5/5}$ ($p=0.016$) and $PER3^{4/5}$ ($p<0.001$) groups following >6 TZD compared to the NTZD environment when each *PER3* group was analysed independently. Likewise, the incidence rate was higher following 2 TZD compared to the NTZD environment in the $PER3^{4/4}$ group ($p=0.002$).

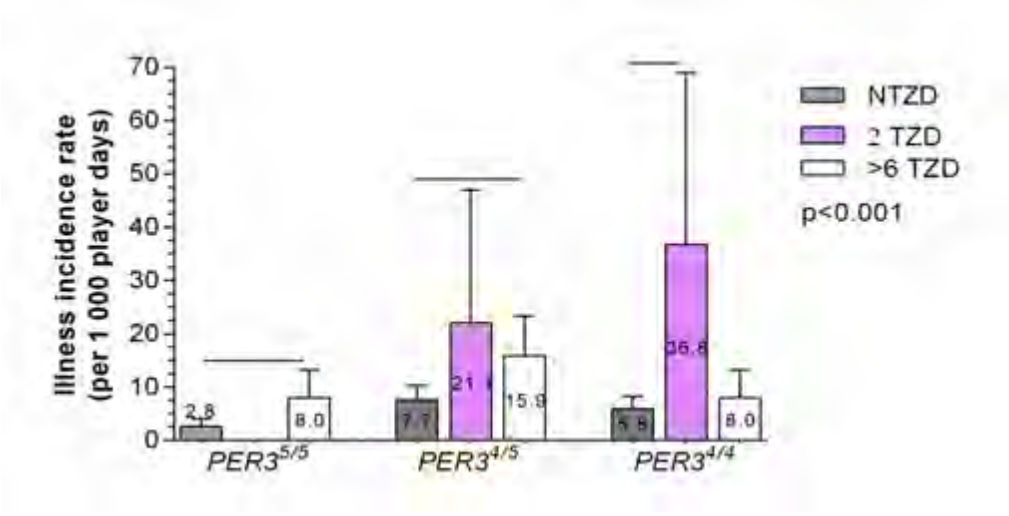


Figure 4.4: Illness incidence rates in players grouped by *PER3* VNTR genotype taking into account the number of time zones crossed immediately prior to onset of symptoms. Data are presented as incidence rates with 95% confidence intervals. Error bars represent the 95% confidence intervals. NTZD: No time zone difference, 2 TZD: Two time zone difference, >6 TZD: Six or more time zone difference. $PER3^{5/5}$: NTZD: n=12, 2 TZD: n=0, >6 TZD: n=9; $PER3^{4/5}$: NTZD: n=36, 2 TZD: n=3, >6 TZD: n=18; $PER3^{4/4}$: NTZD: n=28, 2 TZD: n=5, >6 TZD: n=9. Significance was determined using a Fisher's exact test. The p-value on the graph represents time zone-by-*PER3* VNTR group comparison. Lines above the graph represent significant differences between groups at either end of the line as determined by *post-hoc* analyses.

4.3.2.5 *PER3* VNTR genotype, direction of travel, number of time zones crossed and illness

Figure 4.5 shows the illness incidence rates in players grouped by *PER3* VNTR genotype when both direction of travel and number of time zones crossed were taken into account. When >6 TZ were crossed, there was a trend for the incidence rate distributions to be different between the genotype groups when direction of travel was considered (p=0.052). Within group analysis showed significant differences in the $PER3^{5/5}$ group (p<0.001), with higher illness incidence rate following W-E travel compared to E-W (p=0.019) and NTZD (p<0.001) groups.

When only 2 TZs were crossed, the incidence rate distributions between the three genotype groups were different (p<0.001). The incidence rates were higher following W-E travel in the $PER3^{4/5}$ group (p=0.014) and E-W travel in the $PER3^{4/4}$ group (p=0.014) compared to NTZD.

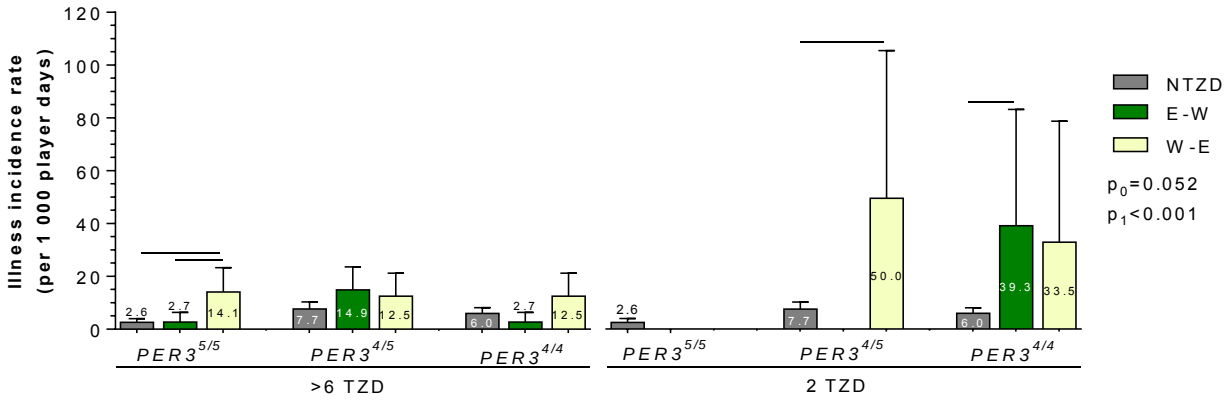


Figure 4.5: Illness incidence rates in players grouped by *PER3* VNTR genotype when direction of travel and number of time zones crossed immediately prior to the onset of symptoms were taken into account. Data are presented as incidence rates with 95% confidence intervals. Error bars represent the 95% confidence intervals. NTZD: No time zone difference, E-W: Westward travel, W-E: Eastward travel, 2 TZD: Two time zone difference, >6 TZD: six or more time zone difference. >6 TZD panel: $PER3^{5/5}$: NTZD: n=12, E-W: n=2, W-E: n=9; $PER3^{4/5}$: NTZD: n=36, E-W: n=11, W-E: n=8; $PER3^{4/4}$: NTZD: n=12, E-W: n=2, W-E: n=8; 2 TZD panel: $PER3^{5/5}$: NTZD: n=12, E-W: n=0, W-E: n=0; $PER3^{4/5}$: NTZD: n=36, E-W: n=0, W-E: n=3; $PER3^{4/4}$: NTZD: n=28, E-W: n=3, W-E: n=2. p_0: represents significance for >6 TZD panel. p_1: represents significance for the 2 TZD panel. Significance was determined using a Fisher's exact test. Lines above the graph represent significant differences between groups at either end of the line as determined by post-hoc analysis.

4.3.3 Injuries during the 2012 tournament

The overall injury incidence rate in South African players in the 2012 Super Rugby tournament was 40.1 per 1 000 exposure hours. Of the 145 players analysed, 77 (53%) players sustained injuries. A total of 160 injuries were recorded in the 77 players who sustained injuries. Of these injuries, 136 (85%) were new injuries. Of all recorded injuries, 138 (86%) happened during a match, 13 (8%) happened during rugby skills training, 4 (3%) occurred during weight training and 5 (3%) occurred during various other activities. Injuries were further classified according to severity. During the tournament, most recorded injuries were of minimal, mild and moderate disturbance to match or training activities as indicated in Table 4.4. The mean time lost from a match or training due to an injury was three days for all injuries. Figure 4.6 shows injury incidence

rates expressed per injury type. Contusions, sprained ligaments, muscle ruptures and lacerations were the most predominant types of injuries recorded.

Table 4.4: Injury severity in the 2012 Super Rugby tournament (n=160).

Severity of injury	n	Incidence rates (per 1 000 exposure hours)	Total (%)
Slight (0-1 day missed)	26	6.6 (4.0-9.1)	13.8
Minimal (2-3 days missed)	44	11.1 (7.8-14.4)	29.7
Mild (4-7 days missed)	37	9.4 (6.3-12.4)	23.9
Moderate (8-28 days missed)	36	9.1 (6.1-12.1)	22.5
Severe (>28 days missed)	16	4.0 (2.1-6.0)	10.1

Data are presented as incidence rates (with the 95% confidence intervals in parentheses) and percentages.

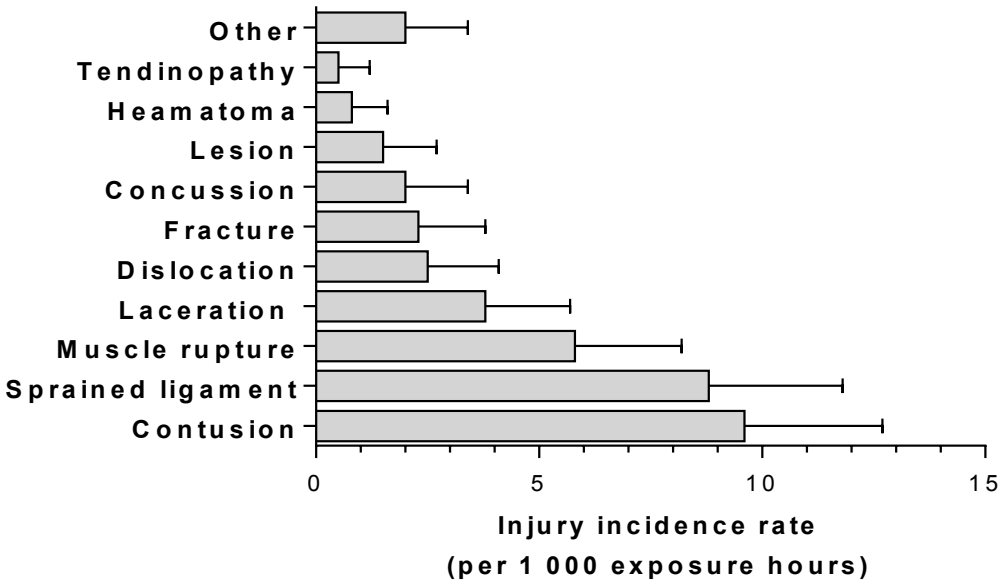


Figure 4.6: Injury incidence rates expressed per injury type. Error bars represent the 95% confidence intervals.

4.3.3.1 Direction of travel and injury

One hundred and twelve injuries were recorded for players in the NTZD environment, 24 following E-W travel and 25 following W-E travel. Injury incidence rates in players grouped by direction of travel undertaken immediately before the occurrence of an injury are presented in Figure 4.7. There were significant differences in the incidence rates between the three groups (p=0.022). A *post-hoc* analysis indicated that the incidence rate was higher following W-E travel compared to NTZD (p=0.010).

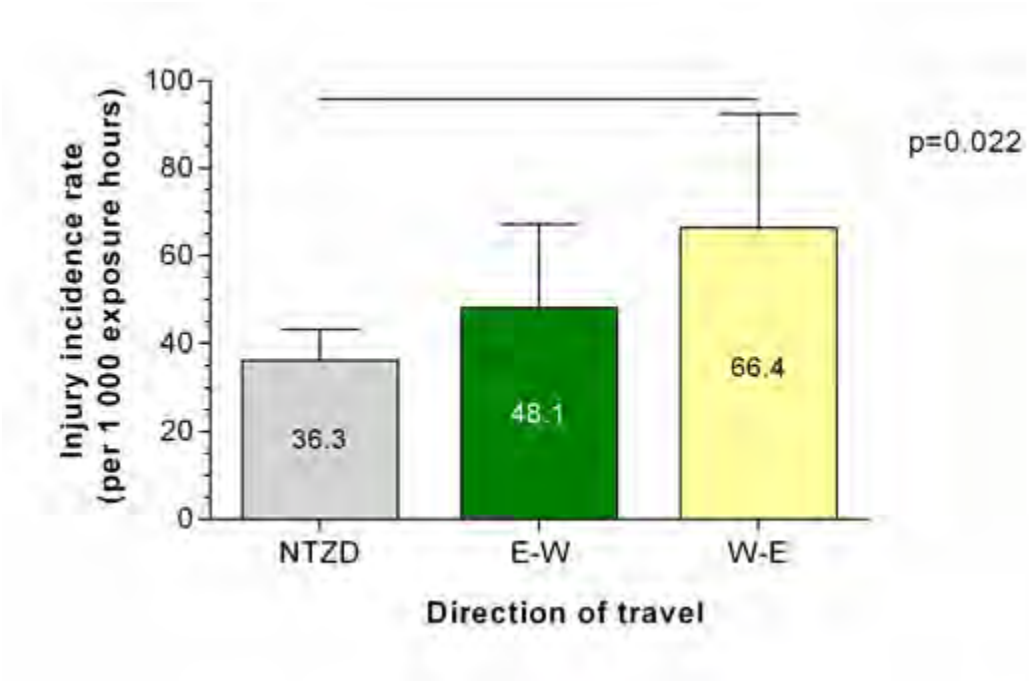


Figure 4.7: Injury incidence rates in players grouped by direction of travel immediately prior to sustaining an injury. Data are presented as incidence rates with 95% confidence intervals. Error bars represent the 95% confidence intervals. NTZD: No time zone difference, E-W: Westward travel, W-E: Eastward travel. Significance was determined using a Chi-squared test. Lines above the graph represent significant differences between groups at either end of the line as determined by *post-hoc* analyses.

4.3.3.2 Number of time zones crossed and injury

One hundred and twelve injuries were recorded for players in the NTZD environment, seven following travel crossing two time zones and 42 following travel crossing six or

more time zones. The injury incidence rates of rugby players grouped by number of time zones crossed immediately prior to the injury are presented in Figure 4.8. The injury incidence rates were different between the three time zone groups ($p<0.001$), with a higher incidence rate occurring following travel across >6 TZs compared to NTZD ($p<0.001$).

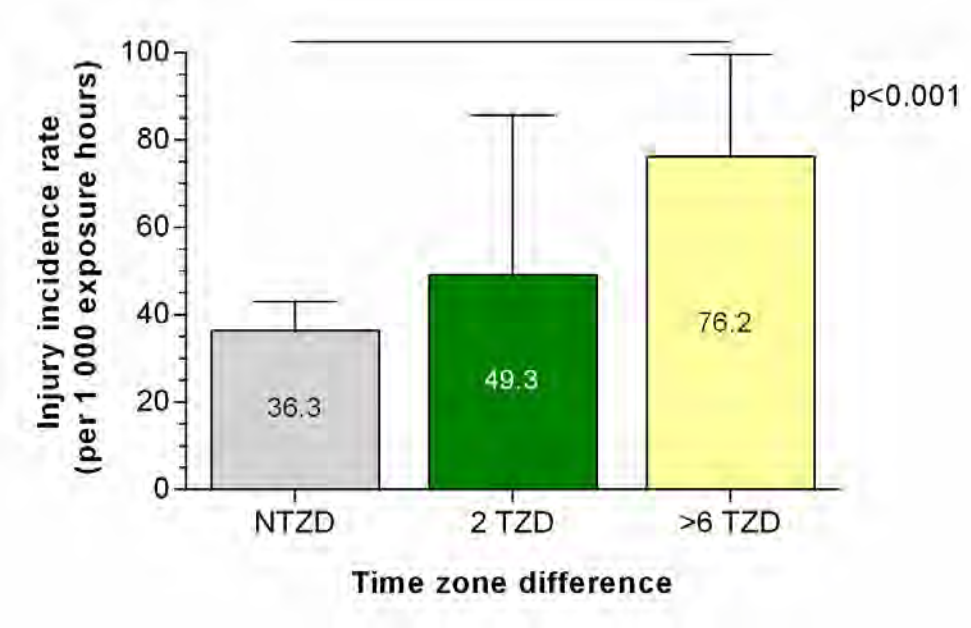


Figure 4.8: Injury incidence rates in players grouped by number of time zones crossed immediately prior to sustaining an injury. Data are presented as incidence rates with 95% confidence intervals. Error bars represent the 95% confidence intervals. NTZD: No time zone difference, 2 TZD: Two time zone difference, >6 TZD: Six or more time zone difference. Significance was determined using a Chi-squared test. Lines above the graph represent significant differences between groups at either end of the line as determined by *post-hoc* analyses.

4.3.3.3 *PER3* VNTR genotype, direction of travel and injury

The injury incidence rates of players grouped by *PER3* VNTR genotype when direction of travel was taken into account are presented in Figure 4.9. The distributions of injury incidence rates were similar for all three groups when direction of travel immediately prior to the injury was considered. The incidence rates were also examined for each group independently, but no significant differences in the rates represented by direction of travel were found.

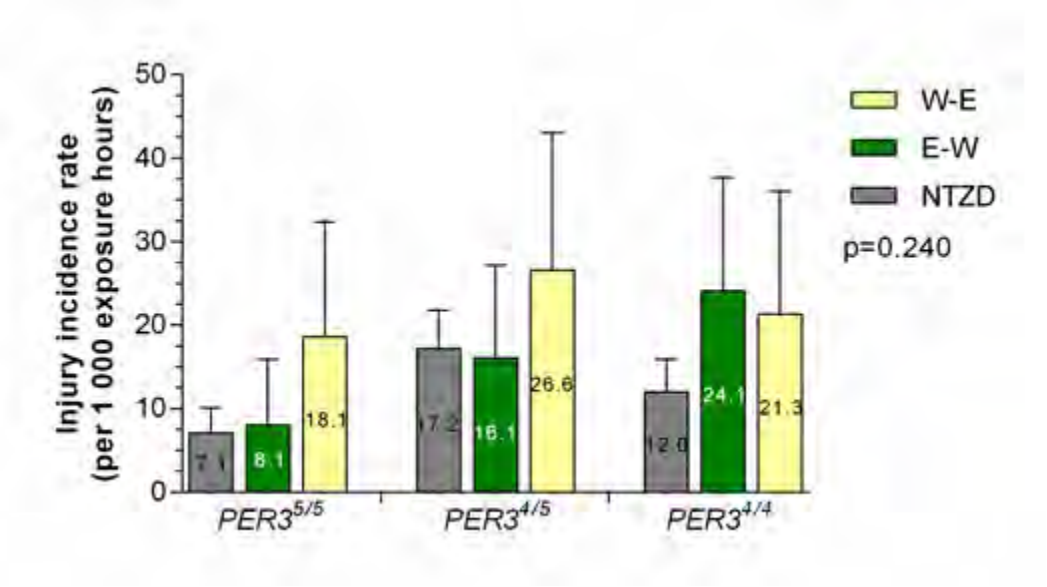


Figure 4.9: Injury incidence rates in players grouped by *PER3* VNTR genotype taking into account direction of travel immediately prior to the injury. Data are presented as incidence rates with 95% confidence intervals. Error bars represent the 95% confidence intervals. NTZD: No time zone difference, E-W: Westward travel, W-E: Eastward travel. $PER3^{5/5}$: NTZD: n=22, E-W: n=4, W-E: n=7; $PER3^{4/5}$: NTZD: n=53, E-W: n=8, W-E: n=10; $PER3^{4/4}$: NTZD: n=37, E-W: n=12, W-E: n=8. Significance was determined using a Fisher's exact test.

4.3.3.4 *PER3* VNTR genotype, number of time zones crossed and injury

The injury incidence rates in players grouped by *PER3* VNTR genotype when the number of time zones crossed was taken into account are presented in Figure 4.10. Injury incidence rates differed significantly between the three groups ($p<0.001$), with higher incidence rates in the $PER3^{4/5}$ group compared to the $PER3^{5/5}$ ($p<0.001$) when there was no change in time zone, and in the $PER3^{4/4}$ group compared to the $PER3^{5/5}$ group ($p=0.013$) following >6 TZD travel. Furthermore, independent analysis of each *PER3* VNTR group showed significant differences in the incidence rates within the $PER3^{5/5}$ ($p<0.001$) and $PER3^{4/4}$ ($p<0.001$) groups. In the $PER3^{5/5}$ group, the incidence rate was higher following 2 TZD compared to NTZD ($p=0.027$). While in the $PER3^{4/4}$ group, the incidence rate was higher following >6 TZD compared to NTZD ($p<0.001$).

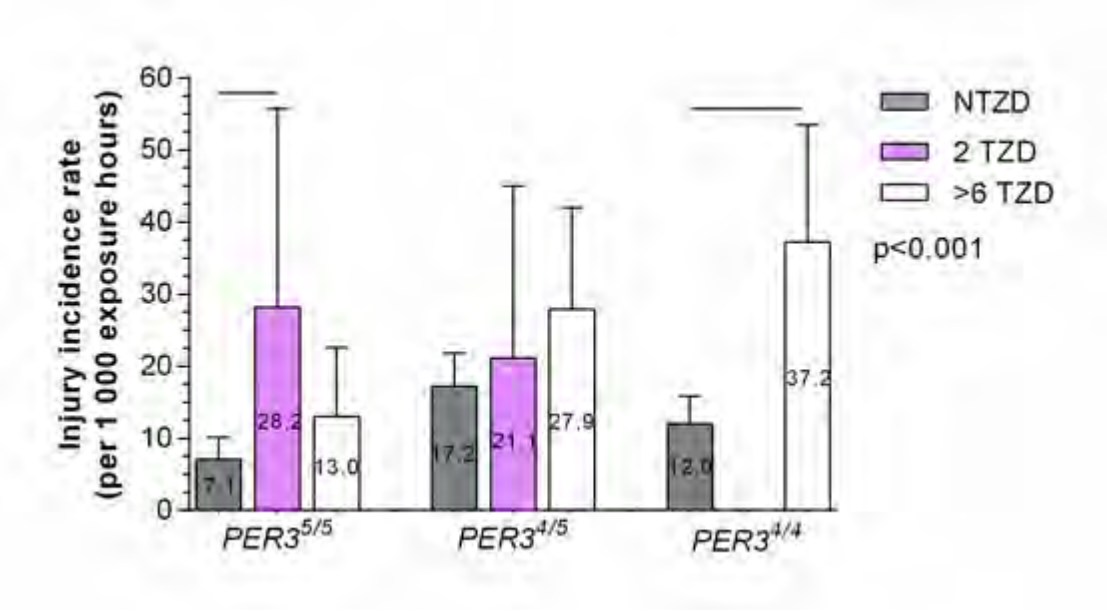


Figure 4.10: Injury incidence rates in players grouped by *PER3* VNTR genotype taking into account number of time zones crossed immediately prior to the injury. Data are presented as incidence rates with 95% confidence intervals. Error bars represent the 95% confidence intervals. NTZD: No time zone difference, 2 TZD: Two time zone difference, >6 TZD: Six or more time zone difference. $PER3^{5/5}$: NTZD: n=22, 2 TZD: n=4, >6 TZD: n=7; $PER3^{4/5}$: NTZD: n=53, 2 TZD: n=3, >6 TZD: n=15; $PER3^{4/4}$: NTZD: n=37, 2 TZD: n=0, >6 TZD: n=20. The p-value on the graph represents the time zone-by-*PER3* genotype group comparison. Significance was determined using a Fisher's exact test. Lines above the graph represent significant differences between groups at either end of the line as determined by *post-hoc* analyses.

4.3.3.5 *PER3* VNTR genotype, direction of travel, number of time zones crossed and injury

The injury incidence rates in players clustered by *PER3* VNTR genotype, number of time zones crossed and direction of travel are presented in Figure 4.11. When the players crossed six or more time zones, there were no significant differences in the incidence rates between the three *PER3* VNTR groups when direction of travel was accounted for. However, independent analysis of each *PER3* VNTR group showed significant within group differences in the $PER3^{4/4}$ group, such that injury incidence rate was higher following E-W travel compared to the NTZD environment (p=0.009).

When the players crossed only two time zones, the incidence rates between the three groups were similar, when direction of travel was accounted for. However, independent analysis of each *PER3* VNTR group showed significant within group differences in the $PER3^{5/5}$ group, such that injury incidence rates were higher following W-E travel compared to NTZD (p=0.006).

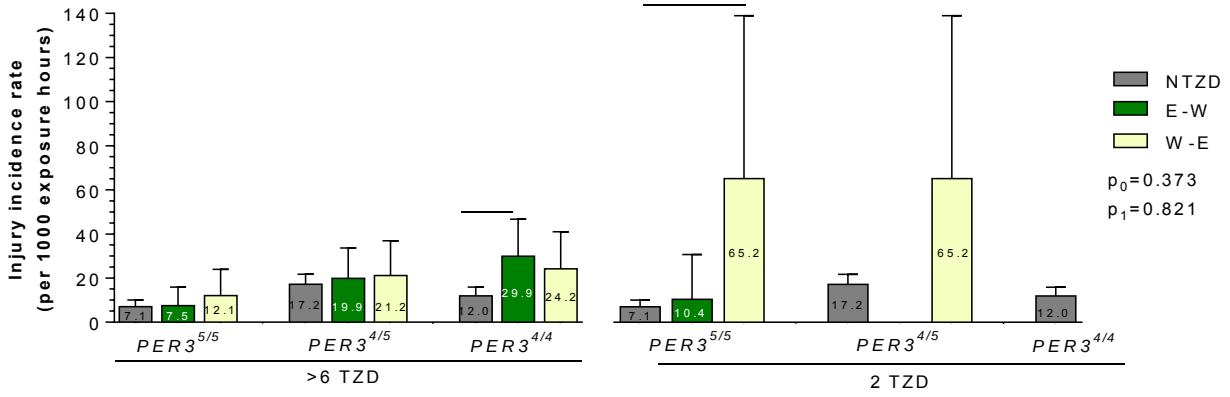


Figure 4.11: Injury incidence rates in players grouped by *PER3* VNTR group when direction of travel and number of time zones crossed were taken into account. Data are presented as incidence rates with 95% confidence intervals. Error bars represent the 95% confidence intervals. NTZD: No time zone difference, E-W: Westward travel, W-E: Eastward travel, 2 TZD: Two time zone difference, >6 TZD: Six or more time zone difference. >6 TZD panel: $PER3^{5/5}$: NTZD: n=22, E-W: n=3, W-E: n=4; $PER3^{4/5}$: NTZD: n=53, E-W: n=8, W-E: n=7; $PER3^{4/4}$: NTZD: n=37, E-W: n=12, W-E: n=8; 2 TZD panel: $PER3^{5/5}$: NTZD: n=22, E-W: n=1, W-E: n=3; $PER3^{4/5}$: NTZD: n=53, E-W: n=0, W-E: n=3; $PER3^{4/4}$: NTZD: n=37, E-W: n=0, W-E: n=0. The p_0: represents overall significance for >6 TZD panel. p_1: represents overall significance for 2 TZD panel Significance was determined using a Fisher's exact test. Lines above the graph represent significant differences between groups at either end of the line as determined by *post-hoc* analyses.

4.4 Discussion

The purpose of the present study was to compare the incidence of illnesses and injuries in rugby players grouped by *PER3* VNTR genotype following trans-meridian travel during the 2012 Super Rugby competition. This study was unique in that it is the first to investigate the association between *PER3* VNTR genotype and the incidence of illnesses and injuries in team sport athletes travelling during a tournament. As the sample sizes of the $PER3^{4/4}$ and $PER3^{4/5}$ groups were nearly

double that of the *PER3*$^{5/5}$ group, the comparison of illness or injury incidence rates between the three genotypes would not have been meaningful. In order to obtain the same number of illnesses or injuries, an individual carrying the *PER3*$^{5/5}$ genotype would have to sustain an injury or experience an illness at least more than once. Equally important, the calculation of illness/injury incidence rates rely on the number of player days or exposure hours, which do not factor the sample size in each group into account. Therefore, this would have affected the outcome of the analysis, as the sample sizes were different.

4.4.1 Illness incidence rate

The overall illness incidence rate of 20.9/1000 player days in this study is similar to the 20.7/1000 player days reported in a 2010 Super 14 rugby study (Schwellnus et al., 2012). A sub-analysis of the data presented by Schwellnuss et al. (2012) indicated an illness incidence rate of 32.6/1000 player days immediately following time zone travel. This was as twice high as the 15.4/1000 player days value reported at baseline when there was no travel across time zones in the same study. This is consistent with findings from a previous study (Newman-Klee et al., 2007) who reported an illness incidence rate of 48.4/1000 person days in a group of adolescent and young adults who attended pre-clinic travel counselling and adhered to prescribed advice following time zone travel. This incidence rate was calculated based on the assumption that 100 person weeks was equivalent to 700 person days.

Using the same assumption, an illness incidence rate of 8.14/1000 person days was reported in the four week period following time zone travel, when the individuals had adapted to the new environment. While the above-mentioned studies suggest an increase in the risk of illness following time zone travel in athletes and general population participants, caution must be exercised when interpreting these findings. Specifically, the definition and classification of illness, nature of travel and population studied in the Newman-Klee et al. (2007) study was different to this study and that of Schwellnus et al. (2012).

The distribution of these illnesses was higher in the respiratory and digestive systems, which concurs with findings from a larger Super Rugby study (Schwellnus et al., 2012), football studies (Dvorak et al., 2011, Theron et al., 2013), and winter Olympic Games studies (Mountjoy et al., 2010, Soligard et al., 2015). In this study, one might hypothesise that changes in the circadian system or stresses associated with the nature of the Super Rugby competition such as repeated trans-meridian travel, demanding travel schedule and high training intensities as noted in other studies leave the respiratory and digestive systems more vulnerable to infections (Thompson et al., 2014, Schwellnus et al., 2012, Castanon-Cervantes et al., 2010). Repeated trans-meridian travel disrupts the circadian system and sleep-wake cycles, resulting in increased anxiety and a suppressed immune system (Nieman, 2000, Neville et al., 2005), leaving individuals susceptible to illnesses.

The high illness incidence rate in this study may also be due to the fact that players were exposed to different populations, pathogens, climatic and environmental conditions (e.g. pollens and humidity) at overseas match venues (Schwellnus et al., 2012). This high illness rate is disturbing bearing in mind that to make a living rugby players rely on being healthy so that they can derive the best possible benefit from training sessions and optimising match performance. Furthermore, so that players can train effectively and are available for as many matches as possible during the tournament. Losing players to illness is a concern because coaches then have fewer players to work with, giving the coach fewer options for line-up changes. This often forces coaches to play players out of position, which may reduce performance and increase injury risk.

4.4.2 Direction of travel, number of time zones crossed and illness

The second finding was that regardless of direction travelled (Figure 4.1) or numbers of time zones crossed (Figure 4.2) illnesses were higher when travelling longhaul compared to no time zone travel. This suggests that it was rather time zone travel, which influenced illness incidence rates. This is consistent with findings which reported no direction of travel effect, but higher illness incidence rates in athletes who travelled across >5 time zones compared to those athletes competing in their domestic leagues (Schwellnus et al., 2012). This is further supported by the

finding that illness incidence rates for both two and six or more time zone crosses were higher compared to when there was no time zone travel, but similar to each other in this study. Although unexpected, this suggests that travelling across two time zones is no better than travelling across six or more time zones. If this were not the case then illness incidence rates between eastward and westward travel, and two and six or more time zones would be expected to be different. This was surprising since previous studies have reported that sleep and circadian disruption are directly proportional to the magnitude of time zone travel, with the degree of disruption increasing as the number of time zones crossed increases (Golombek et al., 2013, Carrier et al., 1996). Likewise, sleep and circadian disruption the two factors that negatively influence immune function are greatly affected following eastward than westward travel (Reilly et al., 2005, Recht et al., 1995, Waterhouse et al., 2007).

In this study, the degree of misalignment between the internal body clock and external environmental time was expected to be greater after travel crossing six or more time zones, and thus a greater degree of immune compromisation was expected. Sleep and the circadian clock cooperate at the systemic (SCN) and cellular (gene) levels to regulate temporal immune functions through neuroendocrine and sympathetic effector mechanisms (Arjona and Sarkar, 2008, Imeri and Opp, 2009), conferring an adaptive immune response during the day. However, circadian and sleep disruption that ensues following time zone travel may lead to altered hormonal levels and immune functions, consequently dampening circadian rhythms and conferring susceptibility to illnesses (McEwen, 2006, Trinder et al., 2015, Voigt et al., 2014).

Circadian and sleep disruption have been reported to disrupt circadian oscillations in colonic motility, production of digestive enzymes, maintenance and repair of the protective barrier (Bron and Furness, 2009, Knutson and Boggild, 2010). For example, maximal colonic motility has been reported to peak during daytime, while minimal motility has been reported to occur during nighttime (Bechtold et al., 2010). Rhythmic expression of clock genes, modulate colonic motility directly and indirectly through clock-mediated transcription of genes such as, acetylcholine transferase and neuronal nitric oxide synthase. Therefore, disruptions to the sleep-wake cycle

and the body clock as a result of the misalignment between the body clock and the day-night cycle in the new time zone may have adverse consequences on the gastrointestinal tract and nutrient transport in the small intestines. Although not directly comparable, this is in line with a recent study which demonstrated significant irregular bowel movement in rotating shift work nurses compared to nurses working either nighttime or daytime shifts only (Nojkov et al., 2010). However, it is also possible that viruses contracted through on-board air circulatory systems, dehydration and close contact with other passengers during international flights may have contributed to the high illness incidence rate in this study (Schwellnus et al., 2012).

Activation of the immune system affects clock gene expression in the SCN and peripheral tissues including, the 24h rhythms of hormones such as cortisol and growth hormone (Nader et al., 2010). Gallagher et al. (2008) reported that vaccination before nocturnal sleep affects the immune system. Specifically, they indicated that antibody titre were significantly higher when inoculation with hepatitis A vaccine was performed in the morning than in the evening. This reflects differences in the speed with which an antigen is processed in combination with the function of nighttime sleep, as a time when the initiation of the adaptive immune system is facilitated. Furthermore, it implies that a complex relationship exists between sleep, the circadian and immune systems, and thus nighttime flights across time zones likely increased players' susceptibility to illnesses.

4.4.3 *PER3* VNTR genotype, number of time zones crossed, direction of travel and illness

The number of time zones crossed and direction of travel seemed to affect each *PER3* VNTR group differently. Specifically, higher illness incidence rates were evident within the $PER3^{5/5}$ group following eastward travel across six or more time zones compared to no time zone travel. In contrast, within the $PER3^{4/5}$ group, higher illness incidence rates were noted following six or more time zone crosses, regardless of travel direction compared to when there was no time zone travel. This suggests that *PER3* genotype may confer protection or create susceptibility to illness when there is significant circadian disruption. Perhaps, individuals carrying the *PER3* 5-repeat allele are more likely to get ill after time zone travel, especially following eastward travel. This is

plausible since circadian and sleep disruption have been reported to be greater in the *PER3*$^{5/5}$ individuals during sleep deprivation studies (Goel et al., 2009, Chellappa et al., 2012b). Specifically, the *PER3*$^{5/5}$ genotype has been shown to experience higher homeostatic sleep pressure, slow wave sleep, and shorter sleep onset latency than the *PER3*$^{4/4}$ genotype during sleep deprivation studies (Viola et al., 2007, Goel et al., 2009, Mongrain and Dumont, 2007). This suggests that *PER3*$^{5/5}$ individuals may experience the effects of jet-lag more than *PER3*$^{4/4}$ individuals, as a result of partial sleep loss, which may be compounded by the misalignment between the internal body clock and the external environment.

These effects of jet-lag are more severe after eastward travel, particularly when the magnitude of time zone travel approaches near maximum further disrupting circadian rhythms and sleep in the *PER3* genotypes. Implementation of appropriate jet-lag alleviation protocols may therefore be useful, especially within the first 72h upon arrival in the new time zone. This will likely reduce the degree of misalignment and sleep disruptions facilitating faster adaptation. The lack of a significant difference in the illness incidence rate of the *PER3*$^{5/5}$ genotype after westward travel should be treated with caution; and can be attributed to the broad 95% confidence intervals, smaller sample size and fewer player days, after westward travel across two time zones. Regardless, this is the first study to assess the effects of *PER3* genotype on the illness incidence rates taking number of time zones crossed and direction of travel into account, and thus further research is warranted.

4.4.4 Injury incidence rate

The injury incidence rate was 40.1/1000 exposure hours, which is similar to the 45.0/1000 exposure hours reported in a single Super 12 Rugby team (Targett, 1998) and the 38.7/1000 exposure hours in semi-professional rugby players (Hodgson et al., 1998). However, it was higher than the incidence rate of 32.0/1000 exposure hours reported during the 1995 Rugby World Cup in South Africa (Jakoet and Noakes, 1998). It is also higher than the 8.0/1000 exposure hours in European professional football players (Hagglund et al., 2009), 2.4/1000 exposure hours in marathon runners (van Mechelen, 1992), 2.5/1000 in triathletes (Burns et al., 2003) and between

6.6-18.0/1000 exposures hours in squash, volleyball and wrestling (Parkkari et al., 2004). In all the above-mentioned studies, athletes competed in their home country or travelled without crossing any geographical time zones between competitions. The differences in the incidence of injuries between this study and others vary and range from use of different protocols, age, level of play to type of sport. For example, methodological differences between this study and other studies make it difficult to directly compare findings across different tournaments or sports. Furthermore, other studies report only match or training injuries (Fuller et al., 2007a, Gabbett et al., 2012b), while this study reports combined match and training injuries. Combining training and match injuries tend to lower the incidence rate compared to the incidence rate of matches alone.

The high injury incidence rate in this study may have been due to jet-lag effects as a result of circadian disruption from the repeated trans-meridian travel compared to studies where athletes are not required to travel. The fact that more than half (53%) of the players sustained an injury is particularly disturbing given that 23% of players sustained a moderate injury (8-28 days missed) and 10% sustained a severe injury (>28 days lost). Thus, nearly one third of players missed at least a match due to injury, a situation that may force the coach to field some players out of position, potentially increasing a player's risk to injury. Of the reported injuries, contusions, sprained ligaments and muscle ruptures were the most predominant a finding which concurs with other Super Rugby studies (Schwellnus et al., 2012, Schwellnus et al., 2014), Olympic Games (Soligard et al., 2015) and Rugby Union studies (Gabbett et al., 2012a, Fuller et al., 2010).

4.4.5 Direction of travel, number of time zones crossed and injury

The direction of travel and number of time zones crossed were both important, as evidenced by the higher injury incidence rates after eastward travel, and following travel across six or more time zones compared to no time zone travel (Figures 4.7 and 4.8). Players seemed to be at an advantage when playing at home or when there was no time zone travel, suggesting that the effects of jet-lag, which may predispose players to injuries, worsen as the magnitude of time zone travel increase, particularly in an eastward direction. Moreover, the necessary exposure to a light

stimulus in order to realign the circadian system does not fit in with the day and night cycle at the destination venue during the first few days. For example, a New Zealand local time of 09h00, the time that most morning training sessions take place is equivalent to 23h00 South African local time, which is bed-time for most people in South Africa. Therefore, instead of phase advancing the circadian clock, exposure to a light stimulus at this time-of-day may phase delay the circadian clock rather than promoting the phase adjustment required. This might exacerbate the effects of jet-lag such as fatigue, daytime sleepiness and sleep disturbances, which influence fine motor skills, alertness, concentration and decision making (Maire et al., 2014, Drust et al., 2005, Goel et al., 2009)**,** potentially increasing the risk of injury. Reilly and Edwards (2007) reported that swimmers who underwent partial (2.5h) sleep deprivation experienced negative mood states, confusion and fatigue. A change in mood state such as that experienced following time zone travel may possibly be another factor associated with increased injury risk in the Super Rugby tournament. This is conceivable, given that high competitive state anxiety, negative mood state and tension were shown to be responsible for the high injury rates in varsity level American Football and rugby players (Lavallee and Flint, 1996)**.**

Sleep is important for psychological and physical restoration of the body and useful for muscle recovery and optimal performance (Skein et al., 2011). Disruption to the sleep-wake cycle could possibly hinder muscle recovery and impair mental faculties (Skein et al., 2013, Reilly and Edwards, 2007); especially since athletes have a stronger rhythmicity than sedentary individuals (Davenne, 2009). It has been demonstrated in both human and animal studies that an increase in physical daily activity is correlated with changes in the period, phase and amplitude of circadian rhythms (Mistlberger et al., 2003, Atkinson et al., 1993, Koteja et al., 2003). In contrast, a sedentary way of life may lead to a reduced circadian period, phase and amplitude (Davenne, 2009). Appropriately-timed physical activity has been reported to play an important role in counteracting circadian desynchronisation either due to nighttime shift work or time zone travel (Waterhouse et al., 2007, Mauvieux et al., 2003, Waterhouse et al., 2000). The stronger rhythmicity in athletes compared to sedentary people may be due to differences in the level of physical activity that these two groups receive. Thus, it is likely that the synchronisation and

amplitude of circadian rhythms in athletes would be higher than in sedentary people. Circadian disruption following time zone travel may be exacerbated by the fact that athletes are highly sensitive to jet-lag (Waterhouse et al., 2000) and also because athletes require more sleep than sedentary people (Davenne, 2009). Another study, Murgia (2013) reported that fatigue as a result of insufficient rest or recovery time following time zone transitions resulted in injuries in hockey players. The failure by fatigued muscles to produce enough force to support movement activity (i.e. decreasing impact force on contact) may increase the risk of future injuries including strains, ligament damage and muscle tears (Takarada, 2003, Murgia, 2013). This may possibly explain the high number of tendon and muscle injuries noted in this study.

4.4.6 *PER3* VNTR genotype, direction of travel, number of time zones crossed and injury

The distribution of injuries was similar in the three *PER3* genotype groups when accounting for direction of travel, but different when number of time zones crossed were considered. Perhaps jet-lag due to lack of synchronisation of the body clock to the new environment may increase the risk of injury following intercontinental travel. Circadian disruption similar to that, which may occur following intercontinental travel, can alter mood and cognitive function, with complex mental performance tasks deteriorating faster than simple tasks (Sack, 2010, Waterhouse et al., 2004, Reilly and Edwards, 2007, Meney et al., 1998). This is consistent with inattention and an increase in errors and injuries, which have been reported in overtime and nighttime shift workers (Dinges et al., 1994, Costa, 1996, Dembe et al., 2005). In another study, a decline in performance involving complex mental activities, lack of energy and a general loss of motivation was reported in athletes immediately following time zone travel (Reilly and Edwards, 2007). Other symptoms of jet-lag that may increase the risk of injury in athletes include reductions in anaerobic power, dynamic strength and capacity (Reilly et al., 2005). Given that rugby is a high contact sport, a reduction in aerobic power, capacity and dynamic strength may negatively affect a player's game involvement and increase the risk of injury.

Higher injury incidence rates were evident in individuals carrying the $PER3^{4/4}$ genotype compared to those with the $PER3^{5/5}$ genotype. This difference in the risk of injury may have arisen due to

the inter-individual variation in sleep pressure and cognitive performance noted between the *PER3*$^{4/4}$ and *PER3*$^{5/5}$ genotypes following sleep deprivation studies (Maire et al., 2014, Viola et al., 2007). While, the *PER3*$^{5/5}$ genotype has been shown to have a faster build-up of sleep pressure than the *PER3*$^{4/4}$ genotype, it is also reported to dissipate sleep pressure faster during a sleep episode compared to the *PER3*$^{4/4}$ genotype (Dijk and Archer, 2010, Goel et al., 2009, Maire et al., 2014, Chellappa et al., 2012b). This suggests active readjustment into the new environment by the *PER3*$^{5/5}$ genotype. Thus, the *PER3*$^{5/5}$ genotype may be able to, for example, restore complex mental functions, dynamic strength, aerobic power and mood to levels prior to intercontinental travel, reducing a player's risk to injury.

The *PER3*$^{5/5}$ genotype has been shown to be more sensitive to blue light exposure than the *PER3*$^{4/4}$ genotype in a laboratory study (Chellappa et al., 2012a). This finding suggest that the two genotypes may entrain differently following time zone transition. For example, the *PER3*$^{5/5}$ genotype might be able to utilise outdoor light faster for entrainment than the *PER3*$^{4/4}$ genotype. Perhaps the *PER3*$^{4/4}$ individuals may have remained jet-lagged, thus predisposing them to injury. This is plausible, since most injuries happened during matches, and matches took place within 3-5 days upon arrival in the new time zone. Therefore, the *PER3*$^{4/4}$ individuals may have been unable to resynchronise rapidly to the new time zone to the extent that the *PER3*$^{5/5}$ individuals had. Within the *PER3*$^{4/4}$ group, higher injury rates were evident after travelling across six or more time zones compared to no time zone travel, further suggesting that number of time zones crossed rather than direction of travel affected the three *PER3* genotypes. It also suggests that the *PER3* genotype may confer protection or create susceptibility to injuries following travel across time zones.

The high injury rates evident within the *PER3*$^{5/5}$ group following travel across two time zones compared to no time zone travel should be treated with caution. This may be explained by the broad 95% confidence intervals, smaller number of injuries and fewer exposure hours, particularly following travel across two time zones. The exact mechanism by which the *PER3* genotype may confer protection or create susceptibility to injuries is not known, however, it is

possible that it acts through differences in the ability to resynchronise in to the new environment. Therefore, affording players adequate rest, proper implementation of jet-lag alleviation protocols and lowering training workloads, especially within the first 72h upon arrival in the new time zone, might be key to reducing the risk of injury. There are no similar studies in the literature with which to compare these findings to at present. Therefore, further research with bigger sample sizes across multiple sports, and seasons is required to determine if the *PER3* genotype interacts with number of time zones crossed or direction of travel to increase injury risk. Specifically, one needs to actually measure the extent of desynchronisation and resynchronisation in the new time zone in order to properly understand the effects of jet-lag on the different *PER3* genotype groups. Without actual data, it is difficult to ascertain if differences in the risk of illness and injury between the three-genotype groups following time zone travel are real.

4.4.7 Limitations

The heavy reliance on team physicians to report all illnesses and injuries experienced by players was a limitation. Specifically, there was no way of determining if there was under-reporting of illnesses or injuries. This is conceivable given that players have a tendency to play with injuries during important matches (e.g. play-offs, finals), instead of resting and undergoing treatment (Kneeland, 2014). Thus, while this study attempts to quantify injuries in the three genotype groups during the Super Rugby tournament, there is no way to fully quantify injuries since players may play through injury if possible. The result is that the injury incidence rate is probably a conservative quantification of injuries in the Super Rugby tournament.

The small sample size of illnesses and injuries when grouped by *PER3* genotype, number of time zones crossed and direction of travel was a limitation. This reduced statistical power, potentially leading to a type 1 statistical error. As a result, it is difficult to tell if the statistical significance observed or the lack thereof was real. While there may be uncertainty in some of the findings due to small simple sizes, trends found in this study are worth exploring further and may serve to stimulate further research in this area of study. These findings warrant further research since

illnesses and/or injuries are modifiable risk factors that can be detrimental to either an individual or a team. Reliability and interpretation of these findings could have been improved by imputing missing data when direction of travel and number of time zones crossed were accounted for. Specifically, building a multivariate imputation model, for example, creating several (say five) imputed values for each missing value, each of which is predicted from a slightly different model and each of which reflects sampling variability. To obtain an overall point estimate for each individual for each of the missing data, the five imputed data sets can then be averaged. This will boost the sample size and improve the reliability and interpretation of the illness and injury data outcome.

4.5 Conclusion

The *PER3* VNTR genotype may have influenced the occurrence of both illnesses and injuries when number of time zones crossed and direction of travel were accounted for during the 2012 Super Rugby tournament. Specifically, the $PER3^{5/5}$ and $PER3^{4/5}$ groups were more susceptible to illnesses than the $PER3^{4/4}$ group after travel across six or more time zones compared to when there was no time zone travel. Furthermore, within the $PER3^{5/5}$ and $PER3^{4/5}$ groups, illness incidence rates were higher following more than six time zone crosses, and eastward travel compared to no time zone travel. This suggests that players carrying one of the *PER3* 5-repeat alleles were more likely to experience an illness after travelling across time zones, especially in an eastward direction.

The $PER3^{4/4}$ group was more susceptible to injuries after travelling across more than six time zones than when there was no time zone travel, suggesting that this group of players may have remained jet-lagged, especially during matches. While carrying a single *PER3* 5-repeat allele was sufficient to predispose players to illnesses after six time zone crosses, it required one to be homozygous for the *PER3* 4-repeat allele to be predisposed to injuries. In this respect, there was some evidence to suggest that the three genotypes may have been affected differently by trans-meridian travel. However, the lack of significant differences between the three genotypes for both illness and injury incidence rates when direction of travel was accounted for suggests that

other genes may be important. Alternatively, it is possible that direction of travel or number of time zones crossed will affect injury more than the *PER3* genotype.

4.6 Perspectives

The *PER3* VNTR genotype may explain the inter-individual variation in infection and injury susceptibility following trans-meridian travel. As such, coaches may want to implement robust entrainment protocols such as timed blue-light exposure and exercise training sessions to resynchronise players into the new environment in order to reduce injury and illness risk in their players. This is necessary so that illness or injury prevention and management strategies for rehabilitation and return to play can be implemented and outcome strategies reviewed. Likewise, it has the potential to improve the longevity of an individual's playing career if properly implemented.

These findings are applicable to other sports, tournaments, recreational and business travellers who undergo frequent trans-meridian travel. They also improve upon the thin collection of studies in literature with respect to the effect of trans-meridian travel on illness and injury incidence rates. However, further studies with larger sample sizes, across multiple tournaments allowing for scrutiny of number of time zones crossed and direction of travel on illness and injury incidence rates in the *PER3* VNTR polymorphism and possibly other polymorphisms are warranted.

CHAPTER 5: THE EFFECT OF BLUE-LIGHT EXPOSURE ON CIRCADIAN RESYNCHRONISATION IN INDIVIDUALS GENOTYPED AS *PER3*$^{4/4}$ AND *PER3*$^{5/5}$.

5.1 Introduction

In the modern day society, trans-meridian travel across multiple time zones is a common phenomenon not only for the general population, but also for many elite sports athletes and business people travelling for work. The primary consequence of trans-meridian travel is a cluster of symptoms known as jet-lag that can impart a physical and emotional burden to those undergoing air travels. Jet-lag is caused by the desynchronisation of the internal body clock from the external environment (Waterhouse et al., 2007, Reilly et al., 2005, Sack, 2010) and recovery from jet-lag symptoms requires resynchronisation to the new time zone. The aim of jet-lag recovery strategies is to realign the innate circadian system with the new environmental time as rapidly as possible. Current strategies designed to enhance recovery from trans-meridian travel include appropriately-timed light exposure, meal times and exercise, manipulation of sleep schedules and the use of sedative or stimulant medication. Light exposure (especially blue light, wavelength 450-495 nm) at an appropriate time-of-day is the strongest entraining stimulus for the human circadian system, making it an ideal protocol for counteracting jet-lag (Duffy and Czeisler, 2009, Roenneberg et al., 2013, Forbes-Robertson et al., 2012).

While the general rule is that for every time zone crossed, 24h of recovery time is required (Manfredini et al., 1998, Youngstedt and O'Connor, 1999, Waterhouse et al., 2007), it appears that this recovery time varies significantly between individuals (Waterhouse et al., 2007, Lee and Galvez, 2012). The variation in the human clock genes, in particular the *PER3* VNTR, may in part explain this inter-individual variation in recovery time given their associations with factors such as sleep timing (Viola et al., 2007, Goel et al., 2009, Archer et al., 2010), cortisol and melatonin hormonal secretion (Wirth et al., 2013, Dijk and Archer, 2010, Chellappa et al., 2012b). A study by Chellappa et al. (2012b) reported that sensitivity to blue light in terms of entrainment may be dependent on *PER3* VNTR genotype, as individuals homozygous for the *PER3* 5-repeat allele appeared more sensitive to blue-light than their *PER3* 4-repeat allele counterparts. Therefore the

PER3 VNTR genotype may influence efficacy of re-entrainment following trans-meridian travel when blue light exposure is used as a recovery modality. There is need for optimal resynchronisation strategies and blue-light is a good candidate, however, no one has used this specific strategy to compare two clock gene genotype groups in order to explain the apparent inter-individual variability in response to resynchronisation. Therefore, the aim of this study was to compare the extent to which individuals genotyped as $PER3^{4/4}$ or $PER3^{5/5}$ respond to appropriately-timed blue light exposure in order to resynchronise their circadian rhythm, following simulated eastward travel, based on changes in dim-light melatonin onset and cortisol circadian phases.

It was hypothesised that individuals carrying the $PER3^{5/5}$ genotype would resynchronise faster into the new environment compared to their $PER3^{4/4}$ counterparts following simulated jet-lag.

5.2 Methods

5.2.1 Participants

Sixteen males genotyped as either $PER3^{5/5}$ (n=8) or $PER3^{4/4}$ (n=8) were recruited for this study. Criteria for inclusion required that participants (i) be between 18-40 years of age, (ii) be free of sleep complaints, (iii) be in good self-reported physical and mental health, (iv) normally sleep between 6 and 10 hours per night, (v) have low habitual levels of physical activity, and (vi) have either the $PER3^{4/4}$ or $PER3^{5/5}$ VNTR genotype. While the National Sleep Foundation in the United States recommends that adults obtain 7-9h of sleep per night (Natale et al., 2009, Ferrara and De Gennaro, 2001), they also note that 6-10h of sleep per night may be appropriate given the inter-individual variation in sleep need. For this reason, individuals sleeping between 6-10h per night were included in this study.

Exclusion criteria were (i) diagnosed chronic medical conditions requiring medication, (ii) any sleep disorder, (iii) any chronic medication known to affect sleep, cortisol, melatonin and/or circadian rhythms (previous six months), (iv) night-time or rotating shift work in the previous three months, (v) trans-meridian travel of three time zones or more within the previous two

months, (vi) smoking, (vii) normal caffeine consumption greater than 300 mg·d^{-1}, (viii) red-green colour blind condition (may affect blue-light response (Smith et al., 2009)), (ix) engaged in any sport/physical activity which requires more than two training sessions per week, (x) a body mass index ≥30 kg·m^{-2} or (xi) *PER3*$^{4/5}$ VNTR genotype. Relatively inactive male participants were used to be matched to the control population in Chapter 2, and because the protocol required long periods of sedentary behaviour. Participants were recruited using flyers, posters and radio advertisements (Appendices 5A and 5B). Prior to formal entry into the study, participants were screened for eligibility (section 5.2.2.1). Descriptive characteristics of the two groups are presented in Table 5.1.

5.2.2 Study design

This intervention trial was designed to compare circadian resynchronisation differences between two groups (*PER3*$^{4/4}$ and *PER3*$^{5/5}$) exposed to blue light following simulated jet-lag. Following the screening visit, eligible participants underwent an 82h intervention. Baseline measures of their innate circadian system were taken, following which jet-lag was induced by manipulating the environmental conditions to mimic an eastward flight across six time zones. On awaking in the new time zone, all participants were exposed to blue light for 3h. The rate at which the innate circadian systems of the two groups resynchronised to the new time zone was compared. The study took place in the Chronobiology and Sleep (CBS) Laboratory at the Division of Exercise Science and Sports Medicine, University of Cape Town (UCT) and was approved by UCT's Faculty of Health Sciences Human Research Ethics Committee (HREC/REF: 360/2014). All participants gave written informed consent (Appendix 5D). The study was performed in accordance with the principles of the Declaration of Helsinki (Fortaleza, Brazil 2013), the International Conference on Harmonisation and South African Good Clinical Practice guidelines.

5.2.2.1 Screening visit

The purpose of the screening visit was to establish eligibility of volunteers for the study. The investigator explained the purpose of the study, as well as the associated procedures, risks and benefits to all volunteers. All volunteers completed a questionnaire (Appendix 5E) to obtain

descriptive characteristics and to establish their diurnal preference, and sleep quality. Volunteers completed the Ishihara colour-blind test to ensure that they were not colour-blind, and the investigator measured their height and weight. The volunteers swabbed the insides of their cheeks. Their DNA was extracted from the cheek cells and they were genotyped for the *PER3* VNTR polymorphism as described previously (sections 2.2.3.2 and 2.2.3.3). The first eight eligible individuals in each of the two genotype categories (*PER3*$^{5/5}$ and *PER3*$^{4/4}$) were invited to continue with the simulated jet-lag phase of the study.

5.2.2.2 Simulated jet-lag trial

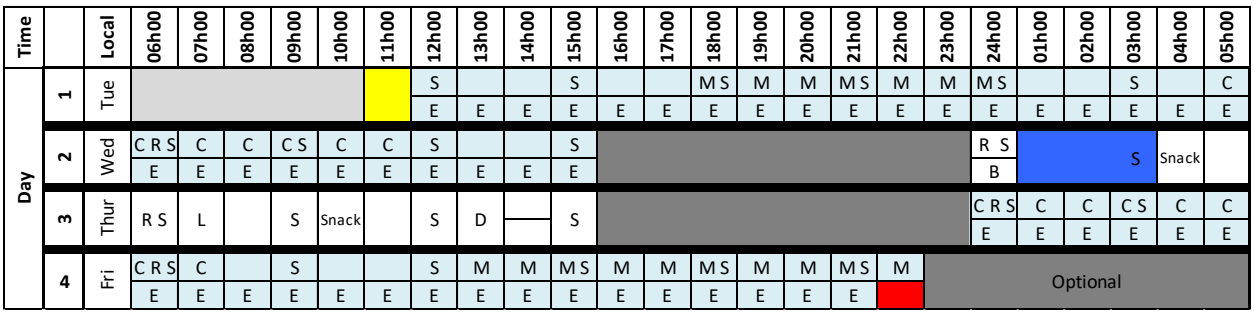
During this 82h intervention trial, participants were housed at the CBS laboratory. This space, which is sound- and light-proof, comprises bedrooms, a lounge area, bathroom and kitchen. Participants reported to the CBS laboratory at 11h30 on Day 1 (D1) of the study. From 11h30 until the end of the trial, participants were not allowed contact with the outside world. That is, all time cues from sources such as environmental light, phones, computers, TV etc. were removed. All times given in the Methods section refer to local actual time (as opposed to phase-shifted environmental time). The first 30 min were used for administrative purposes and familiarisation. Figure 5.1 shows the protocol for the trial.

The simulated jet-lag protocol began at 12h00 on D1. The aim of the first 36h of the protocol (constant routine (CR) 1) was to phase-advance the participants by 6h. Light was controlled to enforce the CR. Sleepiness was assessed every 3h using the Stanford Sleepiness Scale (SSS) and participants donated hourly saliva samples for melatonin and cortisol analysis as illustrated in Figure 5.1. CR1 ended at 16h00 on D2 when the participants were given their first 8h sleep opportunity.

Participants were woken at midnight on D2 (06h00 phase-shifted environmental time). The investigator measured their resting metabolic rate (RMR) in a fasted state. Participants then ate a standardised breakfast, following which they underwent 3h of blue-enriched light therapy. For the remainder of the waking hours on D2/3, participants were kept under normal indoor light

conditions of 80 lux from incandescent bulbs. Participants were given a second sleep opportunity on D3 starting at 16h00. They were then kept awake for a second 24h CR period to assess their endogenous circadian phases of melatonin and cortisol. Participants were allowed to go home after collection of the last sample, in the company of family or friends.

A maximum of three participants were tested at one time, and at least one investigator remained with them at all times. Participants who shared the laboratory were introduced to one another by first name only. During periods of wakefulness, participants interacted with one another in the lounge area, but they retired to individual or partitioned bedrooms for sleep opportunities.



Key:

	Trial starts		28h Constant routine		3h light therapy		Sleep in darkness
	Trial ends	B	Breakfast	C	Saliva sample for cortisol	D	Dinner
E	Ensure meal	L	Lunch	M	Saliva sample for melatonin	R	Resting metabolic rate
S	Stanford sleepiness scale						

Figure 5.1: Raster plot of the simulated jet-lag protocol.

5.2.3 Detailed testing procedures

5.2.3.1 Questionnaire

Participants completed a questionnaire detailing demographics, medical history, medication and supplement use, work, travel, sleep, exercise and dietary history (Appendix 5E). These questions were designed to determine the eligibility and obtain descriptive characteristics of volunteers for the intervention phase of the study. The questionnaire also contained the Horne-Östberg (HÖ) Morningness-Eveningness Personality Questionnaire to determine chronotype (Horne and Ostberg, 1976) as described previously in Chapter 2 (section 2.2.3.1), the Sleep Timing

Questionnaire (Monk et al., 2003), and the Pittsburgh Sleep Quality Index Questionnaire to assess sleep quality (Buysse et al., 1989).

Sleep Timing Questionnaire

This is a published, validated tool used to establish normal sleep patterns (Monk et al., 2003). Specifically, it distinguishes between workday and non-workday sleep habits and outcome variables include workday and non-workday bedtime, wake-up time and sleep latency (Appendix 5G). The outcome variables include estimated sleep latency, habitual sleep- and wake-times.

Pittsburgh Sleep Quality Index Questionnaire

This is a validated and widely used tool comprising 19 questions which are scored to give a global sleep quality score as well as scores for seven sub-components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleep medication and day-time dysfunction (Appendix 5F) (Buysse et al., 1989). The outcome variable used for this study was the global sleep quality score.

5.2.3.2 Ishihara test for colour blindness

Participants were tested for colour blindness as this might have negatively influenced the efficacy of blue light therapy (Ruger et al., 2013, Lavric and Pompe, 2014). They completed the Ishihara test online and responded to 24 different plates. Based on their responses, the test indicated whether participants had (i) normal colour vision, (ii) red green colour blindness or (iii) total colour blindness. This test has been used in previous studies similar to this one studying the effects of blue light exposure on phase shifting (Smith et al., 2009, Smith and Eastman, 2009). None of the participants recruited for this study tested positive for red green colour blindness or total colour blindness.

5.2.3.3 Buccal cell sample collection and *PER3* VNTR genotyping

These methods have been described in Chapter 2 (sections 2.2.3.2 and 2.2.3.3). Only participants who were genotyped as $PER3^{4/4}$ or $PER3^{5/5}$ were eligible for this study.

5.2.3.4 Pre-trial standardised "run-in"

The aim of this standardised two-week "run-in" was to (i) ensure participants were not sleep deprived, (ii) verify supplement and medication use pre-trial, (iii) confirm reported physical activity levels, and (iv) obtain dietary intake information for each participant. All participants were asked to adhere to a regular sleep schedule at home that was in line with their normal sleep habits, with fixed bedtimes and wake times, for two full weeks prior to each trial. Specifically, they were required to go to sleep and wake-up within one hour of their normal weekday sleep/rise times. They were also asked to aim for a "time-in-bed" length that is one hour longer than their habitual weekday sleep length. For example, a person who normally sleeps 7.5h per night during the week was asked to aim to spend 8.5h in bed each night for this two-week period. Participants remained in bed in the dark even if they could not sleep; they were not allowed to listen to music, talk, read, or watch TV during "time-in-bed". The reason for the additional hour was to try to lengthen sleep time marginally to overcome any recent sleep debt.

To confirm that participants adhered to this and to document their normal sleep habits, participants kept a daily logbook (Appendix 5K) for the two weeks prior to the start of the trial and wore wrist actigraphy monitors (Actiwatch-2, Philips Respironics, Bend, OR, USA) continuously for the second week of the run-in period. The actigraphy data were used as an objective measure of bedtime, wake-up time, time-in-bed, sleep latency, efficiency and length. The logbook documented their wake-up and bed times; medications and supplement use; consumption of alcohol- and caffeine-containing products; meal timing; and any exercise sessions. Thus the actigraphy and logbook information were used together primarily to establish and verify their normal sleep habits prior to jet-lag simulation.

5.2.3.5 Resting metabolic rate (RMR) test

RMR was determined using the ventilated hood technique (Quark RMR, Cosmed, Rome, Italy). Participants were asked to recline quietly while oxygen uptake and carbon dioxide production were measured at 10 s intervals for 20 min, or until a steady state was achieved (<5% change in respiratory exchange ratio). To ensure accuracy, the gas analyser was calibrated before each trial

was undertaken with a 3 L syringe and standard gas mixtures of oxygen (26% O_2 with the balance nitrogen) and carbon dioxide (4% CO_2, 16% O_2 and the balance nitrogen) (BOC Special Gas, Afrox, Cape Town, South Africa). RMR on the screening visit served as a familiarisation test, as well as to calculate participants' RMR so that the caloric content of their meals during the trial could be determined. The purpose of taking these measurements during the intervention trial was to observe phase shifting in a physiological system. Baseline RMR measurements were taken between 07h00-08h00 one day prior to the start of each trial, with participants in a fasted state (10-12 hours).

Participants were not permitted to consume alcohol and caffeine or to use non-steroidal anti-inflammatories 24h prior to beginning the trial. Subsequent measurements were taken at 06h00 on D2, D3 and D4, and 00h00 on D2 and D3 of the trial. The 06h00 (actual local time) measurements were taken 1h after the previous hourly snack and the 00h00 measurements were in the fasted state just before breakfast was served in the "new time zone". Outcome variables from this test included total daily energy expenditure ($kcal \cdot d^{-1}$), respiratory exchange ratio (RER- a marker of predominant fuel utilisation), fat and carbohydrate oxidation ($g \cdot day^{-1}$).

5.2.3.6 Meals

All meals were provided for participants in the CBS lab for the duration of each trial (Figure 5.1). For the two CR periods (12h00 D1 - 16h00 D2; 24h00 D3 - 22h00 D4) participants were given hourly snacks as opposed to regular meals. The reason for this was to ensure that the timing and composition of meals did not provide external time cues. The snacks were prepared using Ensure Powder (Abbott Nutrition, Johannesburg, South Africa) mixed with water, which is a complete meal replacement supplement designed to deliver approximately 55% carbohydrate, 30% fat and 15% protein per serving. Each hourly snack was isocaloric, with the exact dosage calculated to be the necessary energy requirement for each participant based on his RMR, body weight and a metabolic equivalent of approximately 1.5 $kcal \cdot kg^{-1} \cdot h^{-1}$ (very light intensity activities such as watching television and writing or desk work) for the 28h period. Standard breakfast (35 g oats, rooibos tea, apple and/or banana), lunch (hot dog rolls with chicken vienna sausages, banana

and/or apple and fruit juice) and dinner (200 g beef lasagne, mixed salad, fruit juice and/or water) meals were scheduled six- or seven-hourly from 24h00 D2 - 24h00 D3 to coincide with appropriate meal times for the "new time zone". Participants had *ad lib* access to water and rooibos tea (without sugar) during the simulated jet-lag trial. No caffeinated products were allowed.

5.2.3.7 Constant routine (CR) protocol

The CR protocol is a standard method used to unmask the endogenous circadian components of the physiological and behavioural rhythms by removing any rhythmic changes due to an individual's lifestyle or environment (Duffy and Wright, 2005, Dallmann et al., 2012, Dijk and Archer, 2010). In this study, this was achieved by keeping participants under constant temperature (25^{o}C), humidity (49%), and dim indoor light conditions (≤10 lux) with an hourly isocaloric nutritional supplement for 28 hours. Light levels at eye level were measured using a handheld digital light meter (Maplin LD 140 Light Meter, model 631, UK). During the CR protocol, participants were restricted to a regimen of semi-recumbent posture, low activity, and continuous wakefulness. They were allowed to engage in activities such as reading, listening to music (no radio), watching TV (not live, only pre-recorded), computer work (no internet), and playing board or card games. Both the TV and computer were fitted with screen filters to block light in the blue wavelength spectrum (LEE filters 767 Oklahoma Yellow, Camquip Trading CC, Johannesburg, South Africa). The clocks within the computers were deactivated so that participants could not obtain time cues from the devices. The investigator on duty enforced wakefulness during the CR.

5.2.3.8 Stanford Sleepiness Scale (SSS)

The investigator administered the SSS every 3h throughout the wake periods. The SSS is an introspective tool used to measure subjective sleepiness (Hoddes et al., 1972) by evaluating sleepiness at a particular moment in time. The scale consists of seven statements with 1 being "Feeling active and vital; alert; wide-awake" and 7 being "almost in reverie; sleep onset soon;

lost struggle to remain awake". Participants choose the statement that best represents their level of perceived sleepiness.

5.2.3.9 Induction of the 6h phase shift

All participants experienced one night of total sleep deprivation (TSD) on D1 - D2, such that they were kept awake from their natural wake-up time on D1 until 16h00 on D2 (±33h). This coincided with the first CR protocol. Following TSD, participants were given their first 8h recovery sleep opportunity from 16h00 - 24h00 on D2. Upon waking at 24h00 on D2 room light levels were increased to 80 lux, and participants were told that it was 06h00 in their new "time zone", thus shifting their environmental time cues by 6h. This simulated eastwards travel crossing six time zones. Participants were not told the degree to which their time zone environment had been shifted (i.e. 6h). The reason for this was to ensure that participants' recovery would be monitored without them being able to second-guess the difference between their innate circadian rhythm and the new environmental time. A clock was provided showing the new time zone time, participants were provided with meals at breakfast, lunch, dinner, and snacks at times appropriate for the new zone times. Light levels at eye level were held at 80 lux during the day in the new time zone.

5.2.3.10 Blue light therapy

Following the first sleep opportunity on D2, participants underwent 3h of blue-enriched light therapy. Cool white fluorescent lights (6500K, model UP-PL3030-18W, China) were mounted on adjustable wooden stands, which delivered blue light (wavelength 450-495 nm) at a brightness of 100 lux measured at eye level. Participants were required to sit at a table for this period with the lights at a distance of 50 cm from their eyes. During this time, participants were allowed to play board or card games, eat breakfast and read novels. Following the 3h blue light therapy intervention, participants were kept in ordinary indoor light (80 lux from incandescent bulbs) for 13h until bed-time on D3 (22h00 new time zone). Napping was not allowed during the light period.

5.2.3.11 Sample collection and analyses

During the two CR periods, saliva was collected from participants for measurement of melatonin (for dim-light melatonin onset, DLMO) and cortisol levels (Duffy and Dijk, 2002).

Saliva collection

Whole saliva was collected by passive drool. The participants tilted their heads forward, allowing saliva to pool on the floor of the mouth and then passed the saliva through a short straw into a polypropylene vial. Samples were immediately placed in a covered icebox to minimise light exposure. All samples were centrifuged for 10 min at 3 000 *g* at 4°C and the supernatants removed and frozen at -20^{o}C within 4h of collection until subsequent analysis.

Saliva samples were collected hourly from each participant for subsequent melatonin and cortisol level analyses. Samples for melatonin were collected between 18h00 - 24h00 D1 (7 x 2ml samples) and 13h00 - 22h00 D4 (10 x 2ml samples). Those for cortisol were collected between 05h00 D1 - 11h00 D2 (7 x 2ml samples) and 24h00 D2 - 07h00 D3 (8 x 2ml samples) (Figure 5.1). To measure DLMO a maximum sampling interval of 1h was required starting ±4h prior to and continuing to at least 4h after habitual sleep onset. Saliva for cortisol measurements were collected approximately 60 min after ingestion of any snack/meal and participants were asked to rinse their mouths out with water 10 min prior to sample collection.

Salivary melatonin and cortisol assays

Melatonin and cortisol were measured using appropriate enzyme immunoassay kits (Salimetrics™, LLC, Newmarket, Suffolk, UK). Specifically, the salivary melatonin and cortisol kits are competitive immunoassays designed and validated for the quantitative measurement of salivary melatonin and cortisol, respectively. A microtiter plate is coated with rabbit monoclonal antibodies to either melatonin or cortisol. Melatonin and cortisol in standards, controls and unknowns compete with components linked to horseradish peroxidase for the antibody binding sites on the microplate. Following incubation, unbound components were washed away. Bound melatonin or cortisol peroxidase was measured by the reaction of the horseradish peroxidase

enzyme with the substrate tetramethylbenzidine. This reaction produced a blue colour. A yellow colour was formed after stopping the reaction with 3 M acid solution. Optical density was read on a standard 96-well microplate reader (Multiscan 355, Thermo Scientific, Shanghai, China) at 450 nm and corrected at 620 nm for melatonin and 630 nm for cortisol. The amount of melatonin- or cortisol-tagged peroxidase detected, as measured by the intensity of colour, was inversely proportional to the number of components present in the sample. Concentrations of unknown melatonin and cortisol samples were determined using a standard curve constructed as per the manufacturer's instructions. All samples collected from one trial were assayed in the same batch to minimise inter-assay variability. DLMO was defined as the point where melatonin concentration reaches 4 $pg \cdot ml^{-1}$ as previously described (Pandi-Perumal et al., 2007, Keijzer et al., 2011).

5.2.4 Data and statistical analyses

Descriptive data are reported as mean ± standard deviation (SD, for normally distributed data) or median with interquartile range (IR, when data are not normally distributed). A Shapiro-Wilks test was used to test for normality of the data. Descriptive characteristics of the two *PER3* VNTR genotype groups were compared using an independent t-test or the Mann-Whitney U test. Repeated data were compared using dependent t-test, Wilcoxon matched pairs, 2-way ANOVA with repeated measures or Friedman's ANOVA tests. Phase shifting for melatonin was determined by subtracting each individual's DLMO time in the first constant routine from that in the second constant routine. A positive difference in DLMO time meant participants had an advanced melatonin phase after the 6h phase shift. Phase shifting for cortisol was determined by calculating the area under the curve (AUC) for each individual during CR1 and subtracting that from the individual's area under the curve for CR2 after the 6h phase shift. *Post-hoc* analyses were performed using Turkey's HSD tests. Data were analysed using Statistica (version 11, StatSoft Inc., Tulsa, Oklahoma, USA). Significance was accepted for $p<0.05$.

5.3 Results

5.3.1 Participant characteristics

Descriptive characteristics of the two *PER3* VNTR polymorphism groups are shown in Table 5.1. There were no differences between any of the variables. Eligibility for this study was based on an individual's *PER3* genotype. Since the *PER3* VNTR polymorphism has been reported not to differ greatly with ethnicity (Ciarleglio et al., 2008), it was not essential to match the two groups for ethnicitiy.

Table 5.1: Participant characteristics.

	***PER3*$^{4/4}$ (n=8)**	***PER3*$^{5/5}$ (n=8)**	**p-value**
Age (y)	23.0 (4.5)	24.5 (4.5)	0.983
Height (cm)	177.6±5.6	178.6±6.7	0.749
Weight (kg)	76.8±10.8	77.2±13.4	0.942
Body mass index (kg·m^{-2})	24.3±3.2	24.1±3.2	0.886
Body fat (%)	18.5±4.2	19.6±4.9	0.669
HÖ-score	45.6±12.3	52.1±9.3	0.253
Pittsburgh sleep quality	4 (2.25)	4 (1.25)	0.804
Training (days·week^{-1})	2.3±1.2	2.2±0.9	0.548
Ethnicity (B:MA:W)	4:1:3	2:0:6	0.253

Data are presented as mean ± SD, median with IR or counts. HÖ: Horne-Östberg, B: black African, MA: mixed ancestry, W: white. Significance was determined using an independent t-test, Mann-Whitney U test or Fisher's exact test.

A summary of the participants' sleep data collected via actigraphy for seven days prior to the start of the trial is shown in Table 5.2. Actigraphy data files for two participants were not downloadable, while data from one participant was incomplete (participant removed actiwatch on retiring to bed). Total sleep time for all participants was longer for the night before the trial (8.7±1.3h) compared to the average sleep time for the seven days before the trial (6.9±1.0h, $p<0.001$). Furthermore, when analysed by genotype, the *PER3*$^{4/4}$ group slept for longer the night before the trial 8.9(1.2h) compared to the seven days prior to the trial 6.3(0.8h). However, no significant differences in total sleep time for the seven days prior to the trial 8.6(2.6h) and the night before the trial (7.2(0.7h), $p=0.438$) were noted in the *PER3*$^{5/5}$ group.

Table 5.2: Sleep characteristics of the *PER3$^{4/4}$* and *PER3$^{5/5}$* groups measured in the week prior to the start of the trial.

	***PER3$^{4/4}$* (n=8)**	***PER3$^{5/5}$* (n=5)**	**p-value**
Bed-time	24h15 (1h12)	23h26 (0h56)	0.057
Wake-up time	08h35 (1h20)	07h32 (0h56)	0.280
Total-time-in-bed (h)	7.3 (0.9)	7.9 (0.2)	0.263
Total sleep time (h)	6.3 (0.8)	7.2 (0.7)	0.255
Sleep onset latency (min)	7.4 (6.1)	1.2 (14.3)	0.881
Sleep efficiency (%)	88.7 (2.5)	92.1 (6.1)	0.487
WASO time (min)	28.3 (7.4)	28.7 (7.4)	0.311

Data are presented as median with IR. WASO: wake after sleep onset. Significance was determined using a Mann-Whitney U test.

5.3.2 Sleep characteristics of participants during the trial

Sleep data for both groups measured using actigraphy during the trial are shown in Table 5.3. Data are presented for sleep opportunity (SO) 1 and SO2 as they took place after two different conditions. SO1 took place immediately after the first 28h CR period, starting at 16h00 and finishing at 24h00 (D2, local time). SO2 was also an 8h sleep opportunity, which took place after a full day (16h) in the new time zone, between 16h00 and 24h00 on D3. During SO1 the *PER3$^{5/5}$* group slept for longer (p=0.031) and had a better sleep efficiency (p=0.031) compared to the *PER3$^{4/4}$* group. During SO2 the *PER3$^{4/4}$* group had a longer sleep latency (p=0.050).

Sleep data were also compared between SO1 and SO2 within each of the two groups. No significant differences were observed between SO1 and SO2 for all variables for the *PER3$^{4/4}$* group. However, the *PER3$^{5/5}$* group slept for longer (p=0.012), had greater sleep efficiency (p=0.012) and less WASO time (p=0.025) during SO1 compared to SO2 (Table 5.3).

Table 5.3: Sleep characteristics of the *PER3*$^{4/4}$ (n=8) and *PER3*$^{5/5}$ (n=8) groups during the first (SO1) and second (SO2) sleep opportunities.

	SO1		SO2		p-values			
	***PER3*$^{4/4}$**	***PER3*$^{5/5}$**	***PER3*$^{4/4}$**	***PER3*$^{5/5}$**	**p_1**	**p_2**	**p_3**	**p_4**
Total sleep time (h)	7.2 (0.2)	7.4 (0.6)	6.8 (1.2)	7.2 (0.4)	0.031	0.208	0.093	0.012
Sleep onset latency (min)	2.0 (1.1)	1.8 (3.3)	7.0 (2.6)	2.6 (3.5)	0.834	0.050	0.161	0.310
Sleep efficiency (%)	92.0 (9.6)	96.0 (3.2)	87.6 (6.7)	91.3 (15.4)	0.031	0.227	0.093	0.012
WASO time (min)	22.5±22.3	17.1±10.3	26.4 (22.2)	25.8 (10.7)	0.114	0.667	0.263	0.025

Data are presented as median with IR. SO: Sleep opportunity, WASO: Wake after sleep onset. Significance was determined using the Mann-Whitney U and Wilcoxon matched pair's tests. p_1: SO1 *PER3*$^{4/4}$ v *PER3*$^{5/5}$, p_2: SO2 *PER3*$^{4/4}$ v *PER3*$^{5/5}$; p_3: *PER3*$^{4/4}$ SO1 v SO2, and p_4: *PER3*$^{5/5}$ SO1 v SO2.

5.3.3 Sleepiness of participants during the trial

Subjective sleepiness data for both groups measured every 3h throughout the trial are presented in Figure 5.2. While there was a time effect in the level of sleepiness ($p<0.001$) during CR1 (Figure 5.2A), there was no *PER3* genotype effect ($p=0.316$) or time-by-genotype interaction ($p=0.504$) effect. In contrast, there were no time ($p=0.516$), genotype ($p=0.394$) or time-by-genotype interaction ($p=0.332$) effects during daytime in the new time zone (Figure 5.2B). Likewise, no significant differences were noted for time ($p=0.532$), genotype ($p=0.468$) or time-by-group interaction ($p=0.944$) effects during CR2 (Figure5.2C). No significant differences were noted in AUC between the *PER3*$^{4/4}$ and *PER3*$^{5/5}$ groups' rate of perceived sleepiness during all conditions (CR1: Figure 5.2D, Daytime: Figure 5.2E and CR2: Figure 5.2F).

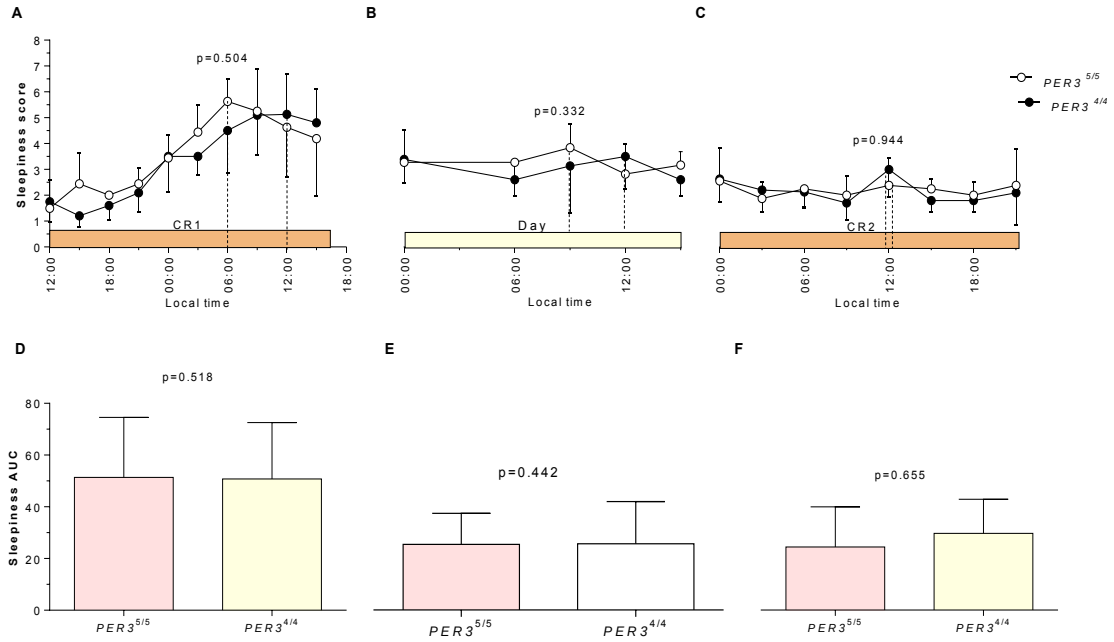


Figure 5.2: **Change in perceived sleepiness levels as measured by the Stanford Sleepiness Scale in the *PER3*$^{5/5}$ and *PER3*$^{4/4}$ groups during CR1 (A), Daytime (B) and CR2 (C) phases of the trial.** Area under the curve (AUC) data are also presented for the CR1 (D), daytime (E) and CR2 (F) phases. Data are presented as mean ± standard deviation. CR: Constant routine and Daytime: 16h day (waking hours) in new time zone. Significance was determined using a two-way ANOVA with repeated measures (A, B and C) and an independent t-test (D, E and F).

5.3.4 Energy expenditure during the trial

Energy expenditure data for the two groups are presented in Tables 5.4 and 5.5. Table 5.4 shows the metabolic data for the two groups at two time points taken at 48h intervals: 06h00 local time on- D2 and D4. The D2 and D4 measurements were during CR1 and CR2, respectively, and thus taken 1h after the previous hourly snack. These time points were chosen for comparison because they reflect time-standardised (i.e. morning) changes in RMR from baseline before (D2 06h00) and after (D4 06h00) blue light therapy.

There were no significant differences in energy expenditure, fat and carbohydrate oxidation rates, and respiratory exchange ratio in the *PER3*$^{5/5}$ group between D2 and D4; and none of these variables were different from baseline at D2 or D4. In the *PER3*$^{4/4}$ group, none of the variables

were different between D2 and D4, nor did they change differently from baseline. There were no significant differences in RER (p=0.756), energy expenditure (p=0.881), carbohydrate (p=0.253) and fat (p=0.955) oxidation rates between groups at 06h00 on D2. Likewise, no significant differences were noted in RER (p=0.782), energy expenditure (p=0.679), carbohydrate (p=0.950) and fat (p=0.733) oxidation rates between groups at 06h00 on D4.

Table 5.4: Resting metabolic rate measurements for the *PER3$^{4/4}$* (n=8) and *PER3$^{5/5}$* (n=8) groups taken at 48h intervals, measured at 06h00 D2 and 06h00 D4 during the trial, as well as within subject change from baseline.

		Baseline	06h00 D2	06h00 D4	p_1	p_2
PER3$^{5/5}$	**EE (kcal·day^{-1})**	1649.3±316.5	1777.6±414.3	1776.2±377.4	0.801	0.178
	Fat (g·day^{-1})	166.3 (40.4)	101.8 (34.0)	99.9 (62.4)	0.297	0.419
	CHO (g·day^{-1})	66.9±59.1	169.1±89.9	195.5±77.9	0.326	0.052
	RER	0.74 (0.09)	0.81 (0.07)	0.83 (0.05)	0.267	0.151
PER3$^{4/4}$	**EE (kcal·day^{-1})**	1605.4±324.5	1749.0±252.2	1980.3±89.6	0.064	0.104
	Fat (g·day^{-1})	122.1 (34.0)	97.9 (156.2)	96.7 (63.3)	0.945	0.531
	CHO (g·day^{-1})	107.2±66.9	139.5±88.9	119.8±63.9	0.096	0.055
	RER	0.78 (0.05)	0.77 (0.06)	0.85 (0.07)	0.219	0.061

Data are presented as mean ± SD and median with IR. Change from baseline. CHO: carbohydrate oxidation rate, D2: day 2 of the trial, D4: day 4 of the trial, EE: energy expenditure, Fat: fat oxidation rate; RER: respiratory exchange ratio. p_1: within group D2 v D4 comparison; p_2: within group change from baseline comparison (Baseline, D2, D4). Significance was determined using dependent t-test or Wilcoxon matched pairs test p_1 and an ANOVA with repeated measures or Friedman ANOVA test p_2.

Table 5.5 shows the waking, fasted metabolic data for the two groups taken at two time points: D2 and D3 upon waking from the two sleep opportunities during the trial (midnight local time, 06h00 new time zone time). These time points were chosen for comparison as they enable comparison of fasted morning energy expenditure measurements between pre-trial (baseline, 06h00), first morning in new time zone (D2, 06h00 new time zone time) and second morning in new time zone (D3, 06h00 new time zone time). The *PER3$^{5/5}$* group oxidised less fat (p=0.017) and more carbohydrate (p=0.015) on D3 compared to D2. Accordingly, their RER was higher on D2 (p=0.003). In addition, within the *PER3$^{5/5}$* group, the change in fat oxidation from baseline was

significantly higher at D2 (p=0.035) while that for carbohydrate oxidation was higher at D3 (p=0.009). Accordingly, RER change from baseline was higher at D3 (p=0.005). In the *PER3*$^{4/4}$ group, none of the variables were different at D3 compared to D2. There were no significant differences in RER (p=0.865), energy expenditure (p=0.622), carbohydrate (p=1.000) and fat (p=0.943) oxidation rates between groups at 24h00 on D2. Likewise, no significant differences were noted in RER (p=1.00), energy expenditure (p=0.486), carbohydrate (p=0.721) and fat (p=0.798) oxidation rates between groups at 24h00 on D3.

Table 5.5: Morning, fasted resting metabolic rate measurements of the *PER3*$^{5/5}$ (n=8) *PER3*$^{4/4}$ (n=8) groups measured at 24h00 D2 and 24h00 D3 during the trial, as well as their respective changes from baseline.

		Baseline	24h00D2	24h00 D3	p_1	p_2
PER3$^{5/5}$	**EE (kcal·day^{-1})**	1649.3±316.5	1691.0±305.3	1677.3±282.6	0.776	0.813
	Fat (g·day^{-1})	146.5±46.5	152.8±46.8	112.5±54.7	0.017	0.035
	CHO (g·day^{-1})	66.9±59.1	71.8±71.6	149.6±89.2	0.016	0.009
	RER	0.75±0.07	0.74±0.06	0.82±0.09	0.003	0.005
PER3$^{4/4}$	**EE (kcal·day^{-1})**	1556.0 (562.0)	1702.0 (377.0)	1620.0 (336.0)	0.313	0.403
	Fat (g·day^{-1})	122.1 (33.5)	167.6 (10.9)	81.3 (68.4)	0.125	0.290
	CHO (g·day^{-1})	95.9 (119.7)	57.6 (33.4)	176.8 (117.0)	0.250	0.273
	RER	0.78 (0.05)	0.73 (0.06)	0.85 (0.09)	0.063	0.117

Data are presented as mean ± SD and Median with IR. CHO: carbohydrate oxidation rate, D2: day 2 of the trial, D3: day 3 of the trial, EE: energy expenditure, Fat: fat oxidation rate; RER: respiratory exchange ratio. p_1: within group D2 v D3 comparison and p_2: within group change from baseline comparison. Significance was determined using dependent t-test and Wilcoxon matched pairs test p_1 and an ANOVA with repeated measures p_2.

5.3.5 Changes in salivary melatonin concentration

Hourly salivary melatonin concentrations for all participants measured during CR1 and CR2 are presented in Figure 5.3A. The dotted red lines represent the time of DLMO. When data from both groups were pooled there was a time-by-condition interaction effect for melatonin (p<0.001) such that there was an advanced increase in melatonin concentration during CR2 (Figure 5.3A). This corresponded with a significantly earlier mean DLMO onset time during CR2 (17h44±0h27)

compared to CR1 (19h28±0h16), indicating a mean phase advance of 1h44min (Figure 5.3B, p=0.026). Figures 5.3C and D show that DLMO phase advanced significantly for the *PER3*$^{4/4}$ (p=0.012) and *PER3*$^{5/5}$ (p=0.011) groups, respectively. Prior to inducing jet-lag, DLMO was similar between the *PER3*$^{4/4}$ (19h34±0h12) and *PER3*$^{5/5}$ (19h23±0h19) groups during CR1 (p=0.224). During CR2, however, mean DLMO time for the *PER3*$^{5/5}$ group (17h31±0h21) occurred earlier than that of the *PER3*$^{4/4}$ group (17h58±0h24) during CR2 (p=0.032). Furthermore, the extent to which DLMO shifted for the *PER3*$^{4/4}$ (01h35) was less than that of the *PER3*$^{5/5}$ (01h52, p=0.021).

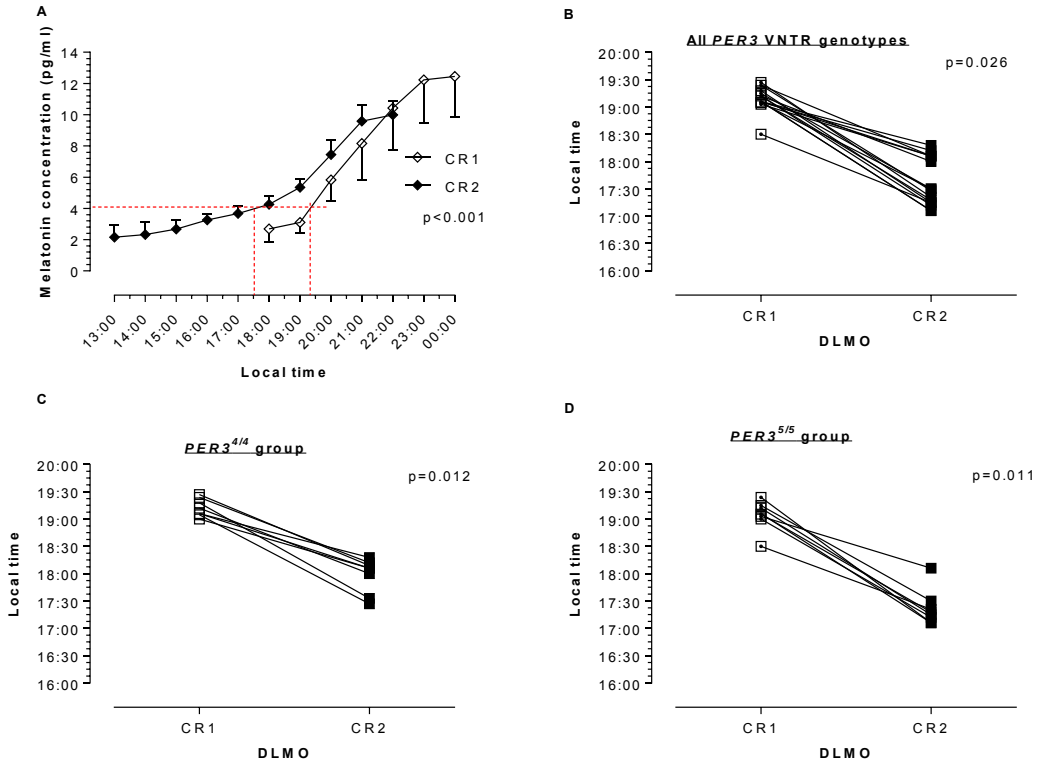


Figure 5.3: **Hourly salivary melatonin concentrations in CR1 and CR2 (A) and dim-light melatonin onset (DLMO) times for CR1 and CR2 for all participants (B) the *PER3*$^{4/4}$ group (C) and the *PER3*$^{5/5}$ group (D)**. Data are presented as mean ± SD (A) and as each individual's DLMO time before and after blue-light treatment (B, C and D). CR: constant routine. The red dotted line indicates DLMO for all participants combined. Significance was determined using a two-way ANOVA with repeated measures (A) and a Wilcoxon matched pairs test (B, C and D).

5.3.6 Salivary cortisol measurements

Salivary cortisol data are presented in Figure 5.4. The pooled hourly cortisol data measured for all participants during CR1 and CR2 are presented in Figure 5.4A. Salivary cortisol peaked at 08h00 during CR1. In contrast, cortisol measured in CR2 shows a small peak at 01h00 local time, where after levels dropped then rose again after 04h00 until the end of the sampling period (07h00). There was no time-by-group interaction effect for cortisol during the three overlapping sampling period. The area under the curve (AUC) for cortisol was determined for CR1 and CR2 for each participant. Figure 5.4B indicate that simulated eastward travel resulted in suppression of morning cortisol AUC in CR2 (0.71±0.24 $\mu g \cdot dL^{-1}$), compared to CR1 (1.04±0.45 $\mu g \cdot dL^{-1}$; p=0.022). Figures 5.4C and D compare the cortisol curves of the two genotype groups during CR1 and CR2, respectively. While there were no time-by-group interaction effects for CR1 (Figure 5.4C) and CR2 (Figure 5.4D), there were significant group effects for both conditions such that cortisol levels were significantly higher in the $PER3^{5/5}$ group compared to the $PER3^{4/4}$ group during CR1 and during CR2. In confirmation of this, Figures 5.4E and F show the cortisol AUCs for the two genotype groups during CR1 and CR2, respectively. The $PER3^{4/4}$ group had lower values during both CR1 (p=0.328) and CR2 (p=0.028).

Lastly, a single cortisol peak representing the cortisol awakening response peak is apparent for both genotype groups during CR1 (indicated by the arrow in Figure 5.4C). In comparison the $PER3^{5/5}$ group shows two peaks during CR2 (Figure 5.4D). The first arrow in Figure 5.4D indicates the original cortisol awakening response peak, while the second arrow indicates the cortisol awakening response peak in the new time zone.

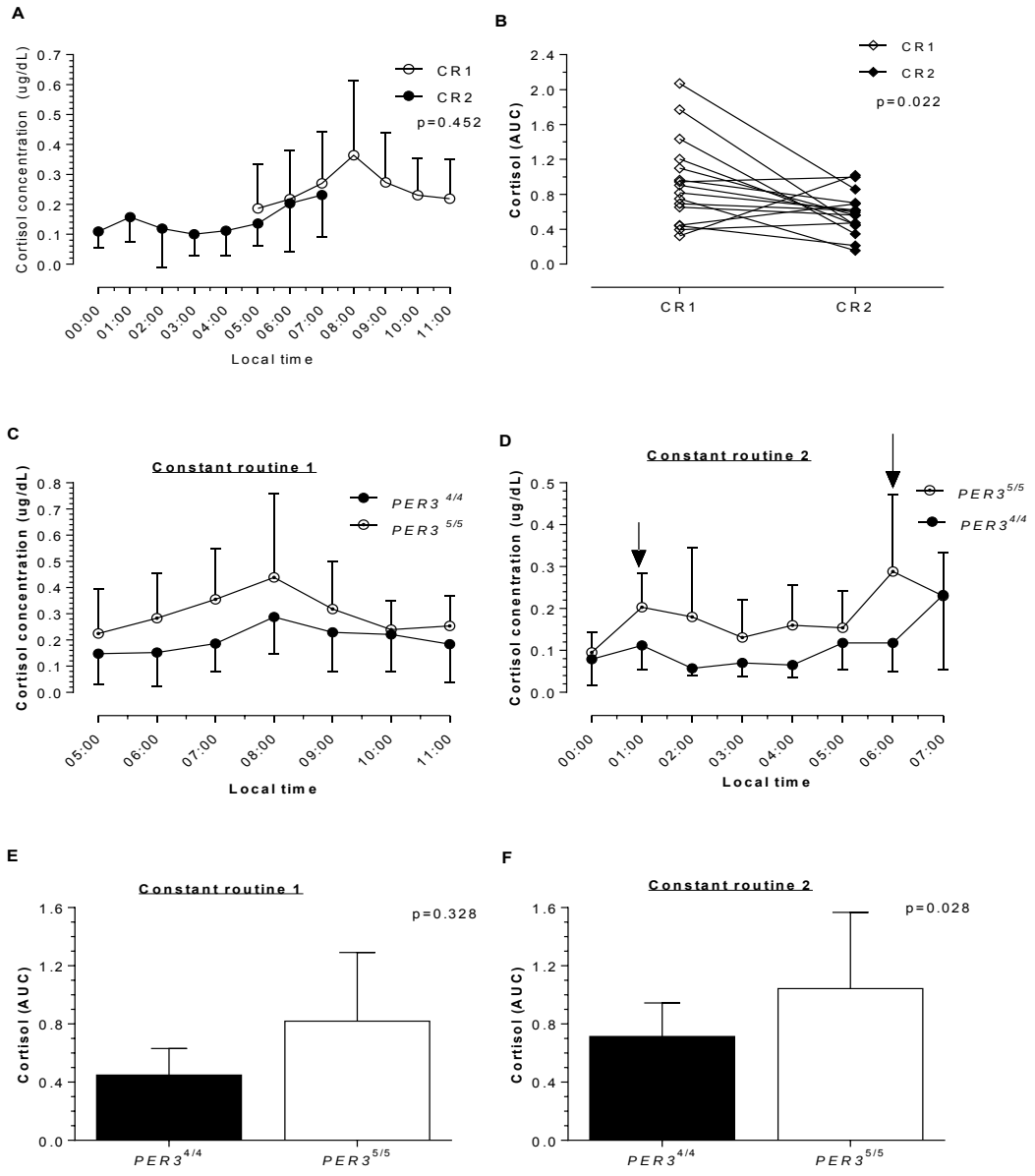


Figure 5.4: Hourly salivary cortisol concentrations of all participants (A) and cortisol AUC for CR1 and CR2 for all participants (B) as well as genotype group comparisons measured during CR1 (C) and CR2 (D), and genotype group comparisons of cortisol AUC in CR1 (E) and CR2 (F). Data are presented as mean ± SD and median with IR. AUC: area under the curve, CR: constant routine, arrows indicate cortisol peaks. Significance was determined using a Wilcoxon matched pair's test (B), ANOVA with repeated measures (C-D) and Mann-Whitney U (E-F) tests, respectively.

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5.4 Discussion

The aim of this study was to compare the extent to which individuals genotyped as *PER3*$^{4/4}$ or *PER3*$^{5/5}$ respond to appropriately-timed blue light exposure in order to resynchronise their circadian rhythm, following simulated eastward travel, based on changes in dim-light melatonin onset and cortisol circadian phases. The melatonin data indicate that while both groups were phase-shifted by the intervention, they entrained to the new time zone at different rates. The main finding was that there was a significant difference in DLMO phase shift between the *PER3*$^{5/5}$ and *PER3*$^{4/4}$ genotype groups during CR2. Specifically, the DLMO of the *PER3*$^{5/5}$ group occurred 27 min earlier than the *PER3*$^{4/4}$ group following the jet-lag induction protocol (Figure 5.3D). This implies that the *PER3*$^{5/5}$ individuals had phase-shifted more than the *PER3*$^{4/4}$ individuals, thus one may hypothesise that *PER3*$^{5/5}$ individuals may feel more tired or fall asleep earlier compared to the *PER3*$^{4/4}$ group in the new time zone.

A recent study by Chellappa et al. (2012) reported that the *PER3*$^{5/5}$ genotype was more sensitive to blue light. This may explain why the *PER3*$^{5/5}$ group in this study had an earlier DLMO compared to the *PER3*$^{4/4}$ group following timed enriched-blue light treatment in CR2. Alternatively, the differences in DLMO between the two *PER3* VNTR genotype groups under the same laboratory conditions may have arisen due to differences in the phase angle of entrainment as a result of differences between the timing of the circadian clock and external environmental time cues (Wright et al., 2013, Roenneberg et al., 2007, Duffy and Czeisler, 2009). For example, Wright et al. (2005) reported phase angle differences using DLMO as a circadian phase marker of the internal circadian period in healthy adults maintaining the same sleep schedules inside and outside the laboratory. Specifically, they reported that individuals who exhibited shorter circadian periods initiated sleep and awakened at an earlier biological time compared to those individuals with longer circadian periods.

It is however unlikely to have been the case in this study because the two *PER3* VNTR groups had similar starting DLMO during CR1. As such, it is likely that the blue light resynchronisation strategy used in this study may actually be more effective in resynchronising the *PER3*$^{5/5}$ genotype.

During CR1, there was an 11 min DLMO difference between the two genotype groups, however, this was not statistically significant. The small sample size and possibly the young age of participants- all still very "night owl" in nature, may have precluded the detection of any significant differences in DLMO between the two genotype groups in this study. This is conceivable given that the melatonin profile has been reported to be closely associated with the internal phase of the sleep-wake cycle which is different between the two genotype groups (Chellappa et al., 2012b). In support of this, Drake et al**.** (2015) demonstrated an earlier DLMO in *PER3*$^{5/5}$ compared to *PER3*$^{4/4}$ individuals in industrial shift workers who worked nighttime shifts only and complied with >5h nightly sleep for two weeks before the start of the controlled laboratory experiment.

5.4.1 *PER3* VNTR genotype and the cortisol circadian phase

Cortisol was used as a second circadian phase marker to ascertain the extent to which re-entrainment had occurred in the two *PER3* VNTR groups. There was a 2h phase advance in the cortisol peak of the *PER3*$^{5/5}$ group, further indicating the effectiveness of the jet-lag induction protocol in phase shifting participants. Of interest was that, amplitude and AUC for cortisol were greater in the *PER3*$^{5/5}$ group compared to the *PER3*$^{4/4}$ group for both CRs, and it was significantly larger in CR2. This is congruent with findings from a recent study by Wirth et al. (2013), who reported higher amplitude and AUC for cortisol in the *PER3*$^{5/5}$ compared to the *PER3*$^{4/4}$ New York police officers who were involved in nighttime shift work. This result is also in line with studies that have demonstrated elevated cortisol levels among morning-types compared to evening-types in general (Kudielka et al., 2007, Griefahn and Robens, 2008).

The differences in amplitude and AUC for cortisol between the two *PER3* VNTR groups during CR2 may be due to phase shifting of the circadian clock and dampening of output rhythms as observed in DLMO and sleepiness levels for the same output and activity. The lower cortisol awakening response during CR2 for both *PER3* VNTR groups may be explained by the fact that these individuals were awoken before the time of their entrained biological awakening (Randler and Frech, 2006). It could also just be an indication of psychological stress arising from changes

in adrenal sensitivity around the process of awakening at this time-of-day (Shibuya et al., 2014, Clow et al., 2010).

5.4.2 *PER3* VNTR genotype and metabolism

The RMR test was used to assess metabolic re-entrainment in the $PER3^{4/4}$ and $PER3^{5/5}$ groups. Fasting morning energy expenditure was similar after 24h in the new time zone compared to awakening on the first morning in each of the genotype groups (Table 5.5). Differences in the predominant type of fuel oxidised by the $PER3^{5/5}$ group were, however, evident. Specifically, the $PER3^{5/5}$ group oxidised less fat and more carbohydrate after 24h in the new time zone. This finding suggests that sleep deprivation, such as that induced by CR1, may have affected adipokines such as leptin (Spiegel et al., 2005, Knutson et al., 2007) in the $PER3^{5/5}$ individuals, but not in the $PER3^{4/4}$ individuals. Specifically, sleep deprivation lowers serum leptin concentration in the blood (Spiegel et al., 2005), which acts as a negative feedback on body fat stores to the appetite centers of the brain (Ahima and Flier, 2000, Tasali et al., 2008).

A reduction in leptin concentration has been shown to promote fatty acid oxidation in the mitochondria (Ruderman and Saha, 2006), which may explain the elevated fat oxidation in the $PER3^{5/5}$ individuals following sleep deprivation in the jet-lag study. Other studies have reported a reduction in glucose uptake by peripheral tissues in favour of fat oxidation following sleep deprivation (Tasali et al., 2008, Knutson et al., 2007), which is consistent with what was observed in the $PER3^{5/5}$ group. The elevated carbohydrate oxidation in the $PER3^{5/5}$ group at 24h00 during D3 compared to D2 implies an up-regulation of hormones involved in gluconeogenesis after two nights of sleep recovery. This suggests that energy production may be affected differently in the two *PER3* VNTR genotypes following circadian disruption.

Alternatively, these variances may simply be due to differences in the function of the circadian clock (Markwald et al., 2013, Reilly and Edwards, 2007) between the 24h00 time points during D2 and D3 in the $PER3^{5/5}$ group. The neuroendocrine and metabolic systems may be co-regulated by the body clock resulting in differential production of RNAs that induce vital factors involved in

metabolism (Hastings et al., 2003, Laposky et al., 2008). The lack of significance in all RMR variables in the *PER3*$^{4/4}$ group further suggests that hormones and adipokines involved in fat and carbohydrate metabolism may be regulated differently in the two genotypes following circadian disruption.

5.4.3 *PER3* VNTR genotype and sleepiness

Sleepiness is characterised by an overwhelming inclination to fall asleep or persistent uncontrollable urge to sleep and a lack of energy (Maire et al., 2014, Shen et al., 2006, Beebe et al., 2007). While there were no amplitude differences in the peaks of sleepiness levels between the two *PER3* VNTR groups during CR1, the *PER3*$^{5/5}$ group reached their highest sleepiness levels earlier compared to their *PER3*$^{4/4}$ counterparts (Figure 5.2A). This could be explained by the fact that there are considerable differences in sensitivity to sleep loss in the *PER3*$^{4/4}$ and *PER3*$^{5/5}$ genotypes as noted in previous sleep deprivation studies (Maire et al., 2014, Goel et al., 2009, Van Dongen et al., 2012, Archer et al., 2008). Specifically, the *PER3*$^{5/5}$ genotype has been shown to have a faster build-up of sleep pressure than the *PER3*$^{4/4}$ genotype, which may explain the earlier peak in sleepiness in this group of individuals. Thus, it is possible that the *PER3* gene may be mediating vulnerability to sleep loss experienced by participants in response to the same laboratory conditions assessed using the Stanford Sleepiness Scale in this study.

Alternatively, these differences may be related to circadian and homeostatic effects (Chellappa et al., 2012b, Van Dongen et al., 2012, Goel et al., 2009), since the body clock generates and maintains changes in physiological rhythms that control the sleep-wake cycle. Thus, interaction between these two regulatory processes may have induced the non-linear progression in sleepiness particularly during CR1. Sleepiness levels dampened considerably in both groups during daytime in the new time zone (Figure 5.2B) and CR2 (Figure 5.2C) possibly as a result of the recovery sleep, which appears to have been beneficial in restoring sleepiness levels to subjective rates at the beginning of the trial. The lack of a significant difference in sleepiness levels between the two genotypes during CR2 implies that neither of the *PER3* VNTR groups recovered better than the other (Figures 5.2C and F). However, it is possible that the duration of

CR2 was not long enough to observe the disentanglement effects of jet-lag on the circadian system in the two *PER3* VNTR groups. Therefore, time extension of the CR2 condition might have enabled observation of any differences between the $PER3^{4/4}$ and $PER3^{5/5}$ groups in sleepiness levels.

5.4.4 *PER3* VNTR genotype and sleep

A minor observation from this study was that the two *PER3* VNTR groups differed significantly in sleep characteristics immediately following the first 28h CR period. Total sleep time and efficiency were significantly greater in the $PER3^{5/5}$ group compared to the $PER3^{4/4}$ group during SO1 of the trial, suggesting that the $PER3^{5/5}$ group was affected to a larger degree by sleep deprivation and required more sleep than the $PER3^{4/4}$ group to recover. It was also noted that total sleep time and quality were greater during SO1 compared to SO2 in the $PER3^{5/5}$ group. This result demonstrates the differences in sleep debt (higher) accumulated prior to SO1, and the reduced sleep debt prior to SO2 combined with sleeping out of phase with circadian sleep time in the $PER3^{5/5}$ group. This is important because it suggests that the greater sleep debt of the $PER3^{5/5}$ group means that they sleep better on the first night in the new time zone, but then struggle with out of phase sleep for subsequent nights. This is indicated by the decrease in total sleep time and an increase in sleep onset latency and wake after sleep onset episodes in the $PER3^{5/5}$ group on the second night of recovery sleep during CR2.

Perhaps the $PER3^{4/4}$ individuals who do not have such great sleep debt begin to realign their circadian sleep phase earlier compared to the $PER3^{5/5}$ individuals. This is conceivable since the $PER3^{4/4}$ group in this study appeared to have not fully offset the sleep debt in the first recovery sleep, thus they may have had sufficient sleep debt during the second recovery sleep to attain good sleep compared to their $PER3^{5/5}$ counterparts. This reflects active re-adjustment in the $PER3^{5/5}$ group whose homeostatic sleep pressure is tightly regulated and is dissipated faster during a sleep episode compared to that of the $PER3^{4/4}$ group (Dijk and Archer, 2010, Goel et al., 2009, Maire et al., 2014, Chellappa et al., 2012b). This is consistent with other previous studies (Viola et al., 2007, Groeger et al., 2008, Lo et al., 2012), which reported a greater deterioration

in total sleep time and cognitive function in the *PER3*$^{5/5}$ genotype following sleep deprivation. The *PER3* VNTR genotype may affect key markers of sleep homeostasis differently (Viola et al., 2007, Dijk and Archer, 2010, Van Dongen et al., 2012), including sleep latency and efficiency in response to sleep deprivation as was the case in this study.

A number of studies have suggested that the *PER3* VNTR genotype may only play a marginal role in influencing circadian processes (Costa et al., 2011, Shearman et al., 2000). More specifically, that the effect of the *PER3* VNTR genotype on sleep homeostasis may differ depending on the duration of sleep deprivation or the strategy used to adjust sleep loss (Goel et al., 2009, Gamble et al., 2011, Wirth et al., 2013), leading to different findings in various studies. For instance, Viola et al. (2007) reported that the *PER3*$^{5/5}$ individuals had poor cognitive performances and high sleep propensities than the *PER3*$^{4/4}$ individuals before and after total sleep deprivation (40h). Goel et al. (2009) reported no differences in cognitive performance and physiological alertness following partial sleep deprivation (4h per night) in *PER3*$^{4/4}$ and *PER3*$^{5/5}$ individuals across five nights of testing. Furthermore, Gamble et al. (2011) reported that, of the five strategies used by nurses to shift back their sleep patterns to nocturnal sleep times on days off, sleep deprivation was the least effective. Specifically, nurses who used this strategy were most poorly adapted compared to those who used napping, night-stay, incomplete and complete switching of daytime and nighttime shifts.

5.4.5 *PER3* VNTR genotype distribution in different ethnic groups

Studies that have assessed the *PER3* VNTR genotype distribution in various ethnic groups around the world have reported no significant differences in the distribution of this gene (Ciarleglio et al., 2008, Nadkarni et al., 2005, Chellappa et al., 2012b). For example, studies by Ciarleglio et al. (2008) reported no significant differences in the *PER3* VNTR genotype distribution in the African American, European American and the Ghanaian populations. Specifically, they reported a high frequency of the *PER3* 4-repeat allele and a lower frequency of the *PER3* 5-repeat allele. Furthermore, a recent study in our research group (Nyambura Shawa, MSc thesis, 2014, Faculty of Science, University of Cape Town, Cape Town, South Africa) found no differences in the *PER3*

VNTR genotype frequencies in black African, mixed ancestry, Caucasian and Indian South African populations. Therefore, males of low physical activity from all ethnicities that were age matched to the Super Rugby population were recruited for this study.

5.4.6 Study limitations

This study had a few limitations. The cortisol peak of the *PER3*$^{4/4}$ group could not be determined during CR2 due to limited sampling points. This limited the scope of cortisol analyses that could be performed. Future research with increased sampling points during CR2 is warranted in order to determine the cortisol peak in the *PER3*$^{4/4}$ group. The sample size used in this study was also small. Therefore, the data must not be over-interpreted, as there is a risk of committing a type 1 error.

This study lacked a control condition. Ideally, a randomised crossover study would have been performed in which participants repeated the trial where the blue-light therapy would be replaced with light of a longer wavelength. This would have provided a full comparison of the effects of blue-enriched light in resynchronising the *PER3*$^{4/4}$ and *PER3*$^{5/5}$ genotypes to a new environment following trans-meridian travel. The effects of simulated jet-lag following westward travel were not investigated.

5.5 Conclusion

Results from this study suggest that the two *PER3* VNTR groups differed significantly in the speed with which they resynchronised to the new environment when blue light therapy was included as a re-entrainment strategy after simulated eastward travel across six time zones. The DLMO of the *PER3*$^{5/5}$ group occurred significantly earlier during CR2 compared to that of the *PER3*$^{4/4}$ group indicating that they were in the process of re-entraining to the new time zone faster. One of the challenges facing individuals after eastward travel is being able to fall asleep early in the new time zone. Practically, having an earlier DLMO suggests that the *PER3*$^{5/5}$ group may be able to sleep earlier since nighttime sleepiness and sleep onset are related to DLMO timing (Keijzer et al., 2014, Martin and Eastman, 2002, Wright et al., 2013).

These results go some way to understanding the inter-individual differences in jet-lag recovery. The *PER3* genotype of an individual, coupled with some yet unidentified factors, may be important in the rate of resynchronisation of circadian rhythms to a new time zone. Knowing the *PER3* genotype of an individual may make predicting how rapidly or effectively they respond to resynchronisation interventions, and thus may have practical implications for team physicians in the way they manage players, in order to maintain proper circadian alignment with the external 24h day. Future studies with larger sample sizes and more sampling points may be warranted to confirm these findings. This was a pilot study demonstrating proof of concept and the next steps would be to repeat the study with a larger cohort and include a crossover study for the simulated westward travel for comparison. This would either confirm or refute findings from this study as well as compare the effect of westward travel in the same individuals.

CHAPTER 6: SUMMARY AND CONCLUSIONS

Trans-meridian travel is a common feature for many elite athletes and is often associated with jet-lag symptoms that influence an athlete's health and physical performance. Understanding the degree to which individuals are affected by jet-lag symptoms is of interest to the sporting community, including the Super Rugby tournament. In this thesis, the role of the *PER3* VNTR genotype as a source of inter-individual variation in response to jet-lag was examined in travelling rugby players and a control group in a laboratory environment.

In this study, when activity rate was used as a marker for game involvement, no significant differences were evident with direction of travel and number of time zones crossed when athletes were considered together or grouped by genotype. Interesting though was the fact that while not significantly different from the other two groups; the $PER3^{5/5}$ group's activity rate was always higher even after periods of international travel. The lack of significance in all activity rate variables may have arisen due to the fact that other factors such as behavioural and situational variables (Nippert and Smith, 2008, Taylor et al., 2008, Rampinini et al., 2007), which influence the extent of an individual's involvement, were not taken into account. This would have allowed better analysis of game activity rate in general, but would have been logistically difficult to achieve given that situational and behavioural variables constantly change from one match to the next. However, it would be worthwhile attempting in future studies.

Quality of play was reduced in the $PER3^{4/4}$ group compared to the $PER3^{4/5}$ and $PER3^{5/5}$ groups when more than ten time zones were crossed in a westward direction. This suggests that the effects of jet-lag were longer lasting in the $PER3^{4/4}$ players or that the $PER3^{4/4}$ players resynchronised more slowly in comparison to their $PER3^{4/5}$ and $PER3^{5/5}$ counterparts. This is plausible, given that while it was a different group and measured under different conditions, the $PER3^{5/5}$ group in the simulated jet-lag study (chapter 5) phase shifted to a greater extent than the $PER3^{4/4}$ group. Chellappa et al. (2012) showed that $PER3^{5/5}$ individuals were more sensitive to blue-light exposure than their $PER3^{4/4}$ counterparts, which may explain why the $PER3^{5/5}$

genotype phase advanced more rapidly in this study. It is therefore conceivable that the *PER3*$^{5/5}$ individuals may be able to effectively use external time cues to phase delay their circadian rhythms and resynchronise into the new time zone faster than their *PER3*$^{4/4}$ counterparts following westward travel.

Alternatively, it suggests differences in the underlying physiology between the two genotype groups, which may be explained in part by the variation in sleep homeostasis of the two groups (Goel et al., 2009, Dijk and Archer, 2010, Chellappa et al., 2012b, Maire et al., 2014). For example, Maire et al. (2014) reported higher sleep quality and nap efficiency across all naps in the *PER3*$^{5/5}$ compared to the *PER3*$^{4/4}$ individuals following sleep deprivation, suggesting greater flexibility in sleep initiation by the *PER3*$^{5/5}$ genotype. The *PER3*$^{5/5}$ genotype has also been reported to have a quicker build-up and subsequent dissipation of the homeostatic sleep pressure than the *PER3*$^{4/4}$ genotype following sleep deprivation studies (Chellappa et al., 2012b, Dijk and Archer, 2010). Thus, the *PER3*$^{5/5}$ individuals are more likely to re-establish normal sleep-wake cycles following trans-meridian travel prior to the matches, giving them an advantage over the other two *PER3* genotype groups.

While the effects of jet-lag are so severe following travel across more than ten time zones (Leatherwood and Dragoo, 2013, Lee and Galvez, 2012, Reilly et al., 2005), it was expected that the quality of play will be better following westward compared to eastward travel across more than ten time zones. It could be argued that the day is lengthened following westward travel which makes it easier for circadian rhythms to extend in line with their natural free running period of nearly 25h (Golombek et al., 2013, Lehnkering and Siegmund, 2007, Duffy et al., 2011). In contrast, the day is shortened following eastward travel, which decreases the homeostatic sleep pressure during subjective night, since sleep (i.e. in Australia and New Zealand) occurs during the player's biological day at least in the first 72h. In particular, failure to initiate and maintain sleep at this time-of-day may further increase circadian disruption worsening the effects of jet-lag. This is in line with studies which have shown that recovery time is longer and jet-lag symptoms worsen after eastward compared to westward travel (Reilly et al., 2007, Reilly

et al., 2005, Manfredini et al., 1998). Thus, it is conceivable that players may have been more jet-lagged after eastward travel since players generally do not have enough time to resynchronise into the new time zone before a match.

Injury incidence rates were lower when there was no time zone travel compared to crossing six or more time zone, especially in an eastward direction. Time zone travel affects both physical and psychological (e.g. mood, cognitive function) risk factors, for example, a dampened mood might ensue following time zone travel. Cumulative influences of both physical and psychological factors deplete perceptual and sensor motor reserves potentially exacerbating the risk of injury (Vandekerckhove and Cluydts, 2010, Evans et al., 2007). Athletes who undergo time zone travel are more likely to have a higher compound allostatic load (i.e. stressor) and an even more diminished threshold for mounting situational appropriate responses. Therefore, players were at an advantage when playing in their home country or when there was local travel with no time zone change. Furthermore, it suggests that travelling across time zones, especially eastward travel in this study may be detrimental to health. This is plausible since travel across time zones, especially after three or more time zones has been shown to shift physiological variables, leading to undesirable jet-lag symptoms (Chapman et al., 2012, Fowler et al., 2015a, Waterhouse et al., 2005b, Forbes-Robertson et al., 2012). Specifically, players may have been more prone to injury when playing/training at the same intensity following eastward travel, by making mistakes as indicated by no change in GA rate, but lower quality of play.

The *PER3*$^{4/4}$ individuals were more predisposed to injuries than the *PER3*$^{4/5}$ and *PER3*$^{5/5}$ individuals after westward travel across six or more time zones, suggesting that they may have remained jet-lagged, particularly during matches. Specifically, a significant proportion of injuries in this study occurred during matches which concurs with previous Rugby Union studies (Fuller et al., 2015, King et al., 2015, Schwellnus et al., 2014). The fact that matches took place within 3-5 days upon arrival in the new time zone, and more injuries were reported further implies slower resynchronisation in the *PER3*$^{4/4}$ group. This is plausible, since in the simulated jet-lag study, the *PER3*$^{4/4}$ individuals were unable to phase advance their internal 24h rhythms as rapidly as the

PER3$^{5/5}$ individuals using timed blue-light exposure and mealtime cues. As a result, sleep and circadian disruption, which influence cognitive and psychomotor functions key to performance, possibly take longer to get back to levels of prior to trans-meridian travel in the *PER3*$^{4/4}$ group. Thus, proper management of individuals homozygous for the *PER3* 4-repeat allele has the potential to improve performance, subsequently reducing the incidence of injuries, which may improve the longevity of players' careers in the Super Rugby competition.

The *PER3*$^{5/5}$ individuals may however be prone to injuries during training sessions in the first 72h upon arrival. This is conceivable given that while it is a separate group and measured under different circumstances, the *PER3*$^{5/5}$ group in the simulated jet-lag study slept for longer, had shorter sleep onset latency and reduced wake-after-sleep-onset during sleep opportunity 2, suggesting these individuals were jet-lagged. This is in line with studies which demonstrated deterioration in cognitive function, measured using a combination of multiple cognitive (Viola et al., 2007) and working memory tasks (Groeger et al., 2008) in the *PER3*$^{5/5}$ individuals under sleep deprivation. The effects of sleep loss on psychomotor vigilance may be worsened by time on task during training sessions (30-60 min). Specifically, time on task has been shown to result in reduced activation in over-lapping brain regions, suggesting neural and psychological causes (Asplund and Chee, 2013). Therefore, reducing training workload while using appropriate phase shifting protocols such as, morning-phased blue-enriched light exposure to facilitate resynchronisation to the new time zone is recommended in order to improve performance, and subsequently reduce injury risk.

While the *PER3*$^{4/5}$ group's ability to resynchronise into the new environment was not measured during the simulated jet-lag trial, it may be advantageous for actual travel to be heterozygous for the *PER3* gene. This is conceivable given that those homozygous for the *PER3* alleles were either more susceptible to illnesses or injuries while those heterozygous were neither susceptible to illnesses or injuries when direction of travel and number of time zones crossed were considered. Furthermore, the game activity rate of the *PER3*$^{4/5}$ group was in between that of the *PER3*$^{4/4}$ and *PER3*$^{5/5}$ groups across the entire tournament, even during periods of international travel. Thus,

carrying the *PER3*^{5} allele may facilitate faster resynchronisation, thereby reducing the risk of injury, while carrying the *PER3*^{4} allele may aid in reducing the risk for illnesses. The exact mechanism by which the *PER3* gene may mediate a reduction in illnesses is however not clear warranting further research in this area.

6.1 Limitations

Numerous confounders, such as physical activity level, society/culture and the lack of flexibility in responses that one can give may influence the scoring on the HÖ-questionnaire (Horne and Ostberg, 1976, Taillard et al., 1999, Levandovski et al., 2013). As such, one might question the validity of assigning chronotype to athletes using the HÖ-questionnaire. In future, it may be useful to validate the HÖ-questionnaire in a sporting population. Alternatively, it may be useful to use a different questionnaire to the HÖ-questionnaire, possibly one that gives flexibility in the answers that an individual can give, although there may be reliability concerns with this.

Interpretation of GA rate data was complicated by the fact that some players did not play in the same position throughout the duration of each tournament. Alternatively, other factors such as team strategy, game location, stage of play (e.g. finals) may have also influenced a player's involvement on a given day. This highlights the difficulty in generalising GA rate data in this study, since game involvement is undoubtedly a combination of multiple factors, of which a single factor may be more important in a particular match than the other.

This study had the special opportunity of using an actively competing Super Rugby population for the injury and illness study, and recognises that not having non-athlete travelling individuals control group was a limitation. However, the interest was on whether the *PER3* VNTR genotype predisposed individuals within the same population differently to illnesses after trans-meridian travel. This was a first step study, and thus future studies can include travelling control individuals. Another limitation was that rugby players were not used for the simulated jet-lag study. However, the interest was on whether individuals homozygous for the two *PER3*

genotypes desynchronise and resynchronise differently following simulated jet-lag; hence, it wasn't necessary to use actual rugby players for this part of the study.

The fact that a typical Super Rugby team consist of 22 players naturally limited the number of travelling players, which led to smaller sample sizes during periods of international travel. This reduced the sample sizes when the injury and illness incidence rates were analysed by direction of travel and number of time zones crossed, and thus this data should be interpreted with caution. Specifically, it is difficult to determine if differences observed when both direction of travel and number of time zones crossed were accounted for are real or the presence of a significant difference is due to a type 1 statistical error.

6.2 Practical applications and future work

Disruption of habitual sleep patterns is likely to occur during and after trans-meridian travel (Lastella et al., 2014b, Fowler et al., 2015b), which may reduce physical performance (Forbes-Robertson et al., 2012, Leatherwood and Dragoo, 2013). Sleep hygiene and re-entrainment recommendations that minimise this disruption in combination with appropriately-timed artificial or natural blue-enriched light exposure in order to phase shift players with various *PER3* VNTR genotypes may perhaps be the most effective way for resynchronising sports people into the new environment. Specifically, knowledge of how each *PER3* VNTR group desynchronises and resynchronises into the new time zone may aid understanding of the inter-individual differences in illness and injury incidence rates as well as adaptation to new time zones. This may subsequently translate into improved physical performance if the risk of injuries and illnesses can be reduced as players can then derive the maximum possible benefit from training sessions.

Observational studies will continue to be a vital source of performance, illness and injury surveillance with a focus on player welfare, especially following trans-meridian travel via individual specific intervention strategies. Ongoing surveillance studies either from individual teams or as combined data over multiple tournaments should be encouraged in the Super Rugby tournament embracing the covariates of trans-meridian travel. The effect of *PER3* VNTR

genotype and trans-meridian travel on game involvement, quality of play, illness and injury incidence rates during a competition is new and adds a different dimension to knowledge in the current literature. Since the Super Rugby tournament provides an ideal platform to study this aspect further, SARU may want to ensure that such studies continue in the future.

In order to better assess the impact of trans-meridian travel on the various *PER3* VNTR genotypes with regards to illness and injury incidence rates as well as game involvement, future studies with more individuals in each *PER3* VNTR group will need to examine additional team sports and across multiple seasons. Furthermore, studies with a higher resolution of assessment using different *PER3* VNTR genotypes to assess sleep quality during adaptation in new time zones, perhaps every 24h in the first week of arrival are warranted.

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Appendix 1A

APPENDICES

PARTICIPANT INFORMATION SHEET

Background

The UCT/MRC Research Unit for Exercise Science and Sports Medicine together with participating South African team physicians would like to study (i) the incidence and nature of medical illness and (ii) the influence of inter-individual variation in circadian rhythm on performance and incidence of illness and injury in rugby players during this tournament. This particular tournament is of interest since the strenuous schedule sees matches spanning 16 weeks (March to August 2012); during which 15 international rugby teams compete at different venues in South Africa, Australia and New Zealand. The competition is also unique in that the players are required to travel between venues in South Africa, Australia and New Zealand - often up to 9 hours across different time zones.

Firstly, we are interested in determining how common injuries and a variety of medical conditions and illness are in rugby players during the tournament. For example, it is known that athletes, who train hard and then participate in strenuous competition, have increased respiratory tract symptoms (runny nose, sore throat, sinusitis, enlarged lymph glands in the neck, and even cough and chest pain with fever and headaches). However, these symptoms may not always be due to an infection but could be as a result of allergies or pollution. It has also been shown that apart from respiratory tract illness, other illnesses are very most common during competitions such as at the Olympic Games. These illnesses include gastro-intestinal symptoms, allergies, skin conditions, and other infections. These patterns of illness have not been studied in rugby players, particularly during competitions.

Secondly, one of the unique aspects of the Super 15 tournament is that the players are required to travel across many time zones between matches. Such travel is known to disrupt circadian (24-hour) rhythm - experienced as jet lag. This in turn may impact performance. Your body's 24-hour rhythm is also partially determined by your genetic makeup. For example, a variant in one of your "clock" genes may determine whether you are a morning or evening person (also known as diurnal preference). We are interested in whether rugby players tends to be morning or evening types, and how travel across time zones might affect performance and/or incidence of injury and illness in rugby players.

Aims of the research

To document (i) the incidence of injuries and (ii) the incidence of medical illness in rugby players participating in the 2012 Super 15 Rugby tournament.

To relate the incidence of injuries and medical conditions/illness to 1) past medical history, 2) training history and load, and 3) environmental conditions (time zone changes, temperature, humidity, pollen counts, and atmospheric pollution) at the time of the 2012 Super 15 Rugby tournament.

To describe the chronotype distribution of the rugby players participating in the 2012 Super 15 Rugby tournament.

To describe the distribution of circadian rhythm gene polymorphisms (such as *PER3* VNTR) of the rugby players participating in the 2011 & 2012 Super 15 rugby tournaments.

To compare the differences in performance of the morning and evening-type rugby players travelling in both East-West and West-East directions

To compare the differences in the incidence of illness and injury of the morning and evening-type rugby players travelling in both East-West and West-East directions.

Your possible involvement

The UCT Research Office has provided your team doctor with all the information regarding the study, the details of which are explained in this document. As a participant in the 2012 Super 15 Rugby tournament, you are given the choice to participate in this research effort. Your participation is entirely voluntary.

Should you agree to participate, you would be asked to do the following:

Prior to the beginning of the tournament:

Complete a medical questionnaire (this can be done together with your team doctor). This questionnaire, which deals with medical, training and circadian rhythm information, will be anonymous and only a coding system will be used to identify your team.

Donate a 5 ml (1 teaspoon) blood sample from a vein in your arm. This will be used for the extraction and analysis of genetic material (DNA). The DNA will only be used for scientific research purposes relating to circadian rhythm. All data will be analysed anonymously and DNA samples will be destroyed on completion of the study

During the competition:

Every day your team doctor will ask you about possible medical conditions and injuries. This information will be recorded anonymously on a sheet (or in electronic format) that will be sent to the investigators. If you suffer from any injury or disease/condition, your team doctor will treat it in the usual fashion.

Potential risks of this study

The completion of a questionnaire is not associated with any risk. Questionnaire and other clinical data (paper and electronic) will be kept confidential and secure, and will not be made available to any party other than the research team without the consent of the individual participants.

The potential risks to participants of blood collection are minimal and are related to 1) blood sample collection technique, and 2) the volume of blood collected prior to a match and the potential risk of a decreased performance in a subsequent match. The potential risks associated with blood collection technique from the ante-cubital veins are: infection, delayed healing, haematoma, physical pain, mental discomfort and injury to a nerve or a vessel. These risks are small and will be minimized by the use of trained phlebotomists, use of sterile techniques and the use of disposable, single-use materials.

Your personal genetic information will not be made known to you, your team mates, team medical team, coaches, trainers or management. The information will only be used for research purposes.

All medical conditions and injuries will be treated by the team doctor.

You may withdraw from this study at any time without question.

Potential benefits of this study

The research questions that will be addressed by this study have been identified to have a direct impact on improving medical care to rugby players in general, and specifically those who visit South Africa during future Super 15 Rugby competitions. The anticipated benefits of this study are that the results will further our understanding of the possible cause/s of medical conditions and injuries, and relationship between circadian rhythm and performance in rugby players who travel to participate in international competitions.

Contact

Please feel free to contact your team doctor, the UCT Research Office or members of the research team should you have any questions related to the study. Your team doctor has the contact details of the UCT Research Office and the research team. You can also call the following numbers of the principal investigators

Lovemore Kunorozva (+27-76-956 4391),
Dr Dale Rae (+27-72-141 3143) or Dr Laura Roden (+27-82-494 1765).
Faculty of Health Sciences - Research Ethics Committee
Room E52-24, Old Main Building, Groote Schuur Hospital, Observatory, 7925
Tel: (021) 406 6338
Fax: (021) 406 6441
Email: nosi.tywabi@uct.ac.za
University of Cape Town Research Ethics approval number: REC REF 008/2011

Appendix 1B

INFORMED CONSENT

ALL participants to read and sign

I, the undersigned, have been fully informed about the University of Cape Town's study entitled Diurnal preference and sports performance: a study of travelling sports teams to be conducted by researchers from the UCT/MRC Research Unit for Exercise Science and Sports Medicine within the Department of Human Biology and the Department of Molecular and Cell Biology.

I agree to complete a questionnaire disclosing my personal details and information relating to my training and racing history. I understand that the following measurements / tests may be conducted on me during this study, as described in Participant Information document:

Body composition assessment - height and weight measurements.
Blood collection using the venepuncture or side cheek cell swab

I have been fully informed about the risks inherent in participation in this trial. I understand that my DNA sample will only be used for the purposes explained to me, namely to determine my genotype for the VNTR polymorphism within the Per3 gene, and will be destroyed on completion of the Circadian rhythm and sports performance study.

I understand that all the information collected during the study will be treated confidentially, will only be used for scientific research purposes and that my name and personal particulars will not be released under any circumstances.

I have been informed that I will be free to withdraw from the study at any time if I so wish without explanation. I also understand that I may request that my samples are destroyed before the completion of the study. I will be free to ask any questions about the procedures and results of the study. I understand that I will receive, where applicable, feedback pertaining to my morning-evening personality type as well as general results of the study once the entire study has been completed.

I agree to participate in the study.

Participant's name: _____
Signature: _____
Investigator's name: _____
Signature: _____
Witness's name: _____
Signature: _____
Date: _____

Appendix 1C

GENERAL INFORMATION AND TRAINING HISTORY

Participant Code:_____

General Questionnaire

Personal Details

Name_____

Surname_____

Postal address_____ Code _____

Email adress_____

Phone number_____ Cell Phone _____

Date of birth _____Age _____

Gender_____ Occupation _____

Ethnic group (only required and used for research purposes)

Black African White Mixed Ancestry (coloured)

Indian Asian Other

Ancestry (Tribal or National background - E.g. Xhosa, Dutch, Italian): _____

Dominant hand: Left • Right •

Dominant leg : Left Right •

Medication and supplement use

What medication, if any are you currently using ?

Name of medication Years taken

_____

_____

_____

What dietary supplements/vitamins, if any are you currently using?

Type of supplement Name Years taken

Anti-oxidant _____

Caffeine _____

Carbohydrate supplement_____

Creatine_____

Immune boosters_____

Do you currently participate in a sport/physical activity on 2 or more days per week?

Yes • No •

Appendix 1C

In the past two years have you ever participated in any team sport or physical activity on 2 or more days per week

Yes • No •

If yes, type of activity or sport _____ No" of years _____

Type of activity of sport _____ No" of years _____

Appendix 1D

HORNE-OSTBERG QUESTIONNAIRE

Name:__

INSTRUCTIONS

a) Please read each question very carefully before answering.

b) Answer ALL twenty questions.

c) Answer questions in numerical order.

d) Each question should be answered independently of others. **DO NOT** go back and check your answers.

e) For some questions, you are required to respond by placing a cross alongside your answer. In such cases, select **ONE** answer only.

f) Please answer each question as honestly as possible. Both your answers and results will be kept in strict confidence.

QUESTION 1

Considering your own feelings about when you are "at your best", at what time would you get up if you were entirely free to plan your day?

Time:

QUESTION 2

Considering only your own "feeling best" rhythm, at what time would you go to bed if you were entirely free to plan your day?

Time:

QUESTION 3

If there is a specific time you have to get up in the morning, to what extent are you dependent on being woken up by an alarm clock?

a. Not at all dependent ☐

b. Slightly dependent ☐

c. Fairly dependent ☐

d. Very dependent ☐

QUESTION 4

Assuming adequate environmental conditions, how easy do you find getting up in the morning?

a. Not at all easy .....

b. Slightly easy .....

c. Fairly easy .....

d. Very easy

QUESTION 5

How alert do you feel during the first half hour after having woken in the morning?

a. Not at all alert .....

b. Slightly alert .....

c. Fairly alert

d. Very alert .....

QUESTION 6

How is your appetite during the first half hour after having woken in the morning?

a. Not at all good .....

b. Slightly good .....

c. Fairly good .....

d. Very good

QUESTION 7

During the first half hour after having woken in the morning, how tired do you feel?

a. Very tired .....

b. Slightly tired

c. Fairly refreshed .....

d. Very refreshed .....

QUESTION 8

When you have no commitments the next day, at what time do you go to bed compared to your usual bedtime?

a. Seldom or never later .....

b. Less than one hour later .....

c. 1-2 hours later

d. More than 2 hours later .....

QUESTION 9

You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him/her is between 7.00-8.00 am. Bearing in mind nothing else but your own inclinations, how do you think you would perform?

a. Would be on good form ☐

b. Would be on reasonable form ☐

c. Would find it difficult ☐

d. Would find it very difficult ☐

QUESTION 10

At what time in the evening do you feel tired and in need of sleep?

Time:

QUESTION 11

You wish to be at your peak for a test which you know is going to be mentally exhausting and last for two hours. You are entirely free to plan your day. When would you do this task?

a. 8.00 am – 10.00 am ☐

b. 11.00 am – 1.00 pm ☐

c. 3.00 pm – 5.00 pm ☐

d. 7.00 pm – 9.00 pm ☐

QUESTION 12

If you went to bed at 11.00 pm at what level of tiredness would you be at that time?

a. Not at all tired ☐

b. A little tired ☐

c. Fairly tired ☐

d. Very tired ☐

QUESTION 13

For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Will you:

a. Wake up at the usual time and not go back to sleep ☐

b. Wake up at the usual time and doze ☐

c. Wake up at the usual time and go back to sleep ☐

d. Wake up later than usual ☐

QUESTION 14

One morning you have to remain awake between 4.00 am and 6.00 am in order to carry out a watch duty. You have no commitments the next day. Which ONE of the following alternatives suits you best?

a. Would NOT go to bed until 6.00 am

b. Nap before 4.00 am and sleep after 6.00 am

c. Sleep before 4.00 am and nap after 6.00 am

d. Only sleep before 4.00 am and remain awake after 6.00 am

QUESTION 15

You have to do 2 hours of hard physical work. If you were completely free to plan your day, and considering only your "feeling best" rhythm, which hours would you prefer to do it between:

a. 8.00 am – 10.00 am

b. 11.00 am – 1.00 pm

c. 3.00 pm – 5.00 pm

d. 7.00 pm – 9.00 pm

QUESTION 16

You have decided to engage in some physical exercise. A friend suggests that you do this between 10.00 pm and 11.00 pm twice a week. How do you think you would perform?

a. Would be on good form

b. Would be on reasonable form

c. Would find it difficult

d. Would find it very difficult

QUESTION 17

Suppose that you can choose your own work hours, but had to work FIVE hours in the day. Assume that your job is interesting and paid by results. Which FIVE CONSECUTIVE HOURS would you choose?

Hours:

QUESTION 18

At what time of day do you feel at your best?

Time:

QUESTION 19

One hears of “morning” and “evening” types. Which do you consider yourself to be?

a. Morning type .....

b. More morning than evening .....

c. More evening than morning

d. Evening type .....

QUESTION 20

At what time(s) of day do you regularly exercise?

Time:

Appendix 3A

KEY PERFROMANCE INDICATORS TABLE

Table 3.A: Definition of Key Performance Indicators used for game statistics analysis

Variable	Definition
Breakdown	The period immediately after a tackle, before and during the ensuing ruck, when teams compete for possession of the ball, initially with their hands and then using their feet in the ruck.
Charge down	When a player makes a defensive clearance kick, but it hits an opponent who has run towards him in an attempt to block it.
Conversion	An opportunity to "convert" for a further two points after a team scores a try by kicking the ball between the posts and above the crossbar.
Drop goal	Scored when a player kicks the ball from hand through the- 'opposition's goal post, but the ball must touch the ground between being dropped and kicked.
Drop kick	When a player kicks the ball from hand and the ball touches the ground between being dropped and kicked.
Goal	When a player kicks the ball through the plane bounded by the two uprights and above the crossbar.
Handling error	Errors incurred by the players, including knock-ons, forward passes, and balls dropped behind which do not result in a penalty.
High tackle	Also known as head-high tackle, is a form of tackle where the tackler grasps the ball carrier above the line of the shoulders.
Line break	When a player running with the ball breaks the line of defence.
Line-out	A maximum of seven and a minimum of two forwards line up parallel with each other between the five-metre and 15-metre lines. The hooker of the team in possession throws the ball in while his opposite number may stand in between the touchline and the five-metre line.
Penalty	Signal awarded for serious infringements like dangerous play, off-sides and handling the ball on the ground in a ruck.
Penalty try	Try awarded under the posts when the opposing team illegally prevents a try from being scored.
Red card	Signal shown to players who have been ordered off the field, which results in the player being removed from the game without being replaced.
Successful pass	Passes made that successfully went to the hand of a supporting player, including off-loads.
Tackle	Takes place when one or more opposition players grasp onto the ball carrier and succeed in bringing him to ground and holding him there.
Tackle break	When a tackle was made against the opponent, but was unsuccessful.
Try	Scored when a player places the ball on the ground with downward pressure in the in-goal area between and including the goal-line and up to, but not including dead ball line of the opposition's half.
Turnover	When a player turns over the ball to an offensive situation from a defensive tackle.
Yellow card	Signal shown to a player who has been cautioned "indicate temporary suspension" for repeated or deliberate infringements of the rules.

Adapted from Fuller et al. (2007)

Appendix 4A

ILLNESS, INJURY AND CIRCADIAN RHYTHM IN ELITE RUGBY PLAYERS DURING THE 2012 SUPER 15 TOURNAMENT

PARTICIPANT INFORMATION SHEET

Background

The UCT/MRC Research Unit for Exercise Science and Sports Medicine together with colleagues from New Zealand and Australia (team physicians of participating teams) would like to study (i) the incidence and nature of medical illness and (ii) the influence of inter-individual variation in circadian rhythm on performance and incidence of illness and injury in rugby players during this tournament. This particular tournament is of interest since the strenuous schedule sees matches spanning 16 weeks (February to May 2012), during which 15 international rugby teams compete at different venues in South Africa, Australia and New Zealand. The competition is also unique in that the players are required to travel between venues in South Africa, Australia and New Zealand - often up to 9 hours across different time zones.

Firstly, we are interested in determining how common injuries and a variety of medical conditions and illness are in rugby players during the tournament. For example, it is known that athletes, who train hard and then participate in strenuous competition, have increased respiratory tract symptoms (runny nose, sore throat, sinusitis, enlarged lymph glands in the neck, and even cough and chest pain with fever and headaches). However, these symptoms may not always be due to an infection but could be as a result of allergies or pollution. It has also been shown that apart from respiratory tract illness, other illnesses are very most common during competitions such as at the Olympic Games. These illnesses include gastro-intestinal symptoms, allergies, skin conditions, and other infections. These patterns of illness have not been studied in rugby players, particularly during competitions.

Secondly, one of the unique aspects of the Super 15 tournament is that the players are required to travel across many time zones between matches. Such travel is known to disrupt circadian (24-hour) rhythm – experienced as jet lag. This is turn may impact performance. Your body's 24-hour rhythm is also partially determined by your genetic makeup. For example, a variant in one of your "clock" genes may determine whether you are a morning or evening person (also known as diurnal preference). We are interested in whether rugby players tend to be morning or evening types, and how travel across time zones might affect performance and/or incidence of injury and illness in rugby players.

Aims of the research

1. To document (i) the incidence of injuries and (ii) the incidence of medical illness in rugby players participating in the 2012 Super 15 Rugby tournament.
2. To relate the incidence of injuries and medical conditions/illness to 1) past medical history, 2) training history and load, and 3) environmental conditions (time zone changes, temperature, humidity, pollen counts, and atmospheric pollution) at the time of the 2012 Super 15 Rugby tournament.
3. To describe the chronotype distribution of the rugby players participating in the 2012 Super 15 Rugby tournament.
4. To describe the distribution of circadian rhythm gene polymorphisms (such as *Per3* VNTR) of the rugby players participating in the 2012 Super 15 rugby tournament.
5. To compare the differences in performance of the morning and evening-type rugby players travelling in both East-West and West-East directions
6. To compare the differences in the incidence of illness and injury of the morning and evening-type rugby players travelling in both East-West and West-East directions.

Your possible involvement

The UCT Research Office has provided your team doctor with all the information regarding the study, the details of which are explained in this document. As a participant in the 2012 Super 15 Rugby tournament, you are given the choice to participate in this research effort. Your participation is entirely voluntary.

Should you agree to participate, you would be asked to do the following:

<u>Prior to the beginning of the tournament:</u>

- Complete a medical questionnaire (this can be done together with your team doctor). This questionnaire, which deals with medical, training and circadian rhythm information, will be anonymous and only a coding system will be used to identify your team.
- Donate a 5ml (1 teaspoon) blood sample from a vein in your arm. This will be used for the extraction and analysis of genetic material (DNA). The DNA will only be used for scientific research purposes relating to circadian rhythm. All data will be analysed anonymously and DNA samples will be destroyed on completion of the study

<u>During the competition:</u>

- Every day your team doctor will ask you about possible medical conditions and injuries. This information will be recorded anonymously on a sheet (or in electronic format) that will be sent to the investigators. If you suffer from any injury or disease/condition, your team doctor will treat it in the usual fashion.

Potential risks of this study

- The completion of a questionnaire is not associated with any risk. Questionnaire and other clinical data (paper and electronic) will be kept confidential and secure, and will not be made available to any party other than the research team without the consent of the individual participants.
- The potential risks to participants of blood collection are minimal and are related to 1) blood sample collection technique, and 2) the volume of blood collected prior to a match and the potential risk of a decreased performance in a subsequent match. The potential risks associated with blood collection technique from the ante-cubital veins are: infection, delayed healing, haematoma, physical pain, mental discomfort and injury to a nerve or a vessel. These risks are small and will be minimized by the use of trained phlebotomists, use of sterile techniques and the use of disposable, single-use materials. The risk of decreased performance as a result of blood collection will be reduced by not subjecting any participant to the collection of a blood volume exceeding 15ml prior to a match.
- Your personal genetic information will not be made known to you, your team mates, team medical team, coaches, trainers or management. The information will only be used for research purposes.
- All medical conditions and injuries will be treated by the team doctor.
- You may withdraw from this study at any time without question.

Potential benefits of this study

The research questions that will be addressed by this study have been identified to have a direct impact on improving medical care to rugby players in general, and specifically those who visit South Africa during future Super 15 Rugby competitions. The anticipated benefits of this study are that the results will further our understanding of the possible cause/s of medical conditions and injuries, and relationship between circadian rhythm and performance in rugby players who travel to participate in international competitions.

Contact

Please feel free to contact your team doctor, the UCT Research Office or members of the research team should you have any questions related to the study. Your team doctor has the contact details of the UCT Research Office and the research team. You can also call the following numbers of the principal investigators Prof Martin Schwellnus (+27-83-4543783) or Dr Dale Rae (+27-72-1413143).

Faculty of Health Sciences - Research Ethics Committee

Room E52-24, Old Main Building, Groote Schuur Hospital, Observatory, 7925

Tel: (021) 406 6338

Fax: (021) 406 6441

Email: nosi.tywabi@uct.ac.za

University of Cape Town Research Ethics approval number: REC REF 008/2011

Appendix 4B

CONSENT FORM

I understand that a study entitled "Illness, Injury and Circadian Rhythm in Elite Rugby Players During the 2012 Super 15 Rugby tournament" will be conducted by the UCT/MRC Research Unit for Exercise Science and Sports Medicine (University of Cape Town).

I understand that my participation in this research project has no direct benefits to me during the 2012 Super 15 Rugby tournament. However, I understand that my participation will advance the medical and scientific knowledge related to rugby. Therefore, information gathered through my participation in this project could advance the future medical care, training advice and performance of rugby players.

I have read the Participant Information Sheet and understand that the study involves the following components:

Completion of a medical questionnaire before the tournament

The completion of the questionnaire is not associated with any risk. All the questionnaire data and other clinical data (paper and electronic) will be kept confidential, secure and will not be made available to any party other than the research team without the consent of the individual concerned.

I agree that the all the information, which will be collected by my team doctor before the tournament, may be used to answer scientific questions about (i) the medical conditions associated with the participation in and completion of a rugby tournament and (ii) inter-individual variation in circadian (24-hour) rhythms of rugby players.

Blood sample collection for genetic studies before the tournament

Prior to the tournament, I have agreed to donate 5mL (1 teaspoon) of venous blood. The sample will be used for the extraction and analysis of genetic material (DNA).

The potential risks associated with the blood collection technique from the veins on my arm (ante-cubital veins) are: infection, delayed healing, blood clot (haematoma), physical pain, mental discomfort and injury to a nerve or a vessel. These risks are small and will be minimized by the use of staff that are trained to take blood samples (trained phlebotomists), use of sterile techniques and the use of disposable, single-use materials. The risk of decreased performance as a result of blood collection will be reduced by not subjecting any participant to the collection of a blood volume exceeding 15 ml prior to a match.

The genetic material (DNA) that is extracted from my blood will only be used for scientific research purposes. I understand that the DNA will be analysed for variations within genes related to circadian rhythm. I also understand that all data will be analysed without revealing any of my personal details (anonymously) and my DNA sample will be destroyed on completion of the study. I realise that I have the right to request that my DNA sample be destroyed at any time.

I understand that whilst there is no direct benefit to myself if a genetic predisposition for diurnal (morning/evening) preference in rugby players can be established, this research may lead to improved adaptation techniques available to travelling sports people to new time zones in the future. I understand that I will receive only the overall results of this part of the study.

Daily information during the rugby tournament

I agree that the all the information, which will be collected by my team doctor on a daily basis during the tournament, may be used to answer scientific questions about the medical conditions and injuries that are associated with the participation in and completion of a rugby tournament.

I have read (or, where appropriate, have had read to me) and understood the information about this study provided in the preceding Participant Information Sheet. Any questions I have asked have been answered to my satisfaction. I agree that research data provided by me or with my permission during the study may be included in a thesis, presented at conferences and published in journals on the condition that neither my name nor any other identifying information is used. I understand that the medical staff and the research team have professional medical insurance.

I understand that I may withdraw from this study at any time without further question.

I hereby consent to participate in this study.

Player accreditation number: _____	Name of the team doctor: _____	
Signature of the player: _____	Signature of the team doctor: _____	
Date: _____	Date: _____	
Name of the investigator: _____	Signature of the Investigator: _____	Date: _____

Appendix 4C

DAILY REPORT ON ILLNESS DURING THE 2012 SUPER 15 RUGBY TOURNAMENT

Country __________________Physician's name _________________Date of report _____/_____2011

Location: South Africa ______New Zealand ___Australia ___ City: _______________

Contact details Tel _______________ Fax ______________email ______________@_______________

Please report all new and recurrent illness in competition or training during the 2010 Super 14 Rugby tournament regardless of the consequences with respect to absence from competition or training. Illness is any medical condition for which the player consulted with the team physician.

Illness **Squad size:___**

Player's accreditation no.		Affected system	code	Final diagnosis	code	Date of illness onset
Main symptoms/signs	code	Cause of illness	code	Treatment (free text)		Absence in days

Player's accreditation no.		Affected system	code	Final diagnosis	code	Date of illness onset
Main symptoms/signs	code	Cause of illness	code	Treatment (free text)		Absence in days

Player's accreditation no.		Affected system	code	Final diagnosis	code	Date of illness onset
Main symptoms/signs	code	Cause of illness	code	Treatment (free text)		Absence in days

Player's accreditation no.		Affected system	code	Final diagnosis	code	Date of illness onset
Main symptoms/signs	code	Cause of illness	code	Treatment (free text)		Absence in days

Player's accreditation no.		Affected system	code	Final diagnosis	code	Date of illness onset
Main symptoms/signs	code	Cause of illness	code	Treatment (free text)		Absence in days

No illness in any player of our team today ☐

Definitions and codes – see reverse. Please use additional forms if needed

Appendix 4C

Codes For Illness

Affected system (codes A to N)

A Respiratory system	H Endocrine, nutritional or metabolic diseases
B Ears and mastoid	I Mental and behavioural disorders
C Digestive system	J Nervous system
D Genitourinary system	K Skin & subcutaneous tissue
E Circulatory system	L Eye and adnexa
F Other infections and parasitic disease	M Specific medical conditions related to sports
G Hematological and immune system	N Other symptoms, signs, abnormal clinical and laboratory findings

Final diagnosis code (according to affected system)

Respiratory system – diagnostic codes: A		
A1 Acute upper respiratory infections	A5 Allergic sinusitis	A9 Exercise induced bronchospasm
A2 Acute infective rhinitis	A6 Influenza	A10 Other acute lower respiratory tract infection
A3 Acute infective sinusitis	A7 Pneumonia	A11 Other disease of the respiratory tract
A4 Allergic rhinitis	A8 Asthma	
Ears and mastoid – diagnostic codes: B		
B1 External ear infections	B3 Middle ear infections	B5 Inner ear disease
B2 Other external ear disease	B4 Other middle ear disease	B6 Other ear disease
Digestive system – diagnostic codes: C		
C1 Diarrhoea and gastroenteritis (infective)	C4 Gastro-oesophageal reflux	C7 Hernia
C2 Diarrhoea and gastroenteritis (non-infective)	C5 Dyspepsia	C8 Liver disease
C3 Vomiting (no diarrhea – non specific)	C6 Abdominal pain (non-specific)	C9 Other digestive system disease
Genito-urinary system – diagnostic codes: D		
D1 Urethritis (infective)	D4 Prostatitis	D7 Proteinuria
D2 Cystitis (infective)	D5 Nephritis (infective)	D8 Hematuria
D3 Testicular disease (infective)	D6 Other genitor-urinary infection	D9 Other genito-urinary disease
Circulatory system – diagnostic codes: E		
E1 Hypertension	E4 Pulmonary embolism	E7 Endocarditis
E2 Arrhythmia	E5 Pericarditis	E8 Peripheral vascular disease
E3 Ischaemic heart disease	E6 Myocarditis	E9 Other circulatory disease
Other infections and parasitic disease – diagnostic codes: F		
F1 Other viral infections	F3 Other fungal infections	F5 Other infections
F2 Other bacterial infections	F4 Other parasitic infections	
Haematology and immune system – diagnostic codes: G		
G1 Anaemia (iron deficiency)	G4 Bleeding disorder	G7 Other immune system disease
G2 Anaemia (other nutritional)	G5 Other hematological disease	
G3 Anaemia (other)	G6 Immune suppression	
Endocrine, nutritional and metabolic disease – diagnostic codes: H		

Definitions and codes – see reverse. Please use additional forms if needed

H1 Thyroid gland disorder	H3 Hypoglycemia	H5 Other metabolic disease
H2 Diabetes mellitus	H4 Other endocrine disease	
Mental and behavioural disorders – diagnostic codes: I		
I1 Depression	I3 Eating disorder	I5 Chronic fatigue
I2 Anxiety	I4 Sleep disorder	I6 Other mental or behavioral disorder
Nervous system disease – diagnostic codes: J		
J1 Headache	J2 Epilepsy	J3 Other nervous system/muscle disease
Skin and subcutaneous tissue – diagnostic codes: K		
K1 Viral skin infection	K4 Other skin infection	K7 Urticaria
K2 Bacterial skin infection	K5 Allergic dermatitis	K8 Sunburn
K3 Fungal skin infection	K6 Pruritis	K9 Other skin/subcutaneous diseases
Diseases of the eye and adnexa – diagnostic codes: L		
L1 Blepharitis (eyelid inflammation)	L3 Conjunctivitis (allergic)	L5 Other inflammatory eye disease
L2 Conjunctivitis (infective)	L4 Keratitis	L6 Other eye disease
Specific medical conditions related to sports participation – diagnostic codes: M		
M1 Hyperthermia (heat exhaustion)	M4 Collapse (post- exercise)	M7 Dehydration
M2 Hyperthermia (heat stroke)	M5 Collapse (during exercise)	M8 Other medical condition with exercise
M3 Hypothermia	M6 Muscle cramping (EAMC)	

Definitions and codes – see reverse. Please use additional forms if needed

Team: _____

Team Physician: _____

Appendix 4D

DAILY REPORT ON INJURY DURING THE 2012 SUPER 15 RUGBY TOURNAMENT

Country _____Physician's name _____Date of report _____/_____ 2011

Location: South Africa ___New Zealand ___Australia ___ City: _____

Contact details Tel _____ Fax _____email _____@_____

Please report: (1) All injuries (traumatic and overuse) newly incurred in match or training and (2) all illness regardless of the consequences with respect to absence from competition or training. The information provided is for medical and research purposes and will be treated confidentially.

(1) Injury:

Player no.		match or training		time / minute of match		date of injury
injured body part, side	code	type of injury	code	cause of injury	code	absence in days

Player no.		match or training		time / minute of match		date of injury
injured body part	code	type of injury	code	cause of injury	code	absence in days

Player no.		match or training		time / minute of match		date of injury
injured body part	code	type of injury	code	cause of injury	code	absence in days

Player no.		match or training		time / minute of match		date of injury
injured body part	code	type of injury	code	cause of injury	code	absence in days

Player no.		match or training		time / minute of match		date of injury
injured body part	code	type of injury	code	cause of injury	code	absence in days

No injury in any player of our team today ☐

How many hours did your team train today? _____hours

Absence from normal training or match play (in days)

Please provide an estimate of the number of days that the player will not be able to undertake his normal training programme or will not be able to play.

0 =	0 days	7 =	1 week	28 =	4 weeks
1 =	1 day	14 =	2 weeks	> 30 =	more than 4 weeks
2 =	2 days	21 =	3 weeks	>180=	6 months or more

For injuries

Injured body part - Location of injury

Head and trunk		Upper extremity		Lower extremity	
1	face (incl. eye, ear, nose)	11	shoulder / clavicle	21	hip
2	head	12	upper arm	22	groin
3	neck / cervical spine	13	elbow	23	thigh
4	thoracic spine / upper back	14	forearm	24	knee
5	sternum / ribs	15	wrist	25	lower leg
6	lumbar spine / lower back	16	hand	26	Achilles tendon
7	abdomen	17	finger	27	ankle
8	pelvis / sacrum / buttock	18	thumb	28	foot / toe

Type of injury - Diagnosis

1	concussion (regardless of loss of consciousness)	11	contusion / haematoma / bruise
2	fracture (traumatic)	12	tendinosis / tendinopathy
3	stress fracture (overuse)	13	arthritis / synovitis / bursitis
4	other bone injuries	14	fasciitis / aponeurosis injury
5	dislocation, subluxation	15	impingement
6	tendon rupture	16	laceration / abrasion / skin lesion
7	ligamentous rupture	17	dental injury / broken tooth
8	sprain (injury of joint and/or ligaments)	18	nerve injury / spinal cord injury
9	lesion of meniscus or cartilage	19	muscle cramps or spasm
10	strain / muscle rupture / tear	20	other

Cause of injury

1	overuse (gradual onset)	3	non-contact trauma	5	contact with another player
2	overuse (sudden onset)	4	recurrence of previous injury	6	foul play (overt & hidden fouls)

Appendix 5A

RECRUITMENT POSTER

PARTICIPANTS WANTED FOR UCT RESEARCH

HOW CAN WE SPEED UP RECOVERY FROM JET-LAG?

The **aims** of this study are (i) to determine to what extent an aspect of your genetic makeup governs the speed and timing of recovery from jet-lag, and (ii) to determine whether exposure to blue and yellow light in the morning can enhance recovery from jet-lag.

We are looking for males with low levels of physical activity.

If you:

- ✓ Are between 18 and 40 years of age
- ✓ Sleep between 6 and 10 hours per night
- ✓ Do no more than two days of physical activity per week

You can volunteer for this study

What is involved?

- Part A: Complete a questionnaire and donate a cheek cell sample to determine your genetic variant for a gene linked to sleep behaviour and preference for mornings or evenings (i.e. lark v owl behaviour)
- Part B: Eligible participants will then be asked to stay in our Chronobiology and Sleep lab for one four-period so that we can simulate jet-lag and then measure your recovery.

What are the benefits?

- **Personal feedback (e.g. personal preference for either mornings or evenings, sleep quality, colour blind test, resting energy expenditure levels, response to jet-lag) as well as the general results of the study**

If you are interested in taking part in the study and would like additional information, please contact:

Lovemore Kunorozva

KNRLOV001@myuct.ac.za

076 956 4391

Appendix 5B

MEDIA RELEASE

SPORTS SCIENCE INSTITUTE OF SOUTH AFRICA

FOR IMMEDIATE RELEASE

MALES REQUIRED FOR UCT STUDY ON RECOVERY FROM JET-LAG

Anyone who has travelled east or west on an airplane may have experienced jet-lag. We are interested in how fast individuals recover from jet lag – that is, how quickly can your internal body clock realign with your new time zone? Are these differences determined by your genetic make-up? And can blue and yellow light exposure in the morning speed up your recovery? The aims of this study are (i) to determine to what extent an aspect of your genetic makeup governs the speed and timing of recovery from jet lag, and (ii) to determine whether exposure to blue light in the morning can enhance recovery from jet lag. Volunteers will be asked to complete a questionnaire and donate a cheek cell sample to determine their personal variant for a gene linked to sleep behavior. Eligible participants will then be asked to stay in our Chronobiology and Sleep lab for a four-period so that we can simulate jet lag and then measure your recovery.

Those interested in volunteering for this research should

- Be males between the ages of 18 and 40 years
- Normally sleep 6 – 10 hours per night
- Not normally be too physically active

Benefits of participating in the study include

- Personal feedback (e.g. personal preference for either mornings or evenings, sleep quality, colour blind test, resting energy expenditure levels, response to jet lag) as well as the general results of the study.

To apply or for more information, please contact:

Lovemore Kunorozva: KNRLOV001@myuct.ac.za· 076 956 4391

Appendix 5C

PARTICIPANT INFORMATION SHEET

RESETTING OUR BODY CLOCKS AFTER SIMULATED JET LAG

PARTICIPANT INFORMATION SHEET

Dear Volunteer,

Thank you for considering participating in this study to be conducted by researchers from the MRC/UCT Research Unit for Exercise Science and Sports Medicine within the Department of Human Biology and the Department of Molecular and Cell Biology, at the University of Cape Town.

Why are we doing this study?

Jet lag is something that anyone who has travelled east or west in an aeroplane may have experienced – especially if more than three time zones have been crossed. Jet lag occurs when you arrive in a new time zone and your body's internal clock is out of synch with your new environment. While one primarily experiences fatigue and disturbed sleep, other symptoms may include gut disturbances, reduced mental function and reduced physical capacity.

A speedy recovery from jetlag is key, not only to the general population, but also to sports teams travelling during tournaments and business people travelling for work. The purpose of jetlag recovery strategies is to realign your body clock with the new environmental time as fast as possible. Current strategies designed to enhance recovery from east-west travel include timed light exposure, manipulation of sleep schedules and the use of sedative or stimulant medication. In this study we are going to compare the effectiveness of enriched blue light exposure on resetting your internal clock following simulated jet lag.

While the general rule is that for every time zone crossed, 24h of recovery time is required, it appears that this recovery time varies significantly between individuals. We believe that this may in part be attributed to our genetic make-up (i.e. differences in our DNA). For example, small differences within our "clock" genes (those genes involved with regulating our bodies' internal clock) have been associated with our tendency to be owls (night people) or larks (morning people). Specifically, we want to know whether 3 hours of blue light therapy following simulated jet lag is more effective in resynchronising the body clock of individuals who carry the *PER3*^{5} gene variant – which is more common in morning people (larks) compared to evening people (owls), who are more likely to have the *PER3*^{4} variant.

What is the aim of the study?

To compare the extent to which individuals genotyped as *PER3*$^{4/4}$ and *PER3*$^{5/5}$ respond to blue light exposure to resynchronise their circadian rhythm following simulated eastward trans-meridian travel, based on changes in dim-light melatonin onset and cortisol circadian phases.

Who can participate in this study?

Twenty-four apparently healthy males who:

- Are between 18 – 40 years of age
- Normally sleep 6 – 10 hours per night
- Are not normally very physically active
- Are genotyped as *PER3*$^{5/5}$ or *PER3*$^{4/4}$

Exclusion criteria - If any of the following criteria apply to you, you will not be eligible for the study:

- Diagnosed chronic medical condition requiring medication
- Any sleep disorder
- Chronic medication known to affect sleep, cortisol, melatonin and/or circadian rhythms (past six months)
- Night time or rotating shift work in past three months
- East-west travel of three time zones or more (past 2 months)
- Smoking
- Normal caffeine consumption greater than 300mg per day (±10 cups instant coffee, or ±3 cups brewed coffee, or ±5 espressos)
- Red-green colour blind condition
- Body mass index >30kg·m^{-2} (divide your body weight in kg by the square of your height in m)

How long will this study last?

This study will span about 3 weeks. Following a screening visit to establish your eligibility for the trial, you will then be required to have a two-week "run-in" period where you regulate your sleep patterns at home. This will be followed by a **98-hour (4 days and 2 hours)** in-laboratory intervention trial.

What do we want you to do?

Screening visit (±1 hour)

First, to determine whether you would like to participate and whether you are eligible for the study, we ask you to visit us for screening. The researcher will explain the study in detail to you, including the risks and benefits associated with participating. You will be free to ask any questions you may have relating to the study. Should you agree to participate, you will sign a consent form and be asked to complete a questionnaire detailing your personal information; medical history; work, travel, sleep and physical activity habits. The questionnaire will also include sections on your sleep quantity and duration, as well as your daytime sleepiness levels and your "diurnal

preference" – that is whether you are more of a morning person (lark) or an evening person (owl). This should take about 30 minutes to complete.

The investigator will measure your height and weight, following which you will complete a test to determine whether or not you are colour blind. Next, we will measure your resting metabolic rate – that is how much energy you use when lying down. To do this, you will be asked to lie down and relax with your head and shoulders beneath a ventilated hood so that the oxygen and carbon dioxide levels in your expired air can be measured. From this, the researchers are able to calculate your energy expenditure (metabolic rate). Lastly, the investigator will use a cotton wool swab to gently obtain a sample of your cheek cells. This is painless and non-invasive. We will extract your DNA from these cells to test for a variant within the *PER3* gene - one of your clock genes. Based on this genetic analysis, 12 volunteers genotyped as $PER3^{5/5}$ (genetic variant associated with "morningness") and 12 with the $PER3^{4/4}$ genotype (associated with "eveningness") will be invited to participate in the intervention phase of this study.

Prior to the intervention visit

We want to ensure that you are well rested and not sleep deprived before you begin the intervention trials in this study. Therefore we ask that for **two full weeks** prior to the trials you maintain a regular sleep/wake schedule. This will be based on your normal wake-up and bed times during the week, such that you go to sleep and wake-up within an hour of these normal times. The catch is, though, that we will ask you to try to spend one **extra hour** in bed each night. To monitor this, we will ask you to wear a simple device on your wrist for the second week of this period that tracks your movement. It is pretty good at determining when you are awake (more active) and asleep (less active). During this week we also ask that you complete a daily logbook documenting your wake-up and bed times and your daytime sleepiness levels. Seven, four and one day prior to the trial, you will also record all that you eat and drink for these three 24h periods using an online tool to do so. We do this so that we can plan for your meals during the interventions! Lastly, we ask that you please refrain from consuming alcohol and caffeine and using non-steroidal anti-inflammatories 24h before the trial begins.

Intervention trial

For this part of the study we require you to stay at our Chronobiology and Sleep (CBS) Lab for a 98-h period (4 days and 2 hours). The CBS lab is a specially designed space, which is sound- and light-proof, and comprises a bedroom, lounge area, bathroom and kitchen. During these four days you will not be allowed contact with the outside world – that is all time cues from sources such as outdoor light, phones, computers, TV etc. will be removed. You will however be in the company of the study investigators at all times, and may share the laboratory with other participants. During periods of wakefulness, participants will be able to interact with each other in a common seating area. You will retire to individual or partitioned bedrooms for sleep opportunities. Ablutions will be carried out in a shared bathroom to which you will have individual access and the option to lock the door. Please note that you have the right to withdraw from the study at any time, including during the intervention trial period. There are two reasons for this isolation. First, we want to measure the natural timing of your body's internal clock. Second, we want to simulate jet lag by putting you in a new "time zone" so that we

can then measure your recovery. We do this by using what is called a "**constant routine**" protocol combined with sleep deprivation. Essentially, we keep you awake for 28 hours in dim light conditions. During this time you will be required to remain awake, but be relatively inactive. You will be able to read, play board games, work on your computer (but no internet and the clocks will be disabled), watch TV (pre-recorded, not live TV) and we will give you hourly snacks. The investigator will ensure that you are awake during scheduled waking periods, and will engage with you regularly to prevent you from sleeping at these times.

After this constant routine period, you will be given an 8h sleep opportunity. When you wake up, you will have a **3h light therapy session**. This will be with blue-enriched light (a special lamp which emits blue wavelength light). The light therapy session is important, as blue light can speed up your recovery from the imposed jet lag.

You will have breakfast during the light therapy session, and for the rest of the day you will remain relatively inactive (as for the previous day) in dim light, and will be served lunch and dinner. At the end of the day you will be given a second 8-hour sleep opportunity, followed by a second 28-h constant routine period (identical to the first one). Prior to leaving the lab, you will have third 8-h recovery sleep opportunity.

Near the beginning of the trial a trained staff member is going to insert a small tube into a vein in your arm (called a cannula, much like a drip) so that we can obtain regular hourly blood samples (less than one teaspoon at a time) from you for a 7h period on day one and a 10h period on days 3 into 4. In addition, we will also ask you to give us hourly saliva samples for two 7h periods on days 2 and 3 into 4. We will analyse these samples for a hormones called melatonin and cortisol – which are both very good markers of the timing of your internal body clock. Based on the levels of these markers that we observe, we will be able to determine how well and/or fast your body's internal clock has recovered following simulated jet lag. Periodically throughout the trial we will also measure your resting metabolic rate so that we can have an indication of whether your body is adjusting to the new time zone on a physiological (i.e. systems) level.

What are the risks of participating?

The completion of a questionnaire is not associated with any risk. Questionnaire and other clinical data (paper and electronic) will be kept confidential, will be kept secured, and will not be made available to any party other than the research team without your consent. Electronic files will also be kept confidential. There are no risks related to donating either cheek cell or saliva samples. The potential risks relating to blood collection from forearm veins include: infection, delayed healing, bruising, physical pain, mental discomfort and injury to a nerve or a vessel. These risks are small and will be minimized by the use of trained phlebotomists (individuals qualified to draw blood samples), sterile techniques and disposable, single-use materials. The blue light in this protocol is actually blue-enriched white light from cool fluorescent light bulbs used for indoor lighting. The spectrum of these bulbs is closer to natural sunlight than other indoor lighting, and thus there are no adverse effects anticipated from exposure to light from this source. There are also no risks associated with measuring your metabolic rate using the ventilated hood technique or with either of the light therapies to be used in this trial.

The combination of undergoing simulated jet lag and one night of total sleep deprivation may cause discomfort (mental and physical). The risk associated with simulating jet lag is that when the trial ends you may be tired and have difficulty sleeping for a few days afterwards. Acute sleep deprivation, such as you will experience during this protocol, is associated with reduced mental performance and impaired coordination, similar to those induced by alcohol consumption. During this time you will be in the care of the study investigators, and will only be able to go home following a recovery sleep – which reverses the effects of sleep deprivation.

What if something goes wrong?

Any medical emergency will be managed based on the basic life support protocol implemented at both ESSM and the Sports Science Institute of South Africa (SSISA) during the four-day intervention trial period. Should a medical emergency arise, the investigator on duty will trigger the emergency alarm in the CBS laboratory, which is connected to the Medical Practice located at SSISA during working hours, and to the SSISA Main Reception front desk after hours. During working hours, a medical doctor within SSISA will immediately respond to the emergency. During after hours, the receptionist on duty at SSISA will contact one of the following: Metro, Netcare, Life healthcare emergency or the Claremont 24h emergency unit to dispatch an ambulance The investigator on duty can also dial the 112 Emergency call from his/her cell phone. There is also an automated external defribillator (AED) and a crash cart with resuscitation equipment on first floor of the SSISA building, which is in close proximity to the CBS lab. The investigators in this study have been trained in basic life support and cardiopulmonary resuscitation. At any given time during a trial, one of them will always be on site and thus able to initiate the emergency plan.

The University of Cape Town (UCT) undertakes that in the event of you suffering any significant deterioration in health or well-being that is caused by your participation in the study, it will provide immediate medical care. UCT has appropriate insurance cover to provide prompt payment of compensation for any trial-related injury according to the guidelines outlined by the Association of the British Pharmaceutical Industry, ABPI 1991. Broadly-speaking, the ABPI guidelines recommend that the insured company (UCT), without legal commitment, should compensate you without you having to prove that UCT is at fault. An injury is considered trial-related if, and to the extent that, it is caused by study activities. You must notify the study doctor immediately of any side effects and/or injuries during the trial, whether they are research-related or other related complications

UCT reserves the right not to provide compensation if, and to the extent that, your injury came about because you chose not to follow the instructions that you were given while you were taking part in the study. Your right in law to claim compensation for injury where you prove negligence is not affected. Copies of these guidelines are available on request

What are the benefits of participating?

On completion of the study you will receive personal feedback (e.g. preference for either mornings or evenings, sleep quality, resting metabolic rate) as well as the general results of the study when completed.

Is there any reward for taking part in this study?

On completion of the intervention phase of the trial you will receive R800.00 as partial compensation for your time and travel.

What are the ethical considerations?

This study will be performed in accordance with the principles of the Declaration of Helsinki (October 2013, Fortaleza, Brazil), International Conference on Harmonisation and the South African Good Clinical Practice guidelines, and the laws of South Africa. This trial will be covered by the University of Cape Town's No Fault Compensation Insurance cover for clinical trials and/or human volunteer studies (Policy 713/SPRGL1400443). You will not be included in the study unless you have signed a consent form, after the investigator has provided substantial verbal and written explanation of the study, including risk factors. Participation in the study is entirely voluntary and you have the right to withdraw from the study at any time without stating a reason. The investigator may also withdraw you from the study at any time. All the information collected during the trial will be stored in a computer database in a secure facility, will be kept confidential and will only be used for scientific purposes. Your anonymity will be ensured should the data be published. The DNA collected for this study will only be used for the purposes described above. The DNA samples will be destroyed at the end of the study. Alternatively, you may request that your DNA sample be destroyed prior to the end of the study should you so desire.

Contact

Please do not hesitate to contact us should you require any additional information. You may contact the HREC if you have any questions or concerns about your rights or welfare as research participants. It is advisable that you inform family/friends that you are taking part in this trial and give them these contact details should they need to contact you in an emergency. Our contact information is listed below.

Faculty of Health Sciences – Human Research Ethics Committee (HREC)

Room E52-24, Old Main Building, Groote Schuur Hospital, Observatory, 7925

Tel: (021) 406 6338 · Fax: (021) 406 6441 · Email: nosi.tywabi@uct.ac.za

Investigators

Principal investigator: Dr. Laura Roden 021 650 5322 Laura.Roden@uct.ac.za
Principal investigator: Dr. Dale Rae 021 650 4577 Dale.Rae@uct.ac.za
Student investigator: Lovemore Kunorozva 021 650 4691 KNRLOV001@myuct.ac.za
Collaborator: Dr Jacolene Kroff 021 650 5126 Jacolene.Kroff@uct.ac.za

Appendix 5D

INFORMED CONSENT

I, the undersigned, have read and understood the above information and have been fully informed verbally about the study entitled **"Resetting our body clocks after simulated jet lag"** to be conducted by researchers from the MRC/UCT Research Unit for Exercise Science and Sports Medicine (ESSM) within the Department of Human Biology and the Department of Molecular and Cell Biology at the University of Cape Town.

I agree to visit the Chronobiology and Sleep (CSB) laboratory on **two occasions**. The first (screening visit) is to determine my eligibility for the study. I agree to donate a **cheek cell sample** to determine my genotype for a variant within the *PERIOD3* gene as described in the participant information section above. Should I be eligible for the intervention phase of the study, I agree to spend one **four-day period** in the CBS lab. I understand that I will be isolated from the outside world for this four-day period and that I will undergo **jet lag simulation** and two nights of **total sleep deprivation**, as well as light therapy to enhance my recovery from jet lag**.** I also understand that regular blood and saliva samples will be collected from me to assess my recovery from jet lag.

I have been fully informed about the risks inherent in participation in this trial. I understand that all the information collected during the study will be treated confidentially, will only be used for scientific research purposes and that my name and personal particulars will not be released under any circumstances.

I have been informed that I will be free to withdraw from the study at any time if I so wish without explanation. I will be free to ask any questions about the procedures and results of the study. I understand that I will receive, where applicable, personal feedback and the general results of the study once the entire study has been completed.

I agree to participate in the study.

Participant:

_____	_____	_____
Full name	Signature	Date

Investigator:

_____	_____	_____
Full name	Signature	Date

Date: _____ Code: _____

Appendix 5E

QUESTIONNAIRE

RESETTING OUR BODY CLOCKS AFTER SIMULATED JET LAG

GENERAL QUESTIONNAIRE

PERSONAL DETAILS

Date of birth: _____ Age _____:_____

Gender: _____ Ethnicity_____: _____

OCCUPATION AND TRAVEL

Do you currently work **shifts**? Yes ☐ No ☐

If no, Have you ever worked shifts? Yes ☐ No ☐

If yes, When did you stop working shifts? _____ (month, year)

Did you work night shifts? Yes ☐ No ☐

Did you work rotating night/day shifts? Yes ☐ No ☐

What are your current work hours? _____ (start time) _____ (end time)

Have you recently **travelled abroad** via plane? Yes ☐ No ☐

If yes, What was your final destination? _____ (City, Country)

When did you arrive back in South Africa? _____ (Date home)

MEDICAL HISTORY, MEDICATION AND SUPPLEMENT USE

Do you currently suffer from any **sleep disorder**? Yes ☐ No ☐

If yes, please list the disorder(s):

Name of condition	Person who diagnosed	Date diagnosed

Do you currently suffer from any **chronic medical condition**? Yes ☐ No ☐

If yes, please list the condition(s):

Name of condition	**Person who diagnosed**	**Date diagnosed**

What **medication**, if any, are you **currently** using?

Name of medication	**Purpose**	**Years taken**

What **medication,** if any, have you taken during the **past six months, but are no longer using**?

Name of medication	**Purpose**	**Years taken**

What **dietary supplements / vitamins**, if any, are you **currently** using?

Name of supplement/Active ingredient	**Purpose**	**Years taken**

Do you consider yourself to be in good health? Yes ☐ No ☐

Do you currently smoke? Yes ☐ No ☐

Have you ever smoked? Yes ☐ No ☐

If yes, when did you stop smoking? _____ (month, year)

Are you colour blind? Yes ☐ No ☐

CAFFEINE CONSUMPTION

How many of the following do you usually have per day?

Instant coffee _____ (cups per day)

Filter coffee _____ (cups per day)

Espresso _____ (cups per day)

Decaffeinated coffee _____ (cups per day)

Ceylon tea _____ (cups per day)

Rooibos tea _____ (cups per day)

Energy drinks _____ (drinks per day) _____ (brand)

Caffeinated soft drinks _____ (drinks per day) _____ (brand)

Any other caffeine-containing products? _____

PHYSICAL ACTIVITY HISTORY

Over the past **three months**, how would you describe your level of **physical activity**?

Inactive		☐
Occasionally active	*(At least 1 – 4 sessions per month)*	☐
Somewhat active	*(At least 1 – 2 sessions per week)*	☐
Reasonably active	*(At least 3 – 4 sessions per week)*	☐
Very active	*(More than 4 sessions per week)*	☐

Do you currently participate in any sports / physical activities? Yes ☐ No ☐

If yes, please describe

Sport / Physical activity	**Days per week**	**Years**

Has your participation included recreational competition / matches / games etc.?

Yes ☐ No ☐

Over the past three months, what has been the **average duration** of your exercise sessions and / or recreational activity?

0-15min ☐ 16 – 30min ☐ 31 – 60min ☐

1 – 2h ☐ >2h ☐

What is your average **total exercise time for the week**?

<60min ☐ 60 – 90min ☐ 90 – 12min ☐

2 – 3h ☐ 3–4h ☐ >4h ☐

Appendix 5F

PITTSBURGH SLEEP QUALITY INDEX QUESTIONNAIRE

INSTRUCTIONS

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions by either giving the precise time or ticking the appropriate box.

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

Usual bed time: _____

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

Number of minutes: _____

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

Usual getting up time: _____

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

Hours of sleep per night: _____

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

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

(a) Cannot get to sleep within 30 minutes

Not during the past month	☐	*Less than once per week*	☐
Once or twice a week	☐	*Three or more times a week*	☐

(b) Wake up in the middle of the night or early morning

Not during the past month	☐	*Less than once per week*	☐
Once or twice a week	☐	*Three or more times a week*	☐

(c) Have to get up to use the bathroom

Not during the past month	☐	*Less than once per week*	☐
Once or twice a week	☐	*Three or more times a week*	☐

(d) Cannot breath comfortably

Not during the past month	☐	*Less than once per week*	☐
Once or twice a week	☐	*Three or more times a week*	☐

(e) Cough or snore loudly

Not during the past month	☐	*Less than once per week*	☐
Once or twice a week	☐	*Three or more times a week*	☐

(f) Feel too cold

Not during the past month	☐	*Less than once per week*	☐
Once or twice a week	☐	*Three or more times a week*	☐

(g) Feel too hot

Not during the past month	☐	*Less than once per week*	☐
Once or twice a week	☐	*Three or more times a week*	☐

(h) Had bad dreams

Not during the past month	☐	*Less than once per week*	☐
Once or twice a week	☐	*Three or more times a week*	☐

(i) Have pain

Not during the past month	☐	*Less than once per week*	☐
Once or twice a week	☐	*Three or more times a week*	☐

(j) Other reason(s), please describe _____

_____

How often during the past month have you had trouble sleeping because of this?

Not during the past month	☐	*Less than once per week*	☐
Once or twice a week	☐	*Three or more times a week*	☐

6. During the past month, how would you rate your sleep quality overall?

Very good	☐	*Fairly good*	☐
Fairly bad	☐	*Very bad*	☐

7. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?

Not during the past month	☐	*Less than once per week*	☐
Once or twice a week	☐	*Three or more times a week*	☐

8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

Not during the past month	☐	*Less than once per week*	☐
Once or twice a week	☐	*Three or more times a week*	☐

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

No problem at all	☐	*Only a very slight problem*	☐
Somewhat of a problem	☐	*A very big problem*	☐

10. Do you have a bed partner or roommate?

No bed partner or roommate ☐

Partner/roommate in other room ☐

Partner in same room, but not same bed ☐

Partner in same bed ☐

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

(a) Loud snoring

Not during the past month ☐ *Less than once per week* ☐

Once or twice a week ☐ *Three or more times a week* ☐

(b) Long pauses between breaths while asleep

Not during the past month ☐ *Less than once per week* ☐

Once or twice a week ☐ *Three or more times a week* ☐

(c) Legs twitching or jerking while you sleep

Not during the past month ☐ *Less than once per week* ☐

Once or twice a week ☐ *Three or more times a week* ☐

(d) Episodes of disorientation or confusion during sleep

Not during the past month ☐ *Less than once per week* ☐

Once or twice a week ☐ *Three or more times a week* ☐

(e) Other restlessness while you sleep: please describe _____

_____

Not during the past month ☐ *Less than once per week* ☐

Once or twice a week ☐ *Three or more times a week* ☐

SLEEP TIMING QUESTIONNAIRE

INSTRUCTIONS

This questionnaire asks about when you normally sleep. We are interested in getting as accurate a picture as we can of the times when you normally go to bed and get up. Please think carefully before giving your answers and be as accurate and as specific as you can be. **Please answer in terms of a recent "normal average week," not one in which you travelled, vacationed or had family crises. Thanks.**

Please think of "GOOD NIGHT" TIME as the time at which you are finally in bed and trying to fall asleep.

1. On the night before a work day or school day:

a. What is your **earliest** GOOD NIGHT TIME? _____ PM/AM

b. What is your **latest** GOOD NIGHT TIME? _____ PM/AM

c. What is your **usual** GOOD NIGHT TIME? _____ PM/AM

d. How **stabl**e (i.e., similar each night) are your GOOD NIGHT TIMES? *(please tick one)*

0-15min	☐	16-30min	☐	31-45min	☐
46-60min	☐	61-75min	☐	76-90min	☐
91-105min	☐	106-120min	☐	2-3h	☐
3-4h	☐	Over 4h	☐		

2. On a night before a day off (e.g. a weekend):

a. What is your **earliest** GOOD NIGHT TIME? _____ PM/AM

b. What is your **latest** GOOD NIGHT TIME? _____ PM/AM

c. What is your **usual** GOOD NIGHT TIME? _____PM/AM

d. How stable (i.e., similar each night) are your GOOD NIGHT TIMES? *(please tick one)*

0-15min	☐	16-30min	☐	31-45min	☐
46-60min	☐	61-75min	☐	76-90min	☐
91-105min	☐	106-120min	☐	2-3h	☐
3-4h	☐	Over 4h	☐		

Please think of "GOOD MORNING" TIME as the time at which you finally get out of bed and start your day.

3. Before a work day or school day:

a. What is your **earliest** GOOD MORNING TIME? _____ AM/PM

b. What is your **latest** GOOD MORNING TIME? _____ AM/PM

c. What is your **usual** GOOD MORNING TIME? _____ AM/PM

d. How stable (i.e. similar each night) are your GOOD MORNING TIMES? *(please tick one)*

0-15min	☐	16-30min	☐	31-45min	☐
46-60min	☐	61-75min	☐	76-90min	☐
91-105min	☐	106-120min	☐	2-3h	☐
3-4h	☐	Over 4h	☐		

4. Before a day off (e.g. a weekend):

a. What is your **earliest** GOOD MORNING TIME? _____ AM/PM

b. What is your **latest** GOOD MORNING TIME? _____ AM/PM

c. What is your **usual** GOOD MORNING TIME? _____ AM/PM

d. How stable (i.e., similar each night) are your GOOD MORNING TIMES? *(please tick one)*

0-15min	☐	16-30min	☐	31-45min	☐
46-60min	☐	61-75min	☐	76-90min	☐
91-105min	☐	106-120min	☐	2-3h	☐
3-4h	☐	Over 4h	☐		

These questions are about how much sleep you lose to unwanted wakefulness:

5. On most nights, how long, on average does it take you to fall asleep after you start trying?

_____min

6. On most nights, how much sleep do you lose, on average, from waking up during the night (e.g. to go to the bathroom)? _____ min

THE EPWORTH SLEEPINESS SCALE

INSTRUCTIONS

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired? This refers to your usual way of life in recent times. Even if you have not done some of these things recently, try to work out how they would have affected you. Use the following scale to choose the **most appropriate number** for each situation:

0 = would **never** doze

1 = **slight chance** of dozing

2 = **moderate chance** of dozing

3 = **high chance** of dozing

Situation	Score (0 – 3)
Sitting and reading	
Watching television	
Sitting inactive in a public place (e.g. theatre or meeting)	
As a passenger in a car for an hour without a break	
Lying down to rest in the afternoon when circumstances permit	
Sitting and talking to someone	
Sitting quietly after a lunch without alcohol	
In a car, while stopped for a few minutes in the traffic	
TOTAL score	

Appendix 5I

HORNE-ÖSTBERG MORNING-EVENING PERSONALITY QUESTIONNAIRE

INSTRUCTIONS

a) Please read each question very carefully before answering.

b) Answer ALL 19 questions.

c) Answer questions in numerical order.

d) Each question should be answered independently of others. **DO NOT** go back and check your answers.

e) For some questions, you are required to respond by placing a cross alongside your answer. In such cases, select **ONE** answer only.

f) Please answer each question as honestly as possible. Both your answers and results will be kept in strict confidence.

1. Considering your own feelings about when you are "at your best", at what time would you get up if you were entirely free to plan your day?

Time: _____ AM/PM

2. Considering only your own "feeling best" rhythm, at what time would you go to bed if you were entirely free to plan your day?

Time: _____ AM/PM

3. If there is a specific time you have to get up in the morning, to what extent are you dependent on being woken up by an alarm clock?

a. Not at all dependent	☐	*b. Slightly dependent*	☐
c. Fairly dependent	☐	*d. Very dependent*	☐

4. Assuming adequate environmental conditions, how easy do you find getting up in the morning?

a. *Not at all easy*	☐	b. *Slightly easy*	☐
c. *Fairly easy*	☐	d. *Very easy*	☐

5. How alert do you feel during the first half hour after having woken in the morning?

a. Not at all alert	☐	*b. Slightly alert*	☐
c. Fairly alert	☐	*d. Very alert*	☐

6. How is your appetite during the first half hour after having woken in the morning?

a. Not at all good	☐	*b. Slightly good*	☐
c. Fairly good	☐	*d. Very good*	☐

7. During the first half hour after having woken in the morning, how tired do you feel?

a. Very tired	☐	*b. Slightly tired*	☐
c. Fairly refreshed	☐	*d. Very refreshed*	☐

8. When you have no commitments the next day, at what time do you go to bed compared to your usual bedtime?

a. Seldom or never later ☐ *b. Less than one hour later* ☐

c. 1-2 hours later ☐ *d. More than 2 hours later* ☐

9. You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him/her is between 7.00-8.00 am. Bearing in mind nothing else but your own inclinations, how do you think you would perform?

a. Would be on good form ☐ *b. Would be on reasonable form* ☐

c. Would find it difficult ☐ *d. Would find it very difficult* ☐

10. At what time in the evening do you feel tired and in need of sleep?

Time: _____ AM/PM

11. You wish to be at your peak for a test, which you know is going to be mentally exhausting and last for two hours. You are entirely free to plan your day. When would you do this task?

a. 8.00 am – 10.00 am ☐ *b. 11.00 am – 1.00 pm* ☐

c. 3.00 pm – 5.00 pm ☐ *d. 7.00 pm – 9.00 pm* ☐

12. If you went to bed at 11.00 pm at what level of tiredness would you be at that time?

a. Not at all tired ☐ *b. A little tired* ☐

c. Fairly tired ☐ *d. Very tired* ☐

13. For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Will you:

a. Wake up at the usual time and not go back to sleep ☐

b. Wake up at the usual time and doze ☐

c. Wake up at the usual time and go back to sleep ☐

d. Wake up later than usual ☐

14. One morning you have to remain awake between 4.00 am and 6.00 am in order to carry out a watch duty. You have no commitments the next day. Which ONE of the following alternatives suits you best?

a. Would NOT go to bed until 6.00 am ☐

b. Nap before 4.00 am and sleep after 6.00 am ☐

c. Sleep before 4.00 am and nap after 6.00 am ☐

d. Only sleep before 4.00 am and remain awake after 6.00 am ☐

15. You have to do 2 hours of hard physical work. If you were completely free to plan your day, and considering only your "feeling best" rhythm, which hours would you prefer to do it between:

a. 8.00 am – 10.00 am ☐ *b. 11.00 am – 1.00 pm* ☐

c. 3.00 pm – 5.00 pm ☐ *d. 7.00 pm – 9.00 pm* ☐

16. You have decided to engage in some physical exercise. A friend suggests that you do this between 10.00 pm and 11.00 pm twice a week. How do you think you would perform?

a. Would be on good form ☐ *b. Would be on reasonable form* ☐

c. Would find it difficult ☐ *d. Would find it very difficult* ☐

17. Suppose that you can choose your own work hours, but had to work FIVE hours in the day. Assume that your job is interesting and paid by results. Which FIVE CONSECUTIVE HOURS would you choose?

Hours: _____

18. At what time of day do you feel at your best?

Time: _____ AM/PM

19. One hears of "morning" and "evening" types. Which do you consider yourself to be?

a. Morning type ☐

b. More morning than evening ☐

c. More evening than morning ☐

d. Evening type ☐

Appendix 5J

EMERGENCY CONTACT DETAILS

CONTACT INFORMATION

Date: _____ Code: _____

PERSONAL DETAILS

First name: _____

Surname: _____

Postal address: _____

_____ Code: _____

Email address: _____

Phone number (h): _____ Cell phone: _____

EMERGENCY CONTACT

Please give us the contact details of two people we may call in case of an emergency.

Name: _____ Relation: _____

Cell phone: _____ Landline: _____

Name: _____ Relation: _____

Cell phone: _____ Landline: _____

Appendix 5K

RESETTING OUR BODY CLOCKS AFTER JET LAG

LOGBOOK

Instructions:

1. Write the date, day of the week, and type of day (Work, School, Day Off, or Weekend).
2. Put a "✓" in the actiwatch column if you wore it and the letter "x" if you did not.
3. Write the letter "W" to show when you woke up, "B" to show when you went to bed and "N" when you napped.
4. Write the letter "C" in the box when you have coffee or any products with caffeine in them; "M" when you take any medicine; "A" when you drink alcohol and "E" when you exercise.
5. *SAMPLE ENTRY BELOW: On a Monday when I worked, I wore my Actiwatch, woke up at about 06h00, had medicine at 07h00 and coffee at 09h00 and 13h00. I went for a walk at 18h00, had a glass of wine at 20h00 and went to bed at 22h00.*

Date	Day	Type	Actiwatch	04h00	05h00	06h00	07h00	08h00	09h00	10h00	11h00	12h00	13h00	14h00	15h00	16h00	17h00	18h00	19h00	20h00	21h00	22h00	23h00	24h00	01h00	02h00	03h00
02/06/14	Mon	Work	Y			W	M		C				C					E		A		B					

Notes:

Please indicate the type of medication you took, as well as the type of exercise done.

Appendix 5K

Date	Day	Medication (type and dose)	Exercise (type and time)
02/06/14	Monday	DS-24, 1 capsule	30min walk with dog