Factors affecting the ability to undertake repeated sprint performance

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Abstract

The aims of this thesis were to: 1) to review research in the area of repeated sprint (RS) performance and diurnal/circadian rhythmicity; 2) to develop a new RS protocol that conforms to field based team sport time-motion analysis and determine its reliability and compare this with a RS protocol previously utilised in the literature; 3) to assess the sensitivity of the RS protocol following acute altitude exposure and simulated soccer specific exercise 4) to investigate diurnal variation of RS performance and assess whether modulating rectal and/or muscle temperatures lead to changes in RS performance. A review of the published research literature investigating the relationship between RS performance and time-of-day variation was conducted. Six studies made it through the whole analysis process for systematic review. It was established that there was evidence to support a late/early afternoon peak in peak power in RS performance around the peak of the rhythm of core temperature. However, there is a clear demand for more rigorous investigations which control factors specifically related to chronobiological investigations. A reliability study was then performed using running as the mode of exercise for the RS test using two different RS protocols to determine the number of trials required to establish high levels of reliability. The first RS test (consisting of a total of 10 sprints, 6-s in duration with 30-s of passive recovery) was a commonly used protocol in the literature and the second was a newly created RSA protocol which is better representative of field based team sports activity (consisting of a total of 10 sprints, 3-s in duration with 30-s of passive recovery). It was established that a number of performance measures of RSA non-motorised treadmill running in both protocols were reliable. However, measures of fatigue were not. Further, it was found that both protocols took 3 sessions to fully familiarise individuals. The main aim of the next study was to investigate the sensitivity of the RSA protocol by examining the effect of altitude and fatigue on RS performance. The first finding was that acute altitude exposure reduces RS performance by 3.1 to 6.5% at 1500-m and 6.2 to 12.8% at 3000-m. The second finding was that RS performance was reduced by 4.6 to 5.8% in a fatigued state. The newly created RS performance protocol is sensitive enough to detect a negative change following altitude acute exposure and a 90-min football-specific intermittent treadmill fatiguing protocol. A diurnal protocol was then employed in order to address the clear demand for more rigorous investigations in chronobiological studies of RS performance. A total of 20 participants took part in this study and it was found that RS performance was significantly higher in the evening compared to the morning ranging from 3.3 to 8.3% in all measures except fatigue index. Diurnal variation now established in RS performance, two studies assessed whether modulating rectal and/or muscle temperature leads to a change in RS performance and further determine how much can be attributed to the influence of an endogenous, temperature-dependent component. The first study (n = 12) established that raising morning rectal temperature to evening values by active warm-up did not increase RS performance to evening values. However, lowering evening rectal or muscle temperatures to morning values by pre-cooling decreased RS performance to values normally observed in the morning. The second study (n = 12) found passively raising morning rectal temperature to evening values, or passively raising morning and evening rectal temperatures to 38.5ºC did not increase RS performance nor offset diurnal variation. Both studies concluded that although central temperature may provide some endogenous rhythm to RSA, the exact mechanism(s) for a causal link between central temperature and human performance are still unclear, and may involve multiple of components and mechanisms.

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Chapter 1: General introduction

1.1: Background

Major sporting events in the modern era are often settled by the smallest of margins. Sporting success or failure can be measured in small increments, milliseconds. A decision by an individual or a single goal can lead to victory or defeat respectively. The 2011/2012 Barclays Premier League campaign saw Manchester City Football Club take a 3-2 victory over Queens Park Rangers Football Club with two goals scored in added time to win the league title on goal difference over their arch rivals Manchester United. The winner of the 400 m Women's final at the 2008 Beijing Olympics crossed the finishing line in a recorded time of 49.62-s. The second placed athlete crossed 0.07-s behind, while third place a further 0.31-s behind (http://www.iaaf.org/results/olympic-games/2008/the-xxix-olympic-games-3659/women/400-metres/final/result, accessed on 12 February 2014). On this occasion gold was determined by a margin of less than 0.14 % over the athlete who won the gold medal and 0.63 % over the athlete who won the bronze medal. Such small margins in the modern era of athletic sports performance is now a common occurrence. Due to the ever increasing importance narrow margins and athletic development play in successful sporting achievement, highly scientific processes are being developed. Chronobiologists are mainly interested in the characteristics and the origins of a particular biological rhythm. More recently, they have looked at describing circadian rhythms in variables that are more sport and exercise relevant whilst also trying to provide information for the underlying mechanisms. Therefore, it is important for chronobiologists to explain the cause of circadian rhythmicity in athletic sports performance and exercise (Atkinson & Reilly, 1996).

Indirect evidence for the existence of circadian variation in sports performance comes from the analysis of times of day at which individuals excel in actual sporting events, by ex post facto examination (Atkinson & Nevill, 2001). Evaluations of world record breaking performances in sports events have usually been found to be broken by athletes competing in the early evening (Drust *et al.,* 2005) thus displaying that world record breaking performances seem to indicate a circadian variation (Atkinson *et al.,* 1999). However, findings should be taken with caution since there is a bias of scheduling of events in major sporting competitions as a result of numerous extraneous factors such as television demand or environmental conditions. The lack of control over extraneous influences on performance is of major concern of the indirect evidence for the existence of circadian variation in sports performance and has resulted in controlled laboratory-based investigations (Drust *et al.,* 2005). In male participants in a temperate environment (around 17-20ºC), human performance shows diurnal variation (Drust *et al.,* 2005; Reilly & Waterhouse, 2009). In several aspects of sensory motor, psychomotor, cognitive function or perceptual function show the presence of circadian rhythms (Reilly *et al.,* 1997; Winget *et al.,* 1985). Rhythms in gross muscular effort, such as muscle force production and power output, are well established and consistently peak in the mid-afternoon or early evening, regardless of the muscle group measured (hand, elbow, leg or back, for example) or speed of contraction (See reviews by Atkinson & Reilly, 1996; Drust *et al.,* 2005; Reilly & Waterhouse, 2009). The effects of time of day on short-term anaerobic performance (<6-s) are also well documented and display similar significant increases in the afternoon (Racinais *et al.,* 2004a; Souissi *et al.,* 2004). Time trial performance of a 16.1 km cycle also shows significant improvement in the evening under laboratory conditions (Atkinson *et al.,* 2005). Research conducted within laboratory settings has tended to separate components of performance and described the circadian characteristics of each. By doing so the ecological validity of the data to the real event is reduced (Drust *et al.,* 2005).

Success in competitive team sports can be attributed to the ability to repeatedly produce the best possible short-term performance (power output and velocity) with minimal recovery intervals (<60-s) between bouts and has been termed RSA (Bishop *et al.,* 2011). It is important for research to be conducted on RS performance which is more relevant to sporting events and real performance. RS performance is a fundamental fitness component of team sports performance – that is, the ability to sprint to the ball, into free space or towards goal with the ball in such cases as football and hockey (Bishop *et al.,* 2011; Spencer *et al.,* 2005; Wragg *et al.,* 2000). Aspects of RS performance such as peak power output are significantly higher in the mid-afternoon or early evening when compared to the morning (Racinais *et al.,* 2005c, 2010; Souissi *et al.,* 2004, 2010). The percentage decrement in power over repeated sprints has also been reported to be significantly higher in the afternoon with differences ranging from 4.0 to 13.1% (Chtourou *et al.,* 2012; Racinais *et al.,* 2005c; Zarrouk *et al.,* 2012). Findings regarding aspects of RS performance must be taken with great caution due to large differences in protocols utilised in the literature. Therefore, the large differences between exercise protocols and the use of cycling as the mode of exercise not only calls into question the validity of RSA protocols but also the sport-specific relevance of these variables to field based team sports. Therefore, it is evident and important that a sport-specific RS protocol is required to be established to further assess daily variation of RS performance in team sports. Once this RSA protocol has been created, research looking at the reliability of this protocol will need to be conducted.

Previous investigations have used a number of different exercise protocols not related to field based team game sports (Spencer *et al.,* 2005). In addition, ‘performance’ in football is hard to quantify in a laboratory (Drust *et al.,* 2005). Attempts have been made to develop laboratory protocols that closely replicate the activity patterns observed in football by using protocols on the non-motorised treadmill or field based protocols, such as the Loughborough Intermittent Shuttle Test (LIST; Drust *et al.,* 2005). These experimental protocols are based on metabolic and physiological responses which do not accurately represent the work-rate pattern observed during football match-play (Drust *et al.,* 2005). There are limitations to laboratory-based soccer-specific intermittent protocols that include a lower frequency of activity changes and the omission of utility movements, and lack of game skills (Drust *et al.*, 2005). More recent evidence in football suggesting that 3-s rather than 6-s sprints mostly replicates the distance covered in a sprint during European Champions League and UEFA Cup football matches (Di Salvo *et al.*, 2010) suggesting that research conducted should use sprints which are 3-s in duration as opposed to 6-s when looking at football. Once deemed reliable, it should also be investigated whether such an RSA protocol is also sensitive to morning – evening variations and other modifying factors, e.g., fatigue, altitude. An area of research which has received little attention is the effects of acute altitude exposure on RSA and field based team game-related sports performance. The 2010 FIFA (Fédération Internationale de Football Association) World Cup was staged in South Africa with games being held at various locations from Cape Town and Durban (0 m) to Johannesburg (1753 m). Furthermore, many International football games take place at levels of moderate altitude (>2000 m-3000-m) and high altitude (>3000-m-5500 m) cities – Bogota, Colombia (2600 m), Quito, Ecuador (2800 m) and La Paz, Bolivia (3700 m). It has been reported that barometric pressure changes as a function of altitude, or simulated altitude using hypoxic air can have a negative effect on physical performance if appropriate acclimatization or exposure prior to competition has not been undertaken (for a more complete review see Levine *et al.,* 2008). Recent research has looked at the effects of RS performance in 5 different conditions with different inspired oxygen fractions (FIO_2: 12 %, 13 %, 14 %, 15 % and 21 %; Bowtell *et al.*, 2013). Participants completed a RSA protocol consisting of 10 repeated sprints of 6-s in duration with 30-s passive recovery in-between each sprint. Physiological responses associated with RS performance were incrementally larger as F_IO_2 decreased to 13 %, and, unexpectedly, sprint performance was relatively resilient to moderate hypoxic exposure. Fatigue development was only significantly accentuated at the lowest F_IO_2 with no significant differences between any of the other conditions (Bowtell *et al.,* 2013). However, the limitation to this study was that the sprints performed were 6-s in duration. Sprints which are 6-s in duration are not representative of any team-game sport making subsequent findings hard to relate to real world performance.

A number of mechanisms for the observed diurnal variation in human performance are still, as yet unknown but have been attributed to a number of factors such as central/neurological factors (Bambaeichi *et al.,* 2005; Racinais *et al.,* 2005a; 2010), peripheral or muscle-related variables (Araujo *et al.,* 2011; Reilly & Waterhouse, 2009) or input from the body clock, proteins and peripheral clocks (Zhang *et al.,* 2009). However, the most common mechanism to which the evening superiority in human performance has been attributed to is that of a causal link between body temperature and performance. Body temperature is typically maintained within narrow margins of around 37.0 ± 1.0°C (Reilly & Waterhouse, 2009; Waterhouse *et al.,* 2005). The various sites of measurement of core temperature all have limitations both in reliability and validity as a true reflection of core body temperature (Craig *et al.,* 2000; Edwards *et al.,* 2013; Morris *et al.,* 2009). It is proposed that the higher evening core body temperature (~0.8ºC in rectal and gut sites, Edwards *et al.,* 2002b; ~1.1ºC in intra-aural [ear] site, Atkinson *et al.,* 2005; and, ~0.60-0.64ºC for oral temperature, Edwards *et al.,* 2008; 2009) and local muscle temperature (≥ ~0.35 °C in vastus lateralis at depths of 3 cm, Edwards *et al.,* 2013; Robinson *et al.,* 2013) produces an increase in force-generating capacity of the muscle (Bernard *et al.,* 1998; Coldwells *et al.,* 1994; Giacomoni *et al.,* 2005; Melhim, 1993) and neural function (reduced twitch time course or increase in speed of contraction, Martin *et al.,* 1999).

The link between core body temperature and human performance has been studied mainly in two ways. The first link which has received some interest in the literature, involves using either active (by means of exercise) or passive (by means of a chamber or water bath) "warm-ups" to increase core body temperature in the morning to evening and examine whether this increases morning performance (See Edwards *et al.,* 2013 for further details). An increase in T_m has been associated with enhanced maximal anaerobic power, with evidence suggesting that for every 1ºC increase in T_m, maximal muscle force output potentially increases 4-6 % (Bergh & Ekblom, 1979). According to Åstrand & Rodahl (1986), physical performance is 'optimal' at a T_{rec} ~38.3-38.5°C with a corresponding T_m of $\geq$ 39.0°C. Previously mentioned investigations that have measured both T_{rec} and T_m have only elicited central temperatures of $\leq$ 37.6°C with corresponding muscle temperature of $\leq$ 38.0°C following a 20 to 40-min active or 40 to 60-min passive warm-up (Edwards *et al.,* 2013). In order to further confirm this 'ceiling' hypothesis, and also examine the central temperature diurnal variation causal factor theory, warm-ups eliciting central temperatures and/or T_m considered 'optimal' are required. The second link which has received far less attention so far, explores whether pre-cooling core body temperature in the evening to values similar, or exactly those observed in the morning, results in a parallel decrease in performance to morning values. Very few studies have sought to investigate the effect of core body temperature on performance with precise modelling of pre performance core body temperature and time of day interaction. Cooling core temperature down resulted in isokinetic and isometric performance levels to be decreased by 8-14% (See Robinson *et al.,* 2013 for further details).

1.2: Statement of the problem

In the first instance, this thesis is concerned with the notion whether an optimal time of day for RS performance exists. Secondly, it is concerned with the methods of measurement which have been utilised for RSA and whether a more appropriate method which is more closely related to field based team sports can be validated. Thirdly, the concern is to determine whether time of day can be established with the newly created RSA method. Finally, what is the size of the contribution of temperature-dependent components on RS performance?

1.2.1: Aims

Summary of the aims of this thesis are:

1. To assess the research (over the last thirty years) in the area of RSA and diurnal/circadian rhythmicity to determine how strong the evidence is supporting the view that there is an optimal time-of-day for RS performance.
2. To develop a new RSA protocol that conforms to field based team sport time-motion analysis and compare this with a RSA protocol previously utilised in the literature and determine the number of trials required to establish high reliability.
3. To investigate the sensitivity of the RSA protocol by examining the effect of altitude and fatigue on RS performance
4. To investigate the diurnal variation in RS performance.
5. To assess whether modulating rectal or muscle temperature leads to a change in RS performance, determining how much can be attributed to the influence of an endogenous, temperature-dependent component.

1.2.2: Objectives

Realisation of the aims will be achieved through several objectives listed below.

1. Aim 1: Objective: A systematic review of RS performance and time-of-day variation, covering research published within the past 3 decades (since 1984) This will be achieved through study 1 (Chapter 3).
2. Aim 2: Objective: To compare two different RS protocols and determine the number of trials required to establish high levels of reliability. The first, a commonly used protocol in the literature, and the second, a newly created RS protocol which is better representative of field based team sports performance. This will be achieved through study 2 (Chapter 4).
3. Aim 3: Objective: To investigate the sensitivity of the RSA protocol by examining the effect of altitude acute altitude exposure, at three different levels, on physiological and psychological responses to RS performance and the effect of fatigue on RS performance using a football-specific intermittent treadmill protocol which replicates the movement pattern in a 90-min football game. This will be achieved through study 3 (Chapter 5).
4. Aim 4: Objective: To use the newly established RS protocol at two times of day (07:00 h and 17:00 h), it will be determined whether a diurnal variation in RS performance can be detected. This will be achieved through study 4 (Chapter 6).
5. Aim 5: Objective: To use the newly established RS protocol to assess whether modulating rectal or muscle temperature leads to a change in RS performance through a number of ways:
    a. By increasing morning rectal temperature values (by an active warm-up) to evening resting values or decreasing evening rectal and/or muscle temperatures

values (by immersion in a cool bath) to morning resting values. This will be achieved through study 5 (Chapter 7).

b. By increasing morning and evening rectal temperatures by passive warm-ups to 38.5˚C, which is deemed to be “optimal” T_{rec} for physical performance, and increasing morning rectal temperature to evening values. This will be achieved through study 6 (Chapter 8).

Chapter 2: A brief review of the literature and theoretical framework for the research

2.1: Structure of the review

The purpose of the literature review is to outline the scope and influence of circadian rhythms in humans. It is not intended to be a comprehensive review of chronobiology (see these references for reviews on this topic; DeCoursey, 1960; Dunlap *et al.*, 2004; Minors & Waterhouse, 1981; Reilly, 1990; Reilly *et al.*, 1997; Touitou & Haus, 1994; Waterhouse *et al.*, 2002) but rather a review to familiarise the reader with the basic framework of chronobiology, with particular reference to the circadian rhythmicity and daily variation of certain biological and physical factors – more specifically those factors related to body temperature and repeated-sprint ability performance. It is also the aim to provide a firm context regarding the relationship between short-term gross muscular performance and circadian rhythmicity, along with a critique of the methods employed within the literature to date which have attempted to approach and investigate this specific area.

The review begins with an introduction to the basic concepts of chronobiology; after which the range and influence of circadian rhythms measured in a number of resting physiological variables is explained. A comprehensive account of the evidence of circadian rhythms in human physical performance is then analysed, with a more detailed section of the review devoted to RS performance. Prior to establishing circadian rhythmicity in RSA; the review will aim to define RSA, discuss the current aspects of RSA and the validity and reliability of current RSA tests in the literature, and the need of establishing a new RSA protocol. The studies looking at the effects of T_{rec} and T_m manipulation through a warm-up or a cool-down and the effects of altitude in RSA will be investigated and discussed.

2.2: Introduction to Chronobiology

Chronobiology is a word that has been derived from three Greek stems: Chronos meaning time, bios meaning life and logos meaning study (Dunlap *et al.*, 2004). Chronobiology has been defined as the scientific study and objective quantification of the mechanisms of living timing processes in animals and plants (Minors & Waterhouse, 1981). Prior to the multi-disciplinary science of chronobiology practised nowadays, the basic observation that leaf movements of a plant persisted cyclically in constant darkness was a precursor.

Life on Earth evolved in an environment that changed cyclically, which was dependent on the relative movement of our planet in the solar system. As a result, from the revolution of the Earth on its axis and the orbit of it around the sun, environmental fluctuations such as the light-dark cycle and recurring seasons take place (Reilly, 1990). It comes to no real surprise that an astronomer, originally investigating relations between the Earth's environment, the life which inhabits it and the movement of the planets, was the first person officially known to document the possibility that organisms might contain a self-sustaining time-keeping mechanism (d'Ortous de Mairan, 1729) necessary for survival (Atkinson, 1994).

In the middle of the nineteenth century, the acceptances of rhythmic manifestations of life were slightly hampered by the French Physiologist Claude Bernard (1813-1878). Bernard noted that land mammals must deal with large-scale environmental changes in temperature, water availability, wind and gravity and assumed that the external environment was less constant than the internal environment of the human body (Dunlap *et al.*, 2004). This led to a belief that the internal environment (milieu intérieur) was constant and resistant to change. In the twentieth century, the concept was expanded and refined by an American physiologist named Walter Cannon (1871-1945) who created the theory of homeostasis for this concept regarding physiological protective mechanisms (Dunlap *et al.,* 2004). Over the last 60 years, the interest in chronobiological research has increased dramatically and it is therefore clear that Bernard underestimated the significance of small changes in the milieu intérieur (such as body temperature).

Homeostasis can be defined as the maintenance of the internal milieu of the body within very narrow limits, in spite of the tendency for an individual's surroundings and activities to change (Dunlap *et al.,* 2004). For human beings, the alternation between night and day, which results from the regular spin of the earth about its central axis, is the most evident environmental change (Reilly, 1990). The solar day oscillates with a frequency of about once every 24 hours, with the amount of sunlight dependant on the geological location of the planet, the tilt of the axis of the earth and the time of year. As the human is essentially a diurnal creature, with habits such as work, social life and activity usually performed in the daytime, while sleep is undertaken at night, it comes as no surprise that many human biological functions exhibited a circadian rhythmic activity cycle, based on 24 h intervals. These rhythms of around 24 h are termed circadian (Reilly, 1990). The term circadian comes from two Latin stems: circa meaning approximately and dian (dien) meaning about a day.

2.3: Definitions

The importance of definitions is among the most important theoretical tools in any field and a complete ‘glossary of terms’ is beyond the scope of this review (for this see Dunlap *et al.*, 2004; Halberg *et al.*, 1977; Touitou & Haus, 1994). A few terms which are frequently used need to be defined and clarified from the outset for the understanding of the text.

What is a rhythm?

According to Minors and Waterhouse (1981) a rhythm represents a recurring oscillation. Oscillation, cycle and periodicity are terms closely related and used interchangeably with the term rhythm. In simple terms, a rhythm can be more easily defined as a sequence of events that repeat themselves over time in the same order and at the same interval (Palmer, 1976). A rhythm commonly utilised in the literature is the rhythm of core temperature and muscle strength, of which both exhibit daily rhythmic activity cycles over a solar day (24 h). The rhythm of rectal temperature is sinusoidal in form, but this is not to say that all rhythms are of this nature. A number of rhythms are asymmetrical or even symmetrical but not specifically sinusoidal (See Conroy & Mills, 1970 for more information). Therefore, we use the examples of core temperature and muscle strength as these are the most relevant to the research analysed in the experiments within this thesis.

Periodicities

The period is defined as the time after which a distinct phase of oscillation recurs or the time required completing one cycle (Halberg, 1959). The periods of biological rhythms are characteristically broken up into ranges of time (Halberg, 1959). A rhythm is classified as ‘circadian’ if its period rests within the range of 20 to 28 h (Halberg, 1959), around a solar day. ‘Ultradian’ rhythms are periods which occur over a time period which is less than 20 h and usually have a high frequency with periods of less than a minute. Prime examples of these are the respiratory and cardiac pacemakers (Minors & Waterhouse, 1981). ‘Infradian’ rhythms occur over a time period longer than 28 h and a prime example for this is the menstrual cycle. Infradian rhythms are then further split up into branches according to period length using the prefix circa in all cases (for more information see Dunlap *et al.,* 2004; Haus & Touitou, 1992).

2.4: The body clock

Previously, it was believed that a fundamental theory for the explanation of biological behaviour even at the level of the cell or organism was a long way away. However, research is at a far more advanced stage than the old published findings in the area of biological rhythms and human performance (Colquhoun, 1971). Following the initial observations by d'Ortous de Mairan (1729) in plants, several pieces of evidence now suggest that circadian rhythmicity in mammals has an internal (endogenous) component and is not just fully dependent upon external (exogenous) factors. Evidence of endogenous components of circadian rhythmicity in human beings has mainly been conducted via protocols which aim to remove or control the influence of exogenous factors, which might mask (hide or discredit) the contribution of the possible endogenous component, often referred to as the biological clock or the body clock (Dunlap *et al.,* 2004).

The site of the endogenous component cannot be directly measured in humans, and its molecular chemistry and genetics cannot be examined by gene manipulation in human beings for ethical reasons. Although a small amount of histological data is available from cadavers, most conclusions are inferred from other animals. Studying cellular processes of circadian pacemaker cells is a very challenging level of research requiring highly sophisticated techniques and procedures. Specialised recording electrodes with computerised oscilloscopes and elaborate light and electron microscopes for fast data collection and analysis are the techniques utilised (Dunlap *et al.,* 2004). Results from such experiments outline that the body's internal time keeper (the body clock) resides in the paired suprachiasmatic nuclei (SCN) located in the anterior hypothalamus, just above where the optic nerves cross prior to their entry into the brain (Ralph *et al.,* 1990). Results also indicated that the body clock receives inputs from the eyes via the retinohypothalmic pathway (Ralph *et al.*, 1990). For further information regarding these experiments, see Dunlap *et al.* (2004).

A number of chronobiological protocols have been used for the separation of exogenous (environmental and time-since-sleep) and internal (clock driven) components in studies. The partial separation of these has been achieved through a number of different protocols. Protocols which have been utilised vary from free-running protocols, where participants are studied in an environment from which external time cues (the environmental component) have been removed, to constant routine protocols, where participants are required to remain awake and sedentary for 24 h in a constant environment (lighting, temperature and humidity) and take identical meals regularly and engage in similar activities throughout. Other studies which have been utilised are forced desynchronisation, ultra-short sleep-wake cycle and changed sleep times. For more information regarding these protocols and which components (internal or external) can and cannot be separated through the various protocols see Reilly and Waterhouse (2009).

Molecular and genetic biochemistry studies have been conducted, by the use of dissociated cell suspension grafts from SCN transplantation and through direct monitoring of neuronal activity of primary cultures of dissociated SCN cells, to question whether rhythmicity is a cellular, multicellular or tissue property of the SCN (Gilette *et al.*, 1995; Ralph *et al.*, 1990). It appears that the underlying mechanism of the body clock is a set of genes which appear to be activated in a cascade-like sequence with negative and positive feedback loops. To touch upon this in too much detail is beyond this review (for this see Dunlap *et al.*, 2004).

2.5: Zeitgebers

A widely used term is zeitgeber, which literally translated means ‘time giver’. This is a term used to define the rhythmic cues which are derived from the external environment and which influence the continual adjustment of the body clock (Aschoff, 1978; Dunlap *et al.,* 2004). The periodicity of the body clock which is usually around a solar day has been found to be longer when individuals are isolated from external cues. Initially, although it was clear social cues played the main responsibility in the normal entrainment or adjustment of human circadian rhythms, it was concluded that other possible zeitgebers, such as the light-dark cycle, the feeding-fasting cycle and the activity-inactivity cycle, all played an insignificant role in entrainment (Aschoff & Wever, 1980). However, it has now been identified that environmental light-dark cycles are the key entrainer of human circadian rhythm (Czeislar *et al.*, 1986). This is a notion which is now fully supported, but further, it was concluded that not only the light-dark cycle, but also the rhythmic secretion of the hormone melatonin influences human circadian rhythm the most (Lewy *et al.,* 1998).

In human beings, the influence of the light-dark cycle has shown to both advance and delay the activity of the body clock. When pulses of bright light (10000 lux) are administered during the 6 h window prior to core body temperature minimum (± 05:00 h), a delay in the activity of the body clock takes place, resulting in the biological rhythm to occur later than usual. When pulses of bright light are administered during the 6 h window post core body temperature minimum, a phase advance of the body takes place, resulting in the biological rhythm to occur earlier than usual. This process is called the phase response curve (PCR) and has three distinct components (see DeCoursey, 1960).

The hormone melatonin is usually secreted in the late evening and at night and supressed by bright light (Reilly *et al.,* 1997). It is produced by the pineal gland and plays an important role in influencing the adjustment of the body clock. In contrast to the influence of bright light on the activity of the body clock, melatonin invokes a delay during the early morning and an advance during the early evening (Wirz-Justice *et al.,* 2004). Light and melatonin work in harmony with one another. If the body clock is running slower than usual, melatonin secretion will occur later and vice versa.

In most instances, it is important to recognise that, there is more than one zeitgeber present influencing a human at any given time (Edwards *et al.,* 2008). Although the light-dark cycle and melatonin have received much interest, there are other non-photic signals that help synchronise the body clock to a solar day. These zeitgebers include social interaction and timing of food (see review by Mistleberger & Skene, 2005). Other zeitgebers include knowledge of time, scheduling of rest and activity, and physical exercise. To touch upon this in too much detail is beyond this review (see Edwards *et al.,* 2008).

2.6: Resting circadian rhythms

The exercise scientist must take into consideration the existence of resting physiological rhythms. Since the body clock cannot be easily or directly measured in human beings, investigators rely on biological markers (Klerman *et al.*, 1999). In this section of the literature review, the causes and characteristics of a number of circadian rhythms in resting physiological functions measured under nychthemeral (24 hrs) conditions will be discussed in detail.

2.6.1: Body temperature

The circadian rhythm of core temperature acts as a marker for the body clock and has often been described as the fundamental variable (Reilly *et al.*, 2000). Many studies, some of which were performed well over a century ago, following the development of reliable mercury-in-glass thermometers (accurate measurement of oral temperature) and the frequency with which measurements could be taken (limited only by subject cooperation) have been conducted (Minors & Waterhouse, 1981). The determination of core body temperature in circadian studies provides an advantage in that it can be measured throughout the 24 h solar day (Waterhouse *et al.*, 2005).

The body can be divided into a central part (core) and a peripheral part (muscle and skin). Not only do both parts vary regarding sites, but so do the mechanisms for markers of heat-loss or heat-gain, the timings at which they occur and the varying responses to exogenous factors. The earliest records showing a distinct cyclic variation in core body temperature throughout the solar day can be attributed to Davy (1845) or Gierse (1842). Observations were made that if a large number of readings were continuously taken, oral temperature was low upon waking, and increased rapidly until mid-morning, remaining pretty stable until the evening, when it started to decrease. Oral temperature suffers from the disadvantage that the oral mucosa does not reflect deep core body temperature accurately. Therefore other sites which are more reliable have been used, such as, rectal, the gut, insulated axilla and oesophageal, although the latter is deemed unacceptable to the subject (Waterhouse *et al.,* 2005). It has been found that deep core body temperature is relatively constant at approximately 37°C and its daily range of oscillation is about 0.6-1.0°C (Reilly *et al.,* 1997). Few studies have actually looked at core body temperature over 24 h. In participants living a normal lifestyle, circadian changes in core body temperature are phased such that the temperature maximum occurs between 14:00 h to 20:00 h and the temperature minimum at about 05:00 h (Waterhouse *et al.,* 2002). A study performed by Edwards *et al.* (2002b) found T_{rec} to peak at 17:00 h, with a mesor of 36.7°C. The gut thermistor pill yielded a similar rhythm, but found temperatures to be consistently higher by 0.2°C than those reported by $T_{rec.}$ When insulated axilla temperature was considered, the comparability with rectal temperature was far less, with differences of approximately 1°C (see Edwards *et al.,* 2002b for more information).

Research conducted in regard to peripheral body temperature such as skin temperature (T_{skin}) and local muscle temperature (T_m) seems far less established and conclusive. It has also only recently been established that local muscle temperature in vastus lateralis at depths of 3 cm vary with time of day with evening values (17:00 h) higher than the morning (07:30h) values, by ~0.35°C (Robinson *et al.,* 2013) in males. As far as the literature is concerned there seems to be no studies conducted looking at its circadian rhythmicity, maybe due to the invasiveness of the technique. A significant circadian rhythm has been established for T_{skin} with time of maximum and time of minimum varying upon time of day and site. However, findings regarding T_{skin} must be taken with consideration as some skin sites are poor markers (see Kräuchi & Wirz-Justice, 1994; Reilly & Brooks, 1986 for more information).

Internal core temperature is controlled by nerve cells based in the hypothalamus with different centres for instigating heat loss or heat gain mechanisms. The gradient between skin temperature and the body's core temperature permits heat to be exchanged with the environment through radiation and convection (Waterhouse *et al.*, 2005). It is also valuable to consider that there does exist a set point at which core temperature is regulated by the hypothalamus (for more information see Edholm *et al.*, 1973). In resting individuals, the observed circadian rhythm in core temperature is achieved predominantly via changes of heat loss (e.g., decreased heat loss in the afternoon/evening), with only 25 % of the observed rhythm attributed to changes in metabolic heat production via the Q_{10} effect (Reilly & Brooks, 1982; Waterhouse *et al.,* 2005). To touch upon this in detail is beyond this review (see Edwards *et al.*, 2002b; Kräuchi & Wirz-Justice, 1994; Waterhouse *et al.*, 2005 for more information).

2.6.2: Circulation and blood

Heart rate function has many parameters which display circadian variation and plays a vital role supplying oxygen throughout the body and removing waste products. Circadian rhythm in heart rate is highly influenced by exogenous factors such as sleep, posture, activity and ingestion of food (Pickering, 1988), thus questioning the endogenous component of heart rate. The (highest point) of heart rate has shown to occur a few hours earlier than that of body temperature and has shown to have amplitude of 6 % (Reilly *et al.*, 1997). Similar rhythms were found for other characteristics such as stroke volume, blood flow, blood pressure and cardiac output (Miller & Hellander, 1979).

Blood composition, such as serum potassium and plasma proteins have also been found to show circadian rhythms. Many of these blood-borne substances displaying a rhythm seem to be closely related to posture, activity and meal ingestion as opposed to the existence of an internal clock (Reilly *et al.*, 1997), such as blood glucose and insulin. Further, plasma volume is also affected by activity, as well as posture and it is therefore vital to control or take both into consideration, along with time of day, prior to blood sampling.

2.7: Circadian rhythms in sports performance

In male participants in a temperate environment (around 17-20ºC), human performance shows diurnal variation (Drust *et al.,* 2005; Reilly and Waterhouse, 2009). The observation of a significant diurnal or circadian rhythm seems to be somewhat dependent on factors such as time on task, motivation of participants to perform and familiarisation of the tasks to be performed (Bambaeichi *et al.,* 2005; Giacomoni *et al.,* 2005). A number of aspects such as body temperature, circulating levels of hormones and metabolic functions when manipulated artificially have shown to play a role in performance.

The exact mechanisms for this observed diurnal variation in human performance are as yet unknown but have been attributed to peripheral or muscle-related variables (contractibility, metabolism and morphology of muscle fibres and local muscle temperature), which can be influenced by hormonal and ionic muscle process variations (Araujo *et al.,* 2011; Bambaeichi *et al.,* 2005; Reilly & Waterhouse, 2009; Tamm *et al.,* 2009), and/or central/neurological factors (central nervous system command; alertness; motivation and mood: Castaingts *et al.,* 2004; Giacomoni *et al.,* 2005; Racinais *et al.,* 2005a; 2010). Further, input from the body clock and proteins and peripheral clocks - that is, an endogenous component to the daily variation in muscle force production - has been suggested to be important (Zhang *et al.,* 2009). However, the evidence relating to the endogenous contribution to sports performance is very limited. The evening superiority in muscle force production and power output has often been attributed to a causal link between body temperature and performance. Human performance has consistently been shown to peak in the early afternoon or early evening, typically around 15:30 to 20:30 h (Reilly & Waterhouse, 2009), which mirrors the pattern of behaviour or rhythm of the observed recordings in daily variation in body temperature. The term "performance" in the context of a sporting action has a broad meaning and usually compromises a mixture of task components (Atkinson, 2002). In this section of the literature review the evidence of such rhythmicity in human performance will be discussed further.

2.7.1: Indirect evidence of circadian variation in sports performance

The indirect evidence for the existence of circadian rhythms in sport performance comes from the analysis of the times of day at which athletes perform best or worst in actual sports events (Drust *et al.,* 2005). In this type of "post facto" investigation, the element which is maximized is the external validity of sample and performances (Atkinson & Nevill, 2001). Previous evaluations of world records in sporting events, such as athletics, are usually broken by athletes competing in the early evening, which is the time of day at which body temperature is at its highest (Reilly *et al.,* 1997), indicating potential circadian variation. It has been stressed that such "post facto" observations should be interpreted with caution. The bias for scheduling finals of track and field competitions in the afternoon or evening due to extraneous influences such as television demand and spectator availability play are reasons for this. The lack of control over environmental factors on performance is also a major issue regarding field based studies. Environmental temperature and circadian fluctuations in meteorological conditions affect performance in a large number of sporting disciplines.

The event-scheduling bias can be controlled for in some sports however. In the discipline of time-trialling (competitive cycling), the frequency of races tend to be more evenly distributed (Reilly *et al.,* 1997). Performances of young cyclists in 16-km races were better in the afternoon/evening compared to those which took place in the morning (Atkinson, 1994). When trial frequency in weight-throwing is standardized at different times of the day in simulated competitions, performances were also better in evening (Conroy & O'Brien, 1974). The lack of control over environmental influences is therefore a major issue with the aforementioned evidence. A tighter control can however be applied to swimming, where water conditions can be held pretty constant throughout the day. It has been reported that swim performance improved in both the 100-m and 400-m swims held in the afternoon or early evening (Baxter & Reilly, 1983). Similar observations have also been made by other investigators (Arnett, 2002; Rodahl *et al.*, 1976).

2.7.2: Laboratory investigations

In order to remove and have more control over extraneous influences, laboratory-based experiments have been conducted as these allow more conclusive evidence of circadian variation in sports performance to be investigated and established. Experiments which have been conducted are, however, associated with both methodological and theoretical issues (Drust *et al.*, 2005; Reilly *et al.*, 1997).

2.7.2.1: General Experimental Design

The main performance component may vary from one athletic event to another and successful performance in events is usually dependent upon a number of these components (Reilly *et al.*, 1997). In laboratory-based investigations researches have tended to isolate separate performance components and described circadian characteristics of each component. However, this reduces the ecological validity of the data to the real event (Drust *et al.*, 2005).

It is crucial that the methodological methods used in laboratory-based investigations approximate the required accuracy needed to enable discrimination between successful and unsuccessful performances within the competitive environment. This difference may well be as small as 1 % (Hopkins *et al.*, 1999). Therefore, research investigators require substantial scientific rigor in the experimental design and data collection with minimal measurement error to enable the detection of such small differences. Further, sample size will also play a role on the conclusions derived (Drust *et al.,* 2005).

Sample size for a study using a valid laboratory or field test is proportional to the square of the within-athlete variation in performance in the test relative to the event; estimates of these variations are therefore crucial and should be determined by repeated-measures analysis of data from reliability studies for the test and event. Enhancements in test and event may differ when factors that affect performance differ between test and event; overall effects of these factors can be determined with a validity study that combines reliability data for test and event. Researchers traditionally are supposed to use a sample size that will permit them to detect (declare statistically significant) the smallest worthwhile effect, with acceptably low rates for detection of non-existent effects (5%) and failed detection of the smallest worthwhile effect (20%; Hopkins *et al.,* 1999.

It has been concluded that circadian rhythm research in performance has been shown to have issues of type II error within its research (Drust *et al.,* 2005) A small sample size and/or too much measurement error are examples of type II issues in research (Atkinson & Nevill, 1998; Atkinson & Reilly, 1999). However, if a circadian rhythm is detected within an experiment, both through statistical and practical significance, it is difficult to criticise the finding on the basis of too small a sample size or too much measurement error. The issue during such an occurrence would then relate to a type I error. Sample size influences on type I error rates are controlled for in the mathematics which underpins statistical interference (Drust *et al.,* 2005). If a sample size is low, this will result in the degrees of freedom in the analysis to be low also. As a result, for a given test to then be deemed as significant or a confidence interval to have sufficient precision, makes it hard. The incorrect choice of a statistical test or the bias of participant sampling is usually the result of a type I error occurring (Atkinson, 2001).

2.7.2.2: Rhythms in psychomotor performance and motor skills

Several components of perceptual, sensory motor, psychomotor and cognitive tasks display circadian rhythmicity. Simple reaction time, a major component of performance in sprinting events, is fastest in the early evening, when body temperature is at its highest (Winget *et al.*, 1985). An inverse relationship exists between speed and accuracy with which simple repetitive tests are performed, making accuracy worse in the early evening (Atkinson & Spiers, 1998). Mood states and subjective alertness are believed to play a role in human performance by altering the predisposition of individuals prior to vigorous physical efforts. Alertness and positive mood states peak in the evening, while sleepiness and fatigue tend to peak in the morning (Reilly *et al.*, 1997).

2.7.2.3: Muscle strength performance

Muscle strength, independent of the muscle group or site measured or speed of contraction, consistently peaks in the mid-afternoon or early evening (Reilly *et al.*, 2000). A much explored variable of muscle strength in chronobiology research is that of hand grip strength, due to its ease of measurement and its reliability (Lagerström & Nordgren, 1998; Reilly *et al.*, 1997) and is therefore often used as a marker of the circadian rhythm of muscle strength performance (Reilly *et al.*, 2000). Hand-grip strength peaks between 14:00 and 19:00 h with an amplitude of 6 % (Reilly *et al.*, 1997). Other muscle groups such as the quadriceps (Callard *et al.*, 2000) and adductor pollicis (Martin *et al.*, 1999) display similar circadian rhythm characteristics, but these are highly dependent upon the mode of muscle contraction, such as eccentric or concentric contractions, (Giacomoni *et al.*, 2005) and the muscle group being tested (Gauthier *et al.,* 1996; Stratton *et al.,* 2003). Further, elbow flexion (Gauthier *et al.,* 1997) and back strength (Coldwells *et al.,* 1994) also vary with time of day, peaking in the early evening. Eccentric and concentric strength using isokinetic dynamometry has been measured at various time-points of the solar day (Atkinson *et al.,* 1995; Cabri *et al.,* 1988). A time of day difference has been found at various speeds (1.05, 3.14, 4.19 and 5.24 rad.s^{-1}) with peak values occurring in the early evening (Souissi *et al.,* 2002; Wyse *et al.,* 1994).

2.7.2.4: Short-term power output

The presence of circadian rhythms within short-term performance (<60-s) is controversial as it is highly believed to be dependent on the muscle group tested and the type of exercise performed (Bernard *et al.,* 1998). Circadian rhythmicity has been established in some conventional investigations of short-term dynamic exercise and in some laboratory measures of anaerobic exercise (Drust *et al.,* 2005). Performance in vertical jumping (Atkinson, 1994; Bernard *et al.,* 1998), jumping length (Reilly & Down, 1986) and anaerobic power output in a stair run (Reilly & Down, 1992) have all been observed to show significant circadian rhythmicity with higher values in the evening. Further, short duration maximal ergometer tests, lasting between 10-30-s in duration, observed significantly higher values in peak and mean power outputs in the evening compared to the morning (Melhim, 1993; Souissi *et al.,* 2002; 2003), however, not all studies have observed this (Down *et al.,* 1985; Reilly & Down, 1986). The effects of time of day on short-term anaerobic performance (<6-s) are also well documented and display similar significant increases in the afternoon (Racinais *et al.,* 2004a; Souissi *et al.,* 2004). However, research conducted more relevant to sporting events and real performance is needed. Sprints of short duration, interspersed with periods of recovery, which are a common occurrence during most field based team sports, are therefore far more relevant and will be discussed in detail in section 2.8.

2.8: Circadian rhythms in repeated sprint (RS) performance

Success in in competitive team sports can be attributed to the ability of being able to repeatedly produce the highest levels of power output or velocity. In field based team sports, such as soccer, rugby and hockey, players exert a large number of maximal or near maximal sprints of short duration throughout a game which are punctuated by a resting period or lower intensity period activity lasting longer in duration than the sprint bout (Bishop *et al.,* 2001). Short term-term performance (power output and velocity) over a series of sprints (<6-s) with minimal recovery intervals (<60-s) between bouts, has been termed RSA (Bishop *et al.,* 2011). Although sprinting has been found to represent less than 10 % of total distance covered during a game (Carling *et al.,* 2012; Reilly & Thomas, 1976), RSA is regarded crucial to the outcome of a game and is also a fundamental fitness component of team sports performance – that is, the ability to sprint to the ball, into free space or towards goal with the ball in such cases as football and hockey (Bishop *et al.,* 2011; Spencer *et al.,* 2005).

Although there has been a major increase in the scientific research regarding RS performance, the investigations regarding the physiology or circadian rhythmicity in RSA is pretty scarce. There seems to be a massive gap in the research literature in this area which vitally needs addressing. Few studies have investigated the impact of daily variation on RSA (Table 2.1). Findings from these studies have consistently reported a time-of-day difference for peak power output, with higher values in the mid-afternoon or early evening when compared to the morning (~3.1 to 7.6 %) for either the first or first few sprints only (Racinais *et al.,* 2005c; Racinais *et al.,* 2010), or for all sprints performed (Souissi *et al.,* 2004, 2010). The percentage decrement in power over the repeated sprints has also been reported to show diurnal differences (Chtourou *et al.,* 2012; Racinais *et al.,* 2005c; Zarrouk *et al.,* 2012), with higher values in the evening when compared to the morning (4.0 to 13.1 %), although this has not been the case for all investigations (Hamouda *et al.*, 2012; Souissi *et al.*, 2008). It has been suggested that the observation of a significant diurnal variation is somewhat dependent on a number of factors - such as type and intensity of task, motivation of participants to perform the task, time on task and subject familiarisation regarding the task to be performed (Reilly *et al.*, 1997; Bambaeichi *et al.*, 2005; Giacomoni *et al.*, 2005). Although all aforementioned studies found evidence of daily variation in RSA on a cycle ergometer, differences in sprint duration, type of recovery, number of sprint repetitions and training status of participants all varied; therefore making it difficult to compare findings between these studies. This will be discussed in further detail in section 2.12. Further, it is not the intention of this literature review to discuss metabolism and fatigue aspects involved within RSA (For this see Girard *et al.*, 2011; Spencer *et al.*, 2005).

Table 2.1. Summary of relevant previous research investigating diurnal variation on repeated sprints.

Author and Date	Participants	Testing time-of-day	Mode of Exercise	Sprint distance/ duration	No. of reps and Recovery Duration/Type	Performance Variable	Diurnal Variation in Performance? (no/yes)
Souissi *et al.* (2004)	N = 19 Males (Physical education students, all M or intermediate types).	02:00, 06:00, 10:00, 14:00, 18:00 and 22:00 h	Cycle ergometer	6-s sprints	6-7 reps, 24-s rest	Maximal power	**Yes** Maximal power displayed a significant peak at 17:24 h and amplitude of 7.6 % ($P < 0.05$).
Racinais *et al.* (2005c)	N = 9 Males (Physical education students, 2 M and 1 E type).	07:00 –09:00 h and 17:00-19:00 h	Cycle ergometer	6-s sprints	5 reps, 24-s rest	Peak power output, power decrement and total work.	**Yes** Significantly higher power output in the E (958 *vs.* 915 W; + 5.3 %) during 1st sprint only ($P < 0.05$). Higher power decrement in the E (11 *vs.* 7 %; $P < 0.05$). **No** No difference for total work ($P > 0.05$).
Giacomoni *et al.* (2006)	N = 12 Males (Active participants).	08:00 –10:00 h and 17:00-19:00 h	Cycle ergometer	6-s sprints	10 reps, 24-s rest (only reps 1, 5 and 10 taken into account).	Peak power output, total mechanical work, pedalling rate and peak efficient torque.	**Yes** All variables significantly higher in E compared to M session ($P < 0.05$) for sprints 1, 5 and 10.
Souissi *et al.* (2007)	N = 12 (Physical education students, all moderately M or intermediate types).	07:00, 17:00 and 21:00 h	Cycle ergometer	6-s sprints	6-7 reps, rest not mentioned	Maximal power and force.	**Yes** Maximal power and force showed a significant time-of-day effect with higher values at 17:00 h ($P < 0.001$).

Souissi *et al.* (2008)	N = 11 (Physical education students, neither type).	07:00 and 18:00 h	Cycle ergometer	6-s sprints	6-7 reps	Maximal power, velocity and force.	**Yes** Maximal power and force showed a significant time-of-day effect with higher values at 18:00 h ($P < 0.001$). **No** No difference found for velocity ($P > 0.05$).
Racinais *et al.* (2010)	N = 12 Males (Diurnally active, 2 M and 1 E types).	08:00 – 10:00 h and 17:00-19:00 h	Cycle ergometer	6-s sprints	10 reps, 30-s rest	Power output and power decrement.	**Yes** Significantly higher power output in the E during sprints 1, 2, 3 and 5, and power decrement for the 10 sprints ($P < 0.05$).
Chtourou *et al.* (2012)	N = 20 Males (Young soccer players).	07:00 h and 17:00 h	Cycle ergometer	6-s sprints	5 reps, 24-s of passive rest	Peak power, power decrement and total work.	**Yes** Peak power was significantly higher in E ($P < 0.05$) only during first 2 sprints, with diurnal gains of 4.8 and 3.1 % respectively. Power decrement and total work were significantly higher in the E than the M ($P < 0.05$) with amplitude of 13.1 and 4.1 % respectively.

Hamouda *et al.* (2012)	N = 10 Males (Junior soccer players, all moderately M or intermediate types).	07:00 h and 17:00 h	Cycle ergometer	6-s sprints	5 reps, 24-s rest	Peak power output, power decrement and total work.	**Yes** Peak power was significantly higher in E (*P* < 0.05) only during first 2 sprints. Total work significantly higher in the E (*P* < 0.05). **No** Power decrement was unaffected by time-of-day (*P* > 0.05).
Zarrouk *et al.* (2012)	N = 12 Males (Physical education students, all intermediate types).	06:00 h and 18:00 h	Cycle ergometer	6-s sprints	5 reps, 30-s rest	Power output, power decrement and total work.	**Yes** Total work and power decrement were significantly higher in the E (*P* = 0.002 and *P* = 0.041 respectively). Power output was significantly higher only for sprints 1, 2 and 3 in the E (*P* < 0.05).
Aloui *et al.* (2013)	N = 12 Males (Amateur soccer players, all moderately M or intermediate types).	07:00 –09:00 h and 17:00-19:00 h	Cycle ergometer	6-s sprints	5 reps, 24-s rest	Peak power and percentage of peak power.	**Yes** Peak power was higher in the E (653 *vs.* 6823W) for the 1st sprint only (*P* < 0.05). The percentage of peak power was significantly higher in the E than the M (*P* = 0.012)

M = Morning, **E** = Evening, **reps =** repetitions.

2.9: Manipulation of thermal variables and diurnal variation in RS performance: warm-up *vs.* cool down

2.9.1: Warm-up

A warm-up is a widely accepted practice which precedes any form of exercise and considered essential to achieve optimal performance, although the latter has little scientific evidence to back this (Bishop, 2003a; 2003b). It has been suggested that warming up affects performance through a number of mechanisms which are predominantly related to temperature, such as decreased stiffness, increased nerve-conduction rate and increased anaerobic energy provision. A number of non-temperature related physiological mechanisms have also been proposed, such as increased post activation potential and increased O_2 delivery (Bishop, 2003a; 2003b). However, psychological mechanisms such as mental preparedness have also been suggested. To touch upon this in too much detail is beyond this review (for this see Bishop, 2003a; 2003b).

Warm-up procedures have been divided into two main categories: an active warm-up or a passive warm-up. An active warm-up involves exercise such as jogging, cycling and swimming and is the most likely used warm-up technique in the literature (Bishop, 2003b). The increase in T_{rec} and T_m achieved by active movements of the major muscle groups following an active warm-up has been attributed as the main proposed benefit (Shellock, Prentice, 1985). A passive warm-up involves raising T_{rec} and T_m through external means such as a chamber or water bath, without depleting energy substrates (Bishop, 2003a).

Research looking at the causal link between temperature and RS performance is very limited. The little research which has been conducted showed that both active and passive warm-ups resulted in an increase in T_{rec}, T_m and skin temperature (T_{skin}). A submaximal active warm-up of 10-min duration at 70 % $\dot{V}O_2$ max on a motorised treadmill and a passive warm up consisting of hot water submersion at 40.1°C resulted in a 0.4°C increase in T_{rec}. Both conditions caused significant increases in peak speed attained within 1-s and mean peak speed when compared to the control (no warm-up) trial. Further, no differences were found between active and passive warm-ups (Brown *et al.,* 2008). However, research has generally reported that RS performance following an active warm-up or passive warm-up compared to no warm-up did not result in an increase in performance, even though increases in temperature values were reported. Performance measures such as, total work, % work decrement and power output reported no differences following an active or passive warm-up (Brown *et al.,* 2008; Sim *et al.,* 2009; Taylor *et al.,* 2013; Yaicharoen *et al.,* 2012). The lack of effectiveness of the aforementioned warm-ups, the lengths of the protocols and the variations in methods utilised to warm-up individuals and the different RSA protocols used contribute to the lack of well-controlled studies conducted and available in the literature.

2.9.2: Cool down

Thermal stress has shown to subsequently reduce exercise performance (Drust *et al.,* 2005; Maxwell *et al.,* 1999; Morris *et al.,* 2005), increase neuromuscular fatigue (Kay *et al.,* 2001) and result in increased metabolic and cardiovascular loads (Duffield & Marino, 2007; Morris *et al.,* 2000). Individuals have been shown to reduce their intensity of work or alter their strategy of pacing so that they can continue exercising with limited health related illness or injury risk during thermal stress This is the case even when participants are trained or acclimated (Marino, 2002). When subjected to hot conditions, the situation is further impaired and ultimately can result in the premature cessation of exercise. As a result, the method of pre-cooling is a commonly used strategy to combat the increased thermal stress, by decreasing body temperature (T_{rec} and T_m) prior to exercise, which in turn increases the margin for metabolic heat production and the time critical limiting temperature, is reached (Marino, 2002; Nielsen *et al.*, 1993). This ensures individuals can work at higher exercise intensity without significant changes in pacing strategies (Marino, 2002).

Research looking at the causal link between temperature and RS performance through cooling is very limited. A single 70-s cycling sprint following pre-cooling resulted in mean power output for the 70-s performance test to be significantly increased by 3.3 % in a warm and humid environment (Cheung & Robinson, 2004). Power output over 10 s sprints interspersed with 5-min recovery periods in standard laboratory conditions, showed no difference when the upper body was pre-cooled compared to no cooling (Cheung & Robinson, 2004). Measures of work done and power output also showed no differences between no cooling and pre-cooling for repeated sprints in a hot and humid environment (Duffield *et al.*, 2003). As expected, both studies did show changes in skin and core temperature, with a decrease present following the cooling procedures. Further, it has been shown that T_m has a positive dose-dependent relationship with cycle sprint performance (T_m in a range of 36 to 41°C, Asmussen & Bøje, 1945) and lower leg muscular performance (T_m in a range of 29.5 to 33.5°C; Oksa *et al.*, 1996, 1997) and in both cases, the rate of deterioration in performance was linked to decreasing T_m (Racinais & Oksa, 2010). This relationship seems to be somewhat dependant on factors such as type of exercise, time on task and the degree to which body temperature decreases.

2.10: Effects of altitude on RS performance

Most sport competitions, including soccer, are performed at near sea level. However, because of varying geographic locations, individuals are often subjected to different environmental conditions, such as altitude (Bartsch *et al.*, 2008). Many international football games take place at moderate (2000-3000-m) or high altitude (>3000-5500 m; Brutsaert *et al.*, 2000) and the 2010 FIFA (Fédération Internationale de Football Association) World Cup was held in South Africa, games being held at various locations from Cape Town and Durban (at sea level, 0 m) to Johannesburg (at an altitude of 1753 m). It has been found that altitude exposures as low as 580 m have detrimental effects on exercise and sports performance (Hamlin *et al.*, 2008), but also result in short to medium-term physiological changes It is not the intention to touch upon this in too much detail and is beyond this review (for this see Bartsch & Saltin, 2008; Gore *et al.*, 2008; Hahn & Gore, 2001).

Success in field-based team sports, such as football, is highly dependent upon a number of characteristics, which includes RSA. Performance during RSA is limited mainly by ionic changes and the ability to match adenosine triphosphate resynthesis rate to utilisation rate (Bowtell *et al.*, 2013). Analysis of ATP regeneration following hypoxia exposure showed a significant deceleration of ATP resynthesis during three bouts of 6-min steady-state submaximal plantar flexion exercise (Haseler *et al.*, 2004). A reduction in muscle reoxygenation as result of acute hypoxic exposure is believed to potentially compromise PCr resynthesis and attenuate central motor drive to the working musculature (Billaut *et al.*, 2012). After 5 and 10 6-s cycle repeated sprints with 30-s recovery periods in-between, it was found PCr levels were 73 % and 84 % lower than resting levels, respectively, (Dawson *et al.*, 1998). Further, non-oxidative glycolysis decreases following RS performance and is believed to result in work capacity and performance deterioration (Gaitanos *et al.*, 1993). Recent research has been conducted looking to determine the effects of RS performance in 5 different conditions with different inspired oxygen fractions (F_IO_2: 12 %, 13 %, 14 %, 15 % and 21 %; Bowtell *et al.*, 2013). Participants completed a non-motorised repeated sprint protocol of 10 repeated sprints of 6-s in duration with 30-s passive recovery. It was found that physiological responses associated with RS performance were incrementally larger as F_IO_2 decreased to 13 %. Unexpectedly, non-motorised sprint performance was relatively resilient to moderate hypoxic exposure, with fatigue development only apparent at the lowest F_IO_2 as indicated by both an increased fatigue index and speed decrement (Bowtell *et al.*, 2013). Current research which has looked at the acute effects of hypoxia on RS performance has been examined in individuals in a non-fatigued state (Bowtell *et al.,* 2013; Sirotic & Coutts, 2008). It has been argued that models that examine repeated sprints in a non-fatigued state poorly reflect the demands of field-based team sports. Therefore, many researchers have investigated repeated sprints with participants in a fatigued state (Bangsbo, 1994). However, it is important RS performance models used to assess the effects of altitude on performance use a protocol which reflects the demands of field based team sports in a fatigued state, which can be achieved through laboratory tests which have now been established that accurately represent the work-rate patterns observed and match fatigue development during a game (Drust *et al.*, 2000).

2.11: Value of Measurement of RSA

2.11.1: Time-motion analysis of repeated sprints

Numerous studies have looked at time-motion analysis (movement patterns) within field-based team sports. Without time-motion analysis, it is problematic to quantify the physiological responses and the requirements of a sport in particular (Spencer *et al.,* 2005). The initial methodological techniques of time-motion analysis studies have developed since the techniques that were previously used. Current studies now utilise techniques which are based on computer-based tracking systems with a computer software programme then analysing all the time-motion data collected (Mohr *et al.,* 2003; Spencer *et al.,* 2004). This provides accurate real time time-motion data and helps reduce errors and the time required to analyse the data (Spencer *et al.,* 2005). The movement patterns of time-motion analysis studies in field based team sports can be categorized into five or more key classifications: sprinting, striding, jogging, walking, standing and potentially activities which are more skill-related, such as tackling, heading and catching. These can then be further broken down and classified into sub-categories (Mohr *et al.,* 2003).

The variety of team sports and the varying methodological differences make it difficult to evaluate time-motion analysis of team sports (Spencer *et al.,* 2005). In Australian football Rugby League it has been found that the mean duration of movements of high intensity (sprinting and striding combined) is ~3-s and occurs every 75-s (Dawson *et al.,* 2004). However, in rugby union this was found to be 2.2 to 2.9-s with a recovery period of 178 to 436-s, respectively. This was found to also be dependent upon position (Duthie *et al.,* 2005). This shows than when sprinting is combined as a movement of high intensity, it varies greatly upon findings. Studies which have looked at separate classification of sprinting have reported sprinting in soccer to cover a distance of 10 to 20 m over a duration of 2 to 3-s (Carling *et al.,* 2012; Di Salvo *et al.,* 2010). Field hockey reported similar values with sprint duration taking place every 1.8 to 3.1-s (Spencer *et al.,* 2004). It appears that although there are variations in the sprint distances and durations within team sports, the average has been found to be 10 to 20 m over the duration of 2 to 3-s (Spencer *et al.,* 2005).

Time-motion analysis data has shown that a 2 to 3-s sprint is performed approximately every 1 to 2-min during a game. It has been established that when short sprints are repeated with a 2-min recovery period in-between, that performance does not decrease following 15 repeated sprints (Spencer *et al.,* 2005). When recovery period is cut down to 90-s, a decrease in performance time was only evident following the 11th sprint (Balsom *et al.,* 1992). However, research investigating repeated sprint activity within team sports shows a paucity and although a paper investigating RSA in hockey has been conducted (Spencer *et al.,* 2004), looking at time–motion analysis of elite field hockey, with special reference to repeated-sprint activity. Other investigations must be conducted and replicate itto establish specific data for other field based team sports and RS performance. To enable the creation of specific RSA tests for specific field based team sports, this is of great importance.

2.12: Factors involved when testing RSA

The assessment of RSA tests on physiological, metabolic and performance parameters has greatly increased over recent years. With the further help of time-motion analysis our understanding of fitness components and requirements of field based team sports is further enhanced. A number of different exercise protocols have been used to investigate RSA (Spencer *et al.,* 2005). Differences between exercise protocols and the use of cycle ergometry as the mode of exercise not only call into question the validity of RSA protocols but also the sport-specific relevance of these variables to team sports (Table 2.2). Therefore, it is evident and important that a sport-specific RSA protocol is established to assess a number of important aspects (such as time-of-day effects, warm-up effects and cooling effects) on RSA in team sport performance.

2.12.1: Sprint duration

The majority of previous research have so far used a RSA protocol that consists of sprints which are 5 or 6-s in duration (Table 2.2). It has been suggested that this replicates and characterises the duration of repeated sprints carried out in team sport games (Spencer *et al.*, 2005). However, findings in regard to time-motion analysis data from field-based team sports suggest that the mean duration of sprints is no longer than 3-s during which the players cover ~15-m (Carling *et al.,* 2012; Di Salvo *et al.,* 2010; Duthie *et al.,* 2005; Mohr *et al.,* 2003; Spencer *et al.,* 2004), accounting to a total of 1-2 % of match-play. Therefore, a repeated sprint protocol using sprints of 3-s duration as opposed to 6-s, is believed to provide a more valid and reliable assessment of team sport RSA.

2.12.2: Number of sprint repetitions

Previous research has a considerable effect on changes in sprint performance in RSA. A number of different recovery duration times have been utilised within the literature (Table 2.2). It has been found that recovery durations of 30-s are short enough to detect changes in sprint performance, with durations any longer than this showing performance can be maintained. Due to the large amount of research using 30-s and this recovery duration deemed short enough to detect changes in RSA, it is believed this is the appropriate duration for a repeated sprint protocol.

2.12.3: Type of recovery

The majority of studies investigating RSA have tended to use passive recovery (Table 2.2). Although time-motion analysis data has shown recovery following bouts of repeated sprints within field-hockey is usually of an active nature, it appears submaximal active recovery causes a reduction in RS performance (Spencer *et al.,* 2004). Further, when collecting data on a non-motorised treadmill, if an individual starts a run having actively recovered as opposed to passively, average values will be higher over the course of the sprint.

2.12.4: Mode of exercise

Mode of exercise used to test RSA, plays a role on performance and studies in the literature have adopted many different modes (Table 2.2). It has been suggested that the mode of exercise adopted should be sport specific. Running is the most sport-specific exercise mode when it comes to team sport games (Fitszimons *et al.,* 1993; Spencer *et al.,* 2005). Therefore, with the development of the non-motorised treadmill, RSA can now easily be assessed and this comes with a number of additional benefits. Not only does the non-motorised treadmill enable research to be carried out within a standardised laboratory condition but also it allows rapid changes in running velocity at levels similar to running to be performed. It also provides greater accuracy for the measurement of mechanical power generated for each sprint, an aspect that is far more difficult to assess when running on a motorised treadmill or over ground (Lakomy, 1984). Further, although this approach has rarely been used for the assessment of RSA, in spite of the obvious advantages this mode of exercise offers in terms of sport-specificity and in terms of experimental control (Gaitanos *et al.*, 1993; Tong *et al.*, 2001; Hughes *et al.*, 2006) and is worth establishing whether results regarding non-motorised treadmill yield the same results as the studies so far performed on cycle ergometry.

2.12.5: Training status

Training status is another likely aspect influencing RS performance. It has been established that elite team-sport athletes achieve significantly greater peak power and total work during RSA on a cycle ergometer with a greater decline present in team sport athletes when compared to well-trained endurance athletes (Bishop & Spencer, 2004). However, on a non-motorised treadmill, which is more specific to field game team sports, there was no difference in decline in mean power output (Hamilton *et al.*, 1991). Therefore, it is important to take into account the changes in performance tests in the literature, potentially due to the training status of individuals (Spencer *et al.*, 2005).

Table 2.2: Summary of relevant laboratory based investigations of RSA protocols in the literature.

Study authors	Exercise mode	Sprint distance (m)	Sprint duration (sec)	No. of reps	Recovery duration (sec)	Recovery mode	Familiarisation sessions	No. of subjects	Gender
Gaitanos *et al*. (1991)	Non-motorised treadmill		6	10	30	Passive	yes, n/m	7	Males
Hamilton *et al.* (1991)	Non-motorised treadmill		6	10	30	Passive	yes, n/m	6	Males
Fitzsimons *et al.* (1993)	Cycle ergometer		6	6	24	Self selected	2	16	Males
Gaitanos *et al*. (1993)	Cycle ergometer		6	10	30	Passive	yes, n/m	8	Males
Signorile *et al.* (1993)	Cycle ergometer		6	8	30	Active or passive	n/m	6	Males
Balsom *et al.* (1994)	Cycle ergometer		6	10	30	n/m	5	7	Males
Dawson *et al.* (1997)	Cycle ergometer		6	5	24	Active	n/m	6	Males
Hautier *et al.* (1998)	Cycle ergometer		5	15	25	Passive	n/m	9	Males
Stathis *et al.* (1999)	Cycle ergometer		10	4	50	Passive	n/m	9	Males
	Cycle ergometer		10	4	80	Passive			
Bishop *et al.* (2001)	Cycle ergometer		6	5	24	Self selected	1	10	Males
Tong *et al*. (2001)	Non-motorised treadmill		6	3	120	Passive	n/m	27	Males
Lakomy *et al.* (2004)	Cycle ergometer	40	~ 6	6	30	Jog	n/m	18	Males
Racinais *et al.* (2005c)	Cycle ergometer		6	5	24	Passive	n/m	9	Males
Connes *et al.* (2006)	Cycle ergometer		6	5	24	Passive	n/m	14	Males
Giacomoni *et al.,* (2006)	Cycle ergometer		6	10	30	n/m	n/m	12	Males
Hughes *et al.* (2006)	Non-motorised treadmill		6	6	30	Passive	n/m	10	Males
Billaut, & Basset, (2007)	Cycle ergometer		6	10	30	Passive	1	13	Males
Racinais *et al.* (2007)	Cycle ergometer		6	10	30	Passive	1	9	Males
Brown *et al.* (2008)	Non-motorised treadmill		6	10	34	Passive	n/m	10	Males
Mendez-Villanueva *et al.* (2008)	Cycle ergometer		6	10	30	Passive	4	8	Males
Billaut, & Smith, (2010)	Cycle ergometer		5	20	25	Passive	1	15	Males
Racinais *et al.* (2010)	Cycle ergometer		6	10	30	n/m	1	8	Males
Smith, & Billaut, (2010)	Cycle ergometer		10	10	30	n/m	1	13	Males
Hammouda *et al.* (2011)	Cycle ergometer		6	5	30	n/m	1	12	Males
Carling *et al.* (2012)	Non-motorised treadmill		6	6	20	Passive	1	12	Males
Zarrouk *et al.* (2012)	Cycle ergometer		6	5	30	n/m	1	12	Males
Alaoui *et al.* (2013)	Cycle ergometer		6	5	24	n/m	1	12	Males
Bowtell *et al.* (2013)	Non-motorised treadmill		6	10	30	Passive	3	9	Males
Zois *et al.* (2013)	Non-motorised treadmill		4	3	14	Passive	2	8	Males

Chapter 3: A systematic review of repeated sprint ability (RSA) and time-of-day variation.

3.1 Introduction

As has previously been mentioned in section 2.7, a convincing body of research has shown that, in a temperate environment (around 17-20°C), many human performance variables display diurnal variation. The effects of time of day on short-term anaerobic performance (<6-s) are also well documented and display significant increases in the afternoon (Racinais *et al.,* 2004a; Souissi *et al.,* 2004). Studies investigating the effects of time of day on repeated-sprint performance, reported a time-of-day difference for peak power output, with higher values in the mid-afternoon or early evening when compared to the morning for either the first or first few sprints only (Racinais *et al.,* 2005c; Racinais *et al.,* 2010), or for all sprints performed (Souissi *et al.,* 2004; 2010), ranging from 3.1 to 7.6%.

The exact mechanisms for this observed diurnal variation in human performance are still, as yet, unknown but have been attributed to: 1) peripheral or muscle-related variables (contractibility, metabolism and morphology of muscle fibres and local muscle temperature), which can be influenced by hormonal and ionic muscle process variations (Araujo *et al.,* 2011; Bambaeichi *et al.,* 2005; Reilly & Waterhouse, 2009; Tamm *et al.,* 2009), and/or 2) central/neurological factors (central nervous system command; alertness; motivation and mood: Castaingts *et al.,* 2004; Giacomoni *et al.,* 2005; Racinais *et al.,* 2005; 2010). 3) Input from the body clock and proteins and peripheral clocks - that is, an endogenous component to the daily variation in muscle force production has been suggested to be important (Zhang *et al.,* 2009). Lastly, 4) the evening superiority in muscle force production and power output has been often attributed to a causal link between ‘resting’ core body and muscle temperatures and performance.

The first aim of this review is to investigate the evidence relating to diurnal variation in RSA performance and to determine the quality of evidence which reports a ‘peak’ time for RS performance. Secondly, the intention was to establish a set of suitable papers, compliant with the criteria used in this review and establish the effect size of the evidence which has observed a diurnal variation or circadian rhythm of RS performance.

3.2 Method

Study identification and selection

Electronic databases (Google Scholar, PubMed and SportsDiscus$^{®}$) were searched in using the following terms: Repeated sprint; Repeated sprint ability; RSA; Circadian; Male; Running; Cycling. The search was limited to papers published in English and research published within the past 4 decades (since 1974), in order to focus on more modern methods of measurement of RSA. In 1975, Woodway created the The Woodway non-motorised treadmill. The inclusion criteria were based on the Cochrane guidelines for conducting systematic reviews (Higgins & Green, 2009).

The titles and abstracts of the articles revealed by the initial search were then assessed for the following inclusion criteria:

1. Population – male (females were excluded so that menstrual implications did not need to be addressed), adult (18+ yrs of age).
2. Measurement – RSA or repeated sprint (ability)

Where both male and female participants took part in a research study, the article was included if the data from male participants could be independently identified. In instances where the title and abstract did not contain sufficient detail to indicate whether an article was relevant to the review, the complete article was obtained and read. This enabled it to be determined whether the paper met the two primary inclusion criteria. In instances where the primary purpose of the article was not an investigation looking at the effects of time-of-day, the papers were excluded from the review.

Quality control

The criteria for inclusion and exclusion was derived and agreed by all members of the supervisory team. Once an article had been identified and met the inclusion criteria of the review it was forwarded to the Director of Studies (Dr. Ben Edwards) who then independently decided whether the paper should be included or not. A system was in place which governed any areas of disagreement regarding inclusion and exclusion of articles according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines. In such instances the article in question would be forwarded to a third academic for a final decision.

Data Extraction

The studies which met the inclusion criteria were summarised under the following headings: randomised; counterbalanced; controlled for light; controlled for meals; controlled for sleep; fitness; cosinor analysis; study type (diurnal or circadian). In most instances, a simple ‘yes’ or ‘no’ was recorded against each of the included studies, other than ‘fitness’ (when the studies were classified as having ‘trained’ or ‘untrained’ participants). In addition, areas of specific interest were: 1) where cosinor analysis had been performed, what was the period of the rhythm?; 2) where cosinor analysis had been performed, what was the number of time points used to record data and determine acrophase?; 3) did the study control room temperature?

Data Analysis

The main aim of this review was to determine the effect size for each of the studies which reported evidence of a time-of-day variation in RS performance. Since the initial assessment of the studies in this review was concerned with those that were more typical of a repeated-measures design and did not have a control group, the effect size was calculated by subtraction of the lowest mean value from the highest recorded mean value (across the 24 hrs) in order to determine a mean gain value (Cohen, 1988). The standard difference of the means, $M_1 – M_2$, divided by the standard deviation(s) of the two groups has been defined as the effect size (Cohen, 1988). The standard deviation of either group could be used when the variances of the two groups are known to be homogenous (Cohen, 1988). In the instance of the present review, the subtraction, $M_1 – M_2$, was performed so that the difference was always positive (in the direction of improvement). As there was no access to the raw data from the studies considered, the mean of the two standard deviations was utilised to derive an effect size (Cohen, 1988).

3.3 Results

The initial response found a total of 19000 papers related to this area of research. After the primary screening (only work published in English over the past three decades) and taking into account all the search terms, this number was further reduced to 182 papers (See Table 3.1). Each of these was electronically retrieved and their titles and abstracts were then assessed against the following criteria:

1. Population – male (females were excluded so that menstrual implications did not need to be addressed), adult (18+ yrs of age).
2. Measurement – RSA or repeated sprint (ability).

This secondary screening process reduced the number of suitable articles to 41 (Table 3.1); these had been published in 15 peer reviewed journals (see Table 3.2). The papers were then analysed in order to determine whether they fully met the initial criteria for inclusion in the review. A third criterion was added: that RS performance or repeated sprint (ability) must have been performed in laboratory conditions, on a cycle ergometer or non-motorised treadmill.

Table 3.1: The number of research articles in each of the electronic databases (Google Scholar, PubMed and SportsDiscus®) related to the inclusion criteria following primary and secondary screening.

Search Engine		
Google Scholar	**PubMed**	**SportsDiscus®**
25	47	110
7	13	21

The next stage of the literature reviewing process revealed what can be termed as a ‘false positive’, whereby the same article has been identified twice; this occurred for two articles in Chronobiology International, one article in International Journal of Sports Physiology and Performance and two articles in Journal of Science and Medicine in Sport. This reduced the number of suitable articles with a potential for systematic review to 36.

Table 3.2: Journals and number of articles which met the three main inclusion criteria of the review.

Journal	Number of Reported Articles
Applied Physiology, Nutrition and Metabolism	1
Asian Journal of Sports Medicine	1
Australian Journal of Science and Medicine in sport	1
Chronobiology International	7
European Journal of Applied Physiology	2
European Journal of Sports Sciences	1
International Journal of Physical Education, Fitness and Sports	1
International Journal of Sports Medicine	6
International Journal of Sports Physiology and Performance	6
Journal International Society of Sports Nutrition	1
Journal of Science and Medicine in Sport	6
Journal of Sports Science and Medicine	1
Journal of Sports Sciences	2
Medicine & Science in Sports & Exercise	1
The Journal of Strength and Conditioning Research	4
Total	**41**

Further reviewing of the papers for suitability for inclusion revealed that the majority of the studies had not used repeated sprints as a direct means of measuring RS performance; instead they were actually studies which were investigating anaerobic capacity or Force development. Another common finding was that a number of studies had performed RSA measures were performed over-ground in the laboratory or in the field. Lastly, a number of studies did not report values for time of day. At this stage, there remained a potential of 6 articles for systematic review (see Table 3.3).

Table 3.3: Revised list of journals and corresponding number of articles which met with all three inclusion criteria for the systematic review.

Journal	Number of Reported Articles
Chronobiology International	3
International Journal of Sports Physiology and Performance	1
International Journal of Sports Medicine	2

Quality of work

The next stage of the analysis was to quantify the steps taken in the published literature to control for aspects relating to research design and factors deemed specifically important in investigations of chronobiological nature. The seven primary areas of interest were: randomization, counterbalancing, record of light intensity, control of meals, control of room temperature, control of sleep and fitness (see Table 3.4). Articles that made no specific reference to any of these primary areas were considered to indicate a negative response and 'no' was marked against the area in question.

Of the six studies, half of the studies had clearly stated the design of their research had been randomized, and four had clearly stated that their protocol had been counterbalanced. Only two studies were both randomized and counterbalanced (see Table 3.4).

Table 3.4: The list of authors and the main quality criteria for the systematic review.

Authors	Randomization	Counterbalancing	Record of light intensity	Control of meals	Control of room temperature	Control of sleep	Fitness
Racinais *et al.* (2005)	no	yes	no	no	yes	yes	trained
Giacomoni *et al.* (2006)	no	yes	no	no	no	yes	trained
Racinais *et al.* (2010)	yes	yes	no	no	no	yes	trained
Hammouda *et al.* (2011)	yes	yes	no	Yes	yes	yes	trained
Zarrouk *et al.* (2012)	yes	no	no	yes	yes	yes	trained
Aloui *et al.* (2013)	no	no	no	no	no	yes	trained

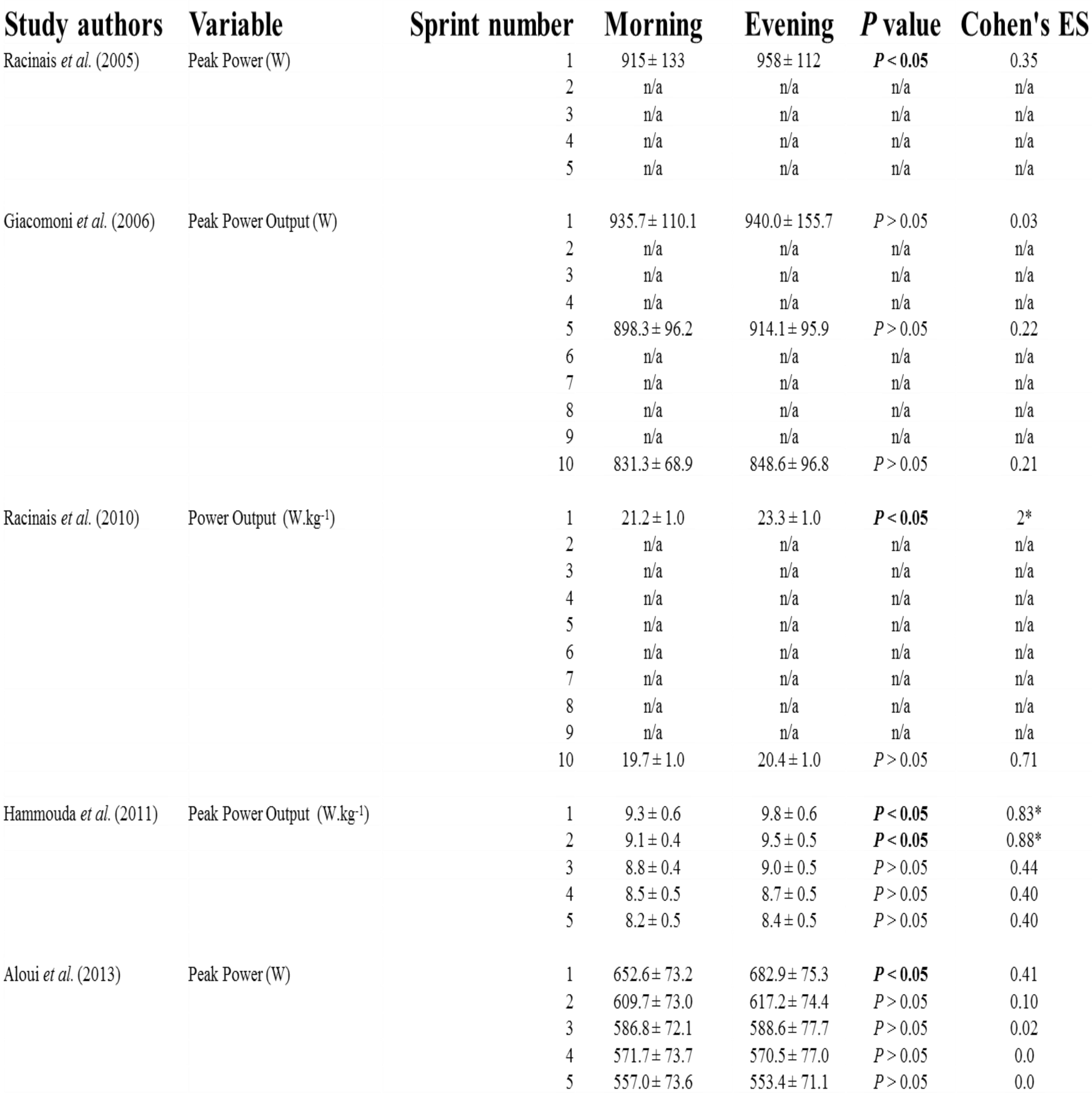
With regard to specific control over matters which are deemed particularly important to investigations of a chronobiological nature, no studies recorded information regarding light, only one had controlled meals (including a food diary) and three controlled for room temperature (all diurnal studies) as part of their research design. All studies controlled for sleep and stated that usual timing and quantities were deemed sufficient. All studies provided specific reference to the amount and type of training their participants underwent. Not one study adhered to the control of all five aforementioned primary chronobiological areas.

The studies were then further assessed to determine whether they were of a diurnal (measures taken within the waking hours of a normal day) or of a circadian (measured taken which cover a 20-28 h period at various time points) nature. It was established that all studies could be described as diurnal, all using two time points during waking hours of a normal day.

Effect size

One of the studies did not contain sufficient information to allow for effect size to be determined; Zarrouk *et al.* (2012) did not provide any mean or standard deviation values, and so was excluded from this stage of the analysis. The remaining five articles provided values for effect size calculations to be conducted. Due to RSA being, short -term performance (power output) over a series of sprints, effect size was determined for every sprint separately, where possible. This revealed that three sprints reported an effect size value classed as "large", eight as "medium" and a further five as "small", according to Cohen's Standard (1998) – see Table 3.5.

Table 3.5: Mean (± SD) values and individual effect sizes for each of the studies of the systematic review for peak power during each sprint, when possible.



Study authors	Variable	Sprint number	Morning	Evening	*P* value	Cohen's ES
Racinais *et al.* (2005)	Peak Power (W)	1	915 ± 133	958 ± 112	**$P < 0.05$**	0.35
		2	n/a	n/a	n/a	n/a
		3	n/a	n/a	n/a	n/a
		4	n/a	n/a	n/a	n/a
		5	n/a	n/a	n/a	n/a
Giacomoni *et al.* (2006)	Peak Power Output (W)	1	935.7 ± 110.1	940.0 ± 155.7	$P > 0.05$	0.03
		2	n/a	n/a	n/a	n/a
		3	n/a	n/a	n/a	n/a
		4	n/a	n/a	n/a	n/a
		5	898.3 ± 96.2	914.1 ± 95.9	$P > 0.05$	0.22
		6	n/a	n/a	n/a	n/a
		7	n/a	n/a	n/a	n/a
		8	n/a	n/a	n/a	n/a
		9	n/a	n/a	n/a	n/a
		10	831.3 ± 68.9	848.6 ± 96.8	$P > 0.05$	0.21
Racinais *et al.* (2010)	Power Output ($W.kg^{-1}$)	1	21.2 ± 1.0	23.3 ± 1.0	**$P < 0.05$**	2*
		2	n/a	n/a	n/a	n/a
		3	n/a	n/a	n/a	n/a
		4	n/a	n/a	n/a	n/a
		5	n/a	n/a	n/a	n/a
		6	n/a	n/a	n/a	n/a
		7	n/a	n/a	n/a	n/a
		8	n/a	n/a	n/a	n/a
		9	n/a	n/a	n/a	n/a
		10	19.7 ± 1.0	20.4 ± 1.0	$P > 0.05$	0.71
Hammouda *et al.* (2011)	Peak Power Output ($W.kg^{-1}$)	1	9.3 ± 0.6	9.8 ± 0.6	**$P < 0.05$**	0.83*
		2	9.1 ± 0.4	9.5 ± 0.5	**$P < 0.05$**	0.88*
		3	8.8 ± 0.4	9.0 ± 0.5	$P > 0.05$	0.44
		4	8.5 ± 0.5	8.7 ± 0.5	$P > 0.05$	0.40
		5	8.2 ± 0.5	8.4 ± 0.5	$P > 0.05$	0.40
Aloui *et al.* (2013)	Peak Power (W)	1	652.6 ± 73.2	682.9 ± 75.3	**$P < 0.05$**	0.41
		2	609.7 ± 73.0	617.2 ± 74.4	$P > 0.05$	0.10
		3	586.8 ± 72.1	588.6 ± 77.7	$P > 0.05$	0.02
		4	571.7 ± 73.7	570.5 ± 77.0	$P > 0.05$	0.0
		5	557.0 ± 73.6	553.4 ± 71.1	$P > 0.05$	0.0

3.4 Discussion

From the six studies that met the criterion for inclusion in this systematic review, it is apparent that the evidence regarding time-of-day variation and RS performance is limited. All studies showed apparent problems when analysed in closer detail. These problems were often due to lack of overall rigour regarding how data had been reported, how the studies were structured, and how the studies were controlled.

Quality

One of the six studies failed to report their data in a manner which allows for greater interpretation and analysis. The study failed to report mean values and standard deviations for peak power. Further to this, whilst three of the six studies represented clearly mentioned that their research design had been randomized, only two of these clearly identified that the protocol employed had been of a counterbalanced design. In all instances where either randomization or counterbalancing was not employed, no explanation or justification was given. This oversight regarding such fundamental aspects of research design is very surprising given that the internal and external validity as well as the enhancement of statistical power are usually the first concerns of any researcher conducting a study (Atkinson & Nevill, 2001).

Control

Of even greater concern is the apparent lack of control of factors which specifically relate to investigations in chronobiological nature. Not one of the studies reported consideration of light or dark exposure of participants. Research as far back as five decades has established the strength of the influence of light-dark cycle as a zeitgeber (DeCoursey, 1960; Lewy *et al.*, 1998; see Section 2.5 for more information); it is difficult to understand why such an important factor would be disregarded. The timing of food, another known zeitgeber (see

Section 2.5), was only reported in two studies. These reported measure of control over meals (either calorific intake or timing), a factor which has been stressed to play an important role in studies of chronobiology (Bougard *et al.*, 2009). These factors are not merely important as external influences, but also directly acting as zeitgebers.

The most surprising observation of this systematic review was that only half the studies reported room temperature. Given the strength of evidence regarding a potential 4 to 6% increase in muscle force development through passive warming of the musculature (Asmussen & Boje, 1945; Ball *et al.*, 1999) and with every 1°C increase in core temperature (Bergh & Ekblom, 1979), it has been suggested as a reason for increases in muscle temperature aligned to core temperature (Martin *et al.*, 1999). Although exact mechanisms for the observed diurnal variation in human performance are still, as yet, unknown, they have been attributed to a number of factors (Edwards *et al.*, 2013) of which the causal link of the temperature rhythm, which might be implicated directly or indirectly in producing an increase in force-generating capacity of the muscle (Bernard *et al.*, 1998; Coldwells *et al.*, 1994; Giacomoni *et al.*, 2005; Melhim, 1993) and neural function (reduced twitch time course or increase in speed of contraction, Martin *et al.*, 1999). Therefore, the decision not to report on all factors relating to aspects of temperature (core and environmental) would seem to be more of an oversight rather than a choice.

All of the studies in this systematic review did comment on measures of sleep such as; keeping standard sleeping habits with a minimum amount of sleep required, no staying up late and no experience of insomnia or sleep deprivation. Of these, only two studies actually quantified timings of retiring or rising, and two others quantified how much sleep/rest was allowed. With great extent of research investigating the effect of sleep (Winget *et al.*, 1985; Waterhouse *et al.*, 2001; Edwards & Waterhouse, 2009) and its negative impact on cognitive performance and central fatigue as a result of time-since-sleep, the extent of this lack of control comes as a major surprise when reviewing articles concerned with time-of-day variation. The known restorative influence of sleep, and fatigue associated with time-since-sleep, would suggest that whilst muscle force output might be parallel to the rhythm of core temperature (see above), cognitive performance and central arousal will decline as time-awake increases (Waterhouse *et al.,* 2001). Given the weight of influence of this factor, although all studies commented on measures of sleep, not one study provided information on both the timings of retiring or rising and how much sleep/rest was allowed. Whilst there is clearly a body of work which observes that many aspects of physical performance are parallel to core temperature, including RS performance, by choosing not to control or report on sleep it is impossible to determine whether sleep-loss had affected these aspects of RSA.

With regard to the previous exercise history of the participants used in the studies, all of the articles recruited people of a 'trained' classification. Choosing to recruit participants with such previous history of exercise should not present a problem with regards to interpretation of findings (Guette *et al,*. 2005; Häkkinen, 1989; Judge *et al.*, 2003). Although all articles recruited people of a 'trained' classification, no study reported their participants to be involved in cycling. Further, it has been suggested that the observation of a significant diurnal variation is somewhat dependent on a number of factors, one of these being subject familiarization regarding the task to be performed (Reilly *et al.,* 1997; Bambaeichi *et al.,* 2005; Giacomoni *et al.,* 2005). All of the studies either reported to have conducted an initial familiarization session or participants came to the laboratory one week prior to the experiment or nothing whatsoever. The lack of control and report on training status and familiarization could have resulted in the neuromuscular adaptations in untrained individuals on cycle ergometry which describes the increases in peak power experienced by them. Further, the interpretation of time-of-day changes in the observed peak power in a number of sprints could be argued as being simply due to acute neuromuscular adaptations associated with familiarisation and the initial learning of motor recruitment pathways as opposed to any endogenously driven rhythm; which is exactly why familiarisation and counterbalancing are such important aspects of the research design, as it is precisely because of familiarisation and counterbalancing that these effects are minimised or removed.

There is clearly a ‘live normal’ diurnal; bias of studies (i.e. the measures taken were typically within normal waking hours and not over six equally spaced time of day time points across the solar 24 h day with cosinor analysis undertaken) and limited to occurring no earlier than 05:00 h, and no later than 22:00 h), which might be argued as a step taken in order to reduce effects due to sleep loss, but is never specifically stated in this manner. Further, only two of the authors stated that they had aligned their time-of-day data collection points to known (based on previous research findings) lows and highs of both the rhythm of core temperature and/or peak power output during the RSA test.

Effect Size

Although there are limitations amidst the evidence provided by the studies included in this systematic review, still there remains evidence which suggests that there is a time-of-day variation of peak power (output) in RS performance which exhibits peak values during the late afternoon/early evening. However, the available statistical evidence suggests that the effect sizes are variable between studies.

Whilst one of the six studies did not present their data in a manner which allowed for effect size to be calculated, only two actually reported complete data to enable effect size to be calculated for all sprints. Effect size was large (greater than 0.8) for the first or first two sprints in two studies (see Table 5.5. Effect size values were greater than 0.3, classed as medium, in 62 % of peak power values. However, 38 % did display peak power to have an effect sizes below 0.3.. This interpretation of findings would therefore imply that there is a considerable inconsistency of the relationship between time-of-day and RS performance, and that for a proportion of the time these phenomena would hold true. The lack of control of factors which specifically relate to chronobiological investigations discussed above, might affect time-of-day and RS performance

3.5: Conclusion

There is evidence to support a late/early afternoon peak in peak power (output) in RS performance at time points which occur around the peak of the rhythm of core temperature. There is a clear demand for more rigorous investigations so that randomization, counterbalancing, record of light intensity, control of meals, control of room temperature, control of sleep and fitness are controlled for. Further, it is of great importance that participants which take part in diurnal studies are fully familiarised with the testing procedures and that the RS test used is sport specific.

Chapter 4: Investigating the reliability of two repeated sprint ability (RSA) protocols on a non-motorised treadmill: 10 x 3-s vs 10 x 6-s.

4.1: Introduction

Sprints of short duration (<6-s), interspersed with periods of brief recovery (<60-s), are a common occurrence during most field game team sports, such as football (Bishop *et al.*, 2011). The ability to reproduce the best possible short-term performance (power output and velocity) over a series of sprints with minimal recovery intervals between bouts is termed repeated sprint ability (RSA; Bishop *et al.*, 2011) and is regarded as important to the outcome of a game and a fundamental fitness component (Bishop *et al.*, 2011; Spencer *et al.*, 2005; Wragg *et al.*, 2000). Various field tests have been utilised to understand physiological mechanisms in RS performance (Fitzsimons *et al.*, 1993; Wadley & Le Rosignol, 1998; Wragg *et al.*, 2000), with similar formats often used. However, in order to control for environmental influences such as temperature and humidity that may affect the outcome of the protocol, laboratory-based experiments have been designed. A number of different exercise protocols have been used to investigate RS performance in males in the literature, varying in aspects such as sprint duration, number of sprint repetitions, mode of exercise, type of recovery type and duration of recovery (See section 2.12 and Table 2.2). The majority of protocols have predominantly consisted of a total of 10 sprints, 6-s in duration with 30-s of passive recovery performed on a cycle ergometer (See Table 2.2).

However, it has been outlined that cycling is not an exercise mode specific to any field game team sport (Hughes *et al.*, 2006). Since running is the most sport-specific exercise mode (Fitszimons *et al.*, 1993; Spencer *et al.*, 2005) and following the development of the non-motorised treadmill (Lakomy, 1984), studies have started to use RSA protocols using running on the non-motorised treadmill as the mode of exercise when assessing repeated sprint performance (Table 2.2) which is deemed to add ecological validity.

The non-motorised treadmill provides far greater accuracy for measuring mechanical power generated as it is fitted with force transducers connected to the running platform that can assess force, velocity, and power performance. The non-motorised treadmill allows for rapid increases in running velocity, similar to sprinting, which the motorised treadmill is unable to replicate (Lakomy, 1984)., it seems that the most sport-specific protocols to field based team sports in the literature are those consisting of a total of 10 sprints, 6-s in duration with 30-s of passive recovery performed on a non-motorised treadmill (Spencer *et al.,* 2004). Findings in regard to time-motion analysis data from field-based team sports suggest that the mean duration of sprints is no longer than 3-s, during which the players cover ~15-m (Carling *et al.,* 2012; Di Salvo *et al.,* 2010; Duthie *et al.,* 2005; Mohr *et al.,* 2003; Spencer *et al.,* 2004). This puts into question the majority of previous researches conducted, which have used 6-s (see Table 2.2).

Research investigating the reliability of the non-motorised treadmill has previously been investigated in repeated sprints (Hopker *et al.,* 2009; Hughes *et al.,* 2006) over a range of performance measures obtained from sprints of 6-s in duration (Hughes *et al.,* 2006) or 20-m in distance (Hopker *et al.,* 2009). Hughes *et al.* (2006) looked at determining the reliability of RS in ten male participants. Each individual attended the laboratory on three separate occasions and performed a RS test consisting of six 6-s sprints with 30-s recovery. Performance measures in RS were determined by maximal speed, average force production, and fatigue across the 6 sprints and were compared between all three trials. Maximal speed and average force were not significantly different between visits ($P < 0.05$) and reliability measures suggested a good agreement (coefficient of variation no more than 5%, low results for bias for the comparison of the ratio 95 LoA). Fatigue indices for maximal speed and for average force were less reliable (coefficients of variation ranging from 22.87 to 49.89%), with fatigue indices for average force also displaying significant differences between trials ($P < 0.05$). Hopker *et al.* (2009) looked at assessing the reliability of sprint performance on a non-motorised treadmill in a cohort of twenty-one male and seventeen female team sport players. Four trials consisting of a 20-m sprint were performed. With the exception of mean force, all parameters (such as peak power, peak force and mean power) showed a reduction in coefficient of variation (CV) across the trial comparisons. Measures of CV ranged from 7.9 to 10.1% between trials three and four. There were no significant differences between trials two and three for any of the performance measures ($P < 0.05$).Whether findings for a RSA protocol consisting of sprints 3-s in duration yield the same findings is unknown as yet.

Therefore, the aim of this study was to investigate the reliability for measures of performance and fatigue from 10 x (3-s or 6-s) with 30-s recovery RSA protocols using non-motorised treadmill ergometry. The second aim was to establish the number of familiarisation trials required until performance measures establish high levels of reliability.

4.2: Methods

Participants

Sixteen field based team sport male participants with age (mean ± SD) 21.1 ± 2.6 yrs, maximal oxygen uptake ($\dot{V}O_2$ max) 59.9 ± 5.1 mL.kg.min^{-1}, height 1.79 ± 0.06 m, body mass 74.4 ± 6.4 kg, respectively, were recruited for RSA protocol 1 (6-s) of this study. Thirty field-based team sport male participants with age (mean ± SD) 21.0 ± 2.2 yrs, maximal oxygen uptake ($\dot{V}O_2$ max) 60.0 ± 4.2 mL.kg.min^{-1}, height 1.79 ± 0.07 m, body mass 79.0 ± 12.3 kg, respectively, were recruited for RSA protocol format 2 (3-s) of this study. The protocol was fully explained to the participants and any questions were answered before their participation in the study. The study was approved by the local ethics committee of the

University. Inclusion in the study required that participants had previous field-based team sport experience, such as soccer, field hockey or rugby (≥ 2 yrs) and a $\dot{V}O_2$ max > 55 mL.kg.min^{-1}. Exclusion criteria included recent shift work or rapid travel across multiple time zones, and extreme chronotype or other personal attributes.

Protocol and measurements

All sessions took place under standard laboratory conditions (lighting, room temperature, humidity and barometric pressure were 200-250 lux, 21.3 ± 1.5°C, 37.8 ± 10.6 %, and 763.8 ± 10.4 mmHg, respectively). Participants were required to participate in five testing sessions and each attendance was separated by at least 3 days. The participants were free to live a ‘normal life’ between sessions, sleeping at home at night and attending lectures and doing light office work in the day. They were told to refrain from drinking alcoholic or caffeinated beverages and from other training or heavy exertion for the 48 h before the experiments or during them. Participants recorded the type, amount and timing of the food they ate for the period of 24 h before and taken during the day of the experimental session, and were asked to replicate this diet for the days before and during the other experimental sessions. The participants then undertook a standardized 5-min warm-up run at 10 km.h^{-1} on a motorised treadmill and then undertook either RSA protocol format 1 (6-s) or format 2 (3-s) of this study.

RSA protocol format 1 (10 x 6-s with 30-s recovery)

This RSA test involved participants to complete a total of 10, 6-s sprints with 30-s of passive recovery. All testing procedures were performed on a Woodway non-motorised treadmill (Woodway Force 3.0; Waukesha, WI, USA). For every session, the position of the subject on the non-motorised treadmill was standardized in accordance with the guidelines set by the manufacturers and also taking into account any adjustment required in accordance to the height of the participant (established during the first familiarization sessions). These guidelines were comparable to those already described elsewhere in the literature (Hughes *et al.,* 2006; Tong *et al.,* 2001) but, in brief, are as follows: participants wore a waist harness attached by a non-elastic tether to a mounted metal strain gauge on an adjustable sliding bracket. To ensure the tether was horizontal throughout the RSA test, the height of the metal strain gauge was adjusted in accordance to the height of the participant. Distance covered was continuously recorded by a tachometer mounted on the treadmill drum, from which continuous velocity time data was calculated. Propulsive force was then measured through the horizontally mounted force transducer (Celtron STC 500 load cell, California, U.S.A.), while four vertical force transducers (Interface SSB-AJ-1000 Sealed beam load cell, Arizona, U.S.A.) recorded vertical force applied to the treadmill bed. Prior to each test, the non-motorised treadmill was calibrated according to the manufacturer's instructions. During the test, treadmill velocity, power output and distance covered were sampled at a rate of 200 Hz to ensure accurate measures, leading to 1200 samples per variable over the 6-s sprint. Sprint data for peak power (PP), average power (AP), peak velocity (PV), average velocity (AV) and total distance covered (DC) was used for subsequent analysis and recorded with a commercially designed software program (Pacer Treadmill Software; Innervations, WA, Australia).

Another key performance outcome from RSA is the ability to resist fatigue and maintain a high performance level throughout the test. As there is no gold standard criterion for the measure of fatigue, we decided to calculate fatigue using the % decrement method as advised by Glaister *et al.* (2008) following their reliability and validity study. This method has been found to be the most suitable method as it considers data from each sprint and has been shown to provide consistent reliability, showing a good construct and logical validity (Glaister *et al.,* 2008) in tests of multiple sprint performance. Therefore, fatigue during each test was calculated using the following formula:

Fatigue = % decrement score (Fitzsimons *et al.,* 1993).

Calculation: Fatigue = (100 x (total Y ÷ ideal Y)) – 100

Where Y = peak velocity, or peak power; and total Y = sum of Y for all sprints, and ideal Y = the number of sprints x the peak Y

During all sessions, participants performed a task-specific warm-up procedure, developed from pilot work, consisting of three sprints at 50, 70 and 80 % of maximum effort, respectively. The task-specific bursts of activity were brief enough not to cause significant fatigue. Standardized strong verbal encouragement was given during each trial. Further, ratings of effort (on a 0 to 10 visual analogue scale; '0' meaning no effort and '10', maximal) were measured after each repeated sprint.

RSA protocol format 2 (10 x 3-s with 30-s recovery)

This RSA test involved participants to complete a total of 10, 3-s sprints with 30-s of passive recovery on a non-motorised treadmill (Woodway, Force 3.0; Waukesha, WI, USA). The method has already been reported previously (See above). During the test, treadmill velocity, power output and distance covered were sampled at a rate of 200 Hz to ensure accurate measures, leading to 600 samples per variable over the 3-s sprint. Sprint data for PP, AP, PV, AV, DC and percentage decrement in power and velocity were recorded with a commercially designed software program (Pacer Treadmill Software; Innervations, WA, Australia) and used in the subsequent analysis. Fatigue was calculated using the % decrement method as explained above. During all sessions, participants performed a task-specific warm-up procedure, developed from pilot work, consisting of three sprints at 50, 70 and 80 % of maximum effort, respectively. The task-specific bursts of activity were brief enough not to cause significant fatigue.

Statistical analysis

Assessment of systematic bias

The first step in reliability assessment is the calculation of the mean (±SD) values to help visualise any systematic bias trends. Data were analysed by means of the Statistical Package for Social Sciences (SPSS) for Windows (SPSS, Chicago, IL, USA); IBM version 20, using a General linear model with repeated measures (GLM; condition [5 levels]) for all RSA variables outlined above in protocols 1 and 2 and rated 'effort'. To correct violations of sphericity, the degrees of freedom were corrected in a normal way, using Huynh-Feldt ($\varepsilon > 0.75$) or Greenhouse-Geisser ($\varepsilon < 0.75$) values for ε, as appropriate (Field, 2000). Graphical comparisons between means and Bonferroni pairwise comparisons were made where main effects were present. The results are presented as the mean ± the standard deviation (SD) throughout the text unless otherwise stated. Following convention, the alpha level of significance was set at 5 %. This is the first step in assessing the reliability of measures of RSA in both protocols and determining the number of familiarization sessions required.

Assessment of random error

The main measure of reliability is the typical percent error: the standard error of measurement typically expressed as a CV (worked out from SD of the differences between trials/mean x 100) and was established to reflect within-subject reproducibility (Hopkins *et al.,* 2001; McGawley & Bishop, 2006). The CV quantifies the degree of absolute reliability or agreement. The CV is a measure equivalent to the standard deviation (SD) of an individual's repeated measurements, then expressed as an individual's mean test score. The CV allows for direct comparison of reliability and the smaller the CV, the better the reliability (Hopkins *et al.,* 2001).

4.3: Results

Protocol 1 (6-s)

Table 4.1 shows the group means (± SD) for all RSA variables recorded in each trial, together with the statistical analyses. There was condition effect for DC, PV, AV and AP with significantly higher values in trials 3 (6.0 to 12.5 %; $P < 0.05$), 4 (7.9 to 15.8 %; $P < 0.05$) and 5 (8.1 to 17.1 %; $P < 0.05$) compared to trial 1 (Table 4.1). Values for DC, AV and AP were also significantly higher in trials 4 (2.7 to 4.2 %; $P < 0.05$) and 5 (3.0 to 5.2 %; $P < 0.05$) compared to trial 2 (Table 4.1). There was no difference between any values for any other conditions ($P > 0.05$). Effort levels were 10 throughout all trials and for all sprints.

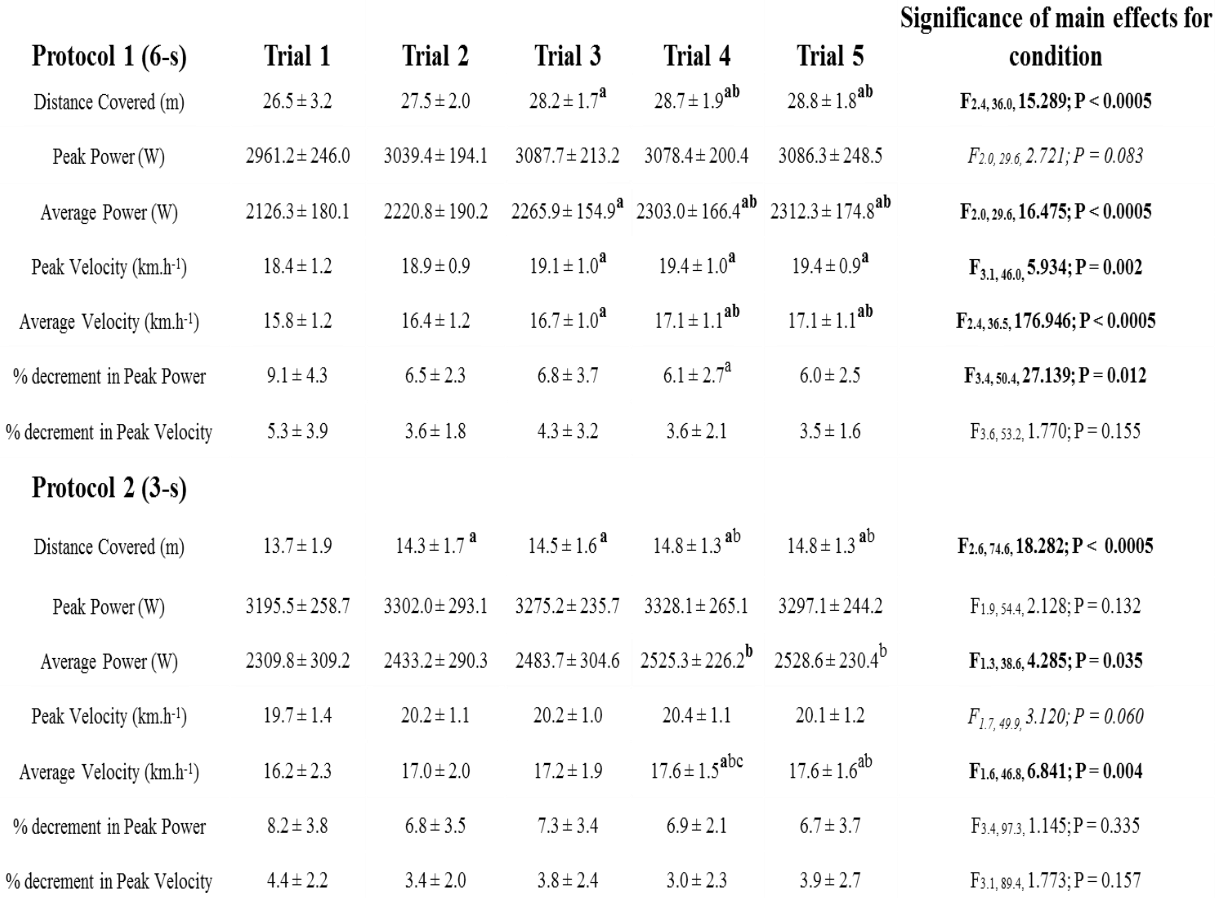
Table 4.2 shows the CV from trial to trial for all variables of RSA. The CV values for DC, PV, AV, PP and AP ranged from 6.3 to 8.2 % between first and second pairs of consecutive trials. For the second and third pairs and the third and fourth pairs of consecutive trials, all values fell below 5 % and the majority of variables, apart from AP showed a reduction of CV across trial comparisons. The CV values for % decrement PP and PV ranged between 32.1 to 87.9 %.

Protocol 2 (3-s)

Table 4.1 shows the group means (± SD) for all RSA variables recorded in each trial, together with the statistical analyses. There was condition effect for DC with significantly higher values in trials 2, 3, 4 and 5 (4.6 to 7.9 %; $P < 0.0005$) compared to trial 1 (Table 4.1). There was also a trend for DC in trial 1 to be lower than trials 4 and 5 ($0.10 > P > 0.05$). Values for AV and AP were significantly higher in trial 4 than trials 1 and 2, respectively (Table 4.1; $P < 0.05$). There was also a trend for a for AP to be higher in trial 5 compared to trial 1 and AV to be higher in trials 4 and 5 compared to trials 2 and 3, and trials 1 and 2, respectively ($0.10 > P > 0.05$). There was no difference between any values for any other conditions ($P > 0.05$). Effort levels were 10 throughout all trials and for all sprints.

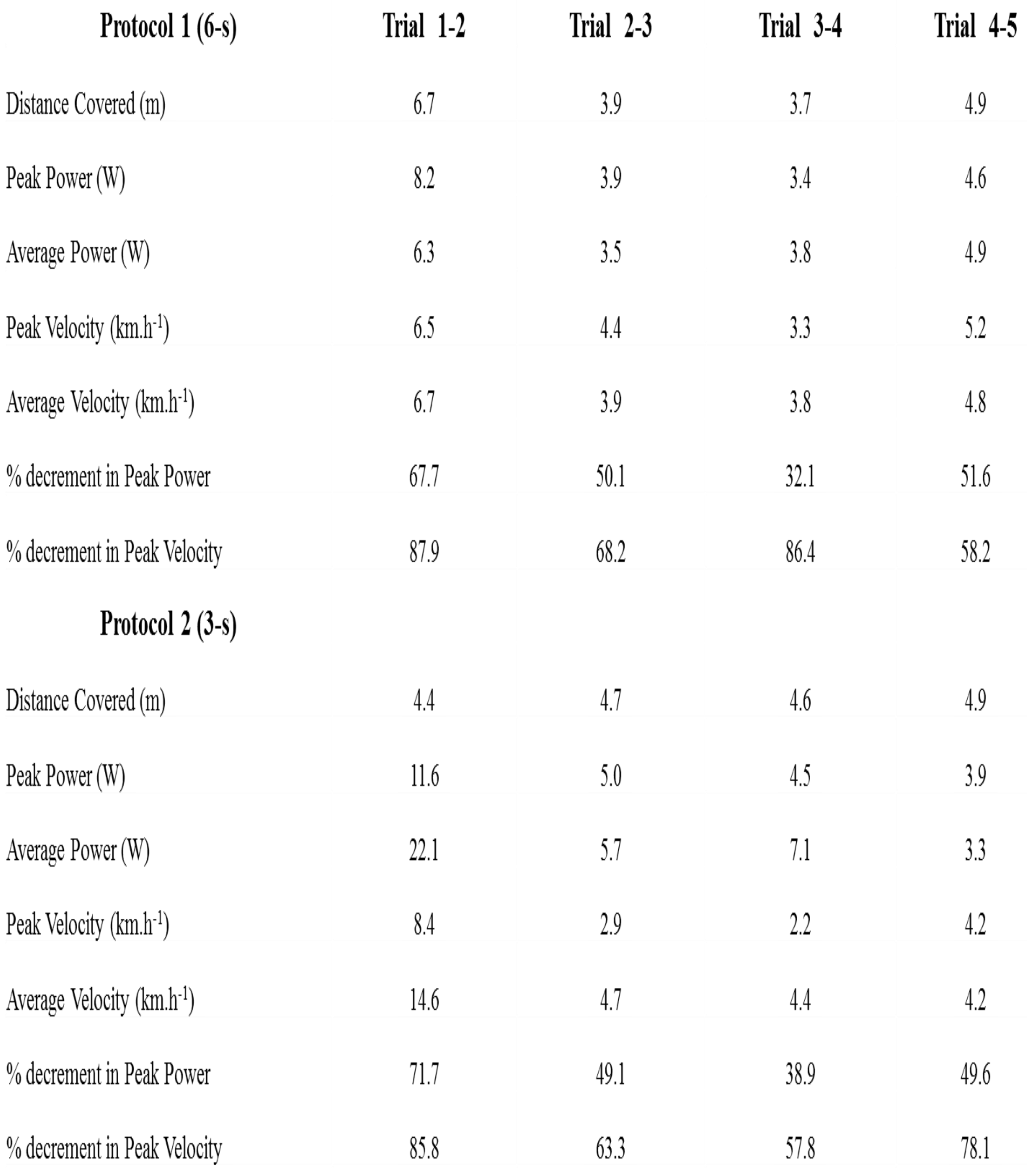
Table 4.2 shows the CV from trial to trial for all variables of RSA. The CV values for DC, PV, AV, PP and AP ranged from 4.4 to 22.1 % between first and second pairs of consecutive trials. For the second and third pairs and the third and fourth pairs of consecutive trials, all values fell below 5 % showed a reduction of the CV across trial comparisons, except for AP. All values for the fourth and fifth pairs of consecutive trials fell below a CV of 5 %. The CV values for % decrement PP and PV ranged between 38.9 to 85.8 %.

Table 4.1: Mean (± SD) for all 10 sprints of RS performance variables in the five trials for protocol 1 and protocol 2. Statistical significance ($P < 0.05$) is indicated in bold, and a trend (where $0.10 > P > 0.05$) is indicated in italics. a - Denotes a significant difference with trial 1 ($P < 0.05$); b –Denotes a significant difference with trial 2 ($P < 0.05$); c – Denotes a significant difference with trial 3 ($P < 0.05$).



Protocol 1 (6-s)	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Significance of main effects for condition**
Distance Covered (m)	26.5 ± 3.2	27.5 ± 2.0	28.2 ± 1.7^{a}	28.7 ± 1.9^{ab}	28.8 ± 1.8^{ab}	**$F_{2.4, 36.0}$, 15.289; P < 0.0005**
Peak Power (W)	2961.2 ± 246.0	3039.4 ± 194.1	3087.7 ± 213.2	3078.4 ± 200.4	3086.3 ± 248.5	*$F_{2.0, 29.6}$, 2.721; P = 0.083*
Average Power (W)	2126.3 ± 180.1	2220.8 ± 190.2	2265.9 ± 154.9^{a}	2303.0 ± 166.4^{ab}	2312.3 ± 174.8^{ab}	**$F_{2.0, 29.6}$, 16.475; P < 0.0005**
Peak Velocity ($km.h^{-1}$)	18.4 ± 1.2	18.9 ± 0.9	19.1 ± 1.0^{a}	19.4 ± 1.0^{a}	19.4 ± 0.9^{a}	**$F_{3.1, 46.0}$, 5.934; P = 0.002**
Average Velocity ($km.h^{-1}$)	15.8 ± 1.2	16.4 ± 1.2	16.7 ± 1.0^{a}	17.1 ± 1.1^{ab}	17.1 ± 1.1^{ab}	**$F_{2.4, 36.5}$, 176.946; P < 0.0005**
% decrement in Peak Power	9.1 ± 4.3	6.5 ± 2.3	6.8 ± 3.7	6.1 ± 2.7^{a}	6.0 ± 2.5	**$F_{3.4, 50.4}$, 27.139; P = 0.012**
% decrement in Peak Velocity	5.3 ± 3.9	3.6 ± 1.8	4.3 ± 3.2	3.6 ± 2.1	3.5 ± 1.6	$F_{3.6, 53.2}$, 1.770; P = 0.155
Protocol 2 (3-s)						
Distance Covered (m)	13.7 ± 1.9	14.3 ± 1.7^{a}	14.5 ± 1.6^{a}	14.8 ± 1.3^{ab}	14.8 ± 1.3^{ab}	**$F_{2.6, 74.6}$, 18.282; P < 0.0005**
Peak Power (W)	3195.5 ± 258.7	3302.0 ± 293.1	3275.2 ± 235.7	3328.1 ± 265.1	3297.1 ± 244.2	$F_{1.9, 54.4}$, 2.128; P = 0.132
Average Power (W)	2309.8 ± 309.2	2433.2 ± 290.3	2483.7 ± 304.6	2525.3 ± 226.2^{b}	2528.6 ± 230.4^{b}	**$F_{1.3, 38.6}$, 4.285; P = 0.035**
Peak Velocity ($km.h^{-1}$)	19.7 ± 1.4	20.2 ± 1.1	20.2 ± 1.0	20.4 ± 1.1	20.1 ± 1.2	*$F_{1.7, 49.9}$, 3.120; P = 0.060*
Average Velocity ($km.h^{-1}$)	16.2 ± 2.3	17.0 ± 2.0	17.2 ± 1.9	17.6 ± 1.5^{abc}	17.6 ± 1.6^{ab}	**$F_{1.6, 46.8}$, 6.841; P = 0.004**
% decrement in Peak Power	8.2 ± 3.8	6.8 ± 3.5	7.3 ± 3.4	6.9 ± 2.1	6.7 ± 3.7	$F_{3.4, 97.3}$, 1.145; P = 0.335
% decrement in Peak Velocity	4.4 ± 2.2	3.4 ± 2.0	3.8 ± 2.4	3.0 ± 2.3	3.9 ± 2.7	$F_{3.1, 89.4}$, 1.773; P = 0.157

Table 4.2: CV (%) for all parameters measured between consecutive trials for protocol 1 (6-s) and protocol 2 (3-s).



Protocol 1 (6-s)	**Trial 1-2**	**Trial 2-3**	**Trial 3-4**	**Trial 4-5**
Distance Covered (m)	6.7	3.9	3.7	4.9
Peak Power (W)	8.2	3.9	3.4	4.6
Average Power (W)	6.3	3.5	3.8	4.9
Peak Velocity (km.h^{-1})	6.5	4.4	3.3	5.2
Average Velocity (km.h^{-1})	6.7	3.9	3.8	4.8
% decrement in Peak Power	67.7	50.1	32.1	51.6
% decrement in Peak Velocity	87.9	68.2	86.4	58.2
Protocol 2 (3-s)				
Distance Covered (m)	4.4	4.7	4.6	4.9
Peak Power (W)	11.6	5.0	4.5	3.9
Average Power (W)	22.1	5.7	7.1	3.3
Peak Velocity (km.h^{-1})	8.4	2.9	2.2	4.2
Average Velocity (km.h^{-1})	14.6	4.7	4.4	4.2
% decrement in Peak Power	71.7	49.1	38.9	49.6
% decrement in Peak Velocity	85.8	63.3	57.8	78.1

4.4: Discussion

The main finding was that the non-motorised treadmill and both RSA protocols used are reliable tools for assessing DC, PV, AV, PP and AP during treadmill sprinting when the appropriate numbers of familiarisation sessions have been performed. The few existing articles which have looked at non-motorised treadmill RS performance showed similar values in measures of PP, PV and % decrement in PP and PV (Cheetham *et al.,* 1986; Hughes *et al.,* 2006; Tong *et al.,* 2001). However, only two articles in the literature have looked at the reliability of RSA in non-motorised treadmill ergometry (Hughes *et al.,* 2006; Hopker *et al.,* 2009). The study conducted by Hughes *et al.* (2006) consisted of 6 repetitions of sprints 6-s in duration with 30-s of passive recovery on three separate occasions. Hopker *et al.* (2009) performed 4 20-m sprints on four occasions. These protocols are not indicative of the majority of studies performed in the literature (see Table 2.2). Therefore, it was decided that this study would use most indicative of those utilised in the literature to ensure an answer regarding the amount of familiarisation sessions required for acceptable levels of reliability could be established.

The issue of reliability in non-motorised treadmill sprinting can be affected by numerous factors. In a test-retest situation, it is important that there is not too large a gap allowed between repeat trials (Batterham, & George, 2003). Intervals of weeks can lead to changes in variables under study and lead to changes which are not due to indications of poor reliability, but poor methodology. A second factor which questions reliability is the inconsistency of test circumstances between repeat trials. To minimize this inconsistency, all participants performed all 5 trials at the same time of day and under standard laboratory conditions. Further, other factors discussed within the inclusion and exclusion criteria were also closely accounted for to ensure reliability was not compromised (Batterham, & George, 2003).

A number of statistical tests have been proposed and utilised in the literature to assess reliability (Atkinson & Nevill, 1998). However, the first step in assessing reliability is to look at systematic bias. Systematic bias refers to a general trend for measurements to be different in a negative or positive direction between repeated tests (Atkinson & Nevill, 1998). Systematic bias can be established if a test displays worse scores in a retest trial potentially due to subject motivation. In this case, subjective ratings recorded after physical effort (on a simple 0-10 VAS scale, 0 being no effort and 10 being maximal effort) were 'maximum', irrespective of trial or protocol. Our ANOVA results demonstrate a significant main effect for condition in measures of total DC, AP, AV, PV and % decrement in PP for protocol 1. No significant differences were present for any of the variables between trials 1 and 2. When looking at data more closely, trials 3, 4 and 5 were significantly better than trial 1 for total DC, AP, AV and PV ($P < 0.05$). And although trials 2 and 3 did not display any significant differences, trials 4 and 5 were also significantly better than in trial 2 for DC, AP and AV ($P < 0.05$). Trials 3, 4 and 5 showed no significant difference for any of the RS performance variables. Protocol 2 also found significant main effects for conditions in measures of total DC, AP and AV. Total DC was significantly lower in trial 1 when compared to subsequent trials, while for AV this was only the case for trials 4 and 5 ($P < 0.05$). Trials 4 and 5 were also significantly better for DC, AP and AV when compared to trial 2 ($P < 0.05$). Both protocols displayed high levels of reliability from trial 3 onwards.

Having tested for and addressed systematic bias from a test to retest across 5 repeat trials in 2 separate protocols, the next step required is quantifying and reporting of random error or noise. This has previously been addressed by correlation in treadmill sprinting (Jaskolski *et al.,* 1996). Although this is usually the most adopted method, it has been deemed highly inappropriate in studies such as this one (Bland & Altman, 1995). Therefore, the CV method was adopted within this study to analyse absolute reliability. CV assumes that the largest test-retest variation occurs in individuals scoring the highest in the RSA test. The magnitude of the CV values in the present study are very similar to those obtained by Hughes *et al.* (2006) and Hopker *et al.* (2009) even though their studies varied slightly to the protocol utilised in the present study. In our study CV values between trials 1 and 2 were higher than subsequent trials. All other CV values were very similar for DC, AP, PP, AV and PV (ranging from 2.2% and 7.1%). Tong *et al.* (2001) established that CV values in non-motorised treadmill sprinting display higher values than other modes of exercise. Therefore, CV values found in this study were small enough to suggest that the present participants were fully familiarised with non-motorised treadmill sprinting.

Although measures of % decrement in PP and PV yielded reliable results in the present study with regard to systematic bias, this was not the case for random error (CV). In agreement with Hughes *et al.* (2005), the CV values of % decrement in PP and PV were poor. CV values ranged from 32.1% to 87.9% in protocol 1 and from 38.9% to 85.8% in protocol 2. It has been suggested that the poor reliability of % decrement in PP and PV is due to the fact that, unlike other recorded values analysed, the assessment of % decrement is derived from a combination of at least two measures (Hughes *et al.*, 2005). Whether indices of fatigue can be used as a reliable indicator of RS performance remains unclear.

4.5: Conclusion

In summary, measures of DC, AP, PP, AV, PV all display high levels of reliability in non-motorised treadmill sprinting in both protocols. For the assessment of fatigue, poor reliability was evident when the % decrement of peak power and peak velocity was adopted. To ensure individual performances are fully reliable, three trials are required to reduce variability in test scores and obtain consistent data.

Chapter 5: Effects of acute altitude exposure and laboratory-based simulated football protocol on repeated sprint performance.

5.1: Introduction

Football is the most popular field-based team sport in the world and is played in a multitude of nations at the professional level (Rampinini *et al.,* 2007). It is an invasive field game characterized by an intermittent activity profile that is multifaceted in its physiological, skill and cognitive demands (Bloomfield *et al.,* 2007; Drust *et al.,* 2000; Hoff & Helgerud, 2004). Many factors determine the outcome of a football match, such as the ability to repeatedly perform maximal sprints during a game without the onset of muscular fatigue (Bangsbo *et al.,* 1991; 2006). Repeated-sprint ability (RSA) is a crucial factor in determining the outcome of a football match and also a fundamental fitness component of team sports performance (Bishop *et al.,* 2011; Spencer *et al.,* 2005).

Recent assessment of time-motion analysis of football has shown that the most football-specific RSA protocols can be investigated using a total of 10 sprints, 3-s in duration with 30-s of passive recovery on the non-motorised treadmill (Pullinger *et al.,* 2013 and Chapter 4). This test has also been shown to be highly reliable if participants undertake the appropriate familiarisation sessions required (see Chapter 4). An important feature of a test of physical performance is that it is sensitive enough to detect changes as a result of factors that could modify performance, for example environmental stressors, fatigue, training status. Simulated altitude using hypoxic air (<21 %) or a decrease in barometric pressure (<760 mmHg) as a function of altitude can have a negative impact on physical performance (Levine *et al.*, 2008). Many international football games take place at moderate (2000-3000-m) or high altitude (>3000-5500 m; Brutsaert *et al.,* 2000) and the 2010 FIFA (Fédération Internationale de Football Association) World Cup was held in South Africa, games being held at various locations from Cape Town and Durban (at sea level, 0 m) to Johannesburg (at an altitude of 1753 m). Altitude exposures as low as 580 m have detrimental effects on sports performance (Hamlin *et al.,* 2008) and result in various short to medium-term physiological changes (see

Gore *et al.,* 2008 for more information). No explicit data on the physiological and performance effects of hypoxia on football performance exists within the literature, however. Recently, the effects of RS performance (10 sprints, 6-s in duration with 30-s of passive recovery on a non-motorised treadmill) in 5 different conditions with different inspired oxygen fractions (F_IO_2: 12, 13, 14, 15 and 21 %) has been investigated (Bowtell *et al.,* 2013). It was established that physiological responses associated with RS performance such as fatigue index and speed decrement were significantly larger at 13 % and 12 % compared to 21 % F_IO_2. Although the aforementioned RSA test utilised by Bowtell *et al.* (2013) has been shown to be a reliable measure of RS performance (see Chapter 4), recent assessment of time-motion analysis of football has shown that the most football-specific RSA protocols can be investigated using a total of 10 sprints, 3-s in duration with 30-s of passive recovery on the non-motorised treadmill (Pullinger *et al.,* 2013; see Chapter 4). It is unclear if the RSA protocol developed in Chapter 4 is sensitive to an environmental stressor such as altitude.

It has been argued that tests that examine repeated sprint performance in a non-fatigued state poorly reflect the demands of football. Therefore, many researchers have investigated repeated sprints with participants in a fatigued state (Bangsbo, 1994). It is very likely that physical fatigue would impair RSA performance but this notion has yet to be investigated. Should RSA performance, assessed using the protocol developed in Chapter 4, be impaired then this would further strengthen the sensitivity and validity of this protocol as an assessment of RSA performance. At the highest level, football players have been reported to cover distances in excess of 10 km during competitive matches. This activity typically consists of periods of walking and low-to-moderate intensity running interspersed with explosive bursts of activity including sprinting, jumping, changes in speed direction and tackling (Drust *et al.,* 2007; Glaister *et al.,* 2008). Laboratory tests have now been established which accurately represent the work-rate patterns observed and match fatigue development during a football game (Drust *et al.,* 2000), with the latter associated with the ability or inability to maintain sprint performance (Krustrup *et al.*, 2010). Such a protocol can be used to mimic soccer-specific exercise and thereby induce physical fatigue consistent with what is experienced during match play. Therefore, the aims of this study were to investigate whether acute altitude exposure and physical fatigue induced via a laboratory based soccer specific protocol were capable of changing RS performance.

5.2: Methods

Participants

Ten male university football players with age (mean ± SD) 20.6 ± 2.0 yrs, maximal oxygen uptake ($\dot{V}O_2\max$) 63.2 ± 3.9 mL.kg.min^{-1}, height 1.8 ± 0.6 m, body mass 75.5 ± 6.2 kg, percentage body fat 13.7 ± 4.3 %, habitual retiring and waking times 23:51 ± 00:25 h:min and 07:58 ± 00:50 h:min, respectively, were recruited for the study. All were physically active, taking part, on average, in three 11-a-side football matches and/or team training sessions per week. The study was approved by the local ethics committee of the University. Inclusion in the study required that participants had previous football experience (> 2 yrs) and a $\dot{V}O_2\max$ > 60 ml/kg/min. Exclusion criteria included recent shift work or rapid travel across multiple time zones, and extreme chronotype or other personal attributes (as assessed *via* the Composite Morningness, Sleep Flexibility/Rigidity [F/R] and Languid/Vigour [L/V] questionnaires; Smith *et al.*, 1989). Mean chronotype score was 33.7 ± 5.5, all intermediate types; F/R score, 44.7 ± 6.1; and L/V score 42.2 ± 4.6. Only participants who habitually trained during a normal week at all times of the day (morning, afternoon and evening) – and hence had no preference for time of training – were selected.

Experimental design

All sessions took place under standardised laboratory conditions (lighting, room temperature, humidity and barometric pressure were 200-250 lux, 20.0 ± 0.8°C, 42.8 ± 9.9 %, and 758.6 ± 7.9 mmHg, respectively). Before taking part in the main experiment, each participant completed four familiarization sessions for RS performance to ensure within-subject reproducibility and high levels of reliability (see Chapter 4). Each attendance was separated by at least 3 days. These sessions ensured that participants were fully familiarized with the RS performance test in the experimental conditions as required for the study. Each participant then completed four experimental sessions which took place 7 days apart. All participants underwent a normoxia trial (reflecting sea level) and two trials with hypoxia (F_IO_2 14.2 % or ~1500-m and F_IO_2 17.2 % or ~3000-m) in a hypoxic chamber creating a normobaric, hypoxic environment (TIS Services UK, Medstead, UK), which consisted of a 90-min football-specific intermittent treadmill fatiguing protocol followed by RS performance; and a further normoxia trial (reflecting sea level); RS performance in a non-fatigued state. The sessions were counterbalanced in order of administration to minimize any learning or order effects, and they were performed at the same time of day (Edwards *et al.,* 2013).

Protocol and measurements

The participants were free to live a ‘normal life’ between sessions, sleeping at home at night and attending lectures and doing light office work in the day. They were told to refrain from drinking alcoholic or caffeinated beverages and from other training or heavy exertion for the 48 h before the experiments or during them. Participants recorded the type, amount and timing of the food they ate for the period of 24 h before, and during the day of, the experimental session, and were asked to replicate this diet for the days before and during the other experimental conditions. For the sessions, participants arrived in a post-absorptive state having undertaken an overnight fast. The protocol is given in Figure 5.1. In the normoxia trial looking at RS performance in a non-fatigued state, participants simply performed a standardized 5-min warm-up run at 10 km.h^{-1} on a motorised treadmill (Pulsar, H/P cosmos, Nussdorf-Traunstein, Germany) and then underwent the RSA test which is explained in further detail below.

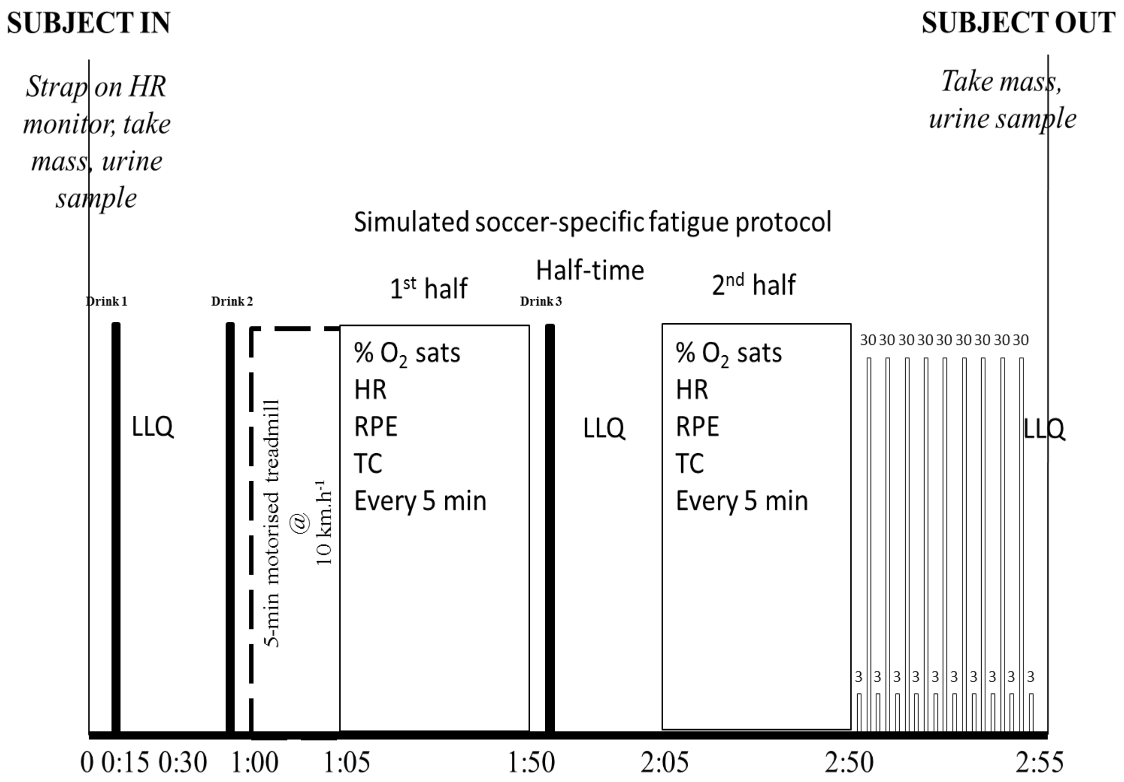


Figure 5.1: Schematic of the protocol for sea level, 1500-m and 3000-m conditions. % O2 sats, TC, HR and ratings of perceived exertion (RPE) taken throughout the warm-up and the simulated soccer-specific fatigue protocol. Rating of effort (0-10 VAS), TC, HR and ratings of perceived exertion (RPE) were taken throughout the repeated sprint ability (RSA) protocol; vertical black bars indicate fluid ingestion at these points. LLQ = Lake Louise Questionnaire.

For the other three conditions, participants arrived at the environmental chamber 1-h before the start of the test to provide a nude body mass measure (Seca 702, Seca GmbH & Co. KG, Hamburg, Germany) and a urine sample (Osmocheck pocket pal OSMO, Vitech Scientific Ltd, Japan). Participants were then required to sit down for 45-min and ingest a 600 ml drink consisting of sodium chloride (0.66 g Table Salt; ASDA, Leeds, UK), sugar-free cordial (140 ml; Vimpto-Nichols plc, Newton Le Willows, UK) and water (460 ml). After the rest period, participants ingested a further 600 ml drink and then undertook a standardized 5-min warm-up run at 10 km.h^{-1} on a motorised treadmill (Pulsar, H/P cosmos, Nussdorf-Traunstein, Germany). After the warm-up participants began the first of two 45-min periods of a football-specific intermittent treadmill fatiguing protocol designed to replicate the forward movement patterns/velocities of a 90-min football game (Drust *et al.*, 2000). During “half time” (15-min), participants ingested the final 600 ml drink. The second 45-min football-specific intermittent treadmill fatiguing protocol was then completed.

Participants then undertook a RSA test (10 sets of 3-s sprints followed by 30-s recovery) on a non-motorised treadmill (Woodway, Force 3.0; Waukesha, WI, USA) in the same environment. The method has already been reported previously (See Chapter 4). Sprint data for peak power (PP), average power (AP), peak velocity (PV), average velocity (AV), distance covered (DC) and percentage decrement in power and velocity (% decrement PP and PV) were recorded with a commercially designed software program (Pacer Treadmill Software; Innervations, WA, Australia) and used in the subsequent analysis. Upon completion of the RSA protocol, nude body mass was measured and urine sample was taken.

Throughout the testing procedures participants wore a heart rate monitor. Heart rate, thermal comfort (Bakkevig & Nielsen, 1994), ratings of perceived exertion (Birk & Birk, 1987) and % saturation of O_2 were measured during the warm-up and every 5-min during the football specific protocol. Heart rate, thermal comfort, ratings of perceived exertion and ratings of effort (on a 0 to 10 cm scale; '0' meaning no effort and '10', maximal) were measured after each repeated sprint. The Lake Louise acute mountain sickness (AMS) score questionnaire (Maggiorini *et al.*, 1998) was given to participants during the rest period, at the half-time interval and at the end of the RSA protocol.

Statistical analysis

The data were analysed by means of the Statistical Package for Social Sciences (SPSS) for Windows (SPSS, Chicago, IL, USA), IBM version 20, by General Linear Models (GLM) with repeated measures was used for the following analyses: fatigue *vs.* non-fatigue RS performance (condition [3 levels] x time [10 levels]); body mass and urine osmolality ([3 levels] x time [2 levels]); for Lake Louise Scores (condition [3 levels] x time [3 levels]); for RPE, TC, heart rate and % O_2 saturation during the warm-up (condition [3 levels] x time [5 levels]); for RPE, rated 'effort', TC, heart rate and performance variables during RS performance (condition [3 levels] x time [10 levels]); for RPE, TC, heart rate, % O_2 saturation during the football-specific protocol (condition [3 levels] x time [18 levels]). To correct violations of sphericity, the degrees of freedom were corrected in a normal way, using Huynh-Feldt ($\varepsilon > 0.75$) or Greenhouse-Geisser ($\varepsilon < 0.75$) values for ε, as appropriate (Field, 2000). Graphical comparisons between means and Bonferroni pairwise comparisons were made where main effects were present. The results are presented as the mean ± the standard deviation (SD) throughout the text unless otherwise stated. Ninety-five percent confidence intervals (CIs) are presented where appropriate. Following convention, the alpha level of significance was set at 5 %.

5.3: Results

Warm-up

Heart rate, rating of perceived exertion (RPE), thermal comfort (TC) and % O_2 saturation

Mean (± SD) values for heart rate during the warm-up were significantly lower during normoxia than both hypoxia trials ($P = 0.034$), while % O_2 saturation levels were significantly higher during normoxia ($P = 0.005$). There was no significant difference between any of the other values between the three conditions ($P > 0.05$). There was a significant time effect for heart rate, TC and RPE, with higher values as the warm-up continued ($P < 0.05$). Percentage of O_2 saturation did not display a significant time effect ($P > 0.05$). There was no significant interaction between any values and condition ($P > 0.05$), values increasing similarly during the warm-up irrespective of condition.

Football-specific intermittent treadmill fatiguing protocol

Hypoxia

Heart rate, rating of perceived exertion (RPE), thermal comfort (TC) and % O_2 saturation

Mean (± SD) values for heart rate, RPE and % O_2 saturation levels were all significantly different between conditions ($P < 0.05$). Pairwise comparisons showed the 3000-m condition to be higher for heart rate and RPE, and lower for % O_2 saturation levels ($P < 0.05$). Further, the 1500-m condition also showed higher heart rate and RPE, and lower % O_2 saturation levels than the normoxia condition ($P < 0.05$). There was no significant difference between conditions for any other values ($P > 0.05$). There was a significant interaction such that profiles of RPE increased more markedly in the 3000-m condition ($P < 0.05$) than the other conditions. There was no significant interaction between any of the other values and condition ($P > 0.05$), such that profiles changed in parallel over time for all conditions (See Figure 5.2).

1st vs 2nd half

Heart rate, rating of perceived exertion (RPE), thermal comfort (TC) and % O_2 saturation

There was a significant half effect for heart rate, RPE and TC with higher values in the 2nd half when compared to the 1st half during the football-specific intermittent treadmill fatiguing protocol ($P < 0.05$; See Figure 5.2). No significant half effect was observed for % O_2 saturation levels, with values the same in the 1st as the 2nd half ($P > 0.05$).

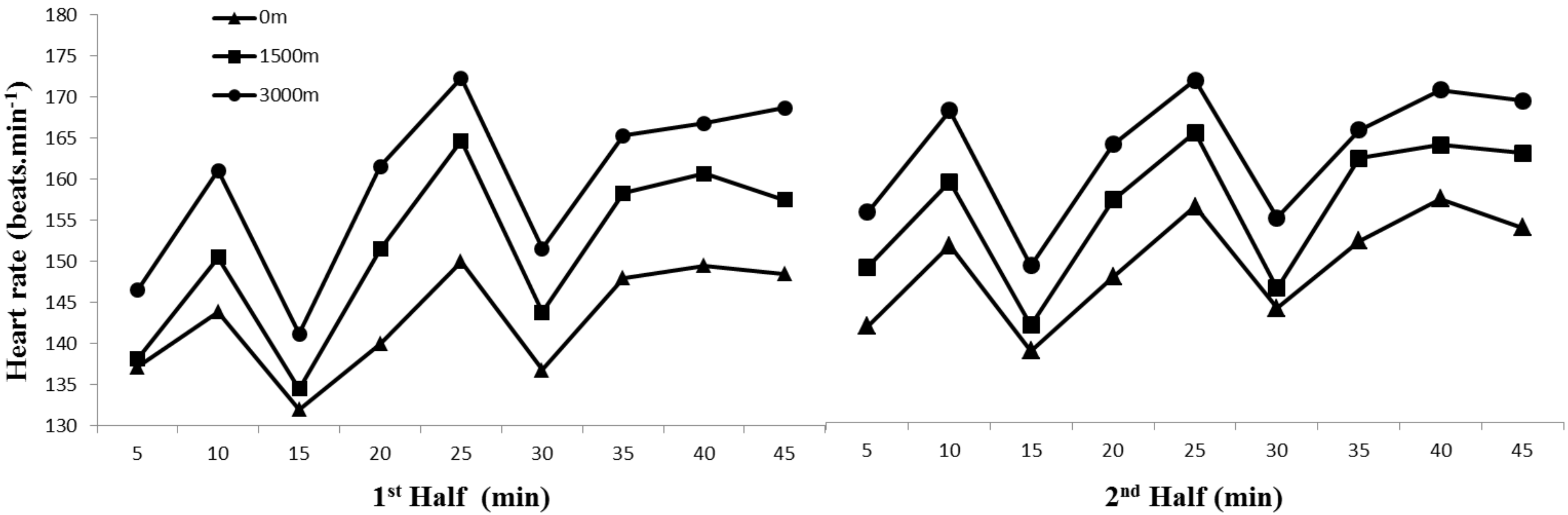


Figure 5.2: Heart rate profiles every 5-min for the 1st and the 2nd half of the football-specific intermittent treadmill fatiguing protocol for sea level (▲), 1500-m (■) and 3000-m (●).

Repeated Sprint Ability (RSA)

Fatigued vs non-fatigued

RS performance measures

Table 1 show the group means (± SD) for all RS performance variables recorded in both normoxia conditions with statistical analyses. Following a 90-min simulated football game DC, AP and AV were significantly reduced (4.6 to 5.8 %; $P < 0.05$; See Table 5.1 and Figure 5.3). There was no difference between any other RSA values ($P > 0.05$). There was a significant sprint effect for all measures of RSA where values generally dropped from sprint 1 to sprint 10 ($P < 0.05$, see Figure 5.3) There was no interaction for any of the measured RSA values between sprints and condition ($P > 0.05$), so that profiles for any of the measured RSA values dropped from sprint 1 to sprint 10 irrespective of condition ($P > 0.05$). In summary, the 90-min football-specific intermittent treadmill fatiguing protocol produced the differences in the main RS performance variables (DC, AP and AP) that were required to test the main research question.

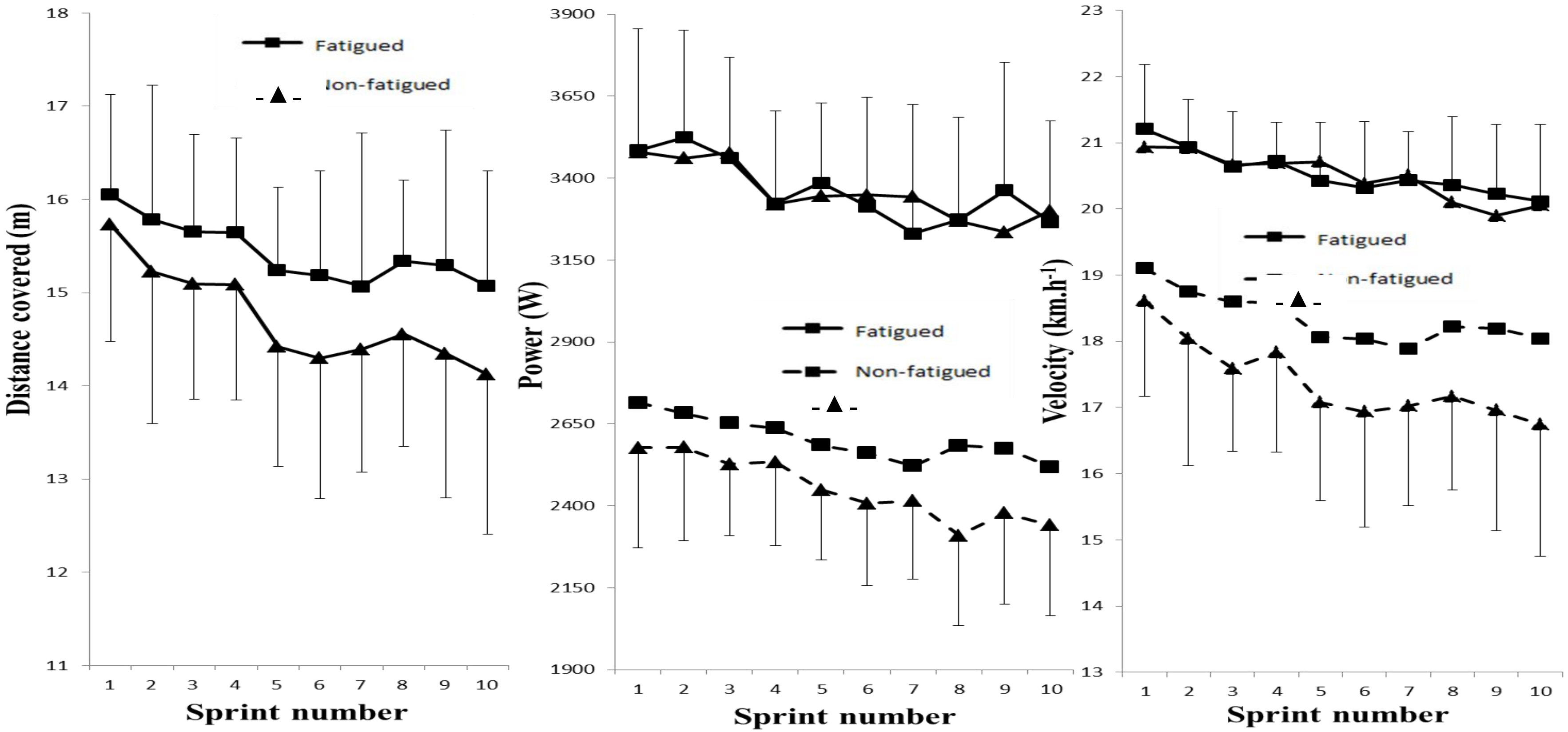


Figure 5.3: Mean and 95% confidence intervals (corrected for between-subject variability) for distance covered, power (peak and average) and velocity (peak and average) from sprint 1 to 10 for fatigued (▲) and non-fatigued (■) sea level conditions. Dashed lines indicate averages and full lines indicate peak variables.

Heart rate, rating of perceived exertion (RPE), thermal comfort (TC) and effort

Mean (±SD) values for heart rate and RPE were significantly lower during RSA in the non-fatigued state (P < 0.05; See Table 5.1). Values of TC were no different between conditions (P > 0.05). There was a significant sprint effect for heart rate, RPE and TC, where values generally increased from sprint 1 to sprint 10 ($P < 0.05$). There was an interaction between sprints and condition ($P > 0.05$), such that profiles for heart rate, TC and RPE increased more significantly in the non-fatigued condition. Effort levels were 10 throughout all conditions and for all sprints.

Hypoxia

RS performance measures

Table 5.2 shows the group means (± SD) for all RS performance variables recorded in each hypoxia condition with statistical analyses. There was an altitude effect for DC, PP, AP, PV and AV with higher values at normoxia than the 3000-m (6.2 to 12.8 %; $P < 0.05$) and 1500-m (3.1 to 6.5 %; $P < 0.05$) conditions (see Figure 5.4 and Table 5.2). There was a significant sprint effect for all measures of RSA ($P < 0.0005$, see Figure 5.4), where values generally dropped from sprint 1 to sprint 10. There was no interaction for any of the measured RSA values between sprints and condition ($P > 0.05$), so that profiles for any of the measured RSA values dropped linearly from sprint 1 to sprint 10 irrespective of condition ($P > 0.05$; see Figure 5.4). There was no difference between any values for any other conditions ($P > 0.05$).

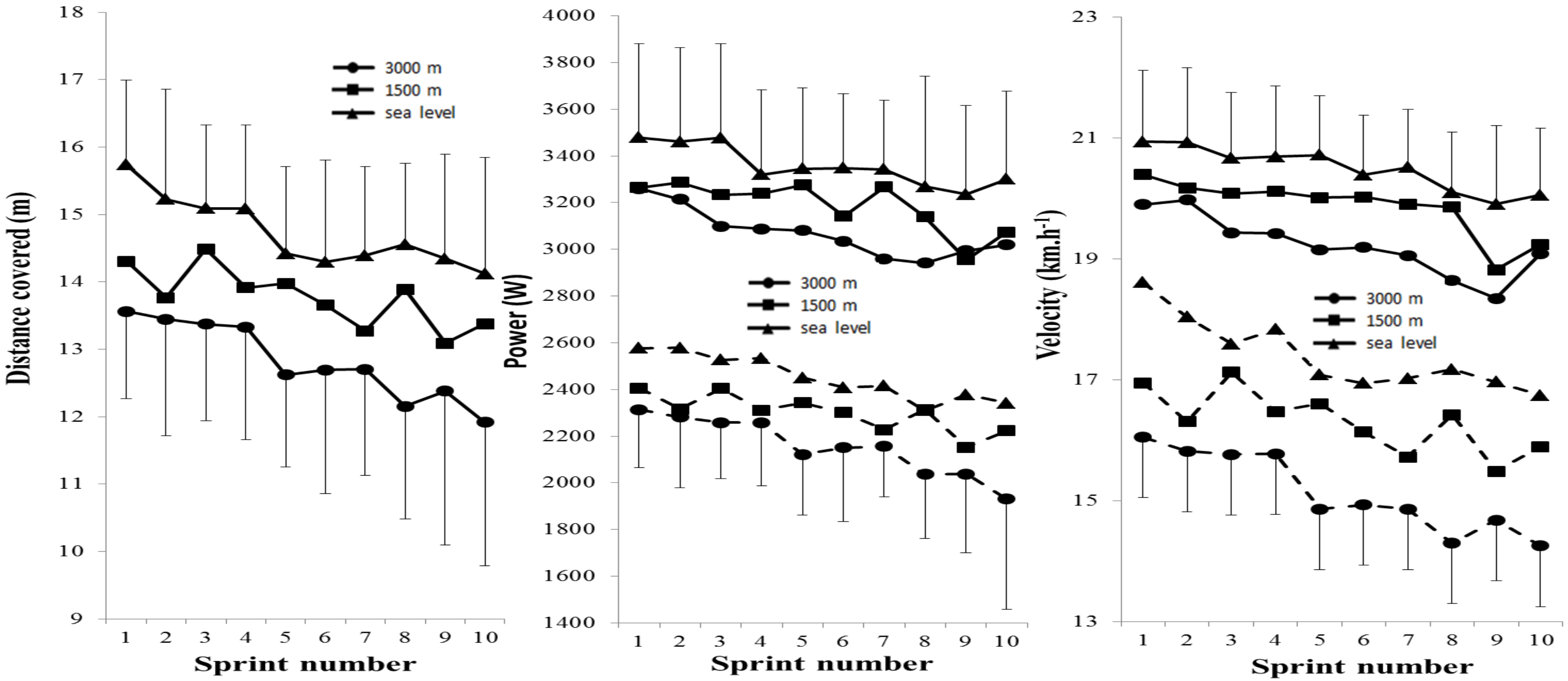


Figure 5.4: Mean and 95% confidence intervals (corrected for between-subject variability) for distance covered, power (peak and average) and velocity (peak and average) from sprint 1 to 10 for RS performance following the football-specific intermittent treadmill fatiguing protocol for sea level (▲), 1500-m (■) and 3000-m (●). Dashed lines indicate averages and full lines indicate peak variables.

Heart rate, rating of perceived exertion (RPE), thermal comfort (TC) and effort

Mean (±SD) values for heart rate were higher in the 3000-m condition than in the normoxia and 1500-m conditions ($P < 0.05$; see Table 5.2). Heart values also displayed a statistical trend for values to be higher in the 1500-m condition than in the normoxia condition ($P < 0.05$; $0.10 > P > 0.05$). There was a significant 'sprint' effect for TC, RPE and heart rate ($P < 0.0005$), where values generally increased from the first to the last sprint. There was no significant interaction between condition and sprint ($P > 0.05$) such that the profiles for RPE, TC and heart rate increased in parallel over sprint number for all three conditions. Effort levels were 10 throughout all conditions and for all sprints.

Lake Louise AMS score Questionnaire

Lake Louise AMS scores were significantly higher at the 3000-m condition than the normoxia (-0.9 %; $P = 0.011$) and the 1500-m (-0.8 %; $P = 0.006$) conditions. There was no difference between any values for any other conditions ($P > 0.05$). There was a significant 'time' effect for Lake Louise AMS scores with lowest values occurring at rest and increasing at half-time and after RS performance ($P = 0.003$). There was a significant interaction between condition and Lake Louise AMS scores ($P = 0.002$), such that the values increased more markedly after the 3000-m condition.

Table 5.1: Mean (± SD) values for RSA variables for all 10 sprints, and subjective measures in a fatigued and non-fatigued state. Statistical significance ($P < 0.05$) is indicated in bold, and a trend (where $0.10 > P > 0.05$) is indicated in italics. * Different than fatigued sea level condition.

Variable	Fatigued	Non-Fatigued	Significance of main effects for condition	Significance of main effects for time	Interaction
Repeated Sprint Ability (RSA)					
Distance Covered (m)	14.7 ± 1.1	15.4 ± 1.0*****	**$F_{1, 9,}$ 8.046, $P = 0.020$**	**$F_{2.9, 26.4,}$ 3.989, $P = 0.019$**	$F_{8.5, 76.1,}$ 0.431, $P = 0.796$
Peak Power (W)	3347.8 ± 350.9	3362.5 ± 261.6	$F_{1, 9,}$ 0.020, $P = 0.963$	**$F_{3.4, 30.2,}$ 5.058, $P = 0.005$**	$F_{2.6, 23.4,}$ 0.548, $P = 0.630$
Average Power (W)	2451.9 ± 213.6	2603.4 ± 164.1*****	**$F_{1, 9,}$ 14.056, $P = 0.005$**	**$F_{4.2, 78.8,}$ 5.409, $P = 0.003$**	$F_{3.4, 30.8,}$ 0.823, $P = 0.505$
Peak Velocity (km.h^{-1})	20.5 ± 1.0	20.7 ± 0.7	$F_{1, 9,}$ 0.233, $P = 0.641$	**$F_{2.9, 25.8,}$ 7.421, $P = 0.001$**	$F_{2.5, 22.3,}$ 1.002, $P = 0.580$
Average Velocity (km.h^{-1})	17.4 ± 1.3	18.4 ± 1.1*****	**$F_{1, 9,}$ 13.050, $P = 0.006$**	**$F_{3.0, 26.7,}$ 4.046, $P = 0.005$**	$F_{9, 81,}$ 0.416, $P = 0.923$
% decrement in Peak Power	6.9 ± 2.6	8.1 ± 3.7	$F_{1, 9,}$ 0.877, $P = 0.374$		
% decrement in Peak Velocity	3.4 ± 1.7	4.4 ± 3.5	$F_{1, 9,}$ 0.678, $P = 0.432$		
Subjective Measures					
Effort (0-10 cm VAS)	10 ± 0	10.0 ± 0	n/a	n/a	n/a
RPE (6-20)	15 ± 2	16 ± 2*****	**$F_{1, 9,}$ 7.682, $P = 0.022$**	**$F_{3.0, 21.5,}$ 126.391, $P < 0.0005$**	**$F_{2.2, 19.9,}$ 4.039, $P = 0.030$**
Thermal comfort (1-9)	8 ± 1	7 ± 1	$F_{1, 9,}$ 1.040, $P = 0.334$	**$F_{2.4, 21.9,}$ 28.765, $P < 0.0005$**	**$F_{4.1, 36.5,}$ 2.831, $P = 0.038$**
Heart rate (beats.min^{-1})	171 ± 8	167 ± 9*****	**$F_{1, 9,}$ 11.943, $P = 0.007$**	**$F_{2.3, 21.1,}$ 63.794, $P < 0.0005$**	$F_{2.7, 23.8,}$ 1.693, P = 0.199

Table 5.2: Mean (± SD) values for RSA variables for all 10 sprints, and subjective measures in at sea level, 1500-m and 3000-m altitude. Statistical significance ($P < 0.05$) is indicated in bold, and a trend (where $0.10 > P > 0.05$) is indicated in italics.

Variable	**Sea Level (0m)**	**17.2 % F_iO_2 (1500m)**	**14.2 % F_iO_2 (3000m)**	**Significance of main effects for condition**	**Significance of main effects for time**	**Interaction**
Repeated Sprint Ability (RSA)						
Distance Covered (m)	14.7 ± 1.1	13.8 ± 1.0	12.8 ± 1.5	**$F_{2, 18,}$ 29.080, $P < 0.0005$**	**$F_{8.0, 71.6,}$ 7.109, $P < 0.0005$**	$F_{4.5, 40.3,}$ 0.770, $P = 0.564$
Peak Power (W)	3347.8 ± 350.9	3187.8 ± 334.5	3069.50 ± 315.6	**$F_{2, 18,}$ 7.363, $P = 0.005$**	**$F_{3.6, 32.4,}$ 6.093, $P < 0.0005$**	$F_{3.5, 31.5,}$ 0.779, $P = 0.532$
Average Power (W)	2451.9 ± 213.6	2299.7 ± 202.9	2154.4 ± 250.3	**$F_{2, 18,}$ 35.778, $P < 0.0005$**	**$F_{8.0, 71.8,}$ 8.159, $P < 0.0005$**	$F_{4.6, 41.6,}$ 1.147, $P = 0.350$
Peak Velocity (km.h^{-1})	20.5 ± 1.0	19.9 ± 1.1	19.2 ± 1.0	**$F_{2, 18,}$ 18.850, $P < 0.0005$**	**$F_{3.4, 30.3,}$ 11.004, $P < 0.0005$**	$F_{3.6, 32.3,}$ 0.683, $P = 0.594$
Average Velocity (km.h^{-1})	17.4 ± 1.3	16.3 ± 1.1	15.2 ± 1.8	**$F_{2, 18,}$ 27.266, $P < 0.0005$**	**$F_{7.1, 64.1,}$ 6.790, $P < 0.0005$**	$F_{4.5, 40.1,}$ 0.896, $P = 0.484$
% decrement in PP	6.9 ± 2.6	7.1 ± 2.2	9.0 ± 2.6	$F_{1.9, 16.9,}$ 2.693, $P = 0.110$		
% decrement in PV	3.4 ± 1.7	4.0 ± 2.7	5.4 ± 2.8	$F_{2, 18,}$ 2.381, $P = 0.121$		
Subjective Measures						
Effort (0-10 cm VAS)	10 ± 0	10 ± 0	10 ± 0	n/a	n/a	n/a
RPE (6-20)	15 ± 2	18 ± 2	18 ± 2	**$F_{1.2, 11.2,}$ 8.503, $P = 0.011$**	**$F_{1.4, 12.9,}$ 35.456, $P < 0.0005$**	$F_{4.0, 35.7,}$ 1.003, $P = 0.418$
Thermal comfort (1-9)	8 ± 1	8 ± 1	8 ± 1	$F_{2.0, 17.6,}$ 1.970, $P = 0.170$	**$F_{1.9, 16.8,}$ 20.540, $P < 0.0005$**	$F_{4.9, 44.2,}$ 0.763, $P = 0.579$
Heart rate (beats.min^{-1})	171 ± 8	167 ± 12	169 ± 11	$F_{1.8, 16.2,}$ 1.429, $P = 0.267$	**$F_{2.5, 22.0,}$ 48.454, $P < 0.0005$**	$F_{4.5, 40.7,}$ 0.892, P = 0.487

5.4: Discussion

The first finding of this study was that RS performance was significantly reduced in a fatigued suggesting that the RS performance protocol is sensitive enough to detect changes as a result of fatigue. . RSA values for DC, AP and AV were all significantly worse when performed in a fatigued state (a range of 4.6 to 5.8 %). Accordingly, it was also established that mean (± SD) values for heart rate and RPE were significantly higher during RS performance in a fatigued state. Fatigue development can therefore be associated with the inability to produce the same levels sprint performance throughout a football game. The 90-min football-specific intermittent treadmill protocol accurately represents the work-rate patterns observed and associated match fatigue development during a football game (Drust *et al.,* 2000). It has shown the physiological strain associated with it to be more accurately relevant to football performance than other laboratory performance tests such as the LIST (Drust *et al.,* 2005). There are limitations to the activity profiles observed due to the lower frequency of activity changes and the omission of utility movements, and lack of game skills however (Drust *et al.,* 2005). Heart rate was lower across the 1st half compared to the 2nd half indicating that the football-specific intermittent treadmill protocol utilised does have a detrimental fatiguing effect upon the individual. Progressive fatigue that occurs during soccer has typically been ascribed to the depletion of muscle glycogen, hyperthermia and the progressive loss of bodily fluids (Mohr *et al.,* 2003). These aspects could have played a role behind the significant decrease in RSA.

The second finding of this study was that physiological and RS performance responses are affected by acute altitude exposure. This shows that the newly created RS protocol is a sensitive enough protocol to detect changes in performance under environmental stressors. As expected, % O_2 saturation levels were significantly different between the 3 conditions. It has been previously found that % O_2 saturation levels previously show significant decreases from altitudes as low as 700 m (Goldberg *et al.*, 2012). Heart rate was increased with increasing altitude, which is a common physiologic effect as a result of altitude exposure (Gore *et al.*, 2008) and is as expected due to the increased demand for O_2 delivery to the active skeletal musculature (Casey & Joyner, 2012). RS performance showed all variables to be significantly higher at sea level than at altitude levels of 1500-m (a range of 3.1 to 6.5 %; See Table 5.2 and Figure 5.4) and 3000-m (a range of 6.2 to 12.8 %; See Table 5.2 and Figure 5.4), respectively. RSA variables were also significantly different between both levels of altitude, with values higher at 1500-m that at 3000-m (3.2 to 7.0 %; See Table 5.2 and Figure 5.4). The inability to match sea level performance during RSA at 2 different levels of hypoxia shows that this RS performance protocol is sensitive to environmental stressors and this altitude-induced impairment is believed to be due to a number of mechanisms, . The attenuation of central motor drive to the active musculature, shown to be potentially due to reductions in % O_2 saturation levels and in O_2 availability at higher altitude levels play a significant role in RS performance reduction at altitude (Billaut & Smith, 2010; Szubski *et al.*, 2006). RPE was significantly higher at 3000-m during both the football-specific intermittent treadmill protocol and the RSA protocol, thus suggesting individuals to perceive both performances to be more difficult at higher levels of altitude. The higher the level of altitude, the higher the reduction in O_2 content resulting in a larger O_2 deficit with each repeated sprint and most likely reflects upon a greater reliance upon non-oxidative adenosine triphosphate synthesis from phosphocreatine stores and a quicker accumulation and a slower clearance rate of inorganic phosphate (Hogan *et al.,* 1999). Motivation cannot be suggested to be a central mechanism that plays a significant role on the decrement of RS performance at altitude as ratings of effort were ‘maximum’, irrespective of condition.

5.5: Conclusion

The newly created RS performance protocol is sensitive enough to detect a negative change following altitude acute exposure and a 90-min football-specific intermittent treadmill fatiguing protocol. The physiological responses associated with RS performance following a football-specific intermittent treadmill fatiguing protocol were incrementally worse at higher altitude in this population of motivated individuals.

Chapter 6: Is there a diurnal variation in repeated sprint ability on a non-motorised treadmill?

Please note this study has been published: Pullinger SA, Brocklehurst EL, Iveson RP, Burniston JG, Doran, D, Waterhouse JM, Edwards BJ. (2013). Is there a diurnal variation in repeated sprint ability on a non-motorised treadmill? *Chronobiol. Int.* Early Online: 1–12. DOI: 10.3109/07420528.2013.865643.

6.1: Introduction

In a temperate environment (around 17-20°C), many human performance variables display diurnal variation. Human performance rhythms consistently peak in the mid-afternoon or early evening regardless of the muscle group measured (hand, elbow, leg or back, for example) or speed of contraction (see Section 2.7). Although short-term anaerobic performance (< 6-s) with minimal recovery intervals (< 60-s) between bouts has also shown time of day effects (Bishop *et al.*, 2011), few studies have investigated the effects of time of day on RSA (see Section 2.8).

A number of studies have consistently reported a time-of-day difference for peak power output (Racinais *et al.*, 2005c; Racinais *et al.*, 2010; Souissi *et al.*, 2004, 2010) and % decrement in power (Chtourou *et al.*, 2012; Racinais *et al.*, 2005c; Zarrouk *et al.*, 2012), with differences of 3.1 to 7.6 % and 4.0 to 13.1 % (see Section 2.8). All aforementioned studies found evidence of daily variation in RSA on a cycle ergometer, but differences in a number of aspects such as sprint duration and type of recovery make it difficult to compare findings (see Section 2.12).

Further, large differences between exercise protocols and the use of cycle ergometry as the mode of exercise not only call into question the validity of RSA protocols but also the sport-specific relevance of these variables to team sports. A RSA protocol using a non-motorised treadmill as the mode of exercise with 10 sprints 3-s in duration and 30-s of passive recovery in-between sprints has been deemed as a reliable protocol (see Chapter 4). Therefore, it is evident and important that this sport-specific RSA protocol is further assessed to look at daily variation on RSA in team sport performance, such as football, in a population of well-trained, familiarised individuals and whether this yields the same results as the studies so far performed on cycle ergometry.

Therefore, the aim of this study is to investigate whether a daily variation in RSA exists in a large sample of motivated participants on a non-motorised treadmill.

6.2: Methods

Participants

Twenty well-trained field based team sport male participants with age (mean ± SD) 21.0 ± 2.2 yrs, maximal oxygen uptake ($\dot{V}O_2$ max) 60.8 ± 4.8 mL.kg.min^{-1} , height 1.79 ± 0.07 m, body mass 77.2 ± 10.5 kg and habitual retiring and waking times 23:43 ± 0:43 h:min and 07:47 ± 0:44 h:min, respectively, were recruited for this study. The protocol was fully explained to the participants and any questions were answered before their participation in the study. Inclusion in the study required that participants had previous field-based team sport experience, such as soccer, field hockey or rugby (≥ 2 yrs) and a $\dot{V}O_2$ max > 55 ml/kg/min. Exclusion criteria included recent shift work or rapid travel across multiple time zones, and extreme chronotype or other personal attributes (as assessed *via* the Composite Morningness, Sleep Flexibility/Rigidity [F/R] and Languid/Vigour [L/V] questionnaires; Smith *et al.*, 1989). Mean chronotype score was 33.8 ± 5.4, all intermediate types; F/R score, 44.4 ± 4.2; and L/V score 39.7 ± 6.3. Only participants who habitually trained during a normal week at all times of the day (morning, afternoon and evening) – and hence had no preference for time of training – were selected.

Protocol and measurements

All sessions took place under standard laboratory conditions (lighting, room temperature, humidity and barometric pressure were 200-250 lux, 21.1 ± 1.0°C, 34.7 ± 4.4 %, and 757.8 ± 7.7 mmHg, respectively). Before taking part in the main experiment, each participant completed three familiarization sessions and each attendance was separated by at least 3 days. These sessions ensured that participants were fully familiarized with the RSA protocol utilised in this study. Following the familiarization process, each participant completed two experimental sessions which took place three days apart; a morning and an evening trial (07:30 and 17:30 h; M and E) after a standardized 5 minute warm-up at 10 km.h^{-1} on a motorised treadmill (Pulsar, H/P cosmos, Nussdorf-Traunstein, Germany). The sessions were counterbalanced in order of administration to minimize any learning effects (Edwards *et al.*, 2013).

The participants were free to live a 'normal life' between sessions, sleeping at home at night and attending lectures and doing light office work in the day. They were told to refrain from drinking alcoholic or caffeinated beverages and from other training or heavy exertion for the 48 h before the experiments or during them. Participants recorded the type, amount and timing of the food they ate for the period of 24 h before and taken during the day of the experimental session, and were asked to replicate this diet for the days before and during the other experimental conditions. For the morning sessions, participants arrived in a fasted state and were asked not to eat in the 4 h prior to the evening session. The protocol is given in Figure 6.1. Participants arrived 1h before the start of the test, inserted a soft flexible rectal probe (Mini-thermistor; Grant instruments Ltd, Shepreth, UK) ~10-cm beyond the external anal sphincter, strapped on a heart rate monitor **(**Polar FT1; Polar Electro Oy, Kempele, Finland**)** and then lay down and relaxed in the laboratory. Rectal temperature (T_{rec}) was then recorded continuously over 30-min by means of a Squirrel 1000 data logger (Grant Instruments Ltd, Shepreth, UK) while participants remained semi-supine but awake. The average value of the last 5 min of recording was defined as resting T_{rec} and used for subsequent analysis. The standard body position (lying down) was used since core body temperature is subject to other influences than that produced by the endogenous 24 h period oscillator, such as muscle activity, feeding and sleep (Edwards *et al.*, 2002b). At this time, 'resting' muscle temperature (T_m) was assessed using a needle thermistor inserted into the right leg vastus lateralis (13050, ELLAB, Hilleroed, Denmark). The area was marked with a permanent marker so as to minimize site variation between testing sessions. Thigh skinfold thickness was measured using Harpenden skinfold callipers (HSK BI, Baty International, West Sussex, UK) and divided by two to determine the thickness of the thigh subcutaneous fat layer over the participant's vastus lateralis (Enwemeka *et al.,* 2002). The needle thermistor was then inserted at a depth of 3-cm plus one-half the skinfold measurement for determination of deep T_m. Muscle temperature was recorded using an ELLAB electronic measuring system (CTF 9004, ELLAB, Hilleroed, Denmark).

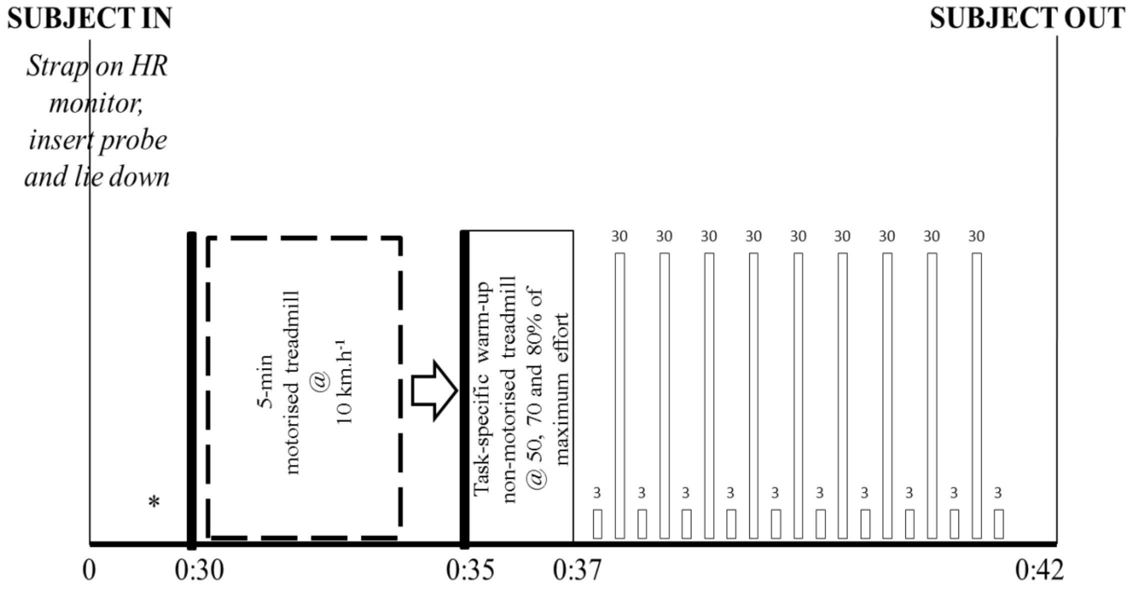


Figure 6.1: Schematic of the protocol the morning (M, 07:30 h) and evening (E, 18:00 h conditions. Rectal (T_{rec}) and muscle (T_m) temperatures, thermal comfort (TC) and profile onset of mood state (POMS) were measured after the participants had reclined for 30 min at the start of the protocol and again after the warm-ups and prior to the RSA measures. * denotes start of 5-min continuous T_{rec} baseline measurement; the average value of these 5 min of recording was defined as resting T_{rec} and used in the subsequent analysis. Rating of effort (0-10 VAS), TC, HR and ratings of perceived exertion (RPE) were taken throughout the repeated sprint ability (RSA) protocol; vertical black bars indicate muscle temperature (T_m) taken at these points.

Following the completion of resting temperature measurements participants completed a Profile of Mood States (POMS) questionnaire (McNair & Lorr, 1971). The participants then undertook a standardized 5-min warm-up run at 10 km.h^{-1} on a motorised treadmill. A second T_m measure was assessed following the warm-up and the rectal temperature probe was removed. Thermal comfort (Bakkevig & Nielsen, 1994), ratings of perceived exertion (Birk & Birk, 1987), ratings of effort (on a 0 to 10 cm scale; ‘0’ meaning no effort and ‘10’, maximal) and heart rate were measured after the warm-ups and after each repeated sprint.

Repeated sprint ability (RSA) protocol

Participants then undertook a RSA test (10 sets of 3-s sprints followed by 30-s recovery) on a non-motorised treadmill (Woodway, Force 3.0; Waukesha, WI, USA) in the same environment. The method has already been reported previously (See Chapter 4). Sprint data for peak power (PP), average power (AP), peak velocity (PV), average velocity (AV), distance covered (DC) and percentage decrement in power and velocity were recorded with a commercially designed software program (Pacer Treadmill Software; Innervations, WA, Australia) and used in the subsequent analysis. Fatigue was calculated using the % decrement method as advised by Glaister *et al*. (2008) (section 4.2).

During all sessions, participants performed a task-specific warm-up procedure, developed from pilot work, consisting of three sprints at 50, 70 and 80 % of maximum effort, respectively. The task-specific bursts of activity were brief enough not to cause significant fatigue.

Statistical analysis

The data were analysed by means of the Statistical Package for Social Sciences (SPSS) for Windows (SPSS, Chicago, IL, USA), IBM version 20, using a General linear model with repeated measures (GLM; condition [2 levels]) for all variables of the POMS questionnaire, 'resting' T_{rec}, 'Pre-RSA' T_{rec}, 'resting' T_m and 'Pre-RSA' T_m. A GLM with repeated measures were used for 'resting' T_m and 'Pre-RSA' T_m, (condition [2 levels] x time [3 levels]), and for RPE, rated 'effort', TC, heart rate and all RSA variables (condition [2 levels] x time [10 levels]). To correct violations of sphericity, the degrees of freedom were corrected in a normal way, using Huynh-Feldt ($\varepsilon > 0.75$) or Greenhouse-Geisser ($\varepsilon < 0.75$) values for ε, as appropriate (Field, 2000). Graphical comparisons between means and Bonferroni pairwise comparisons were made where main effects were present. The results are presented as the mean ± the standard deviation (SD) throughout the text unless otherwise stated. Ninety-five percent confidence intervals (CIs) are presented where appropriate. Following convention, the alpha level of significance was set at 5 %. The meaningfulness of the results was estimated in each group for all RSA parameters by calculating the within-factor effect size (ES) for ANOVA, according to the formulae reported by Mullineaux *et al.* (2001). Effect sizes of 0.2, 0.5 and 0.8 represented small, moderate and large differences, respectively (Mullineaux *et al.*, 2001). Absolute data through the Bland and Altman Method (1995) was used for correlation calculation

6.3: Results

Rectal and muscle temperature at rest

Rectal temperature (*T_{rec}*)

There was a significant diurnal variation present for T_{rec} with higher resting values in the E *vs.* the M condition (mean difference +0.46°C; see Table 6.1 and Figure 6.2). The CI for this comparison was 0.39 to 0.54°C ($P < 0.0005$).

Muscle temperature

T_m values at 3 cm depths were lower in the M condition than the E condition (-0.57°C, CI = 0.34 to 0.80°C; $P < 0.0005$; see Table 6.1 and Figure 6.2).

Rectal and muscle temperature after warm-up (before RSA).

Rectal temperature (*T_{rec}*)

There was a main effect for condition ($F_{1, 19,}$ 78.259, $P < 0.0005$; see Table 6.1 and Figure 6.2), where pairwise comparisons showed T_{rec} values to be lower in the M condition than in the E condition following the warm-up (-0.42°C, CI = 0.32 to 0.52°C).

Muscle temperature

T_m values at 3-cm depths were lower in the M condition than the E condition (-0.38°C, CI = 0.14 to 0.62°C; $P = 0.003$, see Table 6.1 and Figure 6.2).

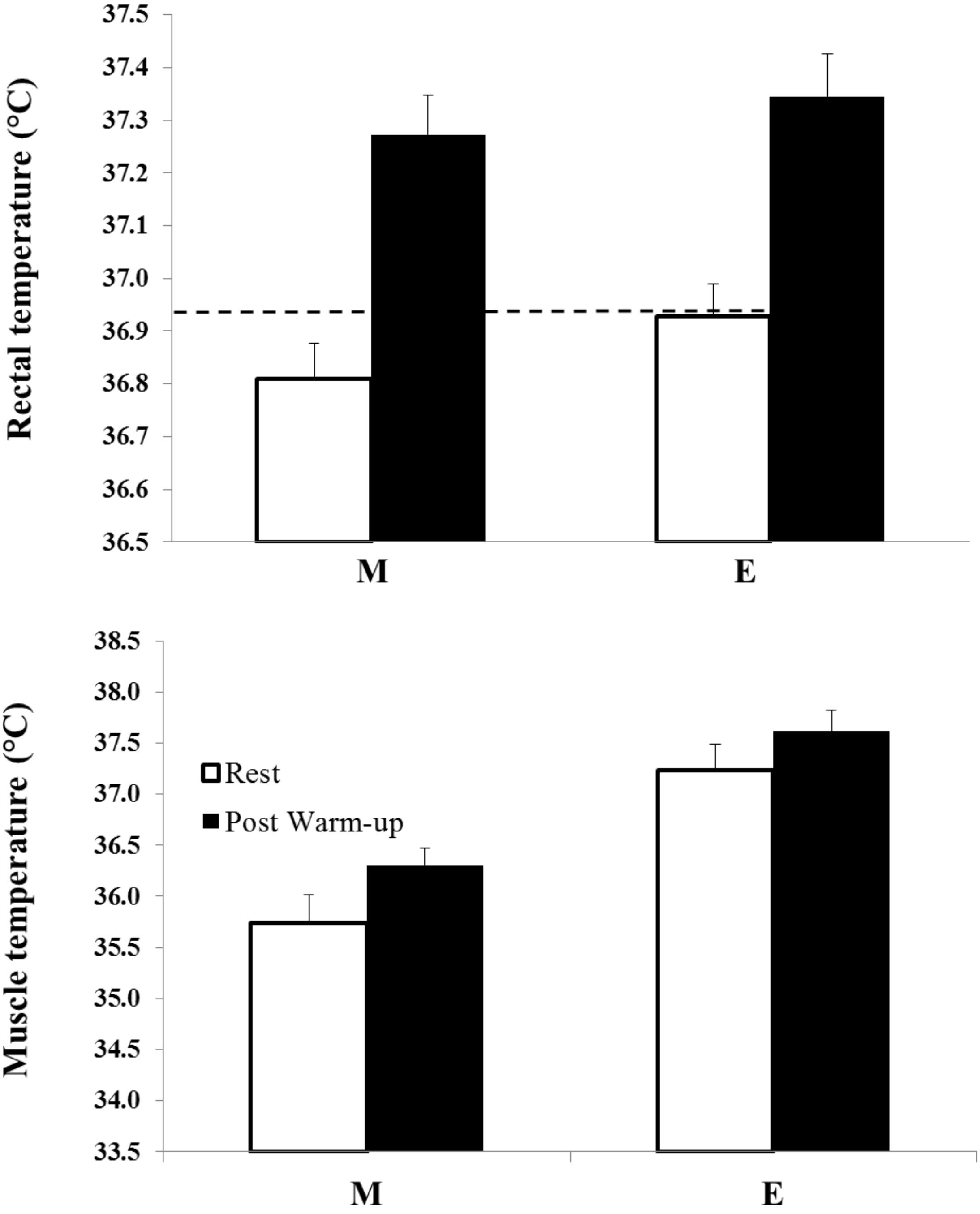


Figure 6.2: Mean and 95% confidence intervals (corrected for between-subject variability) for rectal temperature and muscle temperature (at 3-cm) at rest (□) and post warm-up (■). (a) Significant diurnal variation in temperature, where the morning value (07:30 h) is lower than the evening value (17:30 h; *P* < 0.05). (b) Temperature values significantly lower at rest than after the warm-up (*P* < 0.05).

Repeated Sprint Ability (RSA) measures

Table 6.1 shows the group means (± SD) for all RSA variables recorded in each condition, together with the statistical analyses. There was a time-of-day effect for peak velocity (PV), average velocity (AV), average power (AP) and distance covered (DC) with higher values in the E condition than the M condition (3.3 to 8.3 %; $P < 0.05$). There was a trend for peak power (PP) in the M condition to be lower than the E condition ($P = 0.058$). Peak power and peak velocity decrements using the % decrement method were not significantly different between M and E conditions ($P > 0.05$). There was a significant sprint effect for all measures of RSA, where values dropped from sprint 1 to sprint 10 ($P < 0.05$, see Figure 6.3). There was no interaction for any of the measured RSA values between sprints and time-of-day ($P > 0.05$), and no interaction so that profiles for any of the measured RSA values dropped from sprint 1 to sprint 10 irrespective of time-of-day ($P > 0.05$; see Figure 6.3). The % decrement for power and velocity were no different between M and E conditions ($P > 0.05$).

Differences between morning and evening were moderate towards the upper end for DC (ES = 0.71), AP (ES = 0.64) and AV (ES = 0.70), moderate towards lower end for PP (ES = 0.38), PV (ES = 0.46) and % PP (ES = 0.32), and small for % PV (ES = 0.15).

Table 6.1. Mean (± SD) values for temperatures, RSA variables for all 10 sprints, subjective measures and blood variables measured in the morning and evening. Statistical significance ($P < 0.05$) is indicated in bold, and a trend (where $0.10 > P > 0.05$) is indicated in italics. *Different than M condition

Variable	Morning (M)	Evening (E)	Significance of main effects for condition	Significance of main effects for time	Interaction
Resting temp (°C)					
T_{rec}	36.8 ± 0.1	37.3 ± 0.1*	**$F_{1, 19,}$ 176.946, $P < 0.0005$**		
T_m (3-cm depth)	35.7 ± 0.6	36.3 ± 0.3*	**$F_{1, 19,}$ 27.139, $P < 0.0005$**		
Post warm-up temps (°C)					
T_{rec}	36.9 ± 0.2	37.4 ± 0.2*	**$F_{1, 19,}$ 78.259, $P < 0.0005$**		
T_m (3-cm depth)	37.2 ± 0.5	37.6 ± 0.4*	**$F_{1, 19,}$ 11.315, $P = 0.003$**		
Repeated Sprint Ability (RSA)					
Distance Covered (m)	14.1 ± 1.5	15.2 ± 1.6*	**$F_{1, 19,}$ 43.973, $P < 0.0005$**	**$F_{6.9, 131.5,}$ 3.014, $P = 0.006$**	$F_{8.9, 169.5,}$ 0.533, $P = 0.848$
Peak Power (W)	3176 ± 378	3316 ± 351$^{\#}$	*$F_{1, 19,}$ 4.067, $P = 0.058$*	**$F_{3.3, 61.7,}$ 3.741, $P = 0.013$**	$F_{8.8, 167.2,}$ 1.408, $P = 0.219$
Average Power (W)	2353 ± 288	2537 ± 286*	**$F_{1, 19,}$ 14.926, $P = 0.001$**	**$F_{4.2, 78.8,}$ 3.741, $P < 0.0005$**	$F_{8.9, 168.1,}$ 0.693, $P = 0.712$
Peak Velocity (km.h^{-1})	19.8 ± 1.2	20.4 ± 1.4*	**$F_{1, 19,}$ 8.161, $P = 0.010$**	**$F_{2.0, 37.5,}$ 9.060, $P = 0.001$**	$F_{3.6, 68.0,}$ 1.002, $P = 0.407$
Average Velocity (km.h^{-1})	16.7 ± 1.8	18.0 ± 1.9*	**$F_{1, 19,}$ 44.065, $P < 0.0005$**	**$F_{6.8, 129.3,}$ 3.148, $P = 0.005$**	$F_{9.0, 170.2,}$ 0.539, $P = 0.844$
% decrement in Peak Power	6.2 ± 2.0	7.1 ± 3.5	$F_{1, 19,}$ 0.831, $P = 0.373$		
% decrement in Peak Velocity	3.1 ± 2.1	3.4 ± 2.0	$F_{1, 19,}$ 0.235, $P = 0.633$		
Subjective Measures					
Effort (0-10 cm VAS)	10 ± 0	10 ± 0	n/a	n/a	n/a
RPE (6-20)	15 ± 2	15 ± 2*	**$F_{1, 19,}$ 5.539, $P = 0.003$**	**$F_{1.6, 31.0,}$ 35.153, $P = 0.010$**	$F_{2.8, 53.3,}$ 0.597, $P = 0.609$
Thermal comfort (1-9)	7 ± 1	7± 1	$F_{1, 19,}$ 0.294, $P = 0.594$	**$F_{3.1, 58.0,}$ 104.414, $P < 0.0005$**	$F_{7.1, 135,}$ 0.274, $P = 0.928$
Heart rate (beats.min^{-1})	163 ± 7	169 ± 7*	**$F_{1, 19,}$ 15.124, $P = 0.001$**	**$F_{2.8, 53.2,}$ 99.166, $P < 0.0005$**	*$F_{3.1, 59,}$ 0.768, $P = 0.0768$*

Table 6.2. Correlations between temperatures (T_{rec} and T_m 3 cm depth) and performance for RSA, RPE, Effort and TC. Method of Bland and Altman (1995) was used. Correlations either negative or positive are denoted by the sign + or –. Where emboldened $P < 0.05$; Italicised values represent a statistical trend ($0.10 > P > 0.05$). If $P > 0.05$, values not emboldened.

Covariate/ Dependent	***T_m (°C)***	***Peak power (W)***	***Ave power (W)***	***Peak velocity* (km.h^{-1})**	***Ave velocity* (km.h^{-1})**	***Distance (m)***	***PP (%)***	***PV (%)***	***RPE***	***EFFORT***	***TC***
T_{rec} (°C)	**+ (0.33)**	**+ (0.25)**	**+ (0.49)**	**+ (0.26)**	**+ (0.57)**	**+ (0.56)**	0 (-0.08)	0 (-0.02)	**+ (0.88)**	0 (0)	0 (-0.83)
T_m (°C)		0 (0.01)	+ *(0.17)*	0 (0.02)	**+ (0.24)**	**+ (0.23)**	0 (-0.05)	0 (-0.07)	0 (0.09)	0 (0)	0 (-0.04)

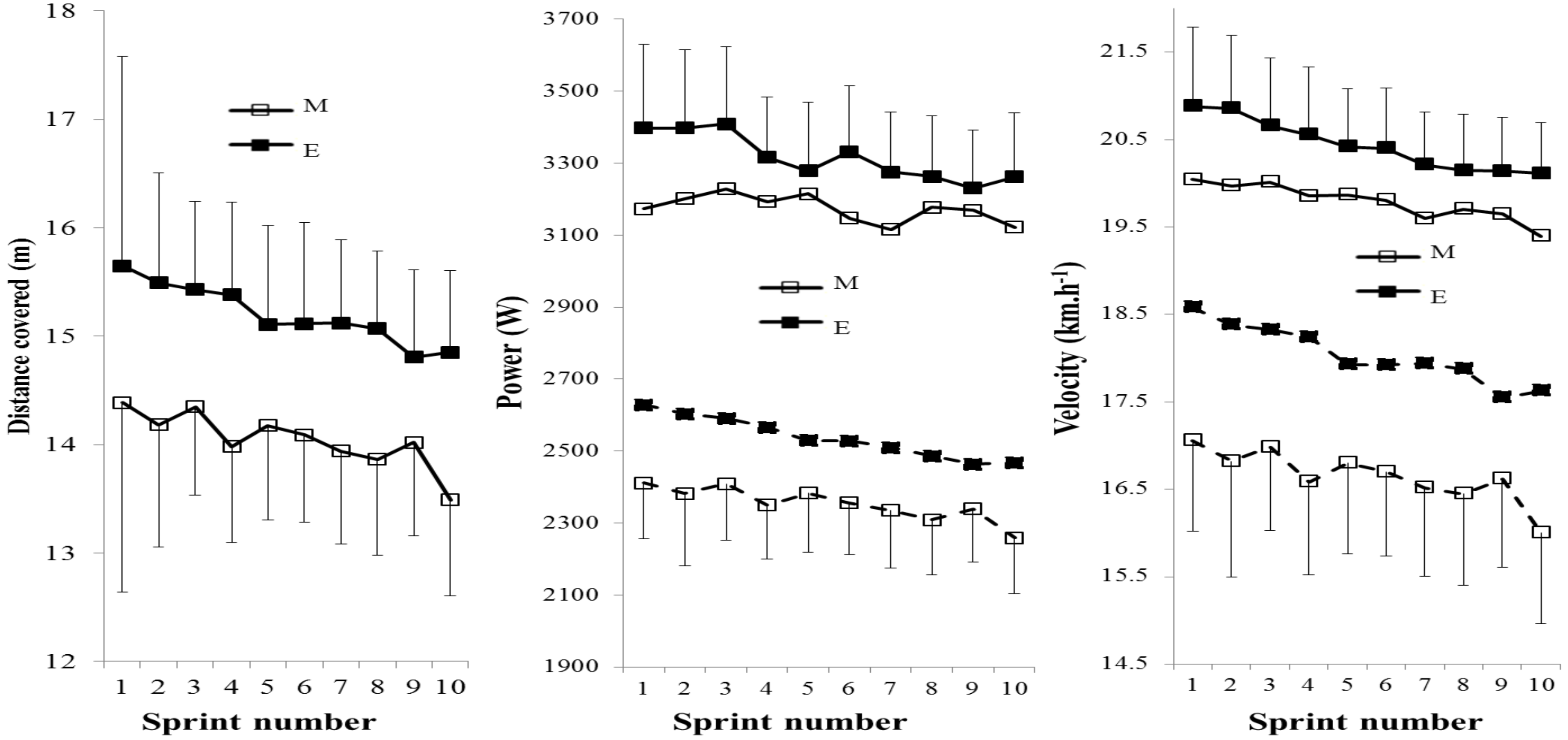


Figure 6.3: Mean and 95% confidence intervals (corrected for between-subject variability) for distance covered, power (peak and average) and velocity (peak and average) from sprint 1 to 10 for morning (□) and evening (■) conditions. Dashed lines indicate averages and full lines indicate peak variables.

Heart rate, rating of perceived exertion (RPE), thermal comfort (TC) and effort levels.

Heart rate and TC (Pre-RSA)

Mean (±SD) heart rate showed a trend for values to be higher in the E condition than the M condition ($F_{1, 19,}$ 4.257, *P* = 0.053). Thermal comfort was no different between conditions (*P* > 0.05). There was a significant time effect for both values of heart rate and TC, with values higher following the warm-up (*P* < 0.0005). There was no interaction such that profiles for heart rate and TC increased irrespective of condition (*P* = 0.668 and *P* = 0.171)

Heart rate, RPE and TC and effort levels (during RSA)

Mean (±SD) values for heart rate for E condition were higher than that of the M condition (*P* = 0.001 see Table 6.1). Mean (± SD) values for RPE were also significantly higher for the E than the M condition (*P* = 0.003; see Table 6.1). Thermal comfort (TC) and effort levels showed no time-of-day effect (*P* > 0.05). There was a significant 'sprint' effect for TC, RPE and heart rate (*P* < 0.0005), where values increased from the first to the last sprint. This rise was not present for self-rated effort, which was rated as 10 or maximal for each sprint and showed the motivation of the participants to perform to the best of their ability in each sprint (*P* > 0.05). There was no significant interaction between condition and sprint (*P* > 0.05) such that the profiles for RPE, TC and heart rate increased in parallel linearly over sprint number for both conditions. Effort levels were 10 throughout both conditions and for all sprints.

Resting POMS questionnaire variables

Resting subjective measures of 'vigour' were lower at rest in the morning than the evening timed session (*P* < 0.0005) and ratings of 'fatigue' were higher at rest in the morning than the evening (*P* < 0.05). There was a trend for resting subjective measures of 'confusion' in the M condition to be lower than the E condition (*P* = 0.069). Resting subjective values for measures of ‘anger’, ‘calmness’, ‘depression’, ‘happiness’ and ‘tensions’ were not significantly different between conditions ($P > 0.05$).

6.4: Discussion

The present study was designed to investigate whether a diurnal variation in RSA can be established in a large cohort of highly motivated individuals [effort scores of ~100] on a non-motorised treadmill. The main finding was that a time-of-day difference was present in RSA. Values for peak velocity, average velocity, average power and distance covered per sprint were significantly higher in the evening than the morning (3.3 to 8.3 % differences; see Table 6.1 and Figure 6.4). Further, values for peak power displayed a trend with values in the evening higher than the morning. In contrast however, no diurnal variation was reported for % decrements for power and velocity. The present finding regarding peak velocity disagrees with previous research (Hamouda *et al.,* 2012; Souissi *et al.,* 2008). They reported no time-of-day differences, while evening values were significantly higher than morning ones (~0.7 km.h^{-1}, 3.3 %). In agreement with previous findings, % decrement in power output is unaffected by time of day (Hamouda *et al.,* 2012; Souissi *et al.,* 2008). However, this was not reported for all studies (Chtourou *et al.,* 2011; Racinais *et al.,* 2005c). This could be due to the number of different protocols utilised within the literature (see Table 2.1). This present study agrees with all previous literature by displaying significant diurnal variation (~141.7 W, 4.5 %). with higher values in the evening than the morning for peak power (Souissi *et al.,* 2004, 2007, 2008; Racinais *et al.,* 2005c, 2010; Giacomoni *et al.,* 2006; Chtourou *et al.,* 2012; Hamouda *et al.,* 2012; Zarouk *et al.,* 2012; Aloui *et al.,* 2013). However, findings regarding peak power in the literature are inconsistent. Although diurnal variation is present in peak power, with higher values in the mid-afternoon or early evening when compared to the morning (~3.1 to 7.6 %), the observed differences occur during either the first or first few sprints only (Racinais *et al.,* 2005c; Racinais *et al.,* 2010), or for all sprints performed

(Souissi *et al.,* 2004, 2010) (Table 2.1). Although all aforementioned studies found evidence of diurnal variation in RSA on a cycle ergometer, differences between the studies make it difficult to compare findings. Furthermore, to the best of our knowledge, the present study is the first to investigate the possible diurnal variation in team-sport specific RSA on a non-motorised treadmill and the first such demonstration of this result. Therefore, it is very important that comparisons made between our study and other studies looking at diurnal variation in RSA (Table 2.1), take into account that previous research has been conducted on the cycle ergometer using a different protocol. We also found that both T_{rec} and T_m (3-cm depth) showed diurnal variation, with higher values in the evening than the morning (0.46 °C and 0.57°C, respectively). These are in agreement with previous research conducted by Edwards *et al*. (2013), who found morning and evening differences ranging from ~0.50°C and ~0.30°C for T_{rec} and T_m (3-cm depth), respectively. Another recent study performed by Robinson *et al*. (2013) also displayed similar diurnal differences in T_{rec} and T_m (3 cm depth), both by about ~0.40°C. The evening superiority in muscle force production and power output has been attributed to a causal link between 'resting' core and muscle temperatures and performance, the higher evening resting core and muscle temperatures producing an increase in the force-generating capacity of the muscle (Bernard *et al.,* 1998; Coldwells *et al.,* 1994; Giacomoni *et al.,* 2005; Melhim, 1993) and neural function (reduced twitch time-course or increase in speed of contraction). In partial support of this link, we found significant positive correlations between T_{rec} and T_m (at 3-cm), T_{rec} and all measures of RS performance (See Table 6.2). There was no correlation between 'fatigue index', 'peak power output' or 'peak velocity' and T_{rec}, probably due to the lack of sensitivity of the performance measure (no diurnal variation being found – Table 6.1). Significant positive correlations between T_m (at 3-cm) and the performance variables of the RSA 'average velocity' and 'distance covered', with a trend for 'average power', were found, but there were no significant correlations between T_m and any other RSA variables. Hence it would appear that T_{rec} rather than T_m may contribute more to the relationship between increase in temperature and RSA. To pursue the contributions made by local or central temperature to RSA would entail manipulation of morning or evening temperature - either by increasing morning temperatures to evening levels or decreasing evening temperatures to morning values – see Edwards *et al*. (2013) and Chapters 7 and 8.

Our findings disagree with those presented by Souissi *et al.* (2010), who looked at the effects of a 5-min *vs.* a 15-min cycle ergometry warm-up at ~50 % peak power output in the morning (08:00 h) and evening (18:00 h) on one 30-s maximal sprint. They reported diurnal variation in rectal temperature of ~0.65°C and in peak and mean powers in the Wingate test. However, direct comparisons between the published results (Table 2.1) and our present ones are very difficult for several reasons due to a number variations in a numerous of underpinning factors that play a role in the observation of significant diurnal variation, with these differences probably resulting in the findings regarding circadian variation of RSA being very conflicting. It has been established that differences in exercise mode, sprint duration, number of sprint repetitions, type of recovery and training status of individuals make it difficult to evaluate and compare studies, and thus question the validity and sport-specific relevance of many of the previously used protocols (Spencer *et al.,* 2005). Therefore, a more externally valid assessment of team-sport RSA (such as football) was used. Additionally, due to the high number of familiarisation sessions performed (three in total), resulting in accurate levels of reliability in this test (see Chapter 4) we believe that the use of our exercise protocol is more reliable than methods used in previous research. Finally, we believe that peak power and peak velocity are less reliable RSA variables to assess on a non-motorised treadmill. The software utilised, a commercially designed powerful Windows software program (Pacer Treadmill Software; Innervations, WA, Australia), permits us to develop a very specific protocol which targets any sport, and also enables collection of multiple samples each second. We utilised a sample rate of 200 Hz, as recommended by the manufacturer, meaning that 200 samples were collected each second. Therefore, peak values only represent 1 time point from the 600 recorded time-points over a 3-s sprint, making it a far less accurate and reliable measure of RSA; we believe this is the reason why only a statistical trend was established for peak velocity.

Differences in core body temperature and muscle temperature are shown to be determinants of (RSA) performance, with several other factors have been suggested to account for circadian variations in performance. A central mechanism that could be involved in the diurnal variation of performance and its effect on task is motivation, easily measured through subjective ratings recorded after physical effort (on a simple 0-10 VAS scale, 0 being no effort and 10 being maximal effort). It has been suggested that motivation could be involved in masking some rhythms in maximal effort. As expected, ratings of effort were ‘maximum’, irrespective of condition. Furthermore, neural activity that is associated with muscle activity is comprised of a number of components (see Edwards *et al.,* 2014 for more information) any of which could play an important role in the determination of diurnal variation in performance. Taken together, the results of this study support the view that morning-evening differences in RSA, when diurnal variation is present in both core and deep muscle temperatures, involve not only peripheral mechanisms, highly dependent on muscle temperature (in agreement with the views of Edwards *et al.,* 2013, 2014; Guette *et al.,* 2005; Martin *et al.,* 1999), but also other factors determined by the environment (exogenous factors) as well as output from the body clock (endogenous factors). Although this has been taken into consideration, direct evidence that there is a large endogenous component to the daily variation in muscle performance is currently unproven (Sargent *et al.,* 2010). As has been stressed, for this internal component to be investigated, time-consuming and highly challenging chronobiological protocols which attempt to standardise or reduce the exogenous component of the rhythm using desynchronisation, constant routines, ultra-short sleep-wake-cycle protocols are required and results from these have not yet been published (Kline *et al.*, 2007; Reilly & Waterhouse, 2009). Such research protocols may further extend our knowledge of the hypothesised 'causal link' between T_{rec} and T_m and RSA.

Considering the applied and chronobiological issues that remain to be addressed, further research questions from the present study can be generated: (1) To what extent can the athlete in a thermoneutral environment overcome the poorer RSA found in the morning? Perhaps through a rigorous warm-up – increasing core and muscle temperatures by a significant physiological amount (either that recorded in the evening or ~38.5°C) - or by using known phase-response curves for light, melatonin or exercise; (2) The strength input of the endogenous component of muscle power output has not yet been investigated; and (3) Further research is required on the effects of temperature modification localised to the exercising limb (using hot baths or diathermy) vs. temperature modifications to the whole body.

6.5: Conclusion

In this population, a diurnal variation in team-sport specific RSA on a non-motorised treadmill was present with higher performance for all measures except fatigue index in the evening compared to the morning. We conclude that, although central temperature may provide some endogenous rhythm to the observed diurnal variation in team-sport specific RSA on a non-motorised treadmill, the causal link that has previously been proposed does not seem to be a simple one – but rather one that is due to a multiplicity of components and mechanisms.

Chapter 7: Modulating rectal and muscle temperatures: can we offset the diurnal variation in repeated sprint ability on a non-motorised treadmill?

7.1: Introduction

In male participants in a temperate environment (around 17-20°C), many human performance variables display diurnal variation. As far as is known, only one study has looked at the effects of time-of-day on RSA on a non-motorised treadmill, an accurate and reliable apparatus sensitive enough to detect time of day differences in RS performance (see Chapter 5; Pullinger *et al.,* 2013). In this study, results from a population of 20 trained male participants, who were familiarised with the protocol 3 times - distance covered, peak power, average power, peak velocity and average velocity all showed significantly higher values in the evening compared to the morning (a range of 3.3 to 8.3 %).

The exact mechanisms for this observed diurnal variation have been attributed to a number of factors (See Edwards *et al.,* 2013 and Pullinger *et al.,* 2013). One factor which has been proposed is the causal link of the temperature rhythm, which might be implicated directly or indirectly, where the higher evening resting core body temperature (~0.8°C in rectal and gut sites, Edwards *et al.,* 2002) and local muscle temperature (≥ ~0.35°C in vastus lateralis at depths of 3-cm, Edwards *et al.,* 2013; Robinson *et al.,* 2013) produce an increase in force-generating capacity of the muscle (Bernard *et al.,* 1998; Coldwells *et al.,* 1994; Giacomoni *et al.,* 2005; Melhim, 1993) and neural function (reduced twitch time course or increase in speed of contraction, Martin *et al.,* 1999). The link between core temperature and performance has been studied mainly in two ways. The first link which has received some interest in the literature, involves using either active (by means of exercise) or passive (by means of a chamber or water bath) "warm-ups" to increase rectal body temperature in the morning, to approximately, or precisely, the temperature found "at rest" in the evening; and examining whether this increases morning performance to evening levels (using cycling or swimming time-trial or muscle force production; See Edwards *et al.,* 2013 for further details). The second link, which has received far less attention so far, explores whether pre-cooling rectal temperature in the evening to values similar, or exactly those observed in the morning, results in a parallel decrease in performance to morning values. Only two studies have sought to investigate the effect of core temperature on isometric and isokinetic performance with precise modelling of pre performance rectal temperature and time of day interaction - they reported cooling core temperature down decreased performance to morning levels but no effect with warming core in the morning to evening levels (See Robinson *et al.*, 2013 for further details).

Therefore, the purpose of the present study was to modulate rectal and/or muscle temperature to investigate whether increasing morning rectal temperature (by an active warm-up) to evening resting values or decreasing evening rectal and/or muscle temperatures (by immersion in a cool bath) to morning resting values leads to a change in RS performance on a non-motorised treadmill in well-trained, familiarised and highly-motivated participants.

7.2: Methods

Participants

Twelve field based team sport male participants with age (mean ± SD) 21.7 ± 2.6 yrs, maximal oxygen uptake ($\dot{V}O_2$ max) 60.6 ± 4.6 mL.kg.min^{-1}, height 1.78 ± 0.07 m, body mass 76.0 ± 6.3 kg and habitual retiring and waking times 23:44 ± 0:38 h:min and 07:43 ± 0:35 h:min, respectively, were recruited for this study. The protocol was fully explained to the participants and any questions were answered before their participation in the study. For inclusion and exclusion criteria see Section 6.1. Mean chronotype score was 35 ± 4, all intermediate types; F/R score, 45 ± 5; and L/V score 37 ± 6. Only participants who habitually trained during a normal week at any time of the day (morning, afternoon and evening) – and hence had no preference for time of training – were selected.

Research Design

All sessions took place under standard laboratory conditions (lighting, room temperature and humidity, and barometric pressure were 200-250 lux, 21.1 ± 1.1°C, 34.6 ± 5.1 %, and 755.3 ± 6.6 mmHg, respectively). Before taking part in the main experiment, each participant completed four familiarization sessions each separated by at least 3 days. These sessions ensured that participants were fully familiarized with the RSA protocol utilised in this study. Following the familiarization process, each participant completed five experimental sessions which took place three days apart; a morning and an evening trial (07:30 and 17:30 h; M and E) after a standardized 5-min warm-up at 10 km.h^{-1} on a motorised treadmill (Pulsar, H/P cosmos, Nussdorf-Traunstein, Germany) which were counterbalanced in order of administration; an active warm-up morning trial (M_E, at 10 km.h^{-1} on a motorised treadmill); two passive cool-down evening trials (E_{PC} and E_{PM}, at 16-17°C in a cold water immersion bath) that were also administered in a counterbalanced design to minimize any learning or order effects (Edwards *et al.*, 2013). In the M_E session, starting rectal temperature was modelled to become equal to that found in the subject in the previous E session at "rest". In the E_{PC} and E_{PM} sessions, starting rectal and muscle temperature was modelled to become equal to that found in the subject in the previous M session at "rest", respectively.

Protocol and measurements

The participants were free to live a "normal life" between sessions, sleeping at home at night and attending lectures and doing light office work in the day. They were told to refrain from drinking alcoholic or caffeinated beverages and from other training or heavy exertion for the 48 h before the experiments or during them. Participants recorded the type, amount and timing of the food they ate for the period of 24 h before and taken during the day of the experimental session, and were asked to replicate this diet for the days before and during the other experimental conditions. Participants then underwent the same testing procedures as the M and E sessions in Chapter 6. The protocol is given in Figure 7.1.

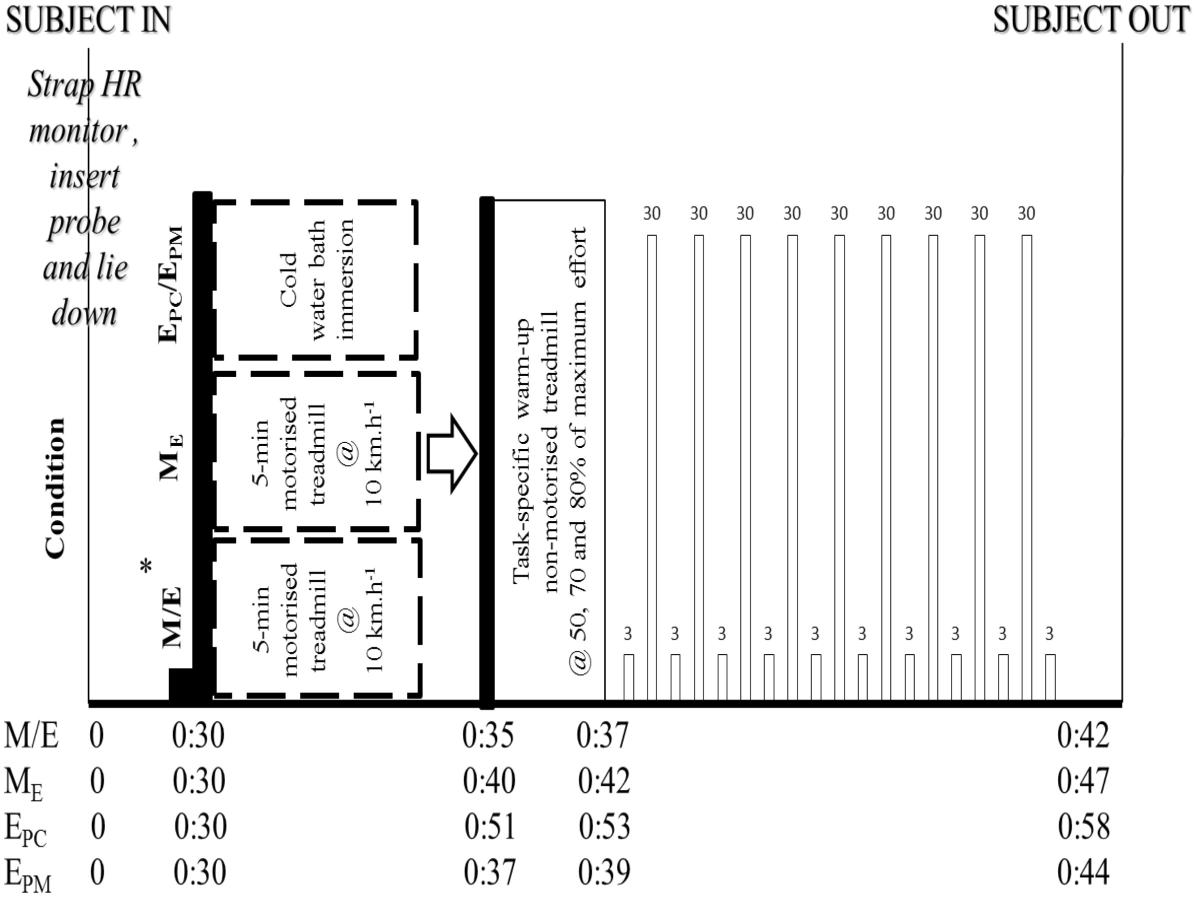


Figure 7.1: Schematic of the protocol for the 5 conditions - the morning (M, M_E 07:30 h) and evening (E, E_{MR}, E_{MM} 18:00 h) conditions. Rectal (T_{rec}) and muscle (T_m) temperatures, thermal comfort (TC) and profile of mood (POMS) were measured after the participants had reclined for 30-min at the start of the protocol and again after the warm-ups and prior to the RSA measures. * denotes start of 5-min continuous T_{rec} baseline measurement; the average value of these 5-min of recording was defined as resting T_{rec} and used in the subsequent analysis. Rating of effort (0-10 VAS), TC, HR and ratings of perceived exertion (RPE) were taken throughout the repeated sprint ability (RSA) protocol; **vertical black bars** indicate muscle temperature (T_m) taken at these points.

Active Warm-up Strategy (M_E)

The active warm-up protocol used in this study was based upon that of Edwards *et al.* (2013), but modified so that the same warming-up effect was obtained using a different exercise mode. Previous active warm-up studies (Atkinson *et al.,* 2005; Edwards *et al.*, 2013) attained increases in core temperature similar or equal to those found in the evening, when participants undertook a cycle ergometry warm-up at either 60 % of peak power output or 150 W. Through multiple trials it was established that it was also possible to attain similar increases in T_{rec} when participants performed an active warm-up at 10 $km.h^{-1}$ on the motorised treadmill. Participants' T_{rec} was measured throughout the warming process. Participants were allowed to stop exercising once their T_{rec} had increased to previous E "resting" values as measured continuously by rectal thermometry (Grant Instruments Ltd, Shepreth, UK) and took 00:10 ± 0:04 h:min on average to complete. Participants T_m was again measured, they removed their rectal probe and the participants then commenced the rest of the experimental protocol. The time between completing the warm-up and beginning the RSA was ~5-min.

Pre-Cooling Strategies (E_{PC} or E_{PM})

The immersion protocol used in this study was based upon that of Robinson *et al.* (2013) who attained decreases in rectal temperature of 0.37°C when immersing participants in water at ~16 to 17°C. Pilot work revealed this temperature to be acceptable in eliciting such a decrease in core temperature. Further, it was also established that water temperature of ~16 to 17°C attenuates any abrupt responses to acute cold stress exposure. Participants lowered themselves into the purpose built water tank (3 m x 2 m x 1.5 m) until semi-reclined and immersed up to their neck (E_{PC}) or waist (E_{PM}), respectively. Water temperature was monitored and maintained at ~16 to 17°C throughout the cooling period (Hitema ECA.002

water chiller, TO1B2620, Italy). Participants' T_{rec} was measured throughout this cooling process. Participants were allowed to exit the water tank once their T_{rec} or T_m had decreased to the value observed during the morning session. This took 21 ± 12 min for the E_{PC} session and 10 ± 4 min for the E_{PM} session. Participants then dried themselves and T_m was again measured and the participants then commenced the rest of the experimental protocol. The time between completing the cooling procedures and beginning the RSA was ~ 5-min.

Repeated sprint ability (RSA) protocol

Participants then undertook a RSA test (10 sets of 3-s sprints followed by 30-s recovery) on a non-motorised treadmill (Woodway, Force 3.0; Waukesha, WI, USA) in the same environment. The method has already been reported previously (See Chapter 4). Sprint data for peak power (PP), average power (AP), peak velocity (PV), average velocity (AV), distance covered (DC) and percentage decrement in power and velocity were recorded with a commercially designed software program (Pacer Treadmill Software; Innervations, WA, Australia) and used in the subsequent analysis. Fatigue was calculated using the % decrement method as advised by Glaister *et al*. (2008) following his reliability and validity study. Therefore, fatigue during each test was calculated using same formula as used in section 4.2.

During all sessions, participants performed a task-specific warm-up procedure, developed from pilot work, consisting of three sprints at 50, 70 and 80 % of maximum effort, respectively. The task-specific bursts of activity were brief enough not to cause significant fatigue.

Statistical analysis

The data were analysed by means of the Statistical Package for Social Sciences (SPSS) for Windows (SPSS, Chicago, IL, USA), IBM version 20, using a General linear model with repeated measures (GLM; condition [5 levels]) for all variables of the POMS questionnaire, resting T_{rec}, Pre-RSA T_{rec}, resting T_m and Pre-RSA T_m. A GLM with repeated measures were used for 'resting' T_m and 'Pre-RSA' T_m, (condition [5 levels] x time [3 levels]), and for RPE, rated 'effort', TC, heart rate and all RSA variables (condition [5 levels] x time [10 levels]). To correct violations of sphericity, the degrees of freedom were corrected in a normal way, using Huynh-Feldt ($\varepsilon > 0.75$) or Greenhouse-Geisser ($\varepsilon < 0.75$) values for ε, as appropriate (Field, 2000). Graphical comparisons between means and Bonferroni pairwise comparisons were made where main effects were present. The results are presented as the mean ± the standard deviation (SD) throughout the text unless otherwise stated. Ninety-five percent confidence intervals (CIs) are presented where appropriate. Following convention, the alpha level of significance was set at 5 %.

7.3: Results

Rectal and muscle temperature at rest

Rectal temperature (*T_{rec}*)

There was a significant diurnal variation for T_{rec} with higher resting values in the E *vs.* the M condition (mean difference +0.45°C; Table 7.1 and 7.2). The CI for this comparison was 0.27 to 0.62°C ($P < 0.0005$). This variation was consistent and in the expected direction for resting values for T_{rec} for the other conditions (E_{MR} *vs.* M +0.44°C, $P < 0.0005$; E_{MM} *vs.* M +0.46°C, $P < 0.0005$; E *vs.* M_E +0.40°C, $P = 0.002$; E_{MR} *vs.* M_E +0.39°C, $P = 0.001$; E_{MM} *vs.* M_E +0.41°C, $P = 0.006$). There was no statistical difference between resting T_{rec} levels in the morning (07:30 h) for M or M_E condition; and T_{rec} levels in the evening (17:30 h) for E, E_{MR} or E_{MM} conditions ($P > 0.05$).

Muscle temperature

T_m values at 3-cm depth were higher in the evening (E, E_{MR} or E_{MM}) conditions than the morning condition (+0.54°C, +0.73 °C and +0.69°C; $P < 0.05$) and M_E condition (+0.54°C, +0.73 °C and +0.69°C; $P < 0.05$). There was no difference between any values for any other conditions ($P > 0.05$; Table 7.1 and Figure 7.2).

Rectal and muscle temperature after warm-up or cool down (before RSA).

Rectal temperature (T_{rec})

T_{rec} post warm-up values for M_E, E and E_{MM} were higher than M (+0.33°C, +0.38°C and +0.22°C; $P < 0.05$) and E_{MR} (+0.45°C, +0.49°C and +0.33°C; $P < 0.05$, see Table 7.1 and Figure 7.2) conditions. Pairwise comparisons showed M values to be higher than E_{MR} values (+0.45°C; $P = 0.032$). Further, M_E and E_{MR} values were exactly the same as resting E and M condition values, respectively. In summary, the protocol produced the changes in T_{rec} (to resting values previously observed in the morning and evening) that were required to test the basic research questions." There was no difference between any values for any other conditions ($P > 0.05$).

Muscle temperature

T_m values at 3-cm depth were lower in the E_{MM} condition than the M, M_E, E and E_{MR} conditions (-3.36°C, -3.76°C, -3.71°C and -1.90°C; $P < 0.05$, see Table 7.1 and Figure 7.2). Pairwise comparisons showed M, M_E and E values to be higher than the E_{MR} condition after the cool down (+1.46°C, +1.86°C and +1.81°C; $P < 0.05$). Further, E_{MM} values were similar to the resting M condition T_m values at 3-cm depth (+0.01°C). In summary, the protocol produced the changes in T_m at 3-cm depth (to resting values previously observed in the morning) that were required to test the basic research question. There was no difference between any values for any other conditions ($P > 0.05$).

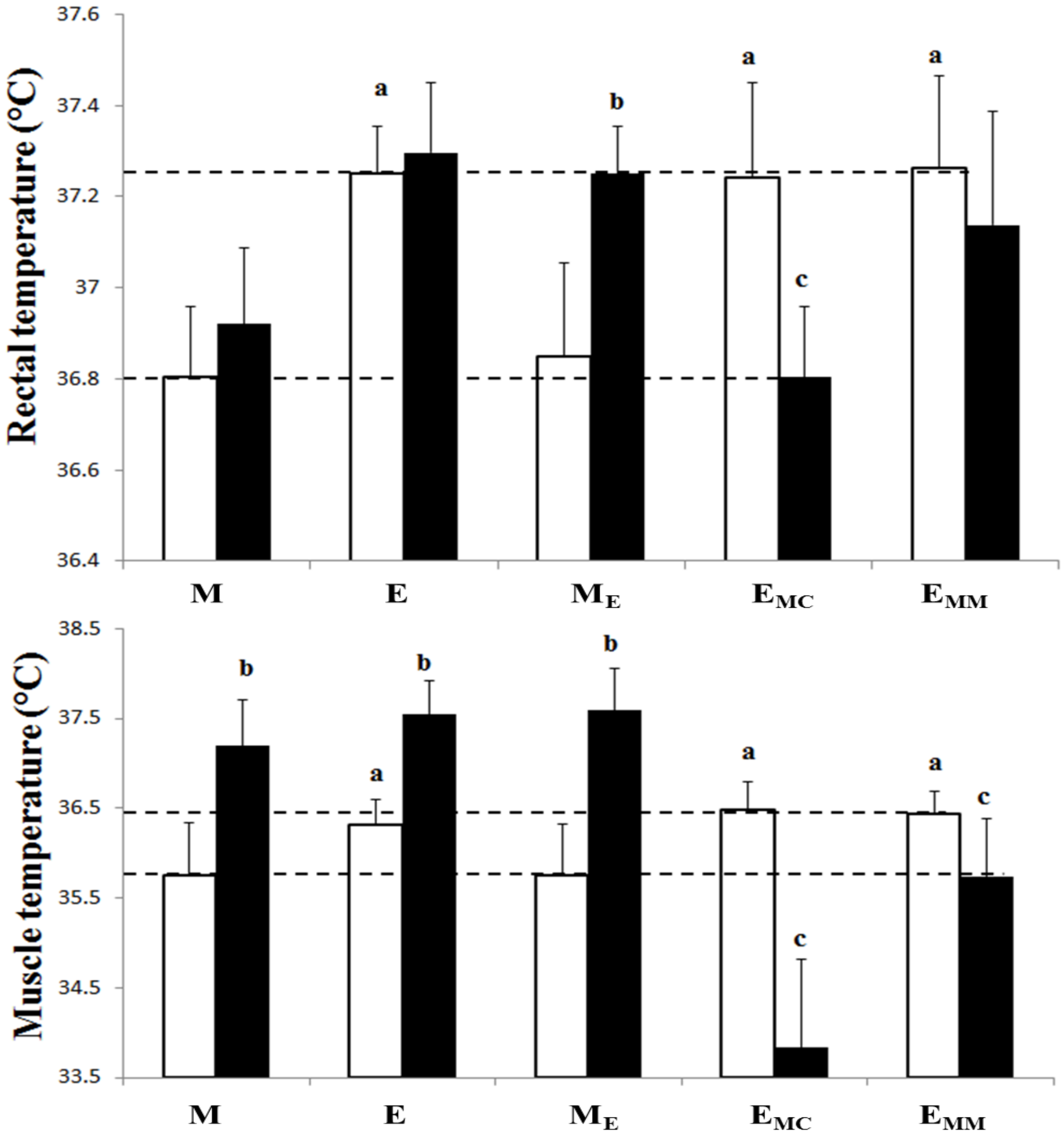


Figure 7.2: Mean and 95% confidence intervals (corrected for between-subject variability) for rectal temperature and muscle temperature (at 3 cm) at rest (□) and post warm-up (■). (a) - Significant diurnal variation in temperature, where the morning value (07:30 h) is lower than the evening value (17:30 h; $P < 0.05$). (b) - Temperature values significantly lower at rest than after the warm-up ($P < 0.05$). (c) - Temperature values significantly higher at rest than after the cool down ($P < 0.05$).

Repeated Sprint Ability (RSA) measures

Table 7.1 shows the group means (±SD) for all RSA variables recorded in each condition with statistical analyses. There were time-of-day effects for distance covered (DC), average velocity (AV) and average power (AP) with higher values in the E condition than the M condition (9.0 to 10.2 %; $P < 0.05$; see Figure 7.3 and Table 7.1). All other measures of RSA were not significantly different between M and E conditions (such as PP, PV, % decrement for power and velocity) conditions. The active warm-up strategy (M_E) in the morning did not significantly increase any RSA values. RSA values for DC, AV and AP all displayed statistical trends for values to be higher in the E condition than the M_E condition ($0.10 > P > 0.05$; see Figure 7.3 and Table 7.1). Pre-cooling core (E_{MR}) in the evening significantly reduced all RSA variables except for % decrement for power and velocity, all of which reported significantly higher values in the E (8.5 to 15.5 %) and M_E (8.9 to 12 %) conditions than the E_{MR} condition ($P < 0.05$; see Figure 7.3 and Table 7.1). Further, E_{MR} values for PV were significantly lower than E_{MM} condition ($P < 0.05$) and displayed a statistical trend for values to be lower than in the M condition ($P < 0.05$; $0.10 > P > 0.05$). E_{MR} values for PP displayed a statistical trend for values to be lower than in the E_{MM} condition ($P < 0.05$; $0.10 > P > 0.05$). Pre-cooling muscle (E_{MM}) in the evening also significantly reduced all RSA variables except for PP, PV, % decrement for power and velocity, all of which reported significantly higher values in the E condition than the E_{MM} (8.8 to 9.5 %; $P < 0.05$; see Figure 7.3 and Table 7.1) condition. There was no difference between any values for any other conditions ($P > 0.05$). There was not a significant sprint effect for any measures of RSA, except DC and AP, which displayed a trend ($P < 0.05$; $0.10 > P > 0.05$). There was no interaction between sprints and condition ($P > 0.05$), values falling from sprint 1 to sprint 10 irrespective of condition ($P > 0.05$; see Figure 7.3).

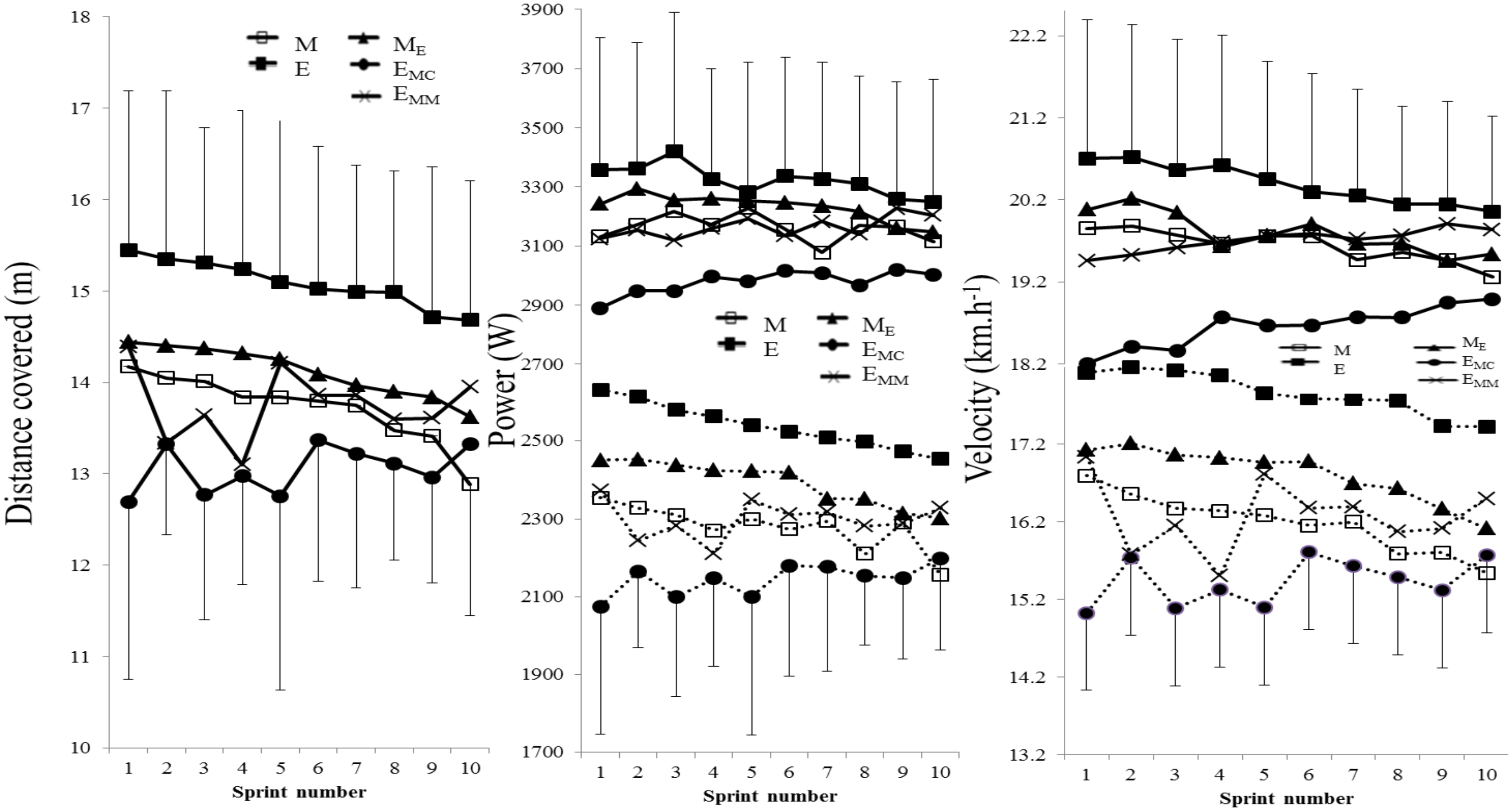


Figure 7.3. Mean and 95% confidence intervals (corrected for between-subject variability) for distance covered, power (peak and average) and velocity (peak and average) from sprint 1 to 10 for morning (□); evening (■); M_E (▲); E_{MR} (●) and E_{MM} (×) conditions. Dashed lines indicate averages and full lines indicate peak variables.

Heart rate, rating of perceived exertion (RPE), thermal comfort (TC) and effort levels.

Heart rate and TC (Pre-RSA)

Heart rate was higher in the M, M_E and E than the E_{MR} condition (+37 beats.min^{-1}, +36 beats.min^{-1} and +39 beats.min^{-1}; $P < 0.0005$) and E_{MM} (+42 beats.min^{-1}, +42 beats.min^{-1} and +45 beats.min^{-1}; $P < 0.0005$, see Table 7.1) conditions, respectively. Thermal comfort was higher in the M, M_E, E and E_{MM} than the E_{MR} (+1.8, +1.9, +1.8 and +0.8; $P < 0.05$). Pairwise comparisons also showed M, M_E and E thermal comfort values to be higher than E_{MM} (+1.0, +1.1 and +1.0; $P < 0.05$, see Table 7.1). There was no difference between any values for any other conditions ($P > 0.05$). There was a significant time effect for heart rate ($P < 0.0005$) with higher or lower values following the warm-up or cool-down, respectively (Table 7.1). Thermal comfort did not display a significant time effect ($P > 0.05$; Table 7.1). There was an interaction such that profiles for heart rate and thermal comfort increased or decreased at different rates dependant on condition ($P < 0.0005$).

Heart rate, RPE and TC and effort levels (during RSA)

Heart rate was lower for E_{MR} condition than M, M_E, E and E_{MM} (+26 beats.min^{-1}, +27 beats.min^{-1}, +30 beats.min^{-1} and +11 beats.min^{-1}; $P < 0.05$) conditions. Heart rate values were also higher in the M, M_E and E conditions compared to the E_{MM} (+16 beats.min^{-1}, +16 beats.min^{-1} and +20 beats.min^{-1}; $P < 0.0005$, see Table 7.1) condition. Values for TC were higher for M, M_E and E conditions than E_{MR} (+2.6, +2.5 and +2.7; $P < 0.0005$) and E_{MM} (+1.3, +1.2 and +1.4; $P < 0.05$) conditions, respectively. TC values were also higher in the E_{MM} than the E_{MR} (+1.2; $P = 0.015$) condition. RPE was higher for M_E and E conditions compared to E_{MM} (+1.3 and +2.0; $P = 0.01$) and E_{MR} (+1.1 and +1.7; $P < 0.05$) conditions, respectively. RPE values were also higher for the M condition than the E_{MR} (+1.3; $P = 0.047$) condition. There was no difference between any values for any of the variables for any other conditions

($P > 0.05$). There was a significant "sprint" effect for TC, heart rate and RPE ($P < 0.0005$), where values increased from the first to the last sprint. This rise was not present for self-rated effort, which was rated as 10 or maximal for each sprint for all conditions and showed the motivation of the participants to perform to the best of their ability in each sprint ($P > 0.05$). There was a significant interaction between condition and sprint (P = 0.035) for heart rate such that profiles did not increase in parallel linearly over sprint number for all conditions. There was no significant interaction between condition and sprint ($P > 0.05$), such that profiles for RPE and TC increased in parallel linearly over sprint number for all conditions.

Resting POMS Questionnaire Variables

Resting subjective measures of 'confusion', 'vigour' and 'happiness' were lower at rest in the morning (M and M_E) than the evening (E, E_{MR} and E_{MM}) timed sessions ($P < 0.05$). Levels of 'fatigue' were higher in the morning (M and M_E) than the evening (E, E_{MR} and E_{MM}) timed sessions ($P < 0.05$). Resting subjective values for measures of 'anger', 'calm', 'depression' and 'tension' were not significantly different between conditions ($P > 0.05$).

Table 7.1. Mean (± SD) values for temperatures, RSA variables for all 10 sprints, subjective measures and blood variables measured in the five conditions. Statistical significance ($P < 0.05$) is indicated in bold, and a trend (where $0.10 > P > 0.05$) is indicated in italics. a - Different than E condition ($P < 0.05$); b – Different than E_{MR} condition ($P < 0.05$); c – Different than E_{MM} condition ($P < 0.05$); d – Different than M_E condition ($P < 0.05$).

Variable	**Morning (M)**	**Evening (E)**	**Active warm-up (M_E)**	**Core cool-down (E_{MR})**	**Muscle cool-down (E_{MM})**	**Significance of main effects for condition**	**Significance of main effects for time**	**Significance for interaction**
Resting temp (°C)								
T_{rec}	36.8 ± 0.2 **a b c**	37.3 ± 0.1 a	36.9 ± 0.2 **a b c**	37.2 ± 0.2	37.3 ± 0.2	**$F_{2.3, 24.8,}$ 25.675, *P* < 0.0005**		
T_m (3cm depth)	35.8 ± 0.6 **a b c**	36.3 ± 0.3	35.8 ± 0.6 **a b c**	36.5 ± 0.3	36.4 ± 0.3	**$F_{3.6, 39.5}$ 13.154, *P* < 0.0005**		
Post warm-up temps (°C)								
T_{rec}	36.9 ± 0.2 **a c d**	37.3 ± 0.2	37.3 ± 0.1 **b**	36.8 ± 0.3 **a**	37.1 ± 0.3 **b**	**$F_{1.8, 20.2,}$ 78.259, *P* < 0.0005**		
T_m (3cm depth)	37.2 ± 0.5 a **b c** d	37.6 ± 0.4	37.6 ± 0.4 **b c**	33.8 ± 1.0 **a**	35.7 ± 0.7 **a b**	**$F_{1.8, 19.5,}$ 80.428, *P* < 0.0005**		
Repeated Sprint Ability								
Distance Covered (m)	13.7 ± 1.3 **a**	15.1 ± 1.5	14.1 ± 1.4 a **b**	13.1 ± 1.2 **a**	13.8 ± 1.5 **a**	**$F_{3.8, 42.0,}$ 12.153, P < 0.0005**	*$F_{4.0, 43.6,}$ 2.151, P = 0.091*	$F_{19.9, 219.0,}$ 1.579, *P* = 0.155
Peak Power (W)	3160 ± 428	3323 ± 390	3230 ± 429 **b**	2978 ± 405 **a**	3163 ± 451 b	**$F_{2.0, 22.5,}$ 6.444, P = 0.006**	$F_{8.2, 50.2,}$ 0.875, P = 0.543	$F_{7.0, 77.1,}$ 1.262, *P* = 0.205
Average Power (W)	2279 ± 241 **a b**	2539 ± 261	2393 ± 256 a **b**	2144 ± 231 **a**	2298 ± 265 **a b**	**$F_{3.6, 39.1,}$ 15.190, P < 0.0005**	*$F_{3.6, 39.3,}$ 2.676, P = 0.051*	**$F_{6.9, 76.4,}$ 2.459, *P* = 0.025**
Peak Velocity (km.h^{-1})	19.6± 1.2 b	20.4 ± 1.2	19.8 ± 1.2 **b**	18.7 ± 1.4 **a**	19.7 ± 1.3 **b**	**$F_{34.0, 33.8,}$ 10.659, P < 0.0005**	$F_{2.5, 27.3,}$ 1.307, *P* = 0.287	**$F_{6.5, 70.9,}$ 4.515, *P* < 0.0005**
Average Velocity (km.h^{-1})	16.2 ± 1.6 **a**	17.9 ± 1.8	16.8 ± 1.5 a **b**	15.4 ± 1.4 **a**	16.3± 1.7 **a**	**$F_{3.9, 423.2,}$ 13.627, P < 0.0005**	$F_{7.0, 78.5,}$ 1.587, *P* = 0.100	$F_{7.1, 78.5,}$ 1.587, *P* = 0.150
% decrement in Peak Power	6.4 ± 2.2	6.4 ± 4.0	6.1 ± 1.4	6.6 ± 2.0	5.5 ± 3.2	$F_{2.2, 24.5,}$ 0.302, *P* = 0.765		
% decrement in Peak Velocity	2.4 ± 1.8	2.4 ± 1.1	3.0 ± 1.5	2.5 ± 1.1	1.9 ± 0.9	$F_{3.5, 38.8,}$ 1.157, *P* = 0.342		
Subjective Measures								
Pre RSA								
Thermal comfort (1-9)	5 ± 1 **b c**	5 ± 1	6 ± 1 **b c**	4 ± 1 **a**	4 ± 0 **a b**	**$F_{3.7, 40.4,}$ 50.371, *P* < 0.0005**	$F_{1, 11,}$ 1.667, P = 0.223	**$F_{4,44}$ 63.983 *P* < 0.0005**
Heart rate (beats.min^{-1})	106 ± 43 **b c**	108 ± 42	105 ± 45 **b c**	69 ± 11 **a**	64 ± 7 **a**	**$F_{3.5, 38.2,}$ 149.789, *P* < 0.0005**	**$F_{1, 11}$ 1323.658 *P* < 0.0005**	**$F_{4, 44,}$ 245.939, *P* < 0.0005**
During RSA								
Effort (0-10 cm VAS)	10 ± 0	10 ± 0	10 ± 0	10 ± 0	10.0 ± 0.0	n/a	n/a	n/a
RPE (6-20)	15 ± 2 **b c**	15 ± 2	15 ± 2 **b c**	13 ± 1 **a**	13.4 ± 1.3 **a**	**$F_{3.7, 40.9,}$ 14.446, *P* < 0.0005**	**$F_{1.6, 17.7,}$ 101.054, *P* < 0.0005**	$F_{1, 19,}$ 5.539, *P* = 0.190
Thermal comfort (1-9)	7 ± 1 **b c**	7 ± 1	7 ± 1 **b c**	5 ± 1 **a**	5.8 ± 0.4 **a b**	**$F_{2.0, 21.8,}$ 39.926, *P* < 0.0005**	**$F_{2.5, 27.2,}$ 90.197, *P* < 0.0005**	$F_{5.1, 56.6,}$ 1.434, *P* = 0.225
Heart rate (beats.min^{-1})	162 ± 7 **b c**	166 ± 8	162 ± 7 **b c**	135 ± 8 **a**	146 ± 10 **a b**	**$F_{3.3, 36.5,}$ 60.753, *P* < 0.0005**	**$F_{2.4, 26.6,}$ 209.010, *P* < 0.0005**	**$F_{6.0, 65.6,}$ 2.438, *P* = 0.035**

7.4: Discussion

The main findings of the study were 1) RSA values for distance covered per sprint, average velocity and average power were all significantly higher in the evening than the morning (a range of 9 to 10.2 %; See Table 7.1 and Figure 7.4). 1) Increasing morning T_{rec} by active warm-up to exactly those found in the evening at rest, a rise of 0.40°C did not result in RSA becoming equal to evening values (See Figure 7.3). 2) Decreasing evening T_{rec} (and T_m) by passive cool-down to exactly those found in the morning at rest, a drop in T_{rec} of 0.40°C, resulted in a decrease in RSA, similar to, and not statistically different from morning values but significantly lower than the E and M_E sessions (a drop of 8.5 to 15.5 % and 5.8 to 10.4 %; See Figure 7.3 and Table 7.1). Lastly 3), decreasing evening T_m by passive cool-down to exactly those found in the morning at rest, a drop of 0.70°C, resulted in a decrease in RSA to those similar to, and not statistically different from morning values (a drop of 3.4 to 9.5 %; See Figure 7.3). To the best of our knowledge, this is the first study to demonstrate these results with precise modelling of pre-exercise temperature (by removing the individual from the warming or cooling stimulus when the required T_{rec} or T_m was reached). Direct comparisons with other studies are therefore difficult for several reasons:

The end criteria for the active warm-up or passive cool-down in our study were based on reaching resting core or muscle temperatures previously observed in individuals in the morning or evening, respectively. The time taken to reach the required temperatures in this study varied between participants and conditions. When using a warm-up (active or passive) or cool-down of standard duration, as used by others, this produces large differences in core and or muscle temperatures within individuals, and results in over- or undershooting the required value.

There is a distinct lack of previous research investigating the effect of a warm-up or a cooling on RS performance. Further, only one has looked at the effect of these on a non-motorised treadmill. Therefore, differences in type and intensity of task, motivation of participants to perform the task, time on task and subject familiarisation regarding the task to be performed might result in conflicting findings (See Pullinger *et al.*, 2013). In addition, no studies have previously investigated precise modelling of pre-exercise temperature in the morning or evening (by removing the individual from the warming or cooling stimulus when the required T_{rec} or T_m was reached) on RS performance.

This study has minimized methodical issues as far as possible (See Edwards *et al.*, 2013 and Robinson *et al.*, 2013). Some implications of our findings – namely, that raising T_{rec} and T_m in the morning does not obliterate the diurnal variation in muscle performance – are considered below. In agreement with Edwards *et al*. (2013), it was established that there was a trend for diurnal variation in vastus lateralis T_m (depth 3-cm) after standardized 5-min warm-up ($P < 0.0005$) and in agreement with those of T_{rec}. When further analysing only the values after the warm-up for T_m for the M, E and M_E conditions, it was established that diurnal variation in T_m and T_{rec} was present. M conditions were lower than E condition and M_E condition for T_m (0.40°C and 0.48°C). However, only T_{rec} values in E conditions were significantly higher than the M condition (0.37°C). There was no difference between any values for any other condition ($P < 0.05$). Although T_{rec} temperatures in the M_E condition were exactly the same post warm-up to those of resting E values, the muscle temperature response after the M_E was similar to that of E condition. Performance change offsetting diurnal variation of RS performance was not associated with an active warm-up (Bishop, 2003b; Edwards *et al.*, 2013). This lack of beneficial increase in RS performance indicates daily variation of muscular function is not fully explained by T_{rec} and T_m.

Whole-body pre-cooling (passively whether by air, a water bath, water-perfused suits or by showering), lowers T_{rec} and T_m, which has been shown to decrease neuromuscular function (Denys, 1991; Racinais & Oksa, 2010). Previous research has shown that muscle force production of the left knee extensor and jump height is greatly reduced following leg immersion in a cold bath, in a small sample size of four (Bergh & Ekblom, 1979; Sargeant, 1987). It has been shown that T_m has a dose-dependent relationship with cycle sprint performance (T_m in a range of 36 to 41 °C, Asmussen & Bøje, 1945) and lower body muscular performance (T_m in a range of 29.5 to 33.5 °C; Oksa *et al.,* 1996, 1997). In both cases, the rate of deterioration in muscle performance was linked to decreasing muscle temperature (Racinais & Oksa, 2010). This relationship seems to be somewhat dependant on factors such as speed of contraction, type of exercise, time on task and the degree at which body temperature decreases. A recent study looked at whether there was of a positive relationship between temperature and movement velocity. It was found in the E_{MM} condition, the range of T_M after cooling in our study (mean 35.7°C with a range of 34.0 to 36.6°C) was equal to that of the M condition at rest and resulted in RS performance to be significantly lower than the E condition for all variables, but no different from the M and M_E conditions. There was no change in rectal temperature in the E_{MM} condition. Further, following the E_{MR} condition, the range of T_M after cooling (mean 33.8°C with a range of 32.1 to 35.1°C) was significantly lower than those found in any of the other conditions and resulted in a significant decrease in the majority of RSA variables (See Table 7.1) when compared to the other 4 conditions. It has previously been stated that in order for T_M to affect muscle performance (peak torque), a critical threshold of <34.0°C is required (Racinais & Oksa, 2010). However, in regards to RSA, previous findings are disagreed with, as it was found that RS performance was affected from T_m values of 35.7°C and below. The mechanisms regarding how and why subnormal muscle temperatures affect RS performance (muscle performance) have been previously reviewed (See Edwards *et al.,* 2013; Falls, 1972; Racinais & Oksa, 2010).

The results of this study support the view that morning-evening differences in RSA could be explained by peripheral mechanisms dependent on muscle temperature (in accord with the views of others: Guette *et al.*, 2005; Martin *et al.*, 1999) and also other factors determined by the environment and outputs from the body clock (endogenous factors) . Therefore, our findings are in agreement from those previously published where an active warm-up in the morning to reach resting evening T_{rec} levels did not offset diurnal variation and where a passive cool-down in the evening to reach resting morning T_{rec} (and T_m) levels did offset performance to morning levels (Edwards *et al.,* 2013; Robinson *et al.,* 2013). These further highlights the different physiological mechanisms involved in cooling versus warming core and muscle and its effects on RS performance (see review by Racinais & Oksa, 2010).

7.5: Conclusion

In this highly motivated population, raising morning rectal temperature to evening values by active warm-up did not increase RS performance to evening values. However, lowering evening rectal or muscle temperatures to morning values by passive pre-cooling, RS performance decreased to values normally observed in the morning. This further highlights the different physiological mechanisms involved in cooling versus warming core and muscle. Therefore, diurnal variation in RS performance could somewhat be attributed, to diurnal changes in core and muscle temperatures. However, the causal link that has been proposed does not seem simple, but rather due to a multiplicity of components and mechanisms.

Chapter 8: Does raising morning rectal temperature to evening levels or an ‘optimal’ level (38.5°C) offset the diurnal variation in repeated sprint ability on a non-motorised treadmill?

8.1: Introduction

Both previous studies showed that in male participants in a temperate environment (around 17-20°C), on a non-motorised treadmill shows effects of time-of-day with higher values in the evening compared to the morning (a range of 3.3 to 10.2 %; Chapters 6 and 7; Pullinger *et al.,* 2013).

The exact mechanism(s) for this observed diurnal variation in RS performance is still, as yet, unknown but has been attributed to a number of factors (See Pullinger *et al.,* 2013). One factor which has been proposed is the causal link of the temperature rhythm, which might be implicated directly or indirectly, where the higher evening resting core body temperature (~0.8°C in rectal and gut sites, Edwards *et al.*, 2002) and local muscle temperature (≥0.35°C in vastus lateralis at depths of 3 cm, Edwards *et al.,* 2013; Robinson *et al.*, 2013; Pullinger *et al.,* 2013) produce an increase in force-generating capacity of the muscle (Bernard *et al.*, 1998; Coldwells *et al.,* 1994; Giacomoni *et al.,* 2005; Melhim, 1993) and neural function (reduced twitch time course or increase in speed of contraction, Martin *et al.*, 1999). The link between core temperature and performance has been studied predominantly using either active (by means of exercise) or passive (by means of a chamber or water bath) "warm-ups" to increase rectal body temperature in the morning, to approximately, or in some cases precisely, the temperature found in the evening; and then examining whether this increases morning performance to evening levels (See Edwards *et al.,* 2013). When raising morning rectal temperature by active warm-up to values precisely found in the evening, RS performance did not increase to evening values (See Chapter 7). However, exposure to hot environments blunts muscle force diurnal variation in performance, and a brief maximal sprint on cycle ergometer (Racinais *et al.,* 2004; 2009). Interestingly, when combining both internal (i.e., afternoon central temperature) and external passive warm-up in the afternoon, evidence points towards a 'ceiling' effect, whereby muscular force cannot be increased further (Racinais *et al.,* 2005a, 2005b, 2009). However, according to Åstrand & Rodahl (1986), gross muscular performance is 'optimal' at a rectal temperature of ~38.3-38.5 °C with corresponding muscle temperature of ≥ 39.0°C. Previously mentioned investigations that have measured both T_{rec} and T_m have elicited central temperatures ≤ 37.6°C with corresponding muscle temperature of ≤ 38.0°C (Edwards *et al.,* 2013; Robinson *et al.,* 2013). In order to further affirm this 'ceiling' hypothesis, (and also examine the central temperature diurnal variation causal factor theory), passive warm-ups eliciting central temperatures considered 'optimal' are required.

Therefore, the present study is designed to investigate whether modulating morning and evening rectal temperatures by passive warm-ups to 38.5°C will lead to 'optimal' RSA values. A second research aim investigates whether a morning passive warm up which raises rectal temperature to evening values offsets diurnal variation in RS performance.

8.2: Methods

Participants

Twelve field based team sport male athlete participants with age (mean ± SD) 21.0 ± 2.4 yrs, maximal oxygen uptake ($\dot{V}O_2$ max) 59.4 ± 3.8 $mL.kg.min^{-1}$, height 1.78 ± 0.06 m, body mass 79.6 ± 11.5 kg and habitual retiring and waking times 23:42 ± 0:46 h:min and 07:50 ± 0:44 h:min, respectively, were recruited for this study. The protocol was fully explained to the participants and any questions were answered before their participation in the study. For inclusion and exclusion criteria see Section 6.1 Mean chronotype score was 33 ± 5, all intermediate types; F/R score, 43 ± 4; and L/V score 41 ± 5. Only participants who habitually trained during a normal week at any time of the day (morning, afternoon and evening) – and hence had no preference for time of training – were selected.

Research Design

All sessions took place under standard laboratory conditions (lighting, room temperature and humidity, and barometric pressure were 200-250 lux, 21.3 ± 0.4 °C, 32.5 ± 2.5 %, and 757.5 ± 6.1 mmHg, respectively). Before taking part in the main experiment, each participant completed three familiarization sessions each separated by at least 3 days. These sessions ensured that participants were fully familiarized with the RSA protocol utilised in this study. Following the familiarization process, each participant completed four experimental sessions which took place three days apart; a morning and an evening trial (07:30 and 17:30 h; M and E) after a standardized 5-min warm-up at 10 km.h^{-1} on a motorised treadmill (Pulsar, H/P cosmos, Nussdorf-Traunstein, Germany) which were counterbalanced in order of administration; and three further trials – a morning passive warm-up trial (M_E, until evening resting T_{rec} were met), a morning ($M_{38.5}$) and an evening ($E_{38.5}$) passive warm-up trial (until T_{rec} levels reached 38.5°C) that were also administered in a counterbalanced design to minimize any learning or order effects (Edwards *et al.*, 2013). Passive warm-up consisted of participants being immersed in a water bath up to the nipple line.

Protocol and measurements

The participants were free to live a “normal life” between sessions, sleeping at home at night and attending lectures and doing light office work in the day. They were told to refrain from drinking alcoholic or caffeinated beverages and from other training or heavy exertion for the 48 h before the experiments or during them. Participants recorded the type, amount and timing of the food they ate for the period of 24 h before and taken during the day of the experimental session, and were asked to replicate this diet for the days before and during the other experimental conditions. Participants then underwent the same testing procedures as the M and E sessions in Chapter 5. The protocol is given in Figure 8.1.

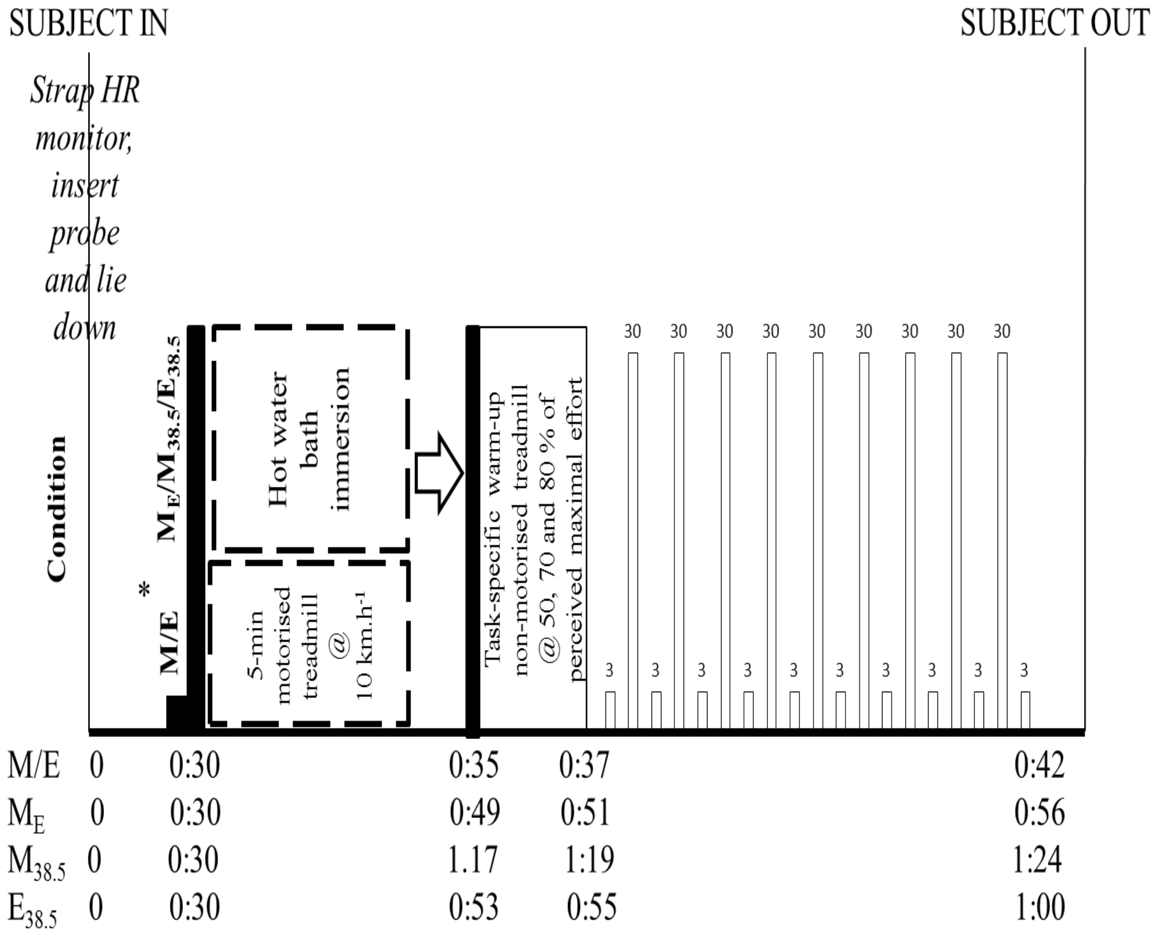


Figure 8.1: Schematic of the protocol for the 5 conditions - the morning (M, M_E, $M_{38.5}$ 07:30 h) and evening (E, $E_{38.5}$ 18:00 h) conditions. Rectal (T_{rec}) and muscle (T_m) temperatures, thermal comfort (TC) and profile of mood (POMS) were measured after the participants had reclined for 30-min at the start of the protocol and again after the warm-ups and prior to the RSA measures. * denotes start of 5-min continuous T_{rec} baseline measurement; the average value of these 5-min of recording was defined as resting T_{rec} and used in the subsequent analysis. Rating of effort (0-10 VAS), TC, HR and ratings of perceived exertion (RPE) were taken throughout the repeated sprint ability (RSA) protocol; vertical black bars indicate muscle temperature (T_m) taken at these points.

Passive warming strategy (M_E, $M_{38.5}$, $E_{38.5}$)

The passive warming protocol used in this study was based upon that of Edwards *et al.* (2013) who attained rectal temperatures of 38.5°C when immersing participants in water at 39.94°C. Further pilot work revealed this temperature to be acceptable in eliciting such an increase in core temperature within one hour. Participants lowered themselves into the purpose built water tank (3 m x 2 m x 1.5 m) until semi-reclined and immersed up to their nipple line. All participants were provided with 150 ml of water to consume every 15-min while immersed. Additionally, body mass measures were obtained pre- and post-immersion (after participants had towel-dried themselves) with previously advised compensatory water intake (Shirreffs & Maughan, 1998). Water temperature was monitored and maintained at 38.74°C throughout the warming period using direct mains water at ~41°C. Participants exited the water tank once their T_{rec} had increased to previous E “resting” values (M_E) or had increased to 38.5°C ($M_{38.5}$, $E_{38.5}$) as measured continuously by rectal thermometry (Grant Instruments Ltd, Shepreth, UK). Participants then towel-dried themselves, T_m was again measured, the rectal probe was removed and participants then commenced the rest of the experimental protocol. The time from completing the passive warming to starting the RSA protocol was ~ 5-min.

Repeated sprint ability (RSA) protocol

Participants then undertook a RSA test (10 sets of 3-s sprints followed by 30-s recovery) on a non-motorised treadmill (Woodway, Force 3.0; Waukesha, WI, USA) in the same environment. The method has already been reported previously (See Chapter 4). Sprint data for peak power (PP), average power (AP), peak velocity (PV), average velocity (AV), distance covered (DC) and percentage decrement in power and velocity were recorded with a commercially designed software program (Pacer Treadmill Software; Innervations, WA, Australia) and used in the subsequent analysis. Fatigue was calculated using the % decrement method as advised by Glaister *et al*. (2008) (section 4.2).

During all sessions, participants performed a task-specific warm-up procedure, developed from pilot work, consisting of three sprints at 50, 70 and 80 % of maximum effort, respectively. The task-specific bursts of activity were brief enough not to cause significant fatigue.

Statistical analysis

The data were analysed by means of the Statistical Package for Social Sciences (SPSS) for Windows (SPSS, Chicago, IL, USA), IBM version 20, using a General linear model with repeated measures (GLM; condition [5 levels]) for all variables of the POMS questionnaire, resting T_{rec}, Pre-RSA T_{rec}, resting T_m and Pre-RSA T_m. A GLM with repeated measures were used for 'resting' T_m and 'Pre-RSA' T_m (condition [5 levels]), and for RPE, rated 'effort', TC, heart rate and all RSA variables (condition [5 levels] x time [10 levels]). To correct violations of sphericity, the degrees of freedom were corrected in a normal way, using Huynh-Feldt ($\varepsilon > 0.75$) or Greenhouse-Geisser ($\varepsilon < 0.75$) values for ε, as appropriate (Field, 2000). Graphical comparisons between means and Bonferroni pairwise comparisons were made where main effects were present. The results are presented as the mean ± the standard deviation (SD) throughout the text unless otherwise stated. Ninety-five percent confidence intervals (CIs) are presented where appropriate. Following convention, the alpha level of significance was set at 5 %.

8.3: Results

Rectal and muscle temperature at rest

Rectal temperature (T_{rec})

There was a significant diurnal variation for T_{rec} with higher resting values in the E *vs.* the M condition (mean difference = +0.49°C; see Table 8.1 and Figure 8.2). The CI for this comparison was 0.32 to 0.64°C ($P < 0.0005$). This variation was consistent and in the expected direction for resting values for T_{rec} for the other conditions where morning values at rest were lower than evening ones (E *vs.* M_E +0.60°C, $P < 0.0005$; E *vs.* $M_{38.5}$ +0.53°C, $P < 0.0005$; $E_{38.5}$ *vs.* M +0.50°C, $P < 0.0005$; $E_{38.5}$ *vs.* M_E +0.61°C, $P < 0.0005$; $E_{38.5}$ *vs.* $M_{38.5}$ +0.54°C, $P < 0.0005$). There was no statistical difference between resting T_{rec} levels in the morning (07:30 h) for M, M_E or $M_{38.5}$ condition; and T_{rec} levels in the evening (17:30 h) for E or $E_{38.5}$ conditions ($P > 0.05$).

Muscle temperature

T_m values at 3 cm were lower in the morning (M, M_E or $M_{38.5}$) conditions than the E condition (-0.69 °C, -0.63°C and -0.60°C; $P < 0.05$) and $E_{38.5}$ condition (-0.74°C, -0.67°C and -0.64 °C; $P < 0.05$). There was no difference between any values for any other conditions ($P > 0.05$; see Table 8.1 and Figure 8.2).

Rectal and muscle temperature after warm-up (before RSA).

Rectal Temperature (T_{rec})

T_{rec} post passive warm-up values for $M_{38.5}$ and $E_{38.5}$ condition were higher than M (+1.60°C), M_E (+1.24°C) and E (+1.15°C) conditions ($P < 0.0005$). Pairwise comparisons showed E values for T_{rec} post warm-up to be higher than M (+0.45°C; $P < 0.0005$) and M_E (+0.09°C; $P = 0.026$) values. Further, M_E values for T_{rec} post warm-up were higher than M condition

(+0.36 °C; *P* < 0.0005). M_E values were exactly the same as resting E condition values and $M_{38.5}$ and $E_{38.5}$ were exactly 38.5°C. “In summary, the protocol produced the changes in T_{rec} (to resting values previously observed in the evening and to 38.5°C) that were required to test the basic research questions.” There was no difference between any values for any other conditions (*P* > 0.05; see Table 8.1 and Figure 8.2).

Muscle temperature

T_m values at 3 cm depth were higher in the $M_{38.5}$ and $E_{38.5}$ conditions than the M (+0.92°C and +1.10°C; *P* = 0.005 and *P* = 0.006, respectively), E (+0.58°C and +0.76°C; *P* = 0.019 and *P* = 0.005, respectively) and M_E (+1.13°C and +1.31°C; *P* < 0.0005) conditions. Pairwise comparisons showed a trend for T_m values post warm up at 3 cm depth to be higher in the E condition than M_E condition (+0.56°C, *P* = 0.072). There was no difference between any values for any other conditions (*P* > 0.05; see Table 8.1 and Figure 8.2).

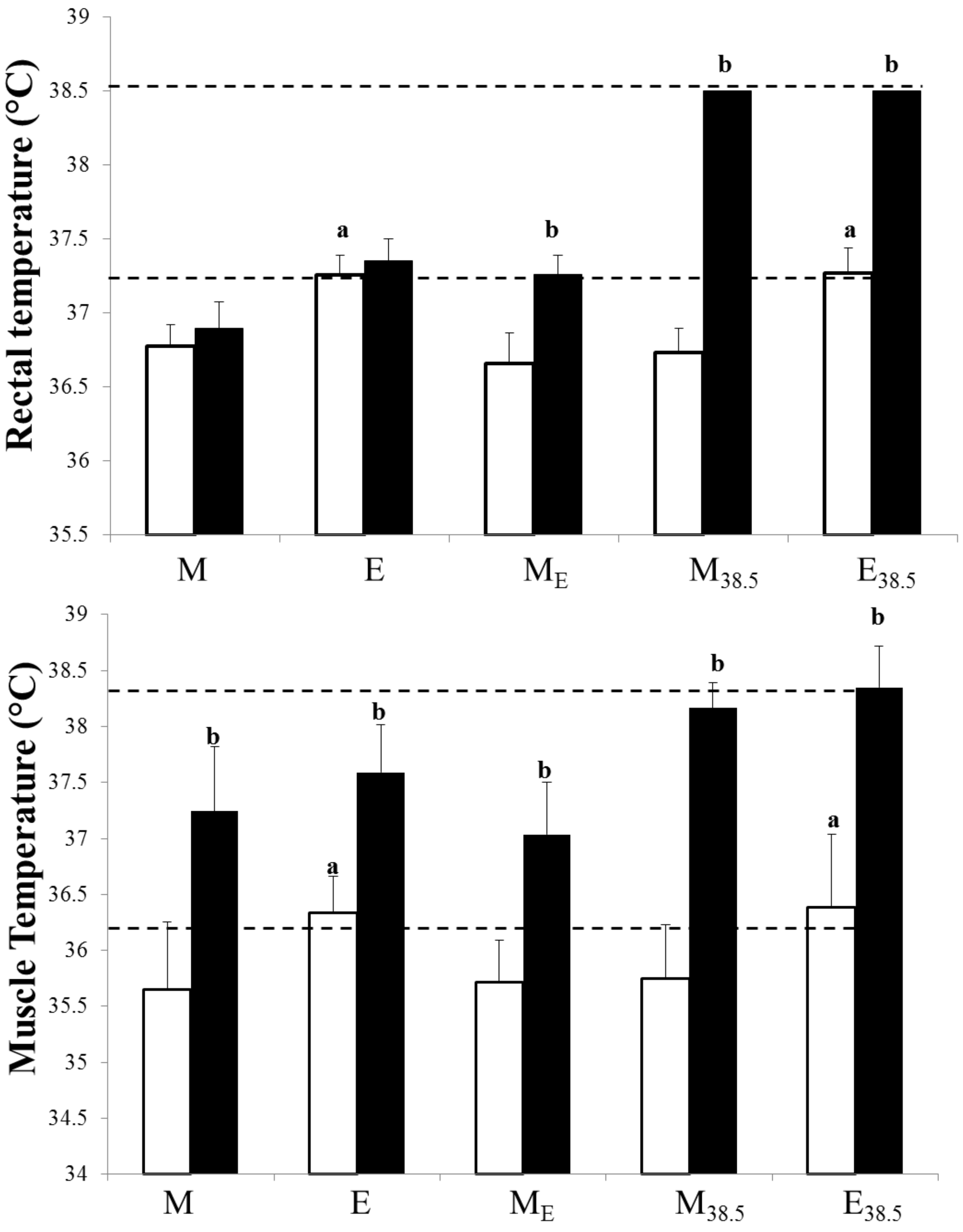


Figure 8.2: Mean and 95% confidence intervals (corrected for between-subject variability) for rectal temperature and muscle temperature (at 3-cm) at rest (□) and post warm-up (■). (a) - Significant diurnal variation in temperature, where the morning values (07:30 h) are lower than the evening values (17:30 h; $P < 0.05$). (b) - Temperature values significantly lower at rest than after the warm-up ($P < 0.05$).

Repeated sprint ability (RSA) measures

Table 8.1 shows the group means (±SD) for all RSA variables recorded in each condition, together with statistical analyses. It is stressed that with regard to the research aim – investigating whether raising morning temperatures to evening values or an optimal level of 38.5°C in the morning or evening would eliminate diurnal variation in muscle performance – the results indicate that such a loss of diurnal change was not found. Thus, there were significant changes in distance covered (DC), peak power (PP), average power (AP), peak velocity (PV) and average velocity (AV; $P < 0.05$; see Figure 8.3 and Table 8.1), but not % decrement for power and velocity, across the 5 conditions ($P > 0.05$). Pairwise analysis showed DC, AP and AV to be higher in the E than the M condition (6.9 to 8.2 %; $P < 0.0005$; See Table 8.1 and Figure 8.3). Values for PP showed a trend for values to be higher in the E than the M condition ($P = 0.080$). Further, DC, AP and AV in the E condition were significantly higher than in the M_E, $M_{38.5}$ and $E_{38.5}$ ($P < 0.05$), except for AP which only showed a trend to be higher in the E condition than the M_E condition ($P = 0.055$). There was a significant sprint effect for all measures of RSA ($P < 0.05$), with values usually decreasing from sprint 1 to 10 (See Figure 8.3). There was no interaction between sprints and condition, values falling from sprint 1 to sprint 10 irrespective of condition ($P > 0.05$).

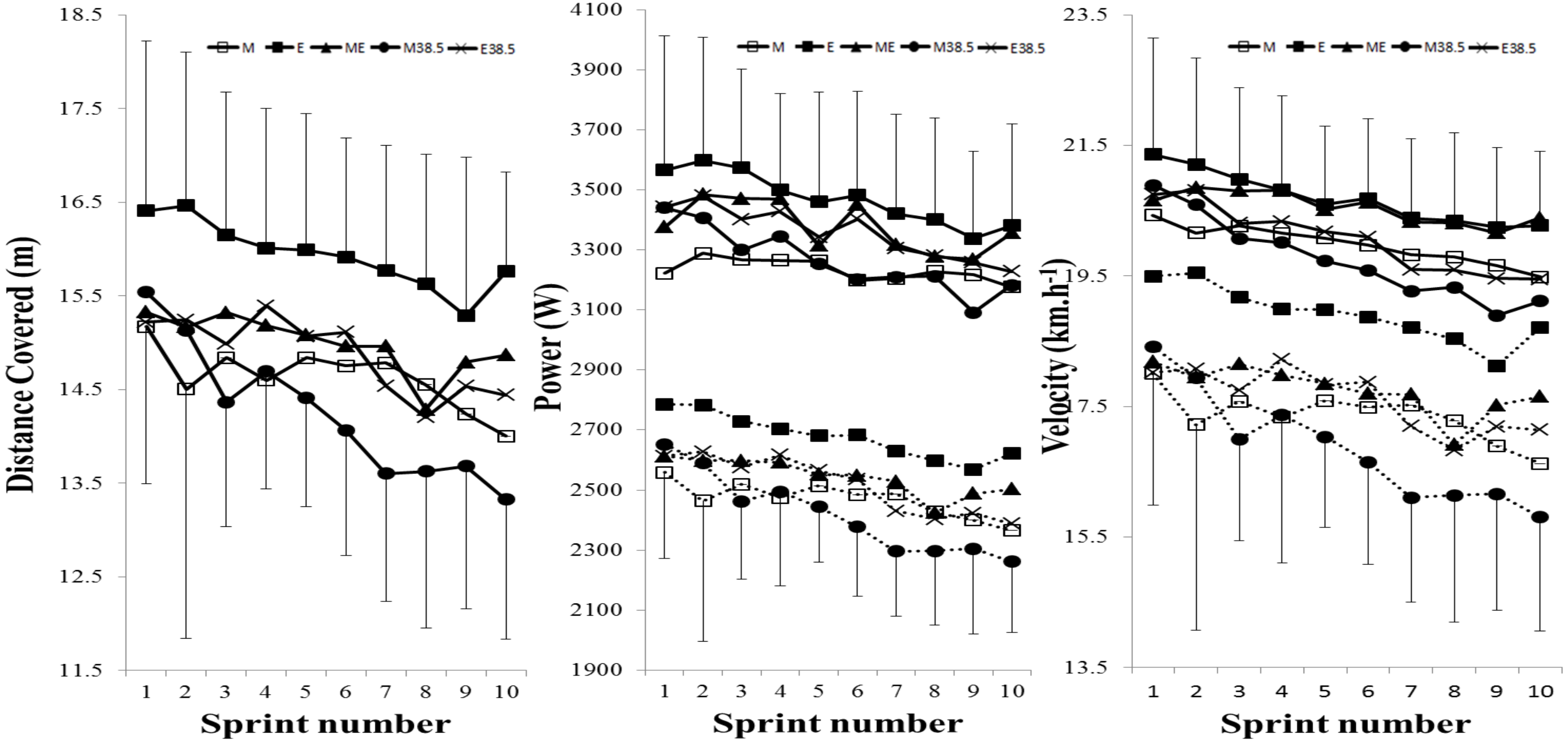


Figure 8.3: Mean and 95% confidence intervals (corrected for between-subject variability) for distance covered, power (peak and average) and velocity (peak and average) from sprint 1 to 10 for morning (□); evening (■); M_E (▲); $M_{38.5}$ (●) and $E_{38.5}$ (×) conditions. Dashed lines indicate averages and full lines indicate peak variables.

Heart rate, rating of perceived exertion (RPE), thermal comfort (TC) and effort levels.

Heart rate and TC (Pre-RSA)

Mean (± SD) values for subjective rating of thermal comfort (TC) and heart rate were significantly different between conditions ($P < 0.0005$; see Table 8.1). Pairwise analysis showed values of TC and heart rate for M and E conditions to be significantly lower and higher, respectively, than M_E, $M_{38.5}$ and $E_{38.5}$ ($P < 0.05$). Further, values of TC and heart rate for M_E conditions were significantly lower and higher, respectively, than $E_{38.5}$ ($P < 0.05$). TC showed a trend to be higher in the $M_{38.5}$ than the M_E condition ($P = 0.059$). There was a significant time effect for both TC and heart rate ($P < 0.0005$), with values increasing from resting to post warm-up. There was an interaction such that profiles for heart rate and TC increased at different rates dependent of condition ($P < 0.0005$).

Heart rate, RPE and TC and effort levels (during RSA)

Mean (± SD) values for heart rate in the M_E were lower during RSA than in the E and $E_{38.5}$ conditions Values for TC during RSA in the $M_{38.5}$ condition were higher than the E and M_E conditions. There was no difference between any values for any other conditions ($P > 0.05$). There was a significant "sprint" effect for RPE, TC and heart rate ($P < 0.0005$), with values increasing from sprint 1 to sprint 10. This rise was not present for self-rated effort ($P > 0.05$), which was rated as 10, or maximal, for each sprint for all conditions and showed the motivation of the participants to perform to the best of their ability in each sprint. There was a significant interaction between condition and sprint for heart rate and RPE ($P < 0.05$), such that that profiles did not increase in parallel linearly over sprint number for all conditions. There was no significant interaction between condition and sprint ($P > 0.05$), such that profiles for TC increased in parallel over sprint number for all conditions.

Resting POMS Questionnaire Variables

Resting subjective measures of ‘vigour’ and ‘fatigue’ differed significantly with condition, where levels of ‘vigour’ were statistically lower at rest in the morning (M, M_E and $M_{38.5}$) than the evening (E and $E_{38.5}$) sessions. Levels of ‘fatigue’ were higher in the $E_{38.5}$ than the M condition only ($P < 0.05$). Pairwise comparisons showed a trend for $E_{38.5}$ levels of ‘fatigue’ to be higher than the M_E and $M_{38.5}$ condition. Resting subjective measures for ‘anger’, ‘calm’, ‘confusion’, ‘depression’, ‘happy’ and ‘tension’ were not significantly different between the conditions ($P > 0.05$).

Table 8.1: Mean (± SD) values for temperatures, RSA variables (for all 10 sprints), subjective measures and blood variables measured in the five conditions. Statistical significance ($P < 0.05$) is indicated in bold, and a trend (where $0.10 > P > 0.05$) is indicated in italics. a - Different than E condition ($P < 0.05$); b – Different than M_E condition ($P < 0.05$); c – Different than $M_{38.5}$ condition ($P < 0.05$); d – Different than $E_{38.5}$ condition ($P < 0.05$).

Variable	**Morning (M)**	**Evening (E)**	**Passive warm-up (M_E)**	**M optimal ($M_{38.5}$)**	**E optimal ($E_{38.5}$)**	**Significance of main effects for condition**	**Significance of main effects for time**	**Significance for interaction**
Resting temp (°C)								
T_{rec}	36.8 ± 0.2 **a d**	37.3 ± 0.1	36.7 ± 0.2 **a**	36.7 ± 0.2 **a**	37.3 ± 0.2 **b c**	**$F_{3.5, 38.0,}$ 71.113, $P < 0.0005$**		
T_m (3cm depth)	35.7 ± 0.6 **a** d	36.3 ± 0.3	35.7 ± 0.4 **a**	35.8± 0.5 a	36.4 ± 0.7 **b c**	**$F_{3.7, 40,3}$ 30.006, $P < 0.0005$**		
T_m (2cm depth)	35.3 ± 0.5 a	35.8 ± 0.4	35.3 ± 0.5 a	35.3 ± 0.5	35.9 ± 0.7 **b**	**$F_{4, 44,}$ 5.632, $P = 0.001$**		
T_m (1cm depth)	34.6 ± 0.6	35.1 ± 0.5	34.7 ± 0.8	34.8 ± 0.7	35.1 ± 0.8	$F_{3.9, 43.1,}$ 1.835, $P = 0.141$		
Post warm-up temps (°C)								
T_{rec}	36.9 ± 0.2 **a b c d**	37.4 ± 0.2	37.3 ± 0.1 **a**	38.5± 0.0 **a b**	38.5± 0.0 **a b**	**$F_{2.3, 24.8,}$ 617.169, $P < 0.0005$**		
T_m (3cm depth)	37.3 ± 0.6 **c d**	37.6 ± 0.4	37.0 ± 0.5 a	38.2 ± 0.2 **a b**	38.4 ± 0.4 **a b**	**$F_{3.4, 37.6,}$ 20.843, $P < 0.0005$**		
T_m (2cm depth)	36.7 ± 0.5 **c d**	37.3± 0.5	37.0 ± 0.3	38.2 ± 0.2 **a b**	38.4 ± 0.4 **a b**	**$F_{3.5, 38.6,}$ 41.306, $P < 0.0005$**		
T_m (1cm depth)	36.0 ± 0.8 b **c d**	36.4 ± 0.7	36.9 ± 0.3	38.1 ± 0.2 **a b**	38.3 ± 0.4 **a b**	**$F_{2.2, 24.1,}$ 43.934, $P < 0.0005$**		
Repeated Sprint Ability								
DC (m)	14.6 ± 1.6 **a**	15.9 ± 1.4	15.0 ± 1.3 **a**	14.2 ± 1.0 **a** b	14.9 ± 1.2 **a**	**$F_{3.5, 38.5,}$ 11.246, $P < 0.0005$**	**$F_{3.2, 34.7,}$ 6.862, $P = 0.001$**	$F_{7.5, 82.6,}$ 1.542, $P = 0.160$
PP (W)	3233 ± 364 a	3472 ± 315	3379 ± 364	3265 ± 405	3357 ± 316	**$F_{4, 44,}$ 4,585, $P = 0.004$**	**$F_{1.7, 18.3,}$ 6.290, $P = 0.011$**	$F_{7.2, 79.5,}$ 1.116, $P = 0.361$
AP (W)	2469 ± 297 **a**	2678 ± 230	2545 ± 250 a	2418 ± 183 **a** b	2518 ± 279 **a**	**$F_{3.1, 34.2,}$ 7.509, $P < 0.0005$**	**$F_{2.4, 26.5,}$ 11.175, $P < 0.0005$**	$F_{7.4, 81.3,}$ 31.569, $P = 0.153$
PV ($km.h^{-1}$)	20.0 ± 1.3	20.7 ± 1.3	20.6 ± 1.3	19.8 ± 1.4	20.1 ± 1.1	**$F_{3.8, 42.2,}$ 2.931, $P = 0.033$**	**$F_{1.3, 15.0,}$ 11.680, $P = 0.002$**	*$F_{5.8, 64.3,}$ 1.924, $P =0.092$*
V_{ave} ($km.h^{-1}$)	17.4 ± 1.9 **a**	18.9 ± 1.6	17.8 ± 1.5 **a**	16.9 ± 1.2 **a** b	17.6 ± 1.4 **a**	**$F_{3.4, 37.9,}$ 11.401, $P < 0.0005$**	**$F_{3.2, 34.6,}$ 6.952, $P = 0.001$**	$F_{7.5, 83.2,}$ 1.577, $P = 0.148$
% decrement in Peak Power	6.5 ± 2.3	6.6 ± 3.1	7.0 ± 2.3	7.9 ± 5.2	7.4 ± 3.7	$F_{3.5, 38.7,}$ 0.378, $P = 0.800$		
% decrement in Peak Velocity	4.0 ± 2.2	3.9 ± 2.4	2.9 ± 1.9	6.0 ± 5.3	5.2 ± 2.4	$F_{1.6, 17.7,}$ 2.728, $P = 0.101$		
Subjective Measures								
Pre RSA								
Thermal comfort (1-9)	5.1 ± 0.6 **b c d**	5.1 ± 0.5	5.8 ± 1.1 **a**	6.5 ± 1.8 **a** b	6.8 ± 1.8 **a b**	**$F_{4.0, 43.5,}$ 51.849, $P < 0.0005$**	**$F_{1, 11,}$ 445.770, $P < 0.0005$**	**$F_{3.2, 35.6,}$ 83.124, $P < 0.0005$**
Heart rate ($beats.min^{-1}$)	105 ± 42 **b c d**	106 ± 41	80 ± 19 **a**	86 ± 24 **a**	90 ± 23 **a b**	**$F_{2.2, 24.1,}$ 88.035, $P < 0.0005$**	**$F_{1, 11}$ 1455.399 $P < 0.0005$**	**$F_{4, 44,}$ 66.436, $P < 0.0005$**
During RSA								
Effort (0-10 cm VAS)	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	n/a	n/a	n/a
RPE (6-20)	14.6 ± 1.3	15.0 ± 1.1	14.4 ± 1.4	15.7 ± 1.3	15.1 ± 1.0	**$F_{4, 44,}$ 3.239, $P = 0.021$**	**$F_{1.4, 14.9,}$ 125.055, $P < 0.0005$**	**$F_{6.0, 65.6,}$ 0.779, $P = 0.021$**
Thermal comfort (1-9)	7.2 ± 0.7	7.1 ± 0.7	6.9 ± 0.9	7.7 ± 0.9 **a c**	7.5 ± 0.8	**$F_{3.1, 25.9,}$ 6.483, $P = 0.001$**	**$F_{2.1, 25.3,}$ 82.258, $P < 0.0005$**	$F_{6.9, 75.8,}$ 1.198, $P = 0.315$
Heart rate ($beats.min^{-1}$)	163 ± 7	169 ± 9	161 ± 10 **a**	165 ± 8	167 ± 10 **b**	**$F_{4, 44,}$ 6.358, $P < 0.0005$**	**$F_{2.4, 26.2,}$ 89.093, $P < 0.0005$**	**$F_{5.6, 61.9,}$ 3.567, $P = 0.005$**

8.4: Discussion

The main finding of the study was that increasing T_{rec} values to 38.5°C in highly motivated individuals [effort scores of ~100 %] by passive immersion (in a water bath at ~40°C) in the morning (increase in T_{rec} ~1.2°C) and evening (increase in T_{rec} ~1.8°C) did not result in RS performance to become equal to, or greater than, evening values (see Figure 8.3 and Table 8.1). It was found that under normal circumstances, RSA values for distance covered, average velocity and average power were all significantly higher in the evening than the morning (a range of 6.9 to 8.2 %; See Figure 8.4 and Table 8.1), similar to those previously reported in the literature (Pullinger *et al.,* 2013). Only one study has previously assessed the 'ceiling' hypothesis (with precise modelling of pre-exercise T_{rec} values by removing the individual stimulus when T_{rec} reached values of 38.5°C both in the morning or evening respectively) and also examined the theory which proposes that the diurnal variation was caused by core temperature ('T_{rec} diurnal variation-causal-factor-theory') in standard laboratory conditions (Edwards *et al.,* 2014). However, they explored short term gross muscular performance - maximal voluntary contraction (MVC) of the quadriceps on an isometric dynamometer (utilizing the twitch-interpolation technique) as opposed to RS performance. It is our view, which other authors have previously voiced (Waterhouse *et al.,* 2005; Souissi *et al.,* 2010; Edwards *et al.,* 2014), that, if an "optimum" core and/or muscle temperature is sought in order to hypothetically overcome diurnal variation, the subject should be warmed up in the morning or evening to values deemed optimal for short term gross muscular performance. It has been well recognised that a passive warm-up of the potentially benefits force development (Asmussen & Boje, 1945; Ball *et al.,* 1999). In a study conducted by Drust *et al*. (2005), individuals completed repeated sprints in a warm environment, preceded by a period of water immersion, which resulted in elevations of core temperature to levels of 39.6°C and T_m to levels of 40.0°C. It was found that such elevations in core temperature seem to negate the beneficial effect of the elevated T_m during repeated sprints. Such elevations in core temperature have been shown to alter and have a detrimental effect on cardiovascular (Gonzalez-Alonso *et al.,* 1995), metabolic (Brooks *et al.*, 1971; Nybo *et al.*, 2002) and physiological (Febbraio *et al.,* 1994; Hancock & Vasmatszidis, 2003) responses to exercise. However, these do not seem to fully explain the decreases in RSA performance and it is believed possible reductions in the central nervous system's drive to the active musculature might be the reason (Drust *et al.*, 2005).

Our primary hypothesis of increasing T_{rec} to 38.5°C in both the M and E conditions, were followed in this study. According to Åstrand & Rodahl (1986), physical performance is 'optimal' at a T_{rec} ~38.3-38.5°C with corresponding T_m of ≥ 39.0°C This would suggest that T_{rec} values of 38.5°C should not result in RS performance to be negatively affected... However, the $E_{38.5}$ condition significantly decreased RSA performance relative to the E condition (despite the raised muscle temperature), suggesting that the higher core temperature may have decreased RSA performance in line with the factors outlined above (e.g., central fatigue). The extended passive warm-up resulted in increases in T_m at 3 cm depth to 38.2°C and 38.4°C.These T_m values were slightly lower than those suggested to be optimal (Åstrand & Rodahl, 1986; Stickler *et al.,* 1990). In spite of these changes, no differences were present between $M_{38.5}$ and $E_{38.5}$ ($P > 0.05$), with diurnal variation between M and E no longer being present. Therefore, the T_{rec} values of 38.5°C which have been suggested to be optimal in the literature (Åstrand & Rodahl, 1986; Stickler *et al.,* 1990) are not, at least for RSA performance. A plateau effect occurred, but not as originally anticipated, such that RS performance values between M and E following passive warm-up to 38.5°C were not different. This indicates that the link between T_{rec} and RSA is not as simple as suggested and this might suggest that a value of 38.5°C in rectal temperature actually causes mild-hyperthermia induced fatigue (Edwards *et al.,* 2014).

A second main finding was that increasing morning T_{rec} to values observed in the evening (producing a rise of 0.6°C in T_{rec} and 1.3°C in T_{m}) in highly motivated participants [effort scores of ~100 %] by passive water immersion (in a water bath at ~40°C) did not result in RSA becoming equal to evening values (see Figure 8.3 and Table 8.1). Our findings disagree with those of Racinais *et al*. (2004a), who exposed participants, to a moderately warm and humid environment (29ºC ± 1 ºC and 74 ± 10 %, respectively) in the morning and evening to determine the diurnal variation in muscular power. Following passive exposure T_{rec} values increased by 0.4ºC (similar to evening T_{rec} values in a normal environment) and 0.2ºC in the morning and evening, respectively. They reported a significant improvement in all muscular power measures following passive exposure in the morning to levels similar to the evening. However, only one study has previously explored the effects of a passive warm-up (with precise modelling of pre-exercise T_{rec} values in the morning by removing the individual stimulus when T_{rec} reached values previously observed in the evening at rest) in standard laboratory conditions (Edwards *et al.,* 2013). They exposed individuals to a warm environment at 35ºC and 45-50 % relative humidity in the morning until T_{rec} reached values previously observed in the evening at rest (producing a rise of 0.6°C in T_{rec}). This did not result in a significant increase in muscle performance. Although these findings agree with the findings of the present study, the protocol they utilised was long (~2 h) and it has been argued that this might partially explain the lack of effectiveness in their warm-up (Edwards *et al.*, 2014). Post warm-up, T_{rec} values were the same as resting evening values. However, T_{m} following passive warm-up did not significantly increase T_{m} values to those observed in the evening condition. Therefore, RS performance change offsetting diurnal variation of RSA was not associated with a passive warm-up. This lack of beneficial increase in RSA indicates that the daily variation in RSA is not fully explained by core temperatures, and this agrees with the view that some other factor(s) contribute to the observed rhythms in RS performance

(Edwards & Waterhouse, 2013). However, direct comparisons with previous studies were difficult for several reasons:

1. Previous protocols utilised are long (~2 h) and it has been argued that this might partially explain the lack of effectiveness in their warm-up (Edwards *et al.,* 2014).
2. An active warm-up with this group of participants was not used, making it hard to compare the different thermal responses between the effects of warm-up on core and/or muscle temperature and how this affects RS performance.
3. A passive warm-up in a hot and humid environment was not used, which challenges the thermoregulatory heat gain/loss mechanisms differently from a hot but non-humid condition (Gagge & Gonzalez, 1996).
4. The end-criteria for the passive warm-ups in our study were previous resting evening rectal temperature or "optimal" temperature (38.5ºC). Using a standard warm-up would result in large individual variation in temperatures, either under- or over-shooting the required value.
5. There is a distinct lack of previous research investigating the effect of a passive warm-up on RS performance. No studies have previously investigated precise modelling of pre-exercise temperature in the morning or evening (by removing the individual from the warming stimulus when the required T_{rec} or T_m was reached) on RS performance.

Taken together the results of this study support the view that morning-evening differences in RSA, where diurnal variations in core and deep muscle temperatures (3 cm) are evident, involve peripheral mechanisms that are not only dependent on muscle temperature (in accord with the views of others: Guette *et al.,* 2005; Martin *et al.,* 1999) but also possibly due to other factors determined by the environment and outputs from the body clock (endogenous factors).

8.5: Conclusion

In this highly motivated population, passively raising morning rectal temperature to evening values, or raising morning and evening T_{rec} to 38.5ºC did not increase RS performance nor offset diurnal variation. In addition, a T_{rec} of 38.5ºC, previously suggested to be optimal for physical performance, did not appear to be optimal for RSA performance. Therefore, T_{rec} (and T_m) cannot fully explain time-of-day oscillations in RSA on a non-motorised treadmill. Although central temperature may provide some endogenous rhythm to RSA, the exact mechanism(s) for a causal link between central temperature and human performance are still unclear, and involve multiple components and mechanisms.

9: Synthesis of findings

9.1: Fulfilment of aims

The aim of this chapter is to summarise each of the experimental chapters within this thesis and further identify how the main aims of this thesis were met and to ultimately provide recommendations for future research based upon this body of work.

The specific aims of this thesis were:

1. To assess the research (over the last thirty years) in the area of RSA and diurnal/circadian rhythmicity to determine how strong the modern evidence is supporting the view that there is an optimal time-of-day for RS performance.
2. To develop a new RSA protocol that conforms to field based team sport time-motion analysis and compare this with a RSA protocol previously utilised in the literature for measures of performance and fatigue and determine the number of trials required to establish high reliability.
3. To investigate the effect of altitude and fatigue on RS performance after a simulated soccer protocol and establish whether the newly created RS protocol was capable of determining a change in RS performance.
4. To investigate the diurnal variation in RS performance.
5. To assess whether modulating rectal or muscle temperature leads to a change in RS performance, determining how much can be attributed to the influence of an endogenous, temperature-dependent component.

Systematic Review

Aim 1 was addressed in Chapter 3, whereby a systematic review of the research literature investigating the relationship between RS performance and time-of-day variation in laboratory investigations in males was conducted. From the six studies which met all the inclusion criteria, it was apparent that the evidence regarding time-of-day variation of peak power in RS performance was scarce and contradictory.

The original intention of Chapter 3 was to conduct a meta-analysis of the published research which had investigated time-of-day variation in RS performance. The initial assessment of the literature in Chapter 3 quickly revealed that the majority of research within this area was more typical of a repeated measures design (M *vs* E) and did not have a control group. Egger *et al.* (1998) stated that a meta-analysis is designed specifically for assessment of randomised controlled trials. As a result, Chapter 3 continued in the form of a systematic review, whereby combining statistical results from studies included would ultimately allow for an overall effect size to be measured and establish evidence relating to diurnal variation based on performance measures in RS performance.

Although six studies met the inclusion criteria for systematic review out of an initial response of 19000, a number of issues within these studies were present when further analysed in closer detail. The main issues being the lack of control of factors which specifically relate to chronobiological investigations and the manner their data was reported, ultimately not allowing for greater interpretation and analysis of studies. Further although whilst three of the six studies represented clearly mentioned that their research design had been randomized, only two of these clearly identified that the protocol employed had been of a counterbalanced design, with others giving no explanation or justification as to why they did not. All the articles considered in this review were of a diurnal structure (i.e. the measures taken were typically within normal waking hours rather than over a 24 h period and occur no earlier than 05:00 h, and no later than 22:00 h).

The main finding of the systematic review was that there is evidence to support a late/early afternoon peak in peak power in RS performance at time points which occur around the peak of the rhythm of core temperature. However, as this was not the case throughout all sprints, there is a clear demand for more rigorous investigations which control chronobiological factors . Further, it is important that participants who take part in such time-of-day studies are fully familiarised with the procedures and that the RSA test utilised is specific to the sport for which inferences are made, such as a RSA protocol consisting of 10 sprints, 3-s in duration with 30-s of passive recovery. The findings of Chapter 3 ultimately influenced Chapter 4 of this thesis, and led to the investigation of a reliable measure of RS performance specific to team sport (football) to be conducted. Further, it also had an influence on all subsequent Chapters regarding experimental design and conducting research to a more rigorous level.

Reliability of the non-motorised treadmill (6-s *vs* 3-s)

Chapter 4 was the first experimental chapter which was built upon the findings of the systematic review and ultimately addressed **the second aim** of this thesis was to develop a new RSA protocol (10 sprints, 3-s in duration with 30-s of passive recovery) that conforms to field based team sport time-motion analysis and compare this with a RSA protocol (10 sprints, 6-s in duration with 30-s of passive recovery) previously utilised in the literature for measures of performance and fatigue and determine the number of trials required to establish high reliability. Further, the mode of exercise was running as this was specific to field based team sports and specific to all of the individuals which took part in the experimental conditions throughout this thesis.

Sixteen field-based team sport male participants completed the assessment of an established RSA protocol (6-s) and a further thirty field-based team sport male participants took part in the newly created sport-specific RSA protocol (3-s). As a result of the findings in Chapter 3, rigorous testing conditions were abided to. Participants were required to participate in five testing sessions. Sprint data for PP, AP, PV, AV, DC, % decrement for PP and PV were used for subsequent analysis. Step 1 of reliability was to assess for systematic bias (through ANOVA) and step two typical percent error (through CV).

The main findings of Chapter 4 were that both RS performance protocols showed high levels of reliability in all measures of RSA bar % decrement for PP and PV. Further, in order to fully familiarise field based team sport individuals for a RSA protocol consisting of 3-s or 6-s sprints in duration, it required 3 sessions. This was to ensure all RSA measures showed no significant differences in the subsequent session(s) in the most important RS performance variables. The findings of Chapter 4 influenced all subsequent Chapters by ensuring that all participants underwent 3 familiarisation sessions to ensure highly reliable data was collected.

Effects of altitude and fatigue on RS performance

The fifth Chapter of this thesis was built directly upon the findings from the previous Chapter and further investigated the sensitivity of the newly developed RSA protocol. T**he aim** of Chapter 5 was to investigate the effect of altitude (as a stressor) and physical fatigue on RS performance and establish whether the newly created RS protocol was capable of determining a change in performance. Chapter 5 was therefore separated into two parts: fatigued *vs* non-fatigued and sea level *vs* 1500-m *vs* 3000-m. A total of ten male University football players took part in this study. Each participant completed a total of three experimental sessions which consisted of two 45-min periods of a football-specific intermittent treadmill fatiguing protocol designed to replicate the forward movement patterns/velocities of a 90-min football game (Drust *et al.*, 2000), followed by the newly established RSA test at 3 levels of altitude.

From this the effects of altitude on the physiological and performance responses to football-specific RS performance were assessed. All participants then performed one more experimental session at sea level which consisted of performing the same RSA test at the same time of day as the other sessions in a non-fatigued state in order to assess the effects of fatigue on RSA performance.

The findings of Chapter 5 were that the newly created RS performance protocol was capable of detecting a negative change following acute altitude exposure and a 90-min football-specific intermittent treadmill fatiguing protocol. The physiological responses associated with RS performance following a football-specific intermittent treadmill fatiguing protocol were incrementally worse at higher altitude in this population of motivated individuals.

Diurnal variation in RS performance

Chapter 6 was built directly upon the combined findings of Chapters 4, 5 and 6 and addressed **the fourth aim** of this thesis by investigating the diurnal and daily variation in RS performance.

A total of 20 field based team sport male participants took part in this study. According to the findings of Chapter 4, each participant completed four familiarization trials. Following this, two experimental sessions took place; a morning and an evening trial, where participants performed the newly created RSA protocol consisting of 3-s sprints. Factors specifically relating to investigations in chronobiological nature, such as counterbalancing, record of light intensity, control of meals, control of room temperature, control of sleep and fitness were all rigorously controlled for.

The main findings of Chapter 6 were that a diurnal variation in team-sport specific RSA on a non-motorised treadmill was present with higher performance for all measures except % decrement in PP and PV in the evening compared to the morning. Further, central temperature may provide some endogenous rhythm to the observed diurnal variation in team-sport specific RSA on a non-motorised treadmill.

The findings of Chapter 6 directly influenced Chapter 7 and 8 of this thesis, with the newly created team-sport specific RSA protocol established as a reliable measure of RS performance and sensitive enough to detect time-of-day variation in measures of RSA.

The causal link of the temperature rhythm with RS performance

Chapters 7 and 8 were built directly upon the combined findings of Chapters 3, 4 and 6, with **the aim** of investigating the effects of modulating rectal or muscle temperature, through a warm-up or cool down leads to a change in RS performance and determining how much can be attributed to the influence of an endogenous, temperature-dependent component. It was also the aim to further establish diurnal variation in RS performance.

Chapters 7 and 8 were ultimately separated, but were conducted to investigate the same aims. Chapter 7 modulated rectal and/or muscle temperatures to investigate whether increasing morning rectal temperature (by an active warm-up) to evening resting values or decreasing evening rectal and/or muscle temperatures (by immersion in a cool bath) to morning resting values leads to a change in RS performance. Chapter 8 examined whether modulating morning and evening rectal temperatures by passive warm-ups to 38.5°C led to ‘optimal’ RSA values. A second research aim investigated whether a morning passive warm up which raises rectal temperature to evening values offsets diurnal variation in RS performance. The main finding for Chapter 7 was that raising morning rectal temperature to evening values by active warm-up did not increase RS performance to evening values. However, lowering evening rectal and muscle temperatures or muscle temperature alone to morning values by passive pre-cooling decreased RS performance to values normally observed in the morning. Chapter 8 found that raising morning rectal temperature to evening values, or raising morning and evening T_{rec} to 38.5ºC (by a water bath) did not increase RS performance nor offset diurnal variation. Both studies concluded that although central temperature may provide some endogenous rhythm to RSA (as found in Chapter 5), the exact mechanism(s) for a causal link between central temperature and human performance are still unclear, and may involve a multiple of components and mechanisms.

9.2: Limitations

Like any published research, limitations were present in all investigations. **Chapter 3** failed to look at abstracts of conferences to see if any studies were presented which are related to diurnal variation in RS performance and more search engines could have been used to perform literature searches.

Chapter 4 used CV and ANOVA calculations to assess reliability. Previous review papers have used other systematic bias and random error measures to assess reliability, such as 95% limits of agreement, standard error of mean and Bland and Altman plots. The difference in population sizes (n=16 *vs.* n=30) could be another issue surrounding this research study, with 16 individuals not sufficient to determine reliability.

Chapter 5 used a laboratory-based football protocol and acute altitude exposure to determine whether the newly created RS performance protocol was sensitive to well-known factors that impair performance. The levels of altitude (1500m and 3000m) may not be representative of levels of where football is played. Further, there are limitations to the activity profile of the laboratory-based soccer-specific intermittent protocol due to the lower frequency of activity changes and the omission of utility movements, and lack of game skills (Drust *et al.,* 2005). It might have also been of interest to examine the psychological effects of the football-specific fatigue and altitude protocol.

Chapter 6 investigated diurnal variation of RS performance. It can be argued that differences were not found in all RS performance measures due to the smaller sample size (n=20), with previous investigations of this chronobiological nature using far larger ones. Ideally, other time points over the course of a 24h period should have been utilised to determine what the rhythm of RS performance truly is and establish where it peaks.

Chapter 7 and 8 examined the effect of passive and active warm-ups and/or cool downs on RS performance. Chapter 7 only examined the effect of active responses upon performance while Chapter 8 investigated the passive effects. Although, it was claimed that individuals warmed up T_{rec} to evening levels or 38.5°C or cooled down T_M and T_{rec}, it is impossible to confirm what the exact temperatures were when participants commenced the RS performance protocol. There was a small window of time between the end of the warm-up or cool-down and the start of the RS performance protocols in ordser to try and obviate any large changes in temperatures. It has been recommended that a rectal temperature of 38.5°C is the optimal temperature for physical performance, although this appears incorrect for RS performance based on the current findings, the optimal temperature for RS performance was not systematically investigated.

9.3: Implications

There has been demand for a specific RS performance protocol to be created which is reliable, sport specific and sensitive. This research has shown that this protocol is reliable, is sport specific, and is sensitive enough to detect changes due to environmental (altitude) or physical effort (fatigue) stressors. What one may ask however, is how any of this actually has any relevance to real life implications and performance? It has successfully been established that RS performance shows diurnal variation, with higher values in the evening than the morning. Therefore, individuals should therefore train for RS performance in the evening as opposed to the morning. This will ensure individuals are always performing at their peak level and result in optimal adaptations for RS performance. An extended active warm-up did not result in any change in RS performance in the morning relative to the evening. There is no need to perform a warm-up longer than is recommended by the guidelines (5 minutes) in the morning prior to RS performance as there is no further improvement.

9.4: Conclusions and recommendations for future research

The published research related to time-of-day variation in RS performance is very scarce and contradictory due the carelessness nature of investigations surrounding control factors. Nevertheless, a sport specific RSA non-motorised treadmill protocol consisting of 10 sprints, 6-s in duration with 30-s of passive recovery is shown to be highly reliable and also displayed a clear time-of-day variation with higher values in the evening. This was in agreement with some of the previously conducted research, but the difference of exercise mode and varying methodologies in the literature make it hard to compare between studies. However, in order to gain a full understanding as to which time-of-day best suits RS performance for field based team sports performance purposes, a study needs to be conducted where RSA measures are taken over six equally spaced time of day time points across the solar 24 h day (a circadian study), with further cosinor analysis undertaken.

With a clear diurnal variation being established in RS performance and the proposed causal link of the temperature rhythm to be one of the mechanisms playing a role in diurnal variation, it was an aim to look at how much RS performance can be attributed to the influence of temperature-dependent component. In agreement with the findings established by Edwards *et al.* (2013) and Robinson *et al.* (2013), albeit both of them looking at muscle force output as opposed to RS performance, it was concluded that although central temperature may provide some endogenous rhythm to RSA, the exact mechanism(s) for a causal link between central temperature and human performance are still unclear, and may involve a multiple of components and mechanisms. As a result, a number of mechanism regarding how and why subnormal (core) and muscle temperatures affect RS performance (muscle performance) have been reviewed previously (Edwards *et al.,* 2013; Falls, 1972; Racinais & Oksa, 2010).

Considering the applied and chronobiological issues that remain to be addressed, further research questions generated from these findings include: 1) What is the optimal rectal/muscle temperature for RS performance 2) Can the athlete in a thermoneutral environment overcome the poorer RS performance normally found in the morning through a thorough and rigorous warm-up that increases core body temperature to a newly deemed optimal level (different to 38.5°C) and increases muscle temperature by a physiologically significant amount but without inducing fatigue? 3) How strong is the endogenous component of RS performance? Studies such as forced desynchronisation, ultra-short sleep-wake cycle and changed sleep times can be utilised. 4) Further research is needed on the effects of temperature modification localized to the exercising limb (using hot baths or diathermery) *vs* temperature modifications to the whole body.

RS performance was significantly worse when performed in a fatigued state and significantly reduced at higher altitude. Future research could investigate these effects on elite individuals and how results might vary with sub-elite individuals. Further, football is characterized by intermittent activity profiles that are multifaceted in its physiological, skill and cognitive demands (Bloomfield *et al.,* 2007; Drust *et al.,* 2000). We have gained an understanding in the effect of this in RS performance; however knowledge surrounding decision making ability, also an important aspect in soccer is small. There is a paucity of literature examining the effect of altitude and/or fatigue on decision making ability. Finally, it would be important to also find out the effects a 90-min football-specific intermittent treadmill fatiguing protocol has on more physiological aspects, such as changes in muscle oxygenation and pulmonary gas exchange.

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Glossary of terms

Adapted from Minors and Waterhouse, 1981

Acrophase: Time of maximum of a fitted cosine curve, given as a delay from a phase reference point. Often expressed in clock time (phase reference, midnight) or degrees (with 360° = a period – q.v. – of a rhythm). The acrophase may also be specified with the phase reference being the point of an environmental cycle (for example, midnight) or the acrophase of another rhythm in the same individual (for example, mid-sleep time).

Amplitude: The measurement of the peak of the rhythm above the mean level estimated by mathematical function (for example, the difference between the maximum and the mesor – q.v. – of a best fitting cosine curve). When a mathematical function has not been fitted to the data, the amplitude usually refers to the range of oscillation – the distance between the crest and through values.

Circadian: relating to biological variations of rhythms (q.v.) with a period (q.v.) of 24 ± 4 h.

Cosinor analysis: The fitting of a cosine curve to a rhythm (q.v.) by the period of least-squares regression. The rhythm amplitude (q.v.) and acrophase (q.v.) are displayed on polar co-ordinates by the length of the angle, respectively, of a directed line shown with a bivariate statistical confidence region.

Cycle: Synonymous with rhythm (q.v.).

Desynchronisation: A steady state in which different rhythms run with different periods (q.v.). When the desynchronisation is between a biological rhythm and an environmental cycle, external desynchronisation is shown. When the desynchronisation is between two biological rhythms in the individual, internal desynchronisation is shown.

Diurnal: Relating to events occurring during the hours of daylight. The opposite of nocturnal (q.v.).

Endogenous (component of a) rhythm: A biological (component of a) rhythm driven by an internal timing mechanism. Such a rhythm will continue self-sustained oscillations in the absence of external rhythms.

Entrainment: The coupling of a biological self-sustained (endogenously generated) rhythm with an external rhythm (Zeitgeber, q.v.), with the result that both rhythms run synchronously with the same period.

Exogenous (component of a) rhythm: Biological (component of a) rhythm driven by an external oscillation. Such a rhythm will not continue oscillating in the absence of the external oscillation.

Free-running rhythm: A biological rhythm which is continually self-sustained oscillations with an inherent frequency at least slightly different from that of known environmental frequencies.

Masking of a rhythm: Alteration of the usual shape and/or parameters of a rhythm due to random or non-random environmental stimuli, persisting for the duration of the stimulus only (without persistent alteration of endogenous rhythm components) *e.g.* change in body temperature after a hot bath. The exogenous component of a rhythm (q.v.).

Mesor: The average value of a rhythmic variable over a single cycle determined as the mean of a fitted cosine curve.

Nycthermal: Relating to the alteration of day and night. A nycthermal rhythm is a biological rhythm observed when the organism is exposed to all the fluctuations associated with the solar day.

Oscillation: Synonymous with cycle and rhythm (q.v.).

Period: The time to complete one cycle of a rhythm. The reciprocal of frequency.

Phase: Instantaneous value of a rhythm at a fixed time. Also used to describe the temporal relationship between two rhythms where a particular phase of one rhythm is consistently temporally related to another rhythm.

Phase reference: Time point chosen by the investigator as reference for estimation of the timing of a rhythm (such as local midnight for circadian rhythms).

Phase response curves: Graphical plot indicating how the amount and duration of a phase shift, induced by a single stimulus, depend on the rhythm's stage at which the stimulus is applied.

Phase-shift: A displacement of a rhythm along the time axis. A phase shift may be qualified as a phase-advance when all aspects of the rhythm occur earlier in time.

Purification techniques: A technique used to remove the exogenous masking effects by a mathematical process.

Rhythm: A sequence of events, which in a steady state repeat themselves in time in the same order and same interval. Synonymous with oscillation and cycle.

Synchroniser: Synonymous with zeitgeber (q.v.), or time-giver.

Zeitgeber: An external oscillation, which is capable of entraining an endogenously generated biological rhythm.