Environmental Stressors and Cooling Interventions on Simulated Soccer Performance

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ENVIRONMENTAL STRESSORS AND COOLING INTERVENTIONS ON SIMULATED SOCCER PERFORMANCE.

By

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A thesis submitted to the University of Bedfordshire, in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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DECLARATION

I declare that this thesis is own unaided work. It is being submitted for the degree of *Doctor of Philosophy* at the University of Bedfordshire. It has not been submitted before any degree or examination in any other University.

I declare that the word count of this thesis is 61,390 words in length from the introduction to the commencement of the bibliography.

Jeffrey Aldous

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PUBLISHED WORK AND CONFERENCE PRESENTATIONS FROM THIS THESIS AND ADDITIONAL COLLABORATIVE PROJECTS

PUBLISHED WORK

- **Aldous, J. W. F.,** Akubat, I., Chrismas, B. C. R., Watkins, S. L., Mauger, A. R., Midgley, A. W., Abt, G. & Taylor, L. (2014). 'The reliability and validity of a soccer-specific non-motorised treadmill simulation (iSPT)', *The Journal of Strength & Conditioning Research,* 28, (7), pp 1971-1980, doi: 10.1519/JSC.0000000000000310.

- **Aldous, J. W. F.,** Chrismas, B. C. R., Akubat, I., Dascombe, B., Abt, G., & Taylor, L. (2015). Hot and hypoxic environments inhibit simulated soccer performance and exacerbate performance decrements when combined. *Frontiers in Physiology, 6.* doi:10.3389/fphys.2015.00421.

CONFERENCE PRESENTATIONS

- **Aldous, J. W.F.,** Akubat, I., Castle, P., Abt, G., Fitch, N., Watkins, S. and Taylor. L. (2012) The Reliability and Validity of a Non-Motorised Treadmill Based Soccer Simulation. 3rd World Conference on Science and Soccer, Ghent, Belgium – Poster Presentation.

- **Aldous, J. W. F.,** Chrismas, B. C., Beel, L., Akubat, I., Dascombe, B. & Taylor, L. (2014) Hot environment mediated decrements in soccer-specific capacity utilising a non-motorised treadmill soccer-specific simulation (iSPT). 4th World Conference on Science and Soccer (WCSS), 2014 Portland, USA. – Oral Presentation.

- Taylor, L., Chrismas, B. C., Beel, L., Akubat, I., Dascombe, B. & **Aldous, J. W. F.** (2014) Hypoxia mediated decrements in soccer-specific capacity utilising a non-motorised treadmill soccer-specific simulation (iSPT). 4th World Conference on Science and Soccer (WCSS), 2014 Portland, USA. – Poster Presentation.

COLLABORATIVE PROJECTS

- Coull, N.A., Watkins, S.L., **Aldous, J.W.F.**, Warren, L.K., Chrismas, B.C., Dascombe, B., Mauger, A.R., Abt, G., and Taylor, L. (2015). Effect of tyrosine ingestion on cognitive and physical performance utilising an intermittent soccer performance test (iSPT) in a warm environment. *European Journal of Applied Physiology* 115**,** 373-386.

- Taylor, L., Fitch, N., Castle, P., Watkins, S., **Aldous, J.W.F.**, Sculthorpe, N., Midgely, A., Brewer, J., and Mauger, A. (2014). Exposure to hot and cold environmental conditions does not affect the decision making ability of soccer referees following an intermittent sprint protocol. *Frontiers in Physiology* 5**,** 1-9.

- Watkins, S.L., Castle, P., Mauger, A.R., Sculthorpe, N., Fitch, N., **Aldous,** J.W.F., Brewer, J., Midgley, A.W., and Taylor, L. (2014). The effect of different environmental conditions on the decision-making performance of soccer goal line officials. *Research in Sports Medicine* 22**,** 425-437.

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Chapter 6. Experiment 3: To Quantify the Influence that Pre-Cooling and Half-Time-Cooling Has on Soccer-Specific Physical and Physiological Performance in a Hot Environment, when utilising iSPT.

LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

%	Percentage
$\%\Delta PV$	Percentage Change in Blood Plasma Volume
$^{\circ}C$	Degrees Celsius
ATP	Adenosine Triphosphate
ATP-PC	Adenosine Triphosphate-Phosphocreatine
$BEAST_{90}$	Ball-Sport Endurance and Sprint Test
Bla	Blood Lactate
$b \cdot min^{-1}$	Beats per minute
CHO-E	Carbohydrate-Electrolyte
cm	Centre metre
CO_2	Carbon Dioxide
CON	Normoxic-Temperate/Control
CST	Copenhagen Soccer Test
CV	Coefficient of Variation
d	Day
FAM	FAM
FIFA	Federation International Football Association
g	grams
GPS	Global Positioning System
h	hour
H^+	Hydrogen Ion
HH	Hot-Hypoxia Condition
HR	Heart Rate
HR_{MAX}	Maximal Heart Rate
HOT	Hot Condition
HSD	High Speed Distance
HYP	Hypoxia Condition
Hz	Hertz
ICC	Intraclass Correlation
iSPT	Intermittent Soccer Performance Test
JD	Jogging Distance
kg	kilogram
km	kilometre
$Km \cdot h^{-1}$	kilometre per hour
LIST	Loughborough Intermittent Shuttle Test
m	metre
MM	Mixed-Method Condition
mmHg	millimetres of Mercury
min	minute
n	number
NMT	Non-Motorised Treadmill
O_2	Oxygen
PACKS	Ice Packs Condition
PCO_2	Partial Pressure of Carbon Dioxide
PCr	Phosphocreatine
PO_2	Partial Pressure of Oxygen
PSA	Peak Speed Assessment
PSS	Peak Sprint Speed

RD	Running Distance
RPE	Rating of Perceived Exertion
RTIPE	Readiness to Invest Physical Effort
RTIME	Readiness to Invest Mental Effort
s	Seconds
$SAFT_{90}$	Soccer-Specific Aerobic Field Test
S_aO_2	Arterial Blood Oxygen Saturation
SD	Sprint Distance
SLURRY	Ice Slurry Ingestion Condition
SMS	Soccer Match Simulation
SSEP	Soccer-Specific Exercise Protocol
SSIET	Soccer-Specific Intermittent Exercise Test
TD	Total Distance
T_{body}	Total Body Temperature
T_{mu}	Muscle Temperature
T_{re}	Rectal Temperature
T_{sk}	Skin Temperature
TS	Thermal Sensation
TSS	Team Sport Simulation
UEFA	Union of European Football Associations
UK	United Kingdom
VT_{2speed}	Second Ventilatory Threshold
$\dot{V}O_2$	Oxygen Uptake
$\dot{V}O_{2max}$	Maximum Oxygen Uptake
VRD	Variable Run Distance
WD	Walking Distance

ABSTRACT

The increasing globalization of elite soccer match-play means that soccer players are likely to compete in hot, hypoxic and hot-hypoxic environments over a season. Soccer match-play studies have identified a marked decline in soccer-specific physical performance in the heat and hypoxia due to increasing body temperatures and a reduction in partial pressure of oxygen (PO_2), respectively. As hot environments are more prevalent in elite soccer match-play, cooling strategies have been assessed within the literature in an attempt to alleviate these heat-induced-decrements. However, utilising a soccer match-play design makes environmental and interventional inferences difficult to ascertain, as a plethora of match factors and adaptive pacing strategies cause high variability in key physical performance measures within soccer match-play. Therefore, the three experiments within thesis aimed to assess the reliability and validity of a non-motorised treadmill (NMT) based soccer-specific simulation [intermittent Soccer Performance Test - (iSPT)], to enable the reliable investigation of environmental stress on soccer performance and the efficacy of pre- and half-time-cooling to attenuate any heat-induced-decrements.

The purpose of experiment 1 was to investigate the reliability and validity of iSPT which utilised a novel speed component called a 'variable run'. This speed component quantified the distance covered at a self-selected speed above the second ventilatory threshold (VT_{2speed}), which attempted to delimit a 'high-intensity' threshold. Twenty male University soccer players completed one maximal oxygen (O_2) uptake ($\dot{V}O_{2max}$) test, three familiarisation (FAM) sessions and one peak speed assessment (PSA) on the NMT, before completing the iSPT twice ($iSPT_1$ and $iSPT_2$). The total distance, sprint distance and high-speed distance covered were $8,952 \pm 476$ m, $1,000 \pm 74$ m and 2156 ± 140 m, respectively. No significant difference ($p>0.05$) was found between repeated trials of the iSPT for all physical performance measures and physiological responses. Reliability measures between $iSPT_1$ and $iSPT_2$ showed good agreement [Coefficient of variation: <4.6%; Intraclass correlation: >0.80] compared with statistical guidelines. Furthermore, the variable run phase showed high speed running capacity was significantly decreased ($p<0.05$) in the last 15 min compared to the first 15 min, showing parity with previous match-play data. Experiment 1 validated the iSPT as a NMT based soccer-specific simulation compared to previous match-play data, and is a reliable tool for assessing and monitoring the physical performance and physiological responses in soccer players.

Successfully completing the aim of experiment 1 facilitated the quantification of hot (HOT), hypoxia (HYP) and hot-hypoxia (HH) mediated decrements on maximal soccer-specific performance in experiment 2. Twelve male University soccer players completed three FAM sessions, one PSA and four randomised crossover experimental trials of the intermittent Soccer Performance Test (iSPT) in normoxic-temperate (CON: 18°C 50% rH), HOT (30°C; 50% rH), HYP (1,000m; 18°C 50% rH) and HH (1,000m; 30°C; 50% rH). Physical performance and its performance decrements, body temperatures [rectal (T_{re}), skin (T_{sk}) and estimated muscle temperature (T_{mu})], heart rate (HR), arterial blood oxygen saturation (S_aO_2), perceived exertion, thermal sensation (TS), body mass changes, blood lactate (Bla) and plasma volume were all measured. Performance decrements were similar in HOT and HYP [total distance (-4%), high-speed distance (~-8%) and variable run distance (~-12%) covered] and exacerbated in HH [total distance (-9%), high-speed distance (-15%) and variable run distance (-15%)] compared to CON. A 4% increase in peak sprint speed was present in HOT compared with CON and HYP and 7% greater in HH. The sprint distance covered was unchanged ($p > 0.05$) in HOT and HYP and only decreased in HH (-8%) compared with CON. Body mass (-2%), temperatures (+2-5%) and TS (+18%) were altered in HOT. Furthermore, S_aO_2 (-8%) and HR (+3%) were changed in HYP. Similar changes in body mass and temperatures, HR, TS and S_aO_2 were evident in HH compared to HOT and HYP, however, Bla ($p < 0.001$) and plasma volume ($p < 0.001$) were only significantly altered in HH. Perceived exertion was elevated ($p < 0.05$) by 7% in all conditions compared with CON. Regression analysis identified that absolute TS and absolute rise in T_{sk} and estimated T_{mu} (r = 0.82, r = 0.84 r = 0.82, respectively; $p < 0.05$) predicted the hot-mediated-decrements in HOT. The hot, hypoxic and hot-hypoxic environments impaired physical performance during iSPT. Future interventions should address the increases in TS and body temperatures, to attenuate these decrements in physical performance.

Experiment 3 of this thesis aimed to identify three pre- and half-time-cooling strategies to attenuate the heat-induced-decrements previously seen in experiment 2. Eight male University soccer players completed four randomised experimental trials of iSPT, three with cooling and one control (i.e. No pre- or half-time cooling: CON). The pre- and half-time-cooling interventions involved were 30-min or 15 min in duration, respectively. Ice slurry ingestion (SLURRY), ice packs (PACKS) covering the upper legs and mixed-methods (MM: PACKS and SLURRY) were utilised as the three cooling interventions. Physical performance and its performance decrements, body temperatures (T_{re}, T_{sk} and estimated T_{mu}), HR, perceived

exertion, TS, body mass changes and Bla were all measured. Compared with CON, both PACKS and SLURRY pre-cooling significantly reduced ($p > 0.05$) central (T_{re}) and peripheral (T_{sk} and estimated T_{mu}) body temperatures prior to iSPT, respectively. However, body temperature and physical performance were unchanged during the first half of PACKS and SLURRY compared with CON. The MM pre-cooling significantly reduced all body temperatures and TS both prior to and during the first half which coincided with an improvement in total distance (+3%), high-speed distance (+4%) and variable run distance (+5%) covered. Half-time-cooling via PACKS, SLURRY and MM had no ergogenic effect ($p > 0.05$) upon physical performance in the second half, compared with CON. The 30 min of mixed-method pre-cooling, via ice packs placed upon the upper legs and ice slurry ingestion, significantly improved simulated soccer performance during the first half, however, future research should identify a valid half-time-cooling strategy to offer further improvements to physical performance in the second half.

The main findings within this thesis revealed that the iSPT showed validity with previous soccer match-play data and strong reproducibility between two tests (iSPT$_1$ and iSPT$_2$). Furthermore, the variable run component showed efficacy as sensitive measure of the decrements in high-speed running capability. As the iSPT demonstrated low test-retest error compared with the statistical guidelines and previous NMT based soccer-specific simulations, any changes to physical performance can be attributed to an intervention and not the variability of the measure, unlike in soccer match-play situations. No difference was seen for all physical performance measures in both HOT and HYP, however, the heat and hypoxic-induced-decrements stem from increasing body temperatures and changes to both S_aO_2 and HR, respectively. Such decrements may have a detrimental effect upon the match outcome. These heat-induced-decrements were attenuated in the first half after 30 min of mixed-methods pre-cooling, however, the 15 min of mixed-methods half-time-cooling did not significantly improve any physical performance measure in the second half. The mixed-method pre-cooling strategy tested within this thesis could go some way in maintaining physical performance during the first half of soccer match-play in hot environments ($\sim30^{\circ}$C). However, future laboratory based research within a controlled environment should look to assess different combinations, times and strategies of cooling which may be applicable to the time constraints associated with elite soccer.

CHAPTER 1: GENERAL INTRODUCTION

1.1. General Introduction

Association football (Soccer) is the most popular football code in the world (Bangsbo, 1994). It is a high-speed, intermittent sport, normally played over 90 min, consisting of two 45 min halves, with a 15 min interval (Russell *et al*, 2011). During match-play, a player's physical performance can be measured by the total distance covered, however, match-play is also interspersed with numerous bouts of high-speed and explosive activity (e.g. sprinting) requiring frequent changes in intensity and power (Buchheit, 2012). Physical performance in soccer is dependent upon the complex interaction of the cardiovascular and muscular systems which support both aerobic and anaerobic energy provision during match-play (Bangsbo, 2014, Mohr *et al*, 2005). For example, the successful integration of high-speed running and straight sprinting are important during soccer match-play, due to their association with game defining moments (Gregson *et al*, 2010) and preceding a goal or assist (Faude *et al*, 2012), respectively.

Research from soccer match-play data has reported a reduction in some physical performance measures and physiological responses in both hot (Ekblom, 1986, Mohr *et al*, 2010, Mohr *et al*, 2012, Nassis *et al*, 2015, Özgünen *et al*, 2010) and hypoxic (Aughey *et al*, 2013, Buchheit *et al*, 2015, Garvican *et al*, 2013, Nassis, 2013) environments. Recent soccer match-play at 43°C has shown that total distance and high-speed distance covered are deteriorated by up to 7% and 26%, respectively, despite no change in sprint distance, heart rate (HR) and blood lactate (Bla) concentration compared to match-play at 21°C (Mohr *et al*, 2012). Furthermore, elite soccer match-play in a hypoxic environment manifests reductions in total distance (3%) and high-speed distance (15%) covered, in tandem with greater perturbations to both the cardiovascular and muscular systems compared to data from sea level environments (Billaut and Aughey, 2013, Buchheit *et al*, 2015, Garvican *et al*, 2013, Nassis, 2013).

The quantification of environmentally-mediated-changes (e.g. heat and/or hypoxia) in elite soccer is important for sport scientists and coaches alike (Billaut and Aughey, 2013, Taylor and Rollo, 2014). Indeed, 8 of the last 19 Federation International Football Association (FIFA) World Cups held since 1930 were located at either low (500-2,000m) or moderate (2,001-3,000m) altitudes (Bartsch *et al*, 2008, Billaut and Aughey, 2013). Recently, the 2010 FIFA World Cup Finals were played at low altitude (1,200-1,700m above sea level) in Johannesburg, South Africa (Nassis, 2013). Furthermore, elite club sides competing in the Union of European

Football Associations (UEFA) Champions and Europa League could also play in altitudes as high as 1,000m above sea level (Taylor and Rollo, 2014). An altitude of 1,000m is categorised as "low" altitude and has been reported to cause decrements on soccer player's aerobic capacity and elongate recovery from high intensity intermittent activity, due to a reduction in partial pressure of oxygen (PO_2) (Bartsch et al, 2008, Billaut and Aughey, 2013, Garvican et al, 2013, Nassis, 2013). Aside from hypoxia, the same elite clubs competing in the UEFA Champions and Europa League may also play in hot environments during both the early and later stages of the season (Duffield et al, 2013). Most recently, the 2014 FIFA World Cup was played in Brazil where temperatures often exceeded 30°C [Maximum: 34°C - (Nassis et al, 2015)]. Furthermore, clubs, national and continental federations have clashed over the issue of a FIFA World Cup during the summer months in Qatar. The result of much discussion and concern is that the 2022 FIFA World Cup in Qatar will now be played in the winter (BBC, 2015). Previous match-play data suggests a hot environment elicits substantial decrements upon soccer performance (e.g. high-speed distance covered), due to increasing body temperatures amongst other multi-factorial mechanisms (Mohr et al, 2010, Mohr et al, 2012, Nybo et al, 2014, Taylor and Rollo, 2014). Finally, examples also exist where extremes of both temperature and altitude (hypoxia) are experienced. Previous research has reported a reduction in steady-state exercise performance via a cycle ergometer within a hot-hypoxic environment, due to a combination of both heat and hypoxia exacerbating the decrements in physical performance compared to their singular effects (Buono et al, 2012, Girard and Racinais, 2014, Van Cutsem et al, 2015). However, no previous match-play data has quantified the combined permutations of heat and hypoxia during soccer match-play. This is important issue due to the frequency of soccer match play, including elite soccer, within environments that combine temperature and hypoxia is only going to increase given the increasing globalisation of soccer (Taylor and Rollo, 2014).

It is notable that recovery from and the ability to complete repeated bouts of high-speed efforts are impaired in both hot and hypoxic environments (Garvican et al, 2013, Mohr et al, 2012) and can influence activities that are central to match outcome (Faude et al, 2012, Gregson et al, 2010). Furthermore, this ability to perform and recover from high-speed exercise is exacerbated when heat and hypoxia are combined (Girard and Racinais, 2014). Therefore, an intervention which can positively influence the physical performance of players during match-play in extreme environments would be useful to coaches and sport scientists (Taylor and Rollo, 2014). A hot environment is more prevalent compared to hypoxia in elite soccer (Taylor and Rollo, 2014). Therefore, a logical interventional strategy could be to increase an

individual's heat storage capacity prior to soccer performance via pre- and half-time-cooling in an attempt to reduce player's body temperatures before the match and at half time (Taylor and Rollo, 2014). Recent soccer match-play data at 30°C identified that 20 min of mixed-method (ice vests, ice towels and ice slurry ingestion) pre-cooling and 5 min half-time-cooling had a moderate effect (d= 0.6) for high-speed distance covered (Duffield *et al*, 2013). However, despite the well-organised and often novel experimental designs utilised by previous environmentally challenging and pre-/half-time-cooling soccer match-play research (Taylor and Rollo, 2014); high variability has been reported between matches for key outcome measures [(e.g. high-speed distance covered - coefficient of variation (CV); 36%)] (Gregson *et al*, 2010). This is likely due to a plethora of match factors including positional role (Bloomfield *et al*, 2007) and tactics (Bradley *et al*, 2011). Furthermore, the adaptive pacing strategies players adopt within extreme environments are likely to exacerbate this variability for key physical performance measures (Buchheit *et al*, 2015, Mohr *et al*, 2012). This could potentially make inferences from environmentally (e.g. hot and hypoxia) mediated changes, and potential interventions (e.g. pre-/half-time-cooling) to attenuate any decrements, difficult to ascertain from soccer match-play data (Taylor and Rollo, 2014).

One solution to this high variability problem is by utilising a laboratory or field based soccer-specific simulation (Taylor and Rollo, 2014). Some fixed distance simulations have limited ecological validity where, the inability to express maximal running capability, are obvious limitations (Small *et al*, 2010). To address such limitations, simulations utilising a non-motorised treadmill (NMT) have been developed, demonstrating good validity compared with soccer match-play data (Abt *et al*, 2003, Oliver *et al*, 2007a). A NMT based soccer-specific simulation can be individualised for each player via their peak sprint speed enabling a true maximal performance capability of a player to be quantified (Abt *et al*, 2003) whilst, still controlling the confounding match factors (Gregson *et al*, 2010). However, the nature of simulations usually means that speed thresholds are set and players are not free to vary their running speed, even if they are capable of running at a faster-speed. In this scenario, a "variable-run" speed component could quantify the distance covered at a self-selected speed above the second ventilatory threshold (VT_{2speed}), by delimiting the 'high-speed' threshold (Abt and Lovell, 2009, Lovell and Abt, 2013). The successful utilisation of the variable run speed component may provide some additional information on high-speed running capability and help display a player's ability or 'willingness' to run above the VT_{2speed} without an external cue.

The aims of this thesis can be summarised as experimental objectives (research questions):

1. To investigate the reliability and validity of a NMT based soccer-specific simulation called the intermittent Soccer Performance Test (iSPT) that involves a novel speed threshold called the variable run and individualised speed thresholds based upon peak sprint speed (Experiment 1).

2. To investigate the changes in simulated soccer performance during hot (30°C; 50% RH), hypoxic (1,000m; 18°C 50% RH), and hot-hypoxic (1,000m; 30°C 50% RH) environments compared to a normoxic-temperate environment (0m; 18°C 50% RH) by utilising the iSPT (Experiment 2).

3. To investigate the impact of three different pre- and half-time-cooling methods; 1) external (ice packs upon the quadriceps and hamstrings); 2) internal (ice slurry ingestion); and 3) mixed methods (internal and external) compared with a control (i.e. no-cooling) as a solution to acquiesce any heat-induced-decrements that were present in experiment 2 (Experiment 3).

CHAPTER 2: LITERATURE REVIEW

In order to investigate the central aims of this thesis, the literature review below has been divided into five main sections. Section one introduces how physical performance measures in soccer match-play are quantified and the match factors that contribute to the variability seen for these measures within the literature. During section two, aerobic and anaerobic energy provision and the physiological responses associated with soccer match-play are introduced. Section three, is divided into three sub-sections: where changes to both physical performance and physiological responses during soccer-specific exercise in hypoxic, hot and hot-hypoxic environments are examined. During section four the potential use of pre- and half-time-cooling to attenuate the expected heat-induced decrements outlined in the previous section is examined. Finally, section five outlines the development of soccer-specific simulations and how a simulation can be utilised to assess changes in soccer performance within extreme environments and the validity of pre-/ half-time-cooling.

2.1. Physical performance in soccer

The following section of this literature review evaluates the research describing the typical physical performance measures seen in soccer match play, the match factors that influence the match-to-match variation for these measures and how a prospective soccer-specific simulation would have to produce similar responses to gain validity.

2.1.1. Physical performance

Soccer is a popular sport that is competed worldwide by millions of participants with different levels of expertise (Bangsbo, 1994, Stolen *et al*, 2005). During soccer match-play, players are required to reproduce skilful actions and maximal or near-maximal efforts in a semi-chaotic fashion (i.e. their frequency and duration are not cyclical in nature), interspersed with a number of recovery periods (Billaut and Aughey, 2013, Bishop and Girard, 2013). Physical performance during soccer match-play is therefore, multi-faceted including endurance, speed, agility, power and muscular strength alongside the ability to repeatedly execute complex motor skills, both of which are often required whilst under pressure and fatigued (Bishop and Girard, 2013, Rampinini *et al*, 2009). Successful match outcomes during match-play are associated with high physical, and technical performance, which in turn, is rewarded with substantial intrinsic (e.g. team selection) and extrinsic (e.g. cup finals) rewards (Faude *et al*, 2012).

Therefore, it is important for coaches, performance analyst's and sport scientists to accurately quantify a player's physical performance (Di Salvo *et al*, 2007, Di Salvo *et al*, 2006).

Over the last 60 years, a plethora of soccer match-play studies have been published to quantify the physical performance of amateur (Van Gool *et al*, 1983), sub-elite (Mohr *et al*, 2003) and elite (Bradley *et al*, 2009) soccer players (Stolen *et al*, 2005). Early methods (1952-1976) to record the physical performance of players during match-play, utilised manual tracking via a scaled plan of the field of play (Winterbottom, 1952) and an audio commentary method (Reilly and Thomas, 1976). However, due to the limitations of these methods a difference in the physical demands (total distance covered: 3.3-8.7 km) was seen between studies. By the 1980's, the use of video cameras was widespread to measure physical performance in soccer match-play (Ekblom, 1986, Withers, 1982), reporting more consistent results (10-12 km), between studies. It is likely the increase in total distance covered could be the result of i) match-play becoming more demanding and ii) increased accuracy in acquiring the data, both of which have likely occurred in semi-scaled chronological manner (i.e. increased and improved respectively across time) (Barnes *et al*, 2014). Furthermore, the aforementioned is not only true for total distance covered, but for many other variables listed in table 2.1, including but not limited to high-speed distance and sprint distance covered.

In recent years, the quantification methods to ascertain a player's physical performance have been developed further through the use of global positioning systems (GPS) (Aughey, 2011), and semi-automated camera systems (e.g. PROZONE) (Di Salvo *et al*, 2006). By utilising these reliable methods, the precision to assess the physical performance of soccer match-play has increased. Therefore, due to the chronological changes and increased precision when measuring a soccer player's physical performance, studies that have not utilised either GPS or semi-automated camera systems to quantify a player's physical performance will be excluded and not included for review and/or critical analysis within this thesis (Table 2.1.).

Table 2.1: The total, high-speed and sprint distance covered of elite soccer players.

Measure	TD Covered (m)	HSD Covered (m)	SD covered (m)
Barnes *et al* (2014)	10,679 – 10,881	890 – 1,157	N/A
Bradley *et al* (2009)	10,714 ± 991	2492 ± 625	255 ± 64
Di Salvo *et al* (2007)	11,393 ± 1,016	602 ± 162	343 ± 136
Di Salvo *et al* (2012)	9,900 – 11,780	721 ± 218	266 ± 123
Gregson *et al* (2010)	N/A	680 ± 141	235 ± 75
Mohr *et al* (2003)	10,860 ± 180	2165 ± 130	262 ± 50
Morgans *et al* (2014)	8840 – 1050	577 – 677	197 – 364
Mugglestone *et al* (2013)	10,163 ± 1 183	1,635 ± 530	320 ± 173

HSD = High Speed Distance; m = metre; SD = Sprint Distance; TD = Total Distance.

Table 2.1 highlights that by utilising these modern methods, it has been estimated that on average, outfield players will cover between 10-12km during a 90 min soccer match (Barnes *et al*, 2014, Di Mascio and Bradley, 2013, Di Salvo *et al*, 2012), with the variation both between and within studies contributed to playing position (Bloomfield *et al*, 2007) and playing standard (Mohr *et al*, 2003), amongst other match factors (Section 2.1.2.). However, due to the intermittent nature of soccer match-play (Gregson *et al*, 2010), players will cover this distance at a variety of speed and intensities, resulting in researchers dividing a player's physical performance into a number of categories (Stolen *et al*, 2005). Table 2.2 shows that a variety of speed thresholds are used within the literature. However, early research failed to use GPS or semi-automated camera systems to record physical performance and instead estimated speed thresholds by stride length (Reilly and Thomas, 1976, Withers, 1982). This resulted in earlier research failing to differentiate between high-speed distance and sprint distance covered so an increased sprint distance covered [(Reilly and Thomas, 1976) - (950 ± 247 m)] was seen compared with modern studies (Table 2.1). Therefore, studies that did not differentiate between high-speed distance and sprint distance covered by the use of speed thresholds by using GPS or semi-automated camera systems were also excluded from analysis in this thesis (Table 2.2.).

Table 2.2: Comparison of the different speed thresholds utilised by soccer match-play studies.

Activity	Mohr *et al* (2003)	Di Salvo *et al* (2007)	Bradley *et al* (2009)	Di Mascio and Bradley (2013)	Carling *et al* (2012)
Standing (Km·h⁻¹)	0 – 5.9	0 – 11	0 – 0.6	0 – 0.6	0 – 0.6
Walking (Km·h⁻¹)	6 – 7.9	0 – 11	0.7 – 7.1	0.7 – 7.1	0.7 – 7.1
Jogging (Km·h⁻¹)	8 – 11.9	0 – 11	7.2 – 14.3	7.2 – 14.3	7.2 – 14.3
Low speed (Km·h⁻¹)	12 – 14.9	11.1 – 14	-	-	-
Running (Km·h⁻¹)	-	-	14.4 – 19.7	14.4 – 19.7	14.4 – 19.7
Moderate speed (Km·h⁻¹)	15 – 17.9	14.1 – 19	-	-	-
High speed (Km·h⁻¹)	18 – 29.9	19.1 – 23	19.8 – 25.1	19.8 – 25.1	19.8 – 25.1
Sprinting (Km·h⁻¹)	>30	>23	>25.1	>25.1	>25.1

Km·h⁻¹ = kilometre performance.

Table 2.3: Comparison of % distances and given intensities reported by soccer match-play studies.

Activity	Mohr *et al* (2003)	Bradley *et al* (2009)	Di Mascio and Bradley (2013)	Carling *et al* (2012)
Standing (%)	18.6	5.6	5.7	1.7
Walking (%)	43.6	59.3	58.8	61.3
Jogging (%)	19.1	26.1	26.2	30.3
Running (%)	9.4	6.4	6.6	6.7
Moderate speed (%)	3.8	-	-	-
High speed (%)	1.9	2.0	2.7	7.1
Sprinting (%)	0.9	0.6	2.1	-
Backwards (%)	2.9	-	-	-

% = Percentage.

Table 2.3 reveals the percentage of time that players spent at each individual speed category. Both standing and walking distance have been reported to make up for 2-19% and 44-61% of the player's physical performance, respectively (Di Mascio and Bradley, 2013, Mohr *et al*, 2003). Furthermore, jogging distance (19-30%) and running distance (6-9%), make up a smaller proportion of a soccer players physical performance compared to walking (Di Mascio and Bradley, 2013, Di Salvo *et al*, 2007). Both high-speed distance (2-7%) and sprint distance (1-2%) are lower in frequency and duration, however, they are central to game defining moments (Gregson *et al*, 2010) and differences between playing standards (Mohr *et al*, 2003). For example, Mohr *et al* (2003) reported that elite soccer players performed 28% more high-speed distance running and 58% more sprinting when compared to sub-elite soccer players. Therefore, both high-speed distance and sprint distance covered have some construct validity as a measure of soccer performance.

The high-speed distance covered from match-play data (Tables 2.1 and 2.3) ranges from 577-2165m (Di Salvo *et al*, 2012, Morgans *et al*, 2014) with each discrete high speed effort covering on average 19.3 ± 3.2 m every 40-70 s during match-play (Di Salvo *et al*, 2007, Mohr *et al*, 2003), making up 2.7-7.1 % (Mohr *et al*, 2003) of a player's activity profile (Bradley *et al*, 2009, Di Mascio and Bradley, 2013). However, table 2.2 reveals that the speed thresholds used to quantify high-speed running by modern methods of physical performance analysis have varied considerably ranging from 13-19.8 $km \cdot h^{-1}$ (Di Salvo *et al*, 2007, Mallo *et al*, 2007, Weston *et al*, 2007). Furthermore, high speed running encompasses sprinting, (100% maximal effort) which is often examined as a separate category (Gregson *et al*, 2010, Mohr *et al*, 2003).

Sprinting is also important during soccer match-play, as a straight sprint often precedes an assist or a goal being scored (Faude *et al*, 2012). Match-play data (Tables 2.1 and 2.3) suggests on average players will sprint 197-320 m during a match (Di Salvo *et al*, 2007, Di Salvo *et al*, 2012, Mohr *et al*, 2003, Rampinini *et al*, 2007), making up to 0.6-2.1 % of a players physical performance (Bradley *et al*, 2009, Di Mascio and Bradley, 2013). During each individual sprinting bout, players will cover between 10–20 m (Bangsbo *et al*, 2006, Buchheit, 2010) occurring every 90-120 s (Mohr *et al*, 2003) and lasting between 2-4 s (Mohr *et al*, 2003, Rampinini *et al*, 2007). Bradley *et al* (2009) suggests that elite players sprint on average 35 times during English Premier League matches, less frequent (Number of sprints = 52) than reported in earlier research (Withers, 1982). This is likely due to the poor precision of speed

thresholds utilised in earlier methods (Di Mascio and Bradley, 2013), highlighting the importance of the exclusion criteria utilised within this thesis.

Therefore, total distance, high-speed distance and sprint distance covered have been assigned as important physical performance measures in soccer match-play and thus, any prospective soccer-specific simulation should approach similar responses to those seen in table 2.1, in order to gain validity.

2.1.2. Variation of physical performance measures in soccer match-play

A plethora of studies, have reported that there is high match-to-match variation in key physical (Gregson *et al*, 2010, Mohr *et al*, 2003, Rampinini *et al*, 2007) and technical (Barnes *et al*, 2014, Liu *et al*, 2016) performance measures during elite soccer match-play due to a number of match factors (Gregson *et al*, 2010), including; positional role (Bloomfield *et al*, 2007), playing standard (Mohr *et al*, 2003) and tactical play (Bradley *et al*, 2011). Mohr *et al* (2003) reported that there was high variability in the total distance covered (CV: 15%) during an Italian Premier League soccer season including domestic cup and UEFA Champions League matches. Rampinini *et al* (2007) also reported a similar variability for high-speed distance covered (CV: 14.4%) throughout an elite soccer season. Whilst these researchers provide a useful insight into the variation of soccer match-play data (Mohr *et al*, 2003, Rampinini *et al*, 2007), the findings from these studies are based over a limited sample size ($n < 30$ players) and number of games ($n < 40$ games). Therefore, longitudinal observations over an extended period of time are required to provide a comprehensive estimation of match-to-match variation in soccer match-play (Gregson *et al*, 2010).

Gregson *et al* (2010) reported that unacceptably large CV's were demonstrated (16 – 30 %) for all high-speed activities, compared to the recommended acceptable level of error [CV: <10%: (Atkinson *et al*, 1999)], in 485 players during 1,140 elite English Premier League soccer matches over 3 seasons, which exceeds all the aforementioned sample sizes employed previously (Mohr *et al*, 2003, Rampinini *et al*, 2007). Therefore, important physical performance measures (e.g. high-speed distance covered) during soccer match-play are susceptible to high variability between matches due to the aforementioned match factors (Lago-Peñas, 2012). This inherent variability means that research requires large sample sizes ($n = 80$) in order to detect a real systematic change in physical performance (Batterham and Atkinson, 2005), making inferences derived from soccer match-play problematic (Taylor and Rollo, 2014). For example, high-speed distance covered has some construct validity (Section 2.1.1.),

but due to the prevalence of the aforementioned match factors, reliably ascertaining a player's maximal physical performance capacity could require a large number of matches (Gregson *et al*, 2010). Thus, minimising these factors is important and could be achieved by using a soccer-specific simulation.

2.2. Energy provision in soccer

The following section of this literature review evaluates the research describing the physiological responses associated with soccer match-play, the source of the energy provision and how a prospective soccer-specific simulation would have to produce similar physiological responses to gain validity.

2.2.1. Aerobic

Aerobic energy provision is important for intermittent exercise, as those who have a superior capacity for aerobic energy transfer during soccer match-play are more likely to excel (Bangsbo, 1993, Bangsbo, 1994). In particular, section 2.1.1 highlighted a high percentage of activities during soccer match-play are completed at low/moderate intensity which could be deemed as aerobic (Bangsbo, 1994). Furthermore, a good aerobic energy system could also contribute in recovery from repeated high speed and intermittent sprint exercise, by increasing the re-synthesis of key phosphates for anaerobic energy provision [e.g. Repeated-sprint activity: (Gaitanos *et al*, 1993)].

The aerobic energy system is highly taxed at an average intensity of 80-90% of their maximal HR (HR_{MAX}) (Ali and Farrally, 1991) and 70-80% of a player's maximum oxygen uptake ($\dot{V}O_{2max}$) (Tonnessen *et al*, 2013), with the magnitude of variation often relative to playing position (Bloomfield *et al*, 2007) and playing standard (Mohr *et al*, 2003, Rampinini *et al*, 2009), amongst other match factors (Section 2.1.2.). Average and peak HR during match-play is around 87.1% and 99.7% of HR_{MAX} respectively, having been reported for second division Portuguese players (Ascensao *et al*, 2008). This indicates that player's exercise at a higher intensity for periods of play but then this in compensated for by a period of lower intensity exercise. Furthermore, Mohr *et al* (2003) revealed that in elite soccer players, high-speed distance covered in the 5 min period immediately following the most intense 5 min period of the match was less than the average for the whole match, supporting the variability in HR during match-play (Ascensao *et al*, 2008).

The need for players to have a well-developed aerobic energy system, is supported by Tonnessen *et al* (2013), who advocated that elite soccer players require a $\dot{V}O_{2max}$ above 60 ml$\cdot$kg$^{-1}\cdot$min^{-1}. However, measuring $\dot{V}O_{2max}$ during match-play can be difficult. Early research by Ogushi *et al* (1993) showed that players failed to cover the same distance when wearing the measurement equipment (Douglas bags), as they did without which has led to the oxygen uptake ($\dot{V}O_2$) being underestimated from match-play. Although newer more portable gas analysers allow more accurate measurements to be obtained, restrictions to successfully measuring $\dot{V}O_2$ with such equipment will always apply (Stolen *et al*, 2005). Therefore, due to these obvious limitations seen during match-play, the measurement of $\dot{V}O_2$ during a soccer-specific simulation is problematic and has not been attempted within this thesis.

Soccer match-play, is interspersed with sequences of short explosive sprints, which are initially operated by anaerobic energy provision via the utilisation and re-synthesis of stored phosphates including phosphocreatine (PCr) and adenosine triphosphate (ATP) (Buchheit, 2010). However, when the duration of anaerobic exercise exceeds 90 s, the skeletal muscles ability to regenerate stored phosphates is severely reduced, placing a greater importance upon aerobic energy provision to continue muscle metabolism via an increase in oxygen (O_2) transport (Gastin, 2001). Gaitanos *et al* (1993) assessed aerobic metabolism over ten 6 s maximal sprints with 30 s recovery on a cycle ergometer. It was reported that by sprint number 10, ATP production was reduced by 64%, due to the curtailment of phosphate re-generation, therefore, energy is mainly derived from an increase in aerobic metabolism. However, the protocol only consisted of one set of ten sprints over a 11 min period, whereas soccer players are often required to complete several bouts of repeated-sprint exercise over a 90 min period (Buchheit, 2010). In turn, the findings by Gaitanos *et al* (1993) are likely to be exacerbated, suggesting that aerobic energy provision has a greater prevalence in repeated-sprint exercise in the latter stages of soccer match-play, highlighting that player's with a greater aerobic capacity could have a larger influence upon the match outcome (Faude *et al*, 2012).

Therefore, the energy provision in soccer match-play is highly dependent on aerobic energy provision and any soccer-specific simulation should produce similar aerobic responses.

2.2.2. Anaerobic

Anaerobic energy provision during match-play cannot be ignored as these often contribute to game defining moments (Gregson *et al*, 2010), such as a straight sprint being the most

frequently observed movement prior to a goal being scored (Faude *et al*, 2012). Furthermore, the earlier review of studies in table 2.1 also showed 7% of match-play activities are at a high-speed, which are dependent upon anaerobic energy provision (Carling *et al*, 2012).

Anaerobic energy provision incorporates two energy systems; i) adenosine triphosphate-phosphocreatine (ATP-PC) system and ii) Anaerobic Glycolysis (Bangsbo, 1993). The ATP-PC system is responsible for intense and explosive exercise movements. During short very intense exercise, the ATP-PC system provides energy for physical performance as O_2 is not apparent so PCr is split to provide inorganic phosphate to donate to adenosine diphosphate and adenosine monophosphate. For example, the ATP-PC system is active whilst sprinting during soccer match-play as these movements are infrequent and often short (2-3.5 s) in duration (Tumilty, 1993). Abt (2002) showed that supplementation with creatine delayed reductions in peak sprint speed highlighting that not only is the ATP-PC taxed, but anaerobic performance is compromised due to a lack of available PCr. During anaerobic glycolysis, there is a lack of O_2, so the breakdown of muscle glycogen sources the formation of pyruvate and then lactic acid, with the latter rapidly converted to Bla, during these moments of anaerobic energy provision (Krustrup *et al*, 2006). Both of these anaerobic systems are relevant during soccer match-play (Tumilty, 1993) and dominate game defining moments (Gregson *et al*, 2010).

Mohr *et al* (2003) observed that elite soccer players perform 150-250 brief intense actions during match-play, a small proportion of the 1,000-1,200 (All discrete movements) seen throughout the duration of match-play (Bangsbo, 1991); hence the 'semi-chaotic' frequency description mentioned in section 2.1 (Bishop and Girard, 2013). The quantification of anaerobic energy provision in soccer match-play has not been studied directly due to obvious experimental limitations (i.e. Access to a player for even minimally invasive procedures and the time sensitive nature of the associated outcome measures). During anaerobic energy provision, Bla is metabolised within active muscles, however, the production of Bla is recorded during low speed movements as it is easier to quantify (Bangsbo, 1994). Several studies between 1980-2006 (Bangsbo, 1994, Bangsbo, 1995, Bangsbo *et al*, 1991, Capranica *et al*, 2001, Ekblom, 1986, Krustrup *et al*, 2006, Smaros, 1980, Smith *et al*, 1993) report a Bla concentration between 2.7 and 15mmol, showing high variability. The high variability in Bla may be caused by the variability in intensity of the exercise in the previous 5 min before the sample was taken (Bradley *et al*, 2009). A recent study on elite soccer players in the English Premier League by Bradley *et al* (2009) reported that high-speed running in the 5 min period immediately after the most intense 5 min period of match-play was significantly decreased,

showing synergy with later research (Di Mascio and Bradley, 2013). Dependent upon what period the Bla sample was derived, it is likely that there may be some variability between samples. Therefore, this shows that anaerobic energy turnover can be increased or decreased during subsequent periods of match-play and this should be approached during any prospective soccer-specific simulation. However, to truly understand the magnitude that the anaerobic system has been taxed via sprint based movements, a Bla sample is required immediately, or as close to as possible, the end of the sprint (Bangsbo, 2014). Match-play does not facilitate such proximity of Bla sample acquisition to sprint termination. Therefore, an appropriately formulated soccer-specific simulation may facilitate such proximity.

In summary, a thorough assessment of the physiological responses and energy provision associated with soccer match-play shows that physical performance during match-play encompasses both aerobic and anaerobic activity (Bangsbo, 2014). Therefore, any well formulated soccer-specific simulation must be able to tax both energy systems.

2.3. Extreme environments and soccer

Sections 2.1 and 2.2 highlight the physical performance measures and physiological responses a well formulated soccer-specific simulation should approach. If these values are approached by a simulation, then it is considered to have good validity and thus, can be used to assess changes in soccer performance. For the purpose of this thesis, a soccer-specific simulation will be used as a valid and reliable tool to answer two further selected research questions. The first of these research questions is to quantify the physical performance measures and physiological responses of soccer players within heat, hypoxic and heat-hypoxic environments relative to appropriate 'control' conditions. This is important due to the high distribution and a number of forthcoming events (e.g. FIFA World Cup 2022 - Qatar) relative to elite soccer that and/or will be played within such extreme environments (Taylor and Rollo, 2014). Therefore, section 2.3 will review the impact that hot, hypoxic and hot-hypoxic environments have upon soccer performance.

Exposure to hot, hypoxic and hot-hypoxic environments during soccer match-play is not only limited to elite soccer players during continental or international tournaments, as sub-elite and recreational soccer players also compete within similar environments. For example, sub-elite soccer matches played in the United Kingdom (UK) during the winter (November-February) can be postponed due to wet and cold weather (e.g. waterlogged or frozen pitches respectively). This can necessitate three matches per week, for a four to six-week period at the end of the

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season (April-May), to 'catch-up' on these postponed fixtures. This can be a problem for sub-elite players as temperatures at the end of the season in the UK can be as high as 25-30°C. Therefore, as recovery time is reduced between matches, fatigue to key physical performance measures (e.g. high-speed distance covered) is likely to be exacerbated (Mohr et al, 2012). Furthermore, sub-elite soccer teams may also compete in an 'end of season tour' to a low altitude country such as South Africa (1,200-1,900m) to play in a number of friendlies. These players are unlikely to be acclimated to low altitudes which can exacerbate performance decrements (Nassis, 2013). Therefore, it seems wise that an acute intervention is needed to offset these environmentally-mediated-decrements in physical performance during soccer match-play, for the sub-elite teams that do not reside at heat and/or hypoxia (n.b. the environmental conditions outlined above are externally valid relative to elite, sub-elite and recreational soccer players). Such an intervention requires reliable empirical data which quantifies said environmental mediated decrements, and provides a tool to test the efficacy of any proposed intervention; as discussed in sections 2.5 an appropriately formulated soccer-specific simulation could achieve both these objectives.

Figure 2.1: The locations of a plethora of FIFA endorsed elite soccer tournaments based at low (500-2,000m) moderate (2,000 m- 3,000 m) or high (3,000 m- 5,000 m) altitudes. A) Copa América 2023: Ecuador (Quilto), 3,000m; B) FIFA U-20 World Cup 2011, Columbia (Bogota), 2,800m; C) FIFA World Cup 2010, South Africa (Johannesburg), 1,200-1,900m; D) UEFA Championships 2008, Switzerland (Sion), 1,000m; E) UEFA Europa League, Norway (Molde FC), 1,000m; F) FIFA U-17 World Cup 2011, Mexico (Mexico City), 2,500m; G) Copa América 1997: Bolivia (La Paz), 3,600m.

2.3.1. Hypoxia and soccer

Several elite soccer stadiums are situated at altitude (Figure 2.1), and these can be sub-categorised as either low (500-2,000m), moderate (2,001-3,000m) and high (3,001m-5,000m) altitudes (Bartsch *et al*, 2008). Low altitudes are more common in elite soccer match-play (Figure 2.1), most notably within the UEFA Champions and Europa League where the world's best elite players and teams can compete in altitudes as high as 1,000m above sea level (Taylor and Rollo, 2014). An altitude as low as 580m above sea level has been reported to exert a significant influence on the aerobic capacity ($\dot{V}O_{2max}$ - ↓5.9 ± 0.9%) of sea level native elite athletes (Gore *et al*, 1997), which as stated in section 2.2.1 is an essential factor to aerobic energy provision (Tonnessen *et al*, 2013) and recovery from repeated sprint activity (Gaitanos *et al*, 1993) during soccer match-play (Bangsbo, 2014). A consensus statement by Bartsch *et al* (2008) explicitly stated that a "*minor impairment*" to aerobic performance is seen during soccer match-play at a low altitude. Therefore, elite soccer players are likely to experience a reduction in physical performance and physiological response during soccer match-play at a low altitude, an environment experienced by teams competing in the UEFA Champions and Europa League.

Most elite soccer teams competing in the UEFA Champions and Europa League mostly reside at sea level, however, despite the impaired aerobic performance experienced by athletes at low altitudes (Bartsch *et al*, 2008), soccer players only have 1-2 d prior to match-play to acclimate to a low altitude environment (Taylor and Rollo, 2014). This is due to the congested calendar elite soccer teams face within season as players are likely to compete in over 50 matches per season, with up to three matches per week (Dellal *et al*, 2013, Odetoyinbo *et al*, 2009). However, Bartsch *et al* (2008) revealed that elite soccer player's competing in a low altitude are recommended to complete 3-5 d acclimation prior to match-play in order to restore a player's physical performance and physiological response close to sea level values. A sufficient acclimation period rarely occurs when competing within the UEFA region and puts a team/player at a greater disadvantage, as they are unprotected against an accelerated onset of fatigue compared to a team which resides at altitude, with the latter acclimatised to hypoxia in excess of Bartsch *et al* (2008) recommendations (Billaut and Aughey, 2013, Gore *et al*, 2008, Taylor and Rollo, 2014). Therefore, section 2.3.1 will review the mechanisms and physiological responses prevalent during an acute hypoxic exposure and how these impact upon the physical performance and physiological response of elite soccer players.

At sea level the standard atmospheric pressure is 760 mmHg and the air we breathe is made up of 20.93% O_2, 78.07% nitrogen and small quantities (~1%) of carbon dioxide (CO_2), argon and helium (West *et al*, 2007). The PO_2 at normoxia is 159 mmHg ($760 \times 0.21 = 159$); which is the value that corresponds to the start of the oxygen cascade (West *et al*, 2007). When exercising or residing at hypoxia, a common misconception is that the percentage of O_2 molecules within the atmosphere is altered (Taylor and Rollo, 2014). However, at sea-level, low, moderate and high altitudes, the relative percentage of O_2 remains at 20.93% (Bartsch *et al*, 2008, Taylor and Rollo, 2014). Instead, as an athlete ascends in altitude, the atmospheric pressure and PO_2 within the atmosphere are progressively decreased (Table 2.4) causing the onset of hypoxia (Taylor and Pouyssegur, 2007). Therefore, a greater area (volume) of air is needed to inspire the same number of O_2 molecules compared to a sea level environment, reducing the total number of O_2 molecules inspired with each breath when at altitude (Taylor and Rollo, 2014).

Table 2.4: The atmospheric pressure and PO_2 from sea level to 5,000m West *et al* (2007).

Altitude (m)	Atmospheric Pressure (mmHg)	PO_2 (mmHg)
0	760	159
1,000	684	143
2,000	608	127
3,000	532	111
4,000	456	96
5,000	380	80

mmHg = Millimetres of Mercury.

Table 2.4 demonstrates that altitudes as low as 1,000m above sea level will reduce the number of O_2 molecules inspired (West *et al*, 2007), and thus, hypoxia-mediated-decrements on soccer performance will ensue (Taylor and Rollo, 2014). Therefore, due to the reduced number of O_2 molecules inspired, the transport of O_2 to the active skeletal muscles is likely to be altered which will compromise aerobic energy provision, whilst hindering recovery from anaerobically dominated activities (Billaut and Aughey, 2013). Evidently, physiological adaptations must occur to allow a 'sea level like' supply of O_2 to the active skeletal muscles during match play (Billaut and Aughey, 2013). However, as discussed, such adaptations are predominately achieved via acclimation (Bartsch *et al*, 2008) but these are not synergistic with typical elite soccer fixture schedules (Taylor and Rollo, 2014). Therefore, it seems wise that an acute intervention is needed to offset the hypoxia-mediated-decrements in physical performance during soccer match-play, for the majority of teams that do not reside at or have

time to, acclimate to hypoxia. Such an intervention requires reliable empirical data which quantifies said hypoxia mediated decrements, and provides a tool to test the efficacy of any proposed intervention; as discussed in sections 2.5 an appropriately formulated soccer-specific simulation could achieve both these objectives.

2.3.1.2. Oxygen cascade
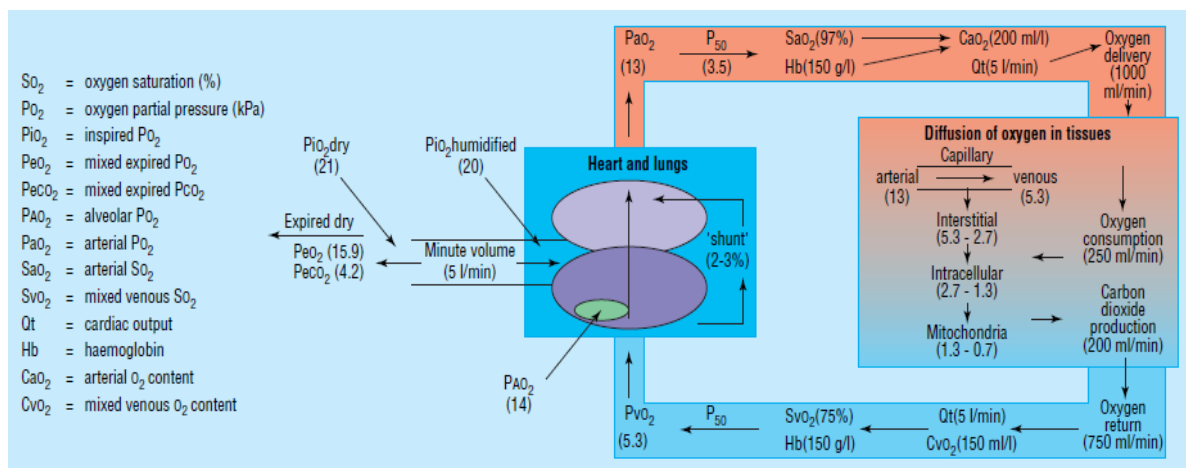
Figure 2.2. Oxygen transport from the atmosphere to the mitochondria (Treacher and Leach, 1998).

The process to transport O_2 from the atmosphere to the cardiovascular and then to the muscular system is known as the oxygen cascade [Figure 2.3: (Treacher and Leach, 1998)]. When O_2 molecules are inspired at sea level during the start of the oxygen cascade, they have a PO_2 of 159mmHg. The O_2 molecules are warmed by the temperature of the body (37°C) and then humidified by water vapour within the trachea, reducing their PO_2 to 150mmHg, before reaching the alveoli (Treacher and Leach, 1998). At a core body temperature of 37°C (rest) the pressure of water vapour in the trachea is 47mmHg, therefore, the inspired PO_2 in the trachea when breathing air at sea level, is 150 mmHg ([760-47] $\times$ 0.21 = 150). Once the O_2 molecules have reached the alveoli, the inspired PO_2 is reduced again to 100mmHg caused by the removal of O_2 by the pulmonary capillaries to supply the alveoli for alveolar ventilation (Treacher and Leach, 1998). During alveolar ventilation, O_2 molecules are moved from the alveolus to the arteries within the lungs in order to be diffused into the systematic arterial blood (Dempsey and Wagner, 1999). The PO_2 within the alveoli provides the driving pressure to allow diffusion of O_2 into the pulmonary capillaries determining the saturation of PO_2 and O_2 within arterial blood (Treacher and Leach, 1998). Treacher and Leach (1998) revealed that a gradient between PO_2 in the alveoli and PO_2 in arterial blood represents the efficiency of the uptake of O_2 from the alveolar gas to the arterial blood in the lungs and this is usually between 5 to 10 mmHg. Thus,

after this period of oxygenation, arterial blood is then diffused from the lungs and into the systematic arteries to activate the working tissues within the skeletal muscle (Treacher and Leach, 1998).

As previously reported, table 2.4 reveals that atmospheric PO_2 is progressively reduced as an athlete ascends in altitude causing a decrease in the availability of O_2 molecules to be transported into the oxygen cascade (West et al, 2007). A reduction in PO_2 will then decrease the number of O_2 molecules to be diffused into the pulmonary and then the systematic arteries, which could compromise the functionality of the cardiovascular and muscular systems, respectively (Treacher and Leach, 1998). Both the cardiovascular and muscular systems work in tandem to provide energy for the physical demands associated with soccer match-play (Bangsbo, 2014). However, due to the prevalence of low altitudes in elite soccer match-play in the UEFA Champions and Europa League, a compromise of these systems may occur, accelerating the curtailment of the physical performance of elite soccer player's (Taylor and Rollo, 2014). As discussed previously the physical performance and physiological impairments (from cardiovascular and muscular system perspectives) due to low altitude environments warrant valid and reliable quantification; within a framework that is externally valid to Elite European soccer-match play. It would seem logical, as discussed in sections 2.5 that such quantification can be achieved by an appropriately formulated soccer-specific simulation.

2.3.1.3. Cardiovascular responses at hypoxia

The cardiovascular responses which occur once an athlete is resting or exercising during an acute hypoxic exposure are well examined, with much of the research assessing the pivotal role the heart has in delivering O_2 to the skeletal muscle (Stembridge et al, 2015a, Stembridge et al, 2015b). To assist the delivery of O_2 to the active muscles at hypoxia, both acute responses and chronic adaptations to the heart will occur, including structure and function (Calbet et al, 2009), depending upon the duration an individual is exposed to the environment (Stembridge et al, 2015b). During an elite soccer season acclimation to hypoxia is unlikely (Taylor and Rollo, 2014). Therefore, only acute cardiovascular responses to hypoxia were reviewed with specificity to elite soccer match-play where possible.

A pivotal factor to consider when examining the cardiovascular response during exercise in a hypoxic environment is the intensity of the exercise bout (Mazzeo, 2008). Ainslie and Burgess (2008) identified that an increase in resting HR initiates an elevation in cardiac output from an altitude of 1,000m above sea level. This occurs via an increase in systemic blood pressure

through the higher activation of arterial chemoreceptors within the pulmonary arteries compared to a sea level environment (Mazzeo, 2008). Sub-maximal exercise in hypoxia causes a similar elevation in both HR and cardiac output compared to an sea level environment at rest (Vogel *et al*, 1967), however, $\dot{V}O_2$ remains unchanged (Mazzeo, 2008). Thus, a higher cardiac output at altitudes of 1,000m and above compared with sea level maintain a similar level of O_2 being transported to and utilised by the working muscles (Mazzeo, 2008). Once maximal exercise is initiated within a hypoxic environment, both lower HR and cardiac outputs are commonly reported when compared to sea level values (Calbet *et al*, 2009, Lundby *et al*, 2001). The reduction in maximal cardiac output at altitude may result from: a regulatory response from the central nervous system, a reduction of maximal pumping capacity of the heart, or a reduced central command (Calbet *et al*, 2009). During soccer match-play the physical demands of soccer players allows fluctuations between rest, low-speed, high-speed and maximal exercise (Stolen *et al*, 2005). Therefore, it is important to consider the mechanistic responses on how HR fluctuates during acute hypoxia compared to normoxia.

During prolonged high-speed intermittent exercise at a low altitude, a significant elevation in HR has been seen during soccer match-play (Garvican *et al*, 2013), a rugby-specific simulation (Hamlin *et al*, 2008) and a cycling intermittent sprint protocol (Turner *et al*, 2014), and is associated with a reduction in high speed and maximal running. An increase in HR during high-speed running has been suggested to occur due to an acute hemodynamic response, which is defined as an increase in the cardiac output of the athlete to counteract a reduction in arterial blood oxygen saturation (S_aO_2) so an adequate delivery of O_2 molecules to the skeletal muscle can occur (Mazzeo, 2008). The up-regulation in maximal cardiac output is likely to be mediated by S_aO_2 sensing mechanisms that adjust the output drive from cardiovascular nuclei within the central nervous system to maintain performance (Piiper and Scheid, 1981). Furthermore, stroke volume has also been shown to decline due to a reduction in plasma volume, venous return and left ventricular filling within the heart (Mazzeo, 2008, Stembridge *et al*, 2015b). This means that the O_2 delivery to the active muscles cannot keep pace with muscle demand, thereby creating a mismatch (Mazzeo, 2008). This mismatch in O_2 delivery from the heart typically manifests itself as a decline in $\dot{V}O_2$ as both altitude and exercise intensity increase, meaning that O_2 delivery to the active muscles is decreased and the given exercise intensity for a task is elevated compared to a sea level environment (Mazzeo, 2008).

Although HR data can be collected throughout match-play, the high variability seen for key physical performance measures, alongside the applied limitations [e.g. moving across time zones - (Fowler *et al*, 2014)] of collecting such cardiovascular data, means that this data lacks reliability (Garvican *et al*, 2013, Taylor and Rollo, 2014). Thus, due to the number of instances of hypoxic situated stadiums within the UEFA Champions and Europa League region outlined in figure 2.1, it is clear that the intensity of soccer performance is increased compared to a sea level environment, disadvantaging teams who are not acclimated (Bartsch *et al*, 2008, Taylor and Rollo, 2014). This means that reliable empirical data of the cardiovascular inferences upon soccer performance at 1,000m above sea level would be advantageous. Therefore, as discussed in sections 2.5 an appropriately formulated soccer-specific simulation could achieve this objective.

2.3.1.4. Exercise induced-arterial-hypoxaemia

Cardiac output, HR and stroke volume are increased during exercise compared to resting values in order to deliver O_2 molecules from the cardiovascular system to the systematic arteries within the active muscles, by binding to the haemoglobin within the red blood cells (Mazzeo, 2008, Stembridge *et al*, 2015a, Stembridge *et al*, 2015b, Wagner, 2008). This equates to blood diffusing from the lungs and then into the systematic arteries at a PO_2 and haemoglobin value of 40mmHg and 75%, respectively (Treacher and Leach, 1998), which correspond to an S_aO_2 of 97-99% (Dempsey and Wagner, 1999). However, section 2.3.1.3 also demonstrates that these cardiovascular responses are elevated by a greater degree during soccer match-play at a low altitude of 1,600m when compared to sea level soccer match-play (Garvican *et al*, 2013). These manifest as a mismatch (Section 2.3.1.3.), reducing the transport of O_2 molecules to the active muscles, contributing to the accelerated onset of fatigue (Saunders *et al*, 2013). This mismatch can be acknowledged by a decrease in the affinity of haemoglobin to O_2 (Teppema and Dahan, 2010, West *et al*, 2007), which is defined as the Bohr Effect as demonstrated in figure 2.3 by the oxyhaemoglobin dissociation curve.
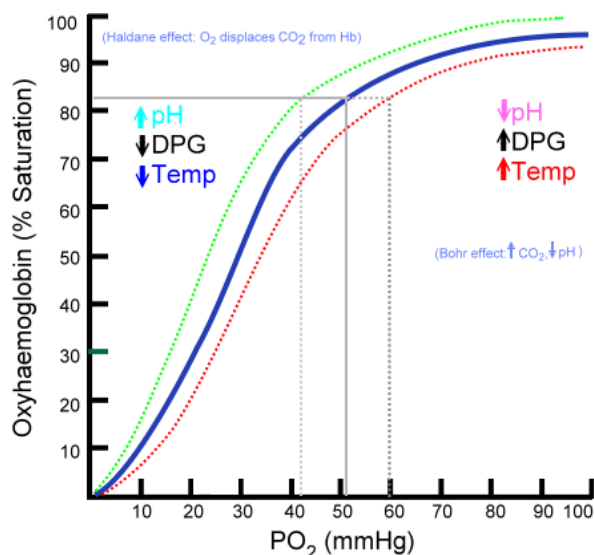
Figure 2.3: Displacement of the oxygen dissociation curve by increases in PCO_2, hydrogen ions (H_+), temperature and 2,3-diphoshoglycerate (DPG).

The oxyhaemoglobin dissociation curve demonstrates a progressive increase in the percentage of haemoglobin bound with oxygen as blood PO_2 increases, commonly referred to as percent saturation of haemoglobin (Beasley *et al*, 2007). However, the Bohr effect (Figure 2.3), shifts the curve to the right meaning that the percentage of haemoglobin bound with O_2 decreases as the PO_2 within the arterial blood increases (Beasley *et al*, 2007). Factors including increases in partial pressure of CO_2 (PCO_2), hydrogen ions (H^+), body temperature, lactic acid (i.e. Bla) and 2,3-diphosphoglycerate in the red blood cells can also decrease the affinity of haemoglobin for O_2 by up to 50% (Teppema and Dahan, 2010, West *et al*, 2007). Furthermore, loading of PO_2 within the lungs is facilitated by the Bohr effect, but the unloading of O_2 molecules to the active tissues is decreased within the skeletal muscle (Bellingham *et al*, 1971). West *et al* (2007) explained that the alterations in O_2 binding is caused by increases in PCO_2 and H^+ at altitude by altering the structure of the haemoglobin molecules. This combination produces an exothermic reaction; however, an increase in body temperatures reverses this reaction (endothermic) by minimising the haemoglobin to O_2 affinity and the transport of O_2 molecules to the active skeletal muscles. Furthermore, when PO_2 is critically low, Bla is built up within the systematic arteries which means that pH of the blood passing through the muscles will be reduced (Jensen, 2004), causing a decrease in S_aO_2 within the systematic arteries (Teppema and Dahan, 2010, West *et al*, 1983).

A reduction in S_aO_2 within the systematic arteries can be defined as an onset of exercise-induced-arterial-hypoxaemia (Dempsey and Wagner, 1999). Dempsey and Wagner (1999) suggested that exercise-induced-arterial-hypoxaemia can be categorised as either mild (95-

93%), moderate (92.9-88%) or severe (<88%), compared to pre-exercise levels. However, this method does not differentiate between sea level and hypoxic environments, as changes in S_aO_2 are acknowledged to occur between sea level and hypoxic situated exercise bouts (Billaut and Aughey, 2013). Therefore, for this thesis: exercise-induced-arterial-hypoxaemia will be defined as a 3% reduction in S_aO_2 compared to sea-level values, as utilised in recent reviews by Billaut and Aughey (2013) and Taylor and Rollo (2014), who investigated the factors responsible for physical performance fatigue and the physiological permutations associated with soccer-specific exercise between normoxic and hypoxic environments.

The onset of exercise-induced-arterial-hypoxaemia can alter the physical performance of athletes via either central (cerebral) or peripheral (muscular) factors alongside a combination of both mechanisms (Billaut and Aughey, 2013, Goodall *et al*, 2014). Central fatigue within hypoxic based exercise can be triggered by a plethora of factors (Goodall *et al*, 2014) including alterations to the central nervous system which can result in; an increase in cerebral de-oxygenation (Chaudhuri and Behan, 2000), and reductions to the neural drive (Amann *et al*, 2007), neuronal activity (Goodall *et al*, 2014) and muscle recruitment (Noakes *et al*, 2001), which cause a reduction in skeletal muscular force and power in acute hypoxia (Smith and Billaut, 2010). However, data collected during prolonged intermittent sprint exercise regarding central fatigue in an acute hypoxic exposure is very limited (Billaut and Aughey, 2013). At altitudes of 1,600-3,600m, a reduction in total work was seen in repeated sprint cycling, due to the 9-fold to 10-fold increase in the de-oxygenation of active muscles during each sprint (Billaut and Smith, 2010, Turner *et al*, 2014). However, research by Smith and Billaut (2012) showed that the de-oxygenation within active muscles during maximal and high-speed exercise bouts are not exacerbated between a hypoxic and normoxic environments. Billaut and Buchheit (2013) revealed that muscle re-oxygenation was attenuated during the recovery periods between maximal sprints by 11%, due to reductions in muscle blood flow (Kime *et al*, 2003) and muscle oxidative enzymes (Puente-Maestu *et al*, 2003). Therefore, as a limited muscle re-oxygenation capacity has been observed during hypoxic exercise, this is likely to be related to the limited muscle O_2 delivery during each sprint, which may have important implications to repeated sprint exercise (Billaut and Buchheit, 2013), which are often central to the game defining moments and the match outcome moments within soccer match-play (Faude *et al*, 2012, Taylor and Rollo, 2014).

PCr re-synthesis is also reduced during the recovery periods between high-speed and maximal exercise bouts (Haseler *et al*, 1999), caused by similar recovery kinetics to the re-oxygenation rate of skeletal muscle (Billaut and Buchheit, 2013). McCully *et al* (1994) reported that an increase in muscle pH resulted in a shift in the oxyhaemoglobin curve causing a reduction in O_2 binding to the haemoglobin which was associated with changes in PCr re-synthesis. As such, the reduction of muscle re-oxygenation and delivery of O_2 to the active muscles is likely to reduce PCr re-synthesis and elongate recovery time between high speed exercise bouts (Garvican *et al*, 2013). In turn, this will hinder the number of high speed and ability to perform constant accelerations, which are central to the match outcome during soccer match-play (Faude *et al*, 2012, Gregson *et al*, 2010), further highlighting the importance of reliable quantification of these exercise types via a soccer-specific simulation. Although PCr re-synthesis was not quantified within this thesis, this can be indirectly measured by Bla as an indication of the Bohr shift (Jensen, 2004). Therefore, utilising a well formulated soccer-specific simulation that facilitates the quantification of Bla post sprint would be advantageous to reliably ascertain the hypoxic induced changes to soccer performance.

Table 2.5: Soccer performance in hypoxia from previous soccer match-play studies

Study	Level	Participants	Design	Physiological	Performance
(**Aughey** *et al*, **2013**)	U20 elite	20 Elite Youth SP Sea Level 'Native' 19 Elite Youth SP 'Altitude Residents'	5 games 2x sea level 3x 3600m	N/A	↓TD and HSD covered in 3 altitude games compared to Sea level in both groups of players.
(**Buchheit** *et al*, **2015**)	U20 elite	7 Elite Youth SP Sea Level 'Native' 6 Elite Youth SP 'Altitude Residents'	5 games 2x sea level 3x 3600m	N/A	HSD In Match-Play and Yo-Yo Reduced After 13 D At 3,600m In Sea Level Residents
(**Garvican** *et al*, **2013**)	U20 elite	20 Elite youth SP	2 matches 1x sea level 2x 1600m	↑8 b·min^{-1} in HR during HSD	HSD (↓15.1%) in G1, TD (↓9.0%) in G1
(**Nassis, 2013**)	Elite Professional players	Outfield Players in the FIFA World Cup 2010 Squads	64 matches 32 teams 0, 660, 1,200-1,400 and 1,401-1,753m	N/A	↓3.1% in TD covered >1200m

U17 = Under 17; U20 = Under 20; SP = soccer players; TD covered = Total distance covered; HSD = High speed distance; b·min^{-1} = beats per min; FIFA = Federation international Football Association

Owing to the large cardiovascular and muscular permutations identified in the previous sections of this literature review, competing at altitude is likely to exacerbate fatigue in soccer match-play compared to a sea-level environment. These can be identified within soccer match-play data (Table 2.5) which demonstrates large inequalities in the probability to win for an away team native to sea level, competing against a home team that reside and have a home ground at moderate/high altitude (Gore *et al*, 2008, McSharry, 2007).

Elite players who are native to a sea level environment have been shown to suffer a marked decline in physical performance when playing soccer in excess of 1,200 m (Nassis, 2013). During the FIFA World Cup 2010 (South Africa), games were played at a variety of "low" altitudes (0 m -1,400 m and 1,401 m-1,753 m). Nassis (2013) revealed that total distance covered by teams was significantly reduced by 3.1% (350 m). However, most interestingly, the player's top running speed and technical ability did not differ across altitudes compared to sea level, suggesting that player's ability to perform at high-speed is maintained during soccer match-play (Nassis, 2013). However, a limitation of the study by Nassis (2013) was that the high-speed distance and sprint distance covered were not quantified despite the knowledge that both these measures are associated with game defining moments (Gregson *et al*, 2010) and most often precede a goal being scored (Faude *et al*, 2012) during soccer match-play, respectively. A further study by Garvican *et al* (2013) collected data in preparation for the 2011 FIFA U 20 World Cup from two soccer matches at altitude (1,600m) compared to a sea level match who were sea level residents. Garvican *et al* (2013) reported a 9% reduction in total distance covered at altitude compared to sea level, supporting earlier soccer match-play research (Nassis, 2013). Furthermore, this study also indicates that high-speed distance covered at altitude for sea level native soccer players is decreased by 15% at 1,600m. Interestingly, despite decrements in the ability to run at high speeds within elite players between 1,200 m – 1,750 m, this is not accompanied by a reduction in technical skill execution success (Nassis, 2013), which is likely to occur due to player's adopting a reduced exercise intensity during match-play.

As shown in table 2.5, soccer matches can occur in altitudes as high as 3,600 m in some FIFA endorsed competitions. Match-play at this altitude also reduces the total distance covered by elite youth soccer players during a match (Aughey *et al*, 2013, Buchheit *et al*, 2015). Aughey *et al* (2013) reported that player's native to sea level (Australia) and altitude (Bolivia) played

two friendly matches at 430 m and three friendly matches at 3,600 m. It was revealed that total distance covered per min and high-speed distance covered per min were reduced in both the Australian and Bolivian soccer players at high altitude (3,600m) when compared to a sea level environment, showing synergy with more recent research (Buchheit *et al*, 2015). However, high altitude locations in elite soccer match-play are lower in frequency during elite soccer and therefore, are only apparent to a small number of the confederations affiliated to world governing bodies (e.g. FIFA).

In May 2007, the high-altitude soccer controversy arose when FIFA introduced a temporary ban on international matches at more than 2,500m above sea level, citing that there were serious health concerns for players and there is an unfair challenge to acclimated teams (Taylor and Rollo, 2014). Yet, despite the series of research detailing the decline in physical performance during match-play at low and high altitudes, in May 2008 the ban was suspended (Gore *et al*, 2008). This section highlights that four soccer match-play studies have attempted to quantify the environmentally mediated decrements at altitude (Aughey *et al*, 2013, Buchheit *et al*, 2015, Garvican *et al*, 2013, Nassis, 2013). However, despite the novelty and well-organised approach of these studies, high variability has been reported for key outcome measures (e.g. total distance and high-speed distance covered) during soccer match-play due to the match factors highlighted in section 2.1.2. Therefore, due to the limitations of match-play data (Section 2.1.2), the lack of time for teams to acclimate to hypoxia (Dellal *et al*, 2013) and the serious doubts that acclimation of anything <13 d has a positive effect on performance (Aughey *et al*, 2013), it is important for the precise decrements in soccer performance to be reliably ascertained (Taylor and Rollo, 2014). The use of a soccer-specific simulation would facilitate such quantification, as proposed elsewhere (Taylor and Rollo, 2014).

Figure 2.4: The locations of a plethora of FIFA endorsed elite soccer tournaments based within hot environments. A) FIFA World Cup 2014 and Copa America 2019, Brazil (Rio de Janeiro), 30-35°C, B) AFC Asian Cup 2015, Australia (Sydney), 30-39°C, C) AFC Asian Cup and FIFA World Cup 2022, Qatar (Doha), 30-45°C, D) FIFA U-17 World Cup, UAE (Abu Dhabi), 30-35°C, E) FIFA U-20 World Cup, Turkey (Istanbul), 25-35°C, F) UEFA Champions League, Spain (Madrid), Portugal (Porto) and Greece (Athens), 25-35°C.

2.3.2. Heat and soccer

Competitive soccer match-play is more commonly played within hot environments (Figure 2.4.) compared with other detrimental extreme environments such as hypoxia [Figure 2.1- (Taylor and Rollo, 2014)]. During the 2012/13 UEFA Champions and Europa League only three stadiums were situated at altitude (800-1,200m) compared with fifteen stadiums that held matches within hot environmental conditions [>25°C (UFEFA, 2013)]. Figure 2.4 reveals the diversity of locations where hot temperatures are encountered within elite soccer match-play, with frequency of exposure determined by fixture scheduling relative to seasonal changes. Temperatures as high as 45°C have been reported to occur in Middle Eastern countries such as Qatar (Matzarakis and Fröhlich, 2014, Mohr et al, 2012). The issue of a FIFA World Cup during the summer months in Qatar was critically discussed across the globe, resulting in the 2022 FIFA World Cup in Qatar being rescheduled to winter months. Although 45°C temperatures could be encountered by players, teams competing in the UEFA region play in temperatures that can, but rarely, exceed 30°C [Figure 2.4 (Taylor and Rollo, 2014)].

Challenging relative humidity's (rH) can be experienced during soccer match-play, which when combined with high environmental temperature increase the environmental stress experienced by players (Maughan et al, 2010). As ambient temperatures increase (above 25°C) decrements in soccer-specific performance indices are also seen in a somewhat linear fashion (Mohr et al, 2012), a similar relationship is seen regarding increasing rH (Maughan et al, 2010) and evidently their combination (increased temperature and rH) exacerbates these performance decrements (Grantham et al, 2010). The rH induced-decrement enhances physiological strain as an athlete's ability to transfer excess heat from their body to the atmosphere is reduced (Cheung, 2009). In line with the described combined environmental challenges, during the 2014 FIFA World Cup in Brazil (Manus) 35°C and 75% rH conditions were seen. Over the 2012/13 UEFA Champions and Europa League seasons, the rH was typically between 35-55% and ambient temperature did not exceed 30°C in the 'hotter' countries within the UEFA region, such as Spain and Greece (Figure 2.4.). Therefore, based on weather conditions seen within previous UEFA Champions and Europa League competitions, the ambient temperature and rH used within relevant experimental designs within this thesis were 30°C and 50 % rH, respectively, to promote external validity.

Unlike hypoxia, high ambient temperatures are most commonly seen in only two stages of an elite club sides season (Duffield *et al*, 2013). For example, a season within the UEFA region

will commence from July to June with both the beginning (July-September) and end (April-June) being the most likely to experience an increase in ambient temperature (Grantham *et al*, 2010). The first example is during pre-season, where each player's training load is increased as coaches/sport-scientists would expect to see the largest training adaptation due to players returning to training with a reduced aerobic and anaerobic fitness compared to the previous season (Bangsbo *et al*, 2006). High ambient air temperatures are also prevalent at the latter stages of the season, when important matches (e.g. cup finals) are often played and residual fatigue in players is increased after a high number of matches in the season (Dellal *et al*, 2013). Similar to hypoxia, acclimation periods prior to competing in matches within a hot environment can take longer than 7 d to maintain a 'temperate like' match-play physical performance (Racinais *et al*, 2012). However, due to the congested calendar soccer teams must contend with, these acclimation periods are unable to implemented (Dellal *et al*, 2013). Therefore, teams that are un-acclimated to a hot environment would be likely to be disadvantaged compared to the home team (As they reside within a hot climate and will likely be acclimatised) when matches are played in the heat (Taylor and Rollo, 2014).

Section 2.1.2 highlights that utilising a soccer match-play design to ascertain the environmentally mediated decrements of elite soccer players within a hot environment is problematic due to the prevalence of a plethora of match factors (Gregson *et al*, 2010). Therefore, to ascertain these hot environment mediated decrements with greater certainty, a soccer-specific simulation should be utilised to increase the understanding of the changes to physical performance and physiological responses associated with soccer-specific exercise within a hot environment.

2.3.2.1. Mechanisms of heat transfer

The fundamental issues regarding soccer within hot environments is a compromised heat exchange process (Grantham *et al*, 2010). Simply, the heat produced by the body has nowhere to go and thus, body temperatures increase (Cheung *et al*, 2000). If these increases are left to progress unchecked, premature exercise fatigue occurs compared to matched exercise within a temperate environment (Nybo *et al*, 2014). Ultimately, if body temperatures continue rise serious pathophysiologies can develop, including exertional heat illness and death (Armstrong and Maresh, 2003). The human body is able to regulate this heat production by maintaining a thermal balance between heat gain and heat loss, to achieve a stable core body temperature of ~37°C at rest (Havenith, 1999). Ultimately, basic functions of the body are maintained via heat

loss mechanisms which dissipate the excess heat gained from the environment (Havenith, 1999). Heat exchange between the body and environment occurs through four main pathways (conduction, convection, evaporation and radiation) in which heat can be gained or lost, and these are defined via a simple heat balance equation, derived from the first law of Thermodynamics as follows:

$$S = M - (\pm W) \pm E \pm R \pm C \pm K$$

S = rate of heat storage, M = metabolic heat production, W = rate of work, E = rate of evaporation, R = rate of radiation, C = rate of convection and K = rate of conduction

Figure 2.5 The heat balance equation (Cheung, 2009)

Heat gain occurs via heat being transferred from active muscles to the surrounding skin and through to the body's trunk and cerebral area via the circulating blood (Crandall and Gonzalez-Alonso, 2010). This process regulates the blood flow within the body, orchestrated by the sympathetic nervous system through vasoconstrictor and vasodilator mechanisms (Charkoudian, 2010). When exercising in a hot environment, vasodilation is activated to divert blood flow closer to the skin so heat has a shorter distance to dissipate down the thermal gradient (Cheung and Sleivert, 2004). This activation of vasodilation is stimulated to increase cutaneous vascular conductance as well as skeletal muscle and skin blood flow (Edholm *et al*, 1957, Kellogg *et al*, 1995). However, high ambient temperatures are particularly problematic for exercising humans, including soccer players (Nybo *et al*, 2014). Humans have poor efficiency when locomotion facilitated by the various energy systems is considered (Maughan, 2010). Indeed, 75-80% of energy production is actually metabolic heat with only a 20% efficiency relative to the work (e.g. movement) that is required (Maughan *et al*, 2010). This metabolically excess heat requires loss from the body's surface (Nadel, 1988). However, when the ambient temperature exceeds skin temperature, heat inevitably is gained within the body's core due to metabolic heat production from the working skeletal muscle (Maughan *et al*, 2010). This is problematic, given human core temperature is restricted to relatively small fluctuations from rest (~37°C) of ~2-3°C (39-40°C). If the heat balance equation (Figure 2.5) becomes unbalanced in favour of heat gain during soccer match-play, core temperature will accelerate beyond 2-3°C past resting values which can mediate reduced physical performance [Namely accelerated fatigue (Mohr *et al*, 2012)] and negatively affect match-play characteristics for fans (Taylor and Rollo, 2014). The body therefore, activates the previously defined heat loss pathways (Figure 2.5.) in an attempt to dissipate this heat production (Havenith, 1999).

Within soccer match-play evaporation can account for high rates of heat dissipation if humidity is low, occurring through the vaporisation of sweat from the skins surface by cooling the blood (Brotherhood, 2008). When water vapour pressure in the air is increased, evaporation via sweat is impaired compared to a hot dry environment. In dry heat, evaporation can account for up to ~98% of heat transfer, whereas in humid environments, it only accounts for ~20% (Armstrong and Maresh, 2003). Additionally, wearing clothing and protective garments will further compromise evaporative heat loss by impeding the natural movement of water vapour, consequently exacerbating the rate of heat gain to potentially dangerous levels (Cheung, 2009, Havenith, 1999).

2.3.2.2. Heat-induced-decrements

If a significant increase in body temperatures is left unchecked during soccer-specific exercise in a hot environment, although highly individualised, this can elicit the onset of premature fatigue upon physical performance and the onset of exertional heat illnesses which could become fatal (Cheung and Sleivert, 2004, Grantham *et al*, 2010, Nybo *et al*, 2014).

Figure 2.6. Integrative model incorporating cardiovascular, respiratory, CNS and peripheral factors that may contribute to heat induced decrements during physical performance (Nybo *et al*, 2014)
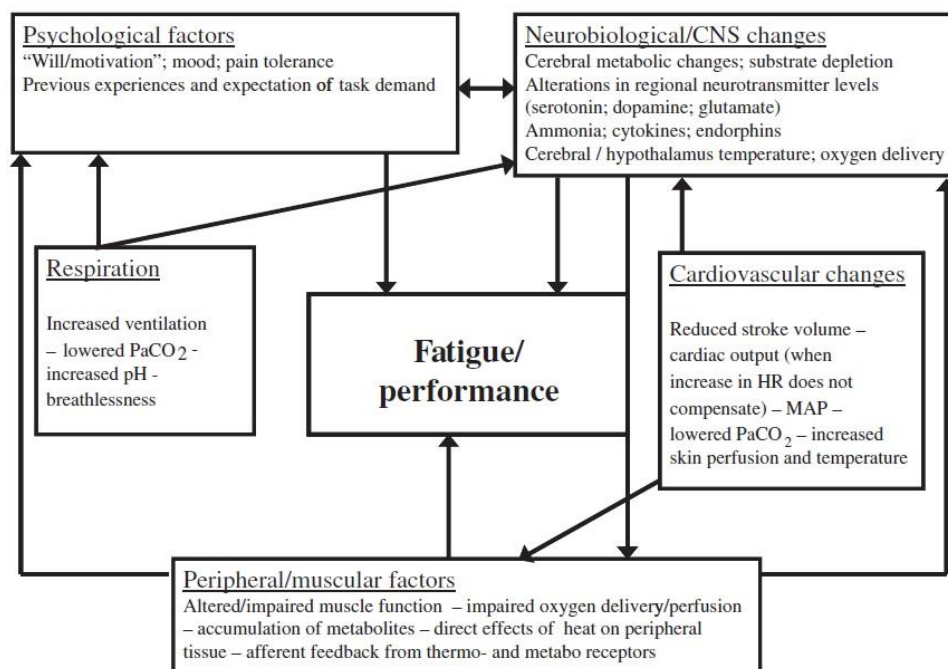
Heat induced decrements (caused by the high internal body temperatures) are known to occur during soccer match-play, whereby decrements in physical performance throughout the 90 min

period are known to be exacerbated compared to a temperate environment (Mohr *et al*, 2010, Mohr *et al*, 2012). In order to understand the mechanistic responses to heat-induced decrements during soccer match-play, it is important to acknowledge the difference between heat-stress and heat-strain (Nybo *et al*, 2014). Heat-stress is defined as the environmental condition (e.g. ambient temperature, humidity etc.) an athlete is competing in, whereas, heat-strain refers to the physiological response (e.g. elevated body temperatures) to heat-stress. Heat-stress exacerbates these decrements in physical performance by increasing the amount of heat strain placed upon a player and thus, these decrements in physical performance are exacerbated at a faster rate than in temperate conditions (Nybo *et al*, 2014). An early study by Cheung and Sleivert (2004) suggested that these heat-induced decrements are a result of the complex interplay between various central and peripheral factors, as supported in a recent review (Nybo *et al*, 2014). Nybo *et al* (2014) presented a detailed model illustrating how these factors integrate and collectively influence fatigue and ultimately performance (Figure 2.6.). The precise mechanism by which a hot environment reduces soccer performance is not clear, with intricate interplay between peripheral (feedback) and central factors (feed forward) known to occur (Nybo *et al*, 2014, Taylor and Rollo, 2014, Taylor, 2014). Therefore, the following sections will focus only on the relevant central, peripheral and psychological factors within this model.

2.3.2.3. Central factors

During the last decade, centrally mediated fatigue has been investigated with several theories being suggested to elucidate the possible mechanisms involved (Nybo *et al*, 2014). Changes in brain temperature, cerebral activity (Nybo, 2012, Nybo *et al*, 2002) and altered brain neurotransmitter (Roelands and Meeusen, 2010) concentrations are regarded as potential aspects of central fatigue, but in humans these are not easily evaluated *in-vivo,* let alone during soccer performance. Core body temperature has also been recognised as a heat-induced central fatigue mechanism, although it is recognised that core temperature is not a reliable predictor of brain temperature (McIlvoy, 2004). Therefore, in order to evaluate these central factors during soccer-specific exercise within a hot environment, changes to core body temperature have been quantified within this thesis (Mohr *et al*, 2012, Nybo and Nielsen, 2001).

Seminal research has identified that a high core body temperature influences an athlete's ability and drive to continue exercising (Nybo *et al*, 2014). It has been demonstrated over recent years that the voluntary exhaustion of an athlete occurs at a similar and consistent body temperature in humans during prolonged high speed exercise (Cheung and Sleivert, 2004, Gonzalez-Alonso

et al, 1999, Nybo and Nielsen, 2001) within a hot environment. It is proposed that the notion of critical core body temperature is a protective mechanism against the attainment of a critically high core and brain temperature (40°C), to prevent heat stroke and cellular apoptosis from occurring (Gonzalez-Alonso *et al*, 1999, Nybo *et al*, 2014). Thus, it was suggested that above this 'critical core body temperature limit', humans are unable to continue exercise (Gonzalez-Alonso *et al*, 1999), as afferent feedback from temperature sensitive sites within the central nervous system, result in a reduction in central neural drive to the exercising muscles, inducing premature exhaustion (Thompson, 2006). Therefore, a high core body temperature between 39-40°C was previously considered one of the main limiting factors of the termination of exercise performance in hot environment (Gonzalez-Alonso *et al*, 1999).

Interestingly, recent research has identified that soccer players have been reported to tolerate a core body temperature exceeding 40.5°C during soccer match-play at 43°C (Mohr *et al*, 2012). The increased core body temperature seen in soccer performance (Mohr *et al*, 2012) past the 'critical core temperature limit' unlike in the aforementioned fixed-paced exercise studies in the heat (Gonzalez-Alonso *et al*, 1999, Nybo and Nielsen, 2001), is likely due to athletes being able to modulate their exercise intensity during soccer match-play to sustain performance for the full exercise period (e.g. Less aggressive closing down of the opposition in soccer match-play) (Taylor and Rollo, 2014). Whereas, during fixed paced exercise the required exercise intensity is unable to be sustained by the athlete and thus, a termination in exercise performance is likely to accrue (Nybo *et al*, 2014). Consequently, the existence of a 'critical core body temperature' limit has lost scientific merit.

Although the termination of exercise performance does not occur from a critically high core temperature during soccer performance at 43°C a marked decline to both total distance (7%) and high-speed distance (26%) covered were seen compared with match-play at 21°C (Mohr *et al*, 2012). Aughey *et al* (2014) also revealed that Australian Rules Football players experienced a core temperature of 40.5°C towards the end of the match played at 30°C, confirming that elite team sports players are able to tolerate a core temperature above 40°C without the onset of exertional heat illnesses. However, the elevated core temperature in the final stages of an Australian Rules Football match, induced a large amount of fatigue to the individual's high speed running capacity in the last 15 min (Aughey *et al*, 2014), as seen in soccer match-play in at 30-43°C (Mohr *et al*, 2010, Mohr *et al*, 2012). This is likely due to an elevated core temperature of 40°C reducing maximum voluntary contractions in a hot environment caused by a reduction in motor drive to the active muscles, as proposed by Nybo

and Nielsen (2001). However, the magnitude of heat induced fatigue from central factors has been considered to be susceptible to high-inter-individual variation (Nybo *et al*, 2014). Furthermore, it is also evident that highly trained individuals are able to tolerate higher core temperatures (~39-40°C) before voluntary exhaustion, compared to their untrained (~38-39°C)counterparts (Cheung and Sleivert, 2004) during a fixed paced exercise bout. Therefore, it is clear that heat-induced central fatigue, may be influenced by other factors including the training and acclimation status of players (Racinais *et al*, 2013), which are influenced by genetic phenotypic variations of favourable traits associated with innate thermal tolerance and its acquirement (Taylor, 2014).

It is clear that core temperature has a crucial role in the development of the heat induced decrements upon soccer performance seen previously in match-play studies (Mohr *et al*, 2010, Mohr *et al*, 2012). However, as players are able to modulate there running performance in the heat to preserve key physical performance measures, the impact that a high core body temperature has on soccer performance is difficult to ascertain (Gregson *et al*, 2010, Nassis *et al*, 2015). Therefore, a soccer-specific simulation will allow reliable inferences on the role core temperature plays in the decrements of soccer performance, and whether future interventions should target internal body temperatures to prolong a temperate like soccer performance in a hot environment.

2.3.2.4. Peripheral factors

It has been reported that despite the high core body temperatures seen at the end of each half in soccer match-play at 43°C compared with 21°C, a multiple regression did not reveal that core temperature was a predictor to the heat induced decrements for total distance and high-speed distance covered between the two conditions (Mohr *et al*, 2012). Therefore, it was concluded that the changes to physical performance at 43°C was not simply a function of high core temperature but may also be dictated by individual responses (Nybo *et al*, 2014) and other peripheral factors including, muscle temperature, skin temperature and several other factors playing an important role as well (Taylor and Rollo, 2014). Therefore, this literature review will now discuss the peripheral factors that are inherent upon the heat-induced decrements seen during physical performance

2.3.2.4.1. Muscular temperature and responses

During moderate intensity exercise, a reduction in muscular blood flow will not occur, however during prolonged high intensity exercise, similar to soccer match-play, a reduction in cardiac output will jeopardise O_2 delivery to the exercising muscles and impair aerobic energy turnover (González-Alonso *et al*, 2008). During the first 60 s aerobic energy turnover is matched between both temperate and heat situated exercise trials, however, $\dot{V}O_2$ after this point is reduced (Wingo *et al*, 2005). In turn, anaerobic energy provision compensates for the reduction in aerobic energy provision to allow the regeneration of ATP. However, over time this will be reduced causing a reduction in ATP regeneration inducing fatigue on an athlete's physical performance. Dimri *et al* (1980) confirmed these findings during repeated sprint exercise at 30°C by reporting that the reduced O_2 delivery to the active muscles towards the end of repeated sprint exercise caused a variety of physiological responses which induced a quicker onset in fatigue. These have been studied in greater detail within recent literature and include a greater decline in muscle ATP, PCr, muscle lactate, H+ accumulation and pH levels, which have all been reported to alter muscular homeostasis, via altering the contractile properties of the skeletal muscles (Nybo, 2008, Nybo and Secher, 2004).

An increase in muscle temperature may also have performance diminishing effects on the contractibility of active skeletal muscle caused by the lower muscle blood flow and O_2 delivery during intense and sub-maximal exercise (Gao *et al*, 2006). Gao *et al* (2006) reported that higher muscle temperatures caused a reduction in skeletal muscle perfusion by limiting O_2 delivery and attenuating muscle contractibility of active skeletal muscle. Furthermore, elevated muscle temperature in a hot environment also affects afferent feedback and can influence the sensation of fatigue during self-paced exercise, meaning athletes will lower their exercise intensity in order to limit the early development of excessive muscle fatigue (Nybo *et al*, 2014). Mohr *et al* (2012) supports these findings by showing that muscle temperature is significantly greater during soccer match-play in 43°C compared to 21°C, which is associated with a 7% and 25% reduction in total distance and high-speed distance covered at 43°C. Therefore, an elevated muscle temperature during 90 min of soccer match-play in a hot environment lowers the intensity of the exercise, highlighted by the reduction in total distance and high-speed distance covered during soccer match-play (Mohr *et al*, 2012).

Similar to the measurement of the muscular responses during soccer-specific exercise in hypoxia, the direct measurement of the muscular inferences upon soccer performance are

difficult to measure as they often require muscle biopsies, which can only be collected post-match or during a prolonged recovery period (Bangsbo, 2014, Taylor and Rollo, 2014). However, as changes in Bla are an indicator of the muscular responses, which are apparent during soccer-specific exercise in the heat, utilising a well formulated soccer-specific simulation that facilitates the quantification of Bla would be advantageous to reliably ascertain the aforementioned muscular responses.

2.3.2.4.2. Skin temperature and responses

During prolonged aerobic exercise in the heat an elevation in skin temperature causes an increase to the skin blood flow of an athlete during both rest and exercise (Crandall and Gonzalez-Alonso, 2010, Sawka *et al*, 2012). Whilst exercising, skin temperature will either increase/decrease dependent upon the ambient air temperature as skin blood flow carries heat from the skeletal muscle towards the skin surface to allow heat exchange with the environment (Sawka *et al*, 2012). Skin blood flow is affected by skin temperature via direct action from the cutaneous blood vessels (Johnson and Kellogg, 2010) and the drive to increase heat gain within the body (Sawka *et al*, 2011). In turn, dependent upon the skin temperature of the athlete cutaneous vasoconstrictor tone will be impacted. Nybo *et al* (2014) defined a hot skin temperature as 35°C and above where vasodilation will be maximal and evaporative heat loss will be increased and skin temperature will be elevated but balanced by the increase in sweat evaporation to cool the skin (Rowell, 1986). During rest, skin blood flow will be ~ 8 L/min at 33°C, however, during prolonged high-speed exercise in a hot environment, skin blood flow will reduce due to the competition between skin and muscle causing vasoconstrictor effects (González-Alonso *et al*, 2008). Thus, skin temperature is important for heat dissipation during exercise, depending on the core-to-skin temperature gradient (Rowell, 1986). Rowell (1986) estimated that skin blood flow is increased as skin temperature is elevated at any given core temperature. However, it is also likely that an elevated core temperature reduces skin blood flow requirements at any given skin temperature by widening the core-to-skin temperature gradient. Therefore, the required skin blood flow for heat exchange is dependent upon the core-to-skin temperature gradient as well as the absolute core temperature (Nybo *et al*, 2014).

The influence of skin temperature on prolonged aerobic exercise similar to soccer is limited, due to the restrictions of measuring skin temperature without compromising exercise intensity. However, as skin temperature warms, it is likely to have great cardiovascular consequences (Section 2.3.2.5.) during exercise (Sawka *et al*, 2011). Therefore, it is clear that fatigue in

soccer is multi-factorial and several complementary multi-factorial mechanisms, besides body temperatures should be considered when explaining soccer-specific fatigue within a hot environment (Taylor and Rollo, 2014).

2.3.2.5. Cardiovascular responses in the heat

During exercise in a hot environment, the cardiac output of an athlete is challenged because of the impaired diastolic filling of the heart, which will reduce lower end diastolic volume and stroke volume, consequently elevating HR to maintain cardiac output (Nybo *et al*, 2014). However, an increase in HR implies that the cardiac cycle is shortened, reducing diastolic filling, which may further compromise stroke volume and cardiac output as HR cannot compensate (Gonzalez-Alonso, 2012). During rest, low and moderate-speed exercise in a hot environment, HR is unchanged as any reduction to stroke volume is compensated for, which increases cardiac output in order to meet the increased requirement for perfusion of the skin (Rowell, 1986). However, during intense prolonged exercise in the heat a process called cardiovascular drift occurs as cardiac output is compromised by the elevated HR and decreased stroke volume caused by the impaired cardiac filling (Nybo and Secher, 2004, Wingo *et al*, 2012, Wingo *et al*, 2005). The impaired cardiac filling occurs because the venous bed of the skin is large and dilates during exercise in a hot environment (Crandall and Gonzalez-Alonso, 2010). Therefore, as skin blood low is increased the blood vessels within the skin expand in size causing large pooling within these vessels which prevent cardiac filling. This process is defined as venous compliance, which is elevated during exercise due to a large increase in core and skin temperature (Nybo *et al*, 2014). Therefore, in order to allow some heat exchange during a hot environment, skin blood flow is increased, although this is not fully compensated for as central blood volume and cardiac filling are compromised (González-Alonso *et al*, 2008).

Due to the prolonged and intense exercise associated with physical performance during soccer match-play, cardiovascular drift is likely to occur during soccer match-play (Taylor and Rollo, 2014). However, much of the research upon cardiovascular drift during prolonged exercise is in steady state exercise. Wingo *et al* (2005) reported that cardiovascular drift was increased in a hot environment over 90 min continuous exercise compared to a temperate environment, however, the exercise type is dissimilar to soccer. Mohr *et al* (2012) revealed that during soccer match-play at 43°C, HR was only slightly increased compared to match-play at 21°C, likely due to the reduction in exercise intensity in a hot environment enabling cardiac filling similar to a temperate environment. Ascertaining the physical performance output of soccer players

via match-play is problematic, as the impact of match factors on performance, means that physical performance may differ between match-play, due to other factors such as the score-line (Gregson *et al*, 2010). Therefore, HR data can be collected throughout match-play, continuous measurement of core and skin temperature in tandem with HR during a soccer-specific simulation will allow greater cardiovascular inferences to be ascertained.

Table 2.6: Soccer performance in the heat from previous soccer match-play studies

Study	Level	Participants	Design	Temperature	Physiological	Performance
(Ekblom, 1986)	Elite	Elite Soccer players	2 matches	HOT: 30°C CON:20°C	N/A	↓4% TD covered
(Mohr *et al*, 2012)	Elite	17 elite male soccer players	2 Soccer matches	HOT: 42°C CON:21°C	↑ 1°C in T_{mu} and T_c for HOT match.	↓7% TD and ↓26% HSD covered in HOT. ↔SD covered ↑4% PSS
(Mohr *et al*, 2010)	Elite	20 soccer players	1 soccer match	31 °C	T_{mu} 40.5°C.	↓2.6% RSA ↓57% in HSD in the last 15 min
(Nassis *et al*, 2015)	Elite	N/A	64 matches	18-34°C	N/A	↔TD covered ↑ Passing success at high ambient temperature
(Özgünen *et al*, 2010)	Sub-Elite	11 semi-professional players	2 matches	HH: 38 °C MH: 34°C	↑T_c at the end of the first half in HH.	↓TD and HSD covered. ↔SD.

CON = Temperate; HOT = Hot environment; HH = High heat; MH = Moderate heat; RSA = Repeated Sprint Ability; RJA = Repeated Jump Ability; TD covered = Total distance covered; HSD = High speed distance; SD = sprint distance; PSS = Peak Sprint Speed

2.3.2.6. Physical performance during soccer in the heat

Only three studies (Table 2.6) have quantified how physical performance during soccer match-play, changes in the heat compared to a temperate environment (Ekblom, 1986, Mohr *et al*, 2012, Nassis *et al*, 2015). Furthermore, two other studies have quantified soccer performance in heat (Mohr *et al*, 2010, Özgünen *et al*, 2010), however, findings from these studies are not compared to temperate environment so it is difficult to understand any environmentally mediated changes in soccer performance. Early research by Ekblom (1986) quantified the changes in physical performance during soccer match-play at 30°C compared with 20°C. It was reported that total distance covered was significantly reduced by 4% at 30°C compared with 20°C. Although, a limitation of this study was that GPS or semi-automated camera systems were not used, violating the exclusion criteria proposed in section 2.1.1. Mohr *et al* (2012), reported that at 43°C, male elite soccer players demonstrated a 26% reduction in high-speed distance covered compared to a match within temperate conditions (~21°C). These findings highlight that the reduction in high-speed distance covered at 43°C (26%) is exacerbated by ~11% compared to a low altitude environment (15%) at 1,600m above sea level (Garvican *et al*, 2013). Therefore, it appears that match-play in the heat, although a very high temperature was utilised (43°C) by Mohr *et al* (2012) which lacks consistent external validity to modern professional soccer, has a greater impact on those variables of soccer performance specifically related to the outcome of the match compared with hypoxia. Furthermore, Mohr *et al* (2012) is the only study to date to access important physical soccer performance measures (high-speed distance and sprint distance) compared to temperate match-play. However, as outlined in section 2.3.2 temperatures exceeding 40°C are uncommon in elite soccer match-play which is a strong rationale for the externally valid temperature of 30°C within this thesis.

Data derived from the 2014 FIFA World Cup in Brazil, revealed that players were able to maintain total running distance in hot conditions (28-34°C) compared with temperate environments below 24°C (Nassis *et al*, 2015). Nassis *et al* (2015) identified that players adopt a protective pacing strategy by reducing high speed running and number of sprints completed in order to modulate the total distance covered during a match and preserve peak sprint speed. Furthermore, as reported during hypoxic based match-play technical skills, such as passing (8%) and crossing (9%), are improved within hot compared to temperate match-play environments (Mohr *et al*, 2012, Nassis *et al*, 2015). This increase in technical skill is likely to be an artefact of the inherent changes in match-play characteristics. For example, hot compared to temperate conditions are associated with a decrease in player duels and turnovers of

possession, with a concomitant increase of time in possession of the ball (Mohr *et al*, 2012). Therefore, prior to a technically challenging skill being attempted within hot compared to temperate conditions, pressure toward the player in possession of the ball is less, (i.e., closing down is less aggressive and proximity is increased), allowing greater attentional focus to the technical skill to be performed. This is the likely explanation for an increase in successful skill execution (Nassis *et al*, 2015).

As discussed in section 2.3.1, there are advantages of investigating performance-related parameters in more controlled conditions (Gregson et al, 2010). Variation in these parameters may be even greater in match-play in the heat ($>30°C$) because there may be an altered "pacing strategy" and distribution of absolute exercise intensity across the game (Mohr et al, 2012). Thus, three previous studies have used field based soccer-specific simulations to quantify the changes in physical performance in a hot environment ($\sim30°C$) compared with a temperate ($\sim20°C$) environment (Hughes et al, 2013, Morris et al, 1998, Morris et al, 2000). An early study by Morris et al (1998) reported a 21% reduction in total distance covered and 40% reduction in sprinting performance at $30°C$ compared with $20°C$ due to a higher rectal temperature and HR. In particular, Morris et al (1998) reported a relationship between the rate of rise in rectal temperature at $30°C$ ($r = 0.94$, $p < 0.01$), suggesting that it was a key factor to the curtailment of exercise. These findings show parity with a later study by Morris et al (2000) who indicated that elevated body temperature (Skin and muscle temperature) was likely a key factor in limiting simulated soccer performance at $30°C$. However, later research by Mohr et al (2012) contradicted these findings by revealing that core and muscle temperature were not predictors of the heat-induced-decrements during match-play at $45°C$. Thus, any heat-induced-decrements on physical performance during soccer-specific exercise could be due to a complex interplay of both these peripheral (thermal sensation, skin and muscle temperature) and central (core temperature) factors, prompting further study of this phenomenon (Taylor and Rollo, 2014).

The field based soccer-specific simulation used within these studies lack external validity with soccer match-play (Morris et al, 1998, Morris et al, 2000), as they employed a run to exhaustion component which meant that some participants were exercising for nearly 105 min, 15 min longer then soccer match-play. Therefore, it is imperative that a 90 min soccer-specific simulation is used to ascertain changes in simulated soccer performance between hot and temperate environments whilst also controlling for heat mediated adaptive pacing strategies. Evidently if the desired experimental outcome is relative to physical and physiological

performance during extra time, a duration of 105 min as utilised by Morris et al (1998) would be externally valid. Such exploration has been seen recently but is beyond the scope of the present thesis (Harper et al, 2015a, Harper et al, 2015b, Harper et al, 2014). Interestingly, there is paucity of data specific to hot and/or hypoxic conditions during simulated or match play soccer which includes a period of extra time; future research designs should address this.

A more recent study Hughes et al (2013) utilised a 90 min field based simulation, reporting an exacerbated decrement in sprint performance between the first and second half at 27°C compared with 19°C. However, a limitation of these findings was the total distance covered was fixed during the simulation to 11.2 km, ensuring that other physical performance measures including high-speed distance covered were unable to be quantified. Furthermore, similar to previous match-play studies (Mohr et al, 2010, Özgünen et al, 2010) a trial within a temperate environment was not included, so changes in simulated soccer performance were unable to be measured between the hot and temperate environment. These findings highlight the importance of using a variable distance soccer-specific simulation to assess the precise decrements in simulated soccer performance between a hot and temperate environment (Taylor and Rollo, 2014), which targeted interventional efficacy can be judged relative too.

2.3.3. Heat-hypoxia and soccer

The combination of both heat and hypoxia has been previously used as an effective training stimulus to improve soccer-specific performance (Buchheit *et al*, 2013). Furthermore, research in endurance cycling has outlined an exacerbated reduction in physical performance (-~51%) in a combination of heat-stress and moderate hypoxia (Buono *et al*, 2012, Girard and Racinais, 2014, Van Cutsem *et al*, 2015). However, the exercise type used within these studies is dissimilar to soccer match-play, where no research assessing the effects of the combination of both extreme heat and hypoxic conditions within soccer has occurred. This could be a possible challenge in some regions specific to where elite soccer match-play can occur (Figure 2.7) as the frequency of soccer match-play, including elite soccer, within environments that combine temperature and hypoxia is only going to increase given the increasing globalisation of soccer (Taylor and Rollo, 2014). An example of a FIFA endorsed tournament where a hot-hypoxic environment is likely to be prevalent is the upcoming 2023 Copa America in Ecuador (30°C, 2800m).

Although no previous match-play data has quantified the combined permutations of heat and hypoxia during soccer match-play, it is thought that a combination of both environments will

exacerbate these decrements in physical performance compared to their singular effects. Figure 2.7 provides further evidence where extremes of both hot and hypoxic environments are likely to occur. UEFA Champions and Europa League match-play is the most prevalent 'European' hot-hypoxic environment seen in elite soccer (Madrid 30°C, 750m). Therefore, the proposed experimental design could provide quantification of the physical performance and physiological responses of soccer players in hot-hypoxic environments.
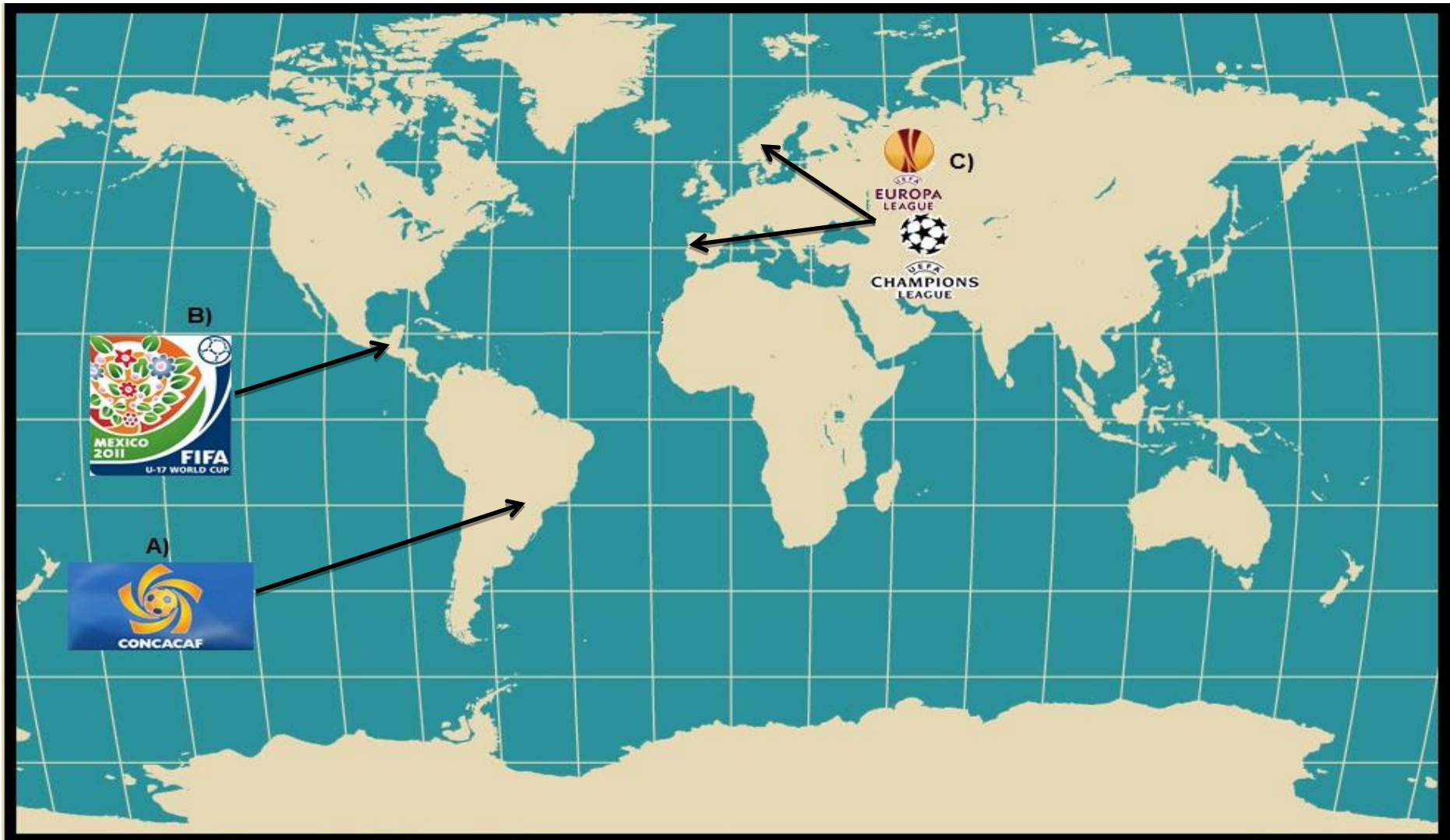
Figure 2.7: The Locations of a plethora of FIFA endorsed elite soccer tournaments based in hot-hypoxic environments. A) Copa América 2023: Ecuador (Quilto), 3,000m, 30-35°C; B) FIFA U-17 World Cup 2011, Mexico (Mexico City), 2,500m, 25-35°C, C) UEFA Champions League, Norway (Molde FC), Spain (Madrid), 1,000m, 25-35°C.

Table 2.7: The influence of pre- and half-time-cooling on soccer performance

Study	Level	Participants	Study Design	Temperature	Pre-Cooling	First Half	Half-Time-Cooling	Second Half
(Clarke *et al***, 2011)**	University	12 Male SP	4 SSS	30°C	60 min VEST CHO-E	↑10% TD	15 min VEST CHO-E	↑10% TD
(Drust *et al***, 2000a)**	University	6 Male SP	2 SSS PC CON	CON - 26°C PC- 20°C	60 min SHOWER	No improvement	N/A	No improvement
(Duffield *et al***, 2013)**	Elite	10 Male SP	1 SM	29°C	20 min SLURRY 350ml VEST TOWEL	No improvement	5 min SLURRY 350ml VEST TOWEL	No improvement
(Price *et al***, 2009)**	Elite	8 Female SP	3 SSS CON PC-VEST PC-HC-VEST	30°C	20 min CON PC-VEST PC-HC-VEST	↓T_{re}, T_{sk}	15 min VEST	↓T_{re}, T_{sk}

PACKS = Ice Packs; VEST = Ice Vest; SLURRY = Ice slurry; TOWEL = Iced Towels; CWI = Cold Water Immersion; T_{mu} = Muscle Temperature; T_{re} = Rectal Temperature; T_{sk} = Skin Temperature; SSS = Soccer-specific simulation; CHO-E = Carbohydrate electrolyte drink; PC = Pre-cooling; MM = Mixed-Methods; SP = Soccer Players; SM = Soccer Match; CON = No-cooling; PC-VEST = Pre-Cooling with Ice Vest; PC-HC-VEST = Pre-Cooling and Half-time-Cooling with ice vest; SHOWER = Cold Shower.

2.4. Pre and half-time-cooling in soccer

As heat induced decrements are more apparent during soccer-specific exercise (Duffield *et al*, 2009, Mohr *et al*, 2010, Mohr *et al*, 2012, Özgünen *et al*, 2010) compared to other extreme environments (e.g. Hypoxia), various strategies have been investigated in an attempt to attenuate these decrements which occur in soccer match-play (Taylor and Rollo, 2014). The most prominent soccer-specific intervention to utilise prior to heat situated match-play is via acclimation protocols (Buchheit *et al*, 2011, Racinais *et al*, 2013, Racinais *et al*, 2012), however, these strategies can often be time consuming (6-13 d) and fail to fit within the time constraints (Towlson *et al*, 2013) and congested calendar associated with an elite soccer season (Taylor and Rollo, 2014). Various cooling interventions have also been developed, differing in duration, method and strategy to be utilised either prior to match-play (pre-cooling) and/or at half-time (half-time-cooling), to attenuate the increased physiological strain seen in hot environments (Bongers *et al*, 2014, Mohr *et al*, 2012). However, to establish the efficacy of pre-cooling can be difficult to achieve from soccer match-play designs, due to the plethora of match factors outlined in section 2.1.2 (Gregson *et al*, 2010). This would be better facilitated by a soccer-specific simulation (Section 2.5.). Therefore, section 2.4 will investigate how pre-cooling and half-time-cooling interventions can be successfully tested for their efficacy via a soccer-specific simulation.

2.4.1. Pre-cooling and half-time-cooling

The fundamental aim of pre-cooling is to reduce body temperatures and increase heat storage capacity prior to exercise (Tyler *et al*, 2013), which in turn will improve an athlete's exercise intensity and/or exercise time to exhaustion (Bongers *et al*, 2014). The majority of pre-cooling research focuses upon endurance exercise (Booth *et al*, 1997, Gonzalez-Alonso *et al*, 1999, Quod *et al*, 2008) and intermittent sprint performance (Castle *et al*, 2006, Duffield *et al*, 2003, Duffield and Marino, 2007) in hot environments. However, over the last 15 years, four specific studies have assessed the use of pre-cooling on soccer performance via simulations (Clarke *et al*, 2011, Drust *et al*, 2000a, Price *et al*, 2009) and match-play designs (Duffield *et al*, 2013), with varying degrees of success (Table 2.7). Section 2.3.2 reveals that during soccer performance in the heat, high body temperatures (e.g. core body temperature) are the primary cause amongst other multi-factorial mechanisms in inducing the exacerbated heat induced decrements seen in soccer match-play (Mohr *et al*, 2010, Mohr *et al*, 2012). Therefore, as pre-cooling has been reported to be a beneficial tool to reduce body temperatures on endurance and

intermittent sprint exercise in the heat (Ross *et al*, 2013), facets relevant to soccer match-play (Buchheit, 2012), it is thought that utilising a well formulated simulation could allow reliable quantification of pre-cooling as an ergogenic aid on soccer-specific performance.

Despite the success of pre-cooling, it is not uncommon for the desired physiological and heat storage capacity alterations induced by pre-cooling to be lost during exercise and the athlete to experience the similar thermal strain, compared to a control trial with no cooling (Booth *et al*, 1997). This has meant that the beneficial effects of pre-cooling upon exercise performance have been negated after 20-45 min of exercise (Bolster *et al*, 1999, Castle *et al*, 2006). In context to soccer, an appropriate pre-cooling strategy would be sufficient to have an ergogenic effect upon soccer performance for one half of soccer match-play (Taylor and Rollo, 2014). However, soccer match-play consists of two forty five min halves (Russell *et al*, 2011), which means that the second half of match-play is likely to be unaffected by any pre-cooling strategy performed before the first half. One option is to utilise a cooling intervention during match-play, which is referred to as per- cooling, derived from the Latin word per meaning 'during' (Bongers *et al*, 2014). However, per-cooling would lack practicality during competitive soccer match-play (Tyler *et al*, 2013), as the extra clothing needed for cooling devices would be outside the regulated clothing players are allowed to wear (Taylor and Rollo, 2014, Tyler *et al*, 2013). Therefore, another cooling option which can be utilised within soccer match-play is a half-time-cooling strategy (Price *et al*, 2009), during the 15 min interval between two halves of soccer match-play.

If successfully utilised, half-time-cooling could attenuate some of the decrements to key physical performance measures in the second half, which would be important to practitioners and coaches in soccer, as this is where most fatigue is seen in soccer match-play (Mohr *et al*, 2003). Furthermore, half-time-cooling could also attenuate the increased body temperatures seen as a result of the first half of soccer match-play in a hot environment (Duffield *et al*, 2013, Mohr *et al*, 2012). A 15 min period of half-time-cooling has been utilised previously in two soccer-specific simulations (Clarke *et al*, 2011, Price *et al*, 2009) and one soccer match-play (Duffield *et al*, 2013) study. Price *et al* (2009) identified that a fifteen minute half time-cooling period via an ice vest for 15 min had a ergogenic effect upon both rectal temperature and HR during the second half of the simulation. However, the use of a fixed distance simulation meant that changes in physical performance were unable to be measured (Price *et al*, 2009). Clarke *et al* (2011) identified a 5% improvement in total distance covered during the second half of a NMT based soccer-specific simulation after participants wore an ice vest for 15 min, although

as the half-time-cooling strategy was combined with carbohydrate supplementation, this meant that inferences specifically from the cooling intervention were unable to ascertained (Clarke *et al*, 2011). In contrast, Duffield *et al* (2013) revealed that 5 min of half-time cooling via an ice slurry drink and ice vest also had no benefit upon soccer match-play performance at 30ºC. Although as match-play design was used, it has been acknowledged that inferences from the intervention were difficult to ascertain, due to the poor reliability of soccer match-play data (Gregson *et al*, 2010). Therefore, half-time-cooling requires reliable empirical data to test its efficacy as an intervention to be utilised with a soccer-specific setting. This could be achieved by utilising an appropriately formulated soccer-specific simulation to achieve this objective.

2.4.2. Cooling strategies and methods

It is well publicised within the literature that both pre- and half-time cooling can be induced via three-specific genres of cooling strategies including external, internal and mixed-methods (Bongers *et al*, 2014, Ross *et al*, 2013, Tyler *et al*, 2013); with several options available for each [See Table 2.8; (Bongers *et al*, 2014)). External cooling is the most popular cooling strategy adopted within the literature, however, internal (Siegel *et al*, 2010) or their combination via a mixed-method cooling strategy (Ross *et al*, 2011), has recently received greater interest recently. These strategies and methods are discussed in detail within their appropriate section of this literature review.

Figure 2.8: Cooling Strategies and methods

External	Internal	Mixed-Method
Whole/part Body water immersion	Ice slurry ingestion	External-External
Ice Vest	Cold Air Inhalation	External-Internal
Ice Packs	Cold Water Ingestion	
Iced towels		
Iced Collars		
Cold Air exposure		

2.4.3. External cooling

External cooling is defined as the application of a cold medium and/or material to the surface of the body via the methods eluded too in table 2.8 (Ross *et al*, 2013). Their relative success relies on the heat-transfer principles of conduction, convection, evaporation and radiation on the skin by offsetting the unbalance between the increased heat gain and reduced heat loss when exercising in a hot environment (Johnson and Kellogg, 2010, Ross *et al*, 2013). In the defence of body temperature, the body and largely the skin, will react to a cold powerful

response through cutaneous vasoconstriction, with consequent reductions in skin perfusion in an attempt to prevent excessive dissipation of heat to the environment (Charkoudian, 2010, Johnson and Kellogg, 2010).

Early external cooling strategies utilised cold-air exposure showing an ergogenic effect on cycling (Hessemer *et al*, 1984, Olschewski and Bruck, 1988) and running (Lee and Haymes, 1995) performance. However, these findings revealed that cold-air exposure pre-cooling can take up to 90 min to gain significant reduction in rectal temperature (1°C) meaning that this method fails to fit within the time constraints of elite soccer, where practitioners have only 30 min prior to match-play to implement a pre-cooling strategy (Towlson *et al*, 2013). Similarly, whole body immersion in water is commonly referred to as the 'gold standard' cooling strategy (Casa *et al*, 2007). This is due to its superior cooling capability compared to cold air exposure as heat loss from water is approximately four times greater than cold air exposure at the same temperature (Ross *et al*, 2013). It has previously been stated that to have significant effect on core temperature during exercise, cold water immersion should be maintained for 30-60 minutes, as a long duration of cold water immersion elicits a greater pre-cooling effect upon body temperatures (Core Body Temperature - 1°C) prior to exercise (Booth *et al*, 1997), but a shorter duration at cooler temperatures still reduces rectal temperature by 0.2°C (Duffield *et al*, 2010). Similarly, a cold shower pre-cooling method has also been investigated; this involved an exposure for 60 min as the water temperature was progressively reduced from 28°C to 24°C (Drust *et al*, 2000a) prior to a 90-min NMT based soccer-specific simulation. Rectal temperature was effectively reduced by 0.6°C which appears meaningful and is similar to that observed in whole-body water immersion protocols (Quod *et al*, 2006, Quod *et al*, 2008). However, this strategy failed to achieve a substantial change in subjective, thermoregulatory, physiological (e.g. HR) or physical performance during exercise, likely due to the simulation being conducted in a temperate environment (20.5°C, 68% RH) (Drust *et al*, 2000a). A further limitation is the practical application of water immersion and cold showers in the field, which may be cumbersome due to the lack of portable equipment, large amounts of time required to effectively cool the athlete and the physical limitations of having an athlete situated in a bath or shower for a prolonged period of time (Ross *et al*, 2013, Tyler *et al*, 2013). In turn, these limitations have reduced the applicability of cold water immersion or cold shower protocols of being used in soccer (Taylor and Rollo, 2014). Therefore, the use of cold water immersion was not considered a viable intervention to answer the experimental aims of this thesis.

Due to its powerful heat transfer capacity, the application of ice directly to the skin has emerged as an effective pre-cooling strategy to assist in the preparation of athletes (Ross *et al*, 2013). Heat from the skin and surrounding tissues is absorbed by the ice and, as a result, the ice changes to water through melting (Merrick *et al*, 2003). Indeed, ice requires 80 times more thermal energy to increase the temperature of water by $1^{o}C$ due to the phase change that must occur when water changes from its solid to liquid state (Poppendieck *et al*, 2013). As a result, cooling with ice may be achieved with lower amounts of integument, at a faster rate (Merrick *et al*, 2003) and to a greater magnitude (Poppendieck *et al*, 2013), when compared with water (Ross *et al*, 2013). Moreover, unlike cold water immersion and shower protocols, ice offers a practical advantage for cooling, as it is highly portable and can be incorporated into a range of garments to target specific regions of the body (Castle *et al*, 2006). Therefore, as ice has a faster pre-cooling rate compared with cold water immersion and cold air exposure, it is more likely to fit within the time constraints practitioners have with soccer players prior to a match (Taylor and Rollo, 2014, Towlson *et al*, 2013).Therefore, the use of ice as pre-/half-time cooling intervention is discussed in the following section.

The most practical method of utilising ice as a pre-cooling strategy is within an ice vest (Castle *et al*, 2006, Quod *et al*, 2008). Furthermore, external cooling via an ice vest is the most prevalent pre-/half-time-cooling strategy within soccer-specific research (Clarke *et al*, 2011, Price *et al*, 2009). However, results proved this method to have a negligible effect at reducing rectal temperature (0.2-$0.7°C$) compared with cold water immersion with the added concern over the vests weight and its use during warm ups adversely affecting necessary mechanisms to pre-cool an athlete (Bongers *et al*, 2014, Tyler *et al*, 2013). Therefore, the development of a more ecologically valid but effective pre-cooling mechanisms utilising ice is essential within soccer (Taylor and Rollo, 2014). A further benefit of ice as that it can be used within a cooling garments for part-body cooling prior to, or during, a subsequent exercise task (Castle *et al*, 2006, Minett *et al*, 2012a, Minett *et al*, 2012b, Minett *et al*, 2011). As such, simple strategies such as the application of ice packs to active (Castle *et al*, 2006) and non-active (Minett *et al*, 2012a, Minett *et al*, 2011) regions of the body have been examined, with relative success, especially when thermoregulation is challenged (Castle *et al*, 2006). A novel finding is that pre-cooling via a 20-min application of ice packs to the thighs increased cycling peak power output over a 45 min period by revealing a 20% increase in work done during each of the 20 sprints observed during an intermittent sprint protocol (Castle *et al*, 2006). Furthermore, a

reduction in rectal and local muscle (quadriceps) temperature throughout the exercise period may be contributed to the increased intermittent sprint performance as a reduction in T_{re} caused a feed forward pacing strategy to occur by increasing muscle activation during each sprint (Drust *et al*, 2005a, Nybo *et al*, 2014). As decrements to intermittent sprint performance are exacerbated in hot environments (Mohr *et al*, 2010), a 20 min period of ice packs pre-cooling based upon previous findings (Castle *et al*, 2006) could attenuate this earlier onset of fatigue. Furthermore, its practicality of being able to be used in a short period of time (5-20 min) makes it a viable option to be used within an elite soccer setting as both a pre- and half-time-cooling strategy (Taylor and Rollo, 2014). Therefore, it seems wise that ice packs could be used as a pre-/half-time-cooling method to offset the heat induced decrements in physical performance and physiological response during soccer match play (Mohr *et al*, 2010, Mohr *et al*, 2012). However, to assess the efficacy of ice packs as an intervention on soccer performance from match-play data can be problematic (Section 2.1.2.), so reliable empirical data is required. Thus, an appropriately formulated soccer-specific simulation could achieve this objective.

2.4.4. Internal cooling

Internal precooling is defined as taking a cold medium into the body through the mouth and can include the inhalation of cold air and the ingestion of cold fluids or ice (Ross *et al*, 2013). The inhalation of cold air is typically not used as a precooling strategy for enhancing sports performance (Geladas and Banister, 1988). However, due to its practical and logistical simplicity, asking an athlete to consume a cold fluid both prior and during exercise, internal cooling has gained recent attention as a method of improving sport performance (Siegel *et al*, 2010, Siegel *et al*, 2012). Cold beverages ingested into the stomach readily gain heat from the body, in order to equilibrate with the surrounding tissues (Burdon *et al*, 2010). The benefits of ingesting cold beverages are that they may provide cooling, hydration and deliver nutrients (fluid, carbohydrate and electrolytes) to the athlete (Ross *et al*, 2013). However, a greater improvement in heat storage capacity has been reported when ice slurry was consumed prior to exercise in hot environments compared with cold water (Ihsan *et al*, 2010, Siegel *et al*, 2010). Therefore, the use of ice slurry ingestion should be considered as a pre- and half-time-cooling strategy to attenuate the heat induced decrements seen in soccer performance.

2.4.4.1. Ice slurry

The ingestion of an ice slurry provides an alternative strategy for inducing a similar magnitude of pre-cooling from a smaller volume of ingested beverage when compared to cold water, as a

larger amount of fluid is required to produce the same cooling effect (Ross *et al*, 2013). Recently, the consumption of 6.8–7.5 g·kg^{-1} of ice slurry, consumed in 10 min blocks during seated rest in the 30 min prior to exercise, improved running capacity (Siegel *et al*, 2010, Siegel *et al*, 2012) and cycling endurance performance (Ihsan *et al*, 2010) in a hot environment. Siegel *et al* (2010) showed an improvement of 9.5 min following ice slurry ingestion in a time to exhaustion trial, when compared with a 4°C beverage. Ice slurry ingestion manifested in a moderate reduction in rectal (Siegel *et al*, 2010, Siegel *et al*, 2012, Stanley *et al*, 2010) gastrointestinal (Ihsan *et al*, 2010) and skin temperatures (Siegel *et al*, 2010), which allowed for a greater heat storage and level of thermal comfort during the respective exercise tasks. These findings are supported in recent meta-analytical reviews (Bongers *et al*, 2014, Tyler *et al*, 2013). However, Siegel *et al* (2012) noted a differing physiological responses to ice slurry ingestion despite similar applied methodologies to their previous study (Siegel *et al*, 2010). The notable difference was the environmental conditions that subjects were exposed to during pre-cooling (25°C vs. 34°C) with larger and more sustained reductions in skin and rectal temperature being achieved when pre-cooling was performed in the cooler environmental conditions (25°C) (Siegel *et al*, 2010). Greater heat exchange between the body and the ice slurry would be expected in cooler conditions due to the lack of competition for absorption of environmental heat (Siegel *et al*, 2012). However, despite the increase in running capacity following the ingestion of the ice slurry, a greater improvement upon physical performance was seen after pre-cooling was performed in a temperate environment (Siegel *et al*, 2010). During elite soccer match-play, there is the availability of air conditioned changing rooms, where players can prepare for the match (Towlson *et al*, 2013). Therefore, based upon the recommendations of Siegel *et al* (2010), ice slurry ingestion should be consumed within a temperate environment, in order to have enhanced physiological effect prior to soccer performance and also allow soccer players to prepare for the match (e.g. Receive instructions from team coaches) simultaneously.

A limitation to previous ice slurry ingestion pre-cooling research is that all findings are based upon short duration exercise between 20-30 min (Siegel and Laursen, 2012, Siegel *et al*, 2010), or endurance cycling protocols (Ihsan *et al*, 2010), which are dissimilar to the physical demands associated with soccer (Buchheit *et al*, 2010). To the authors knowledge, no pre-cooling ice slurry ingestion research has been completed upon simulated or soccer match-play designs, although, one previous study has utilised ice slurry ingestion alongside other external cooling measures (Duffield *et al*, 2013). However, the experimental effect is masked somewhat by

multiple treatment interference. Therefore, it appears that the singular use of ice slurry ingestion is unexamined upon soccer performance and due to the high variability associated with soccer match-play designs (Gregson *et al*, 2010), the use of a soccer-specific simulation could provide reliable empirical data on ice slurry ingestion to attenuate the heat induced decrements seen in soccer match-play data (Mohr *et al*, 2010, Mohr *et al*, 2012).

2.4.5. Mixed-methods

Combining two or more practical precooling methods is defined as mixed-method cooling (Bongers *et al*, 2014) and can provide mutually potentiating effects on performance through enhanced heat storage and decreased thermoregulatory and cardiovascular strain (Ross *et al*, 2013). Mixed-method cooling can be achieved via utilising two or more external cooling strategies (Minett *et al*, 2012a, Minett *et al*, 2011) or via utilising both external and internal cooling strategies in a concurrent (Duffield *et al*, 2013, Ross *et al*, 2011) or sequential (Clarke *et al*, 2011) manner. The use of multiple external cooling methods has been increasingly studied within research by the application of ice garments including iced towels (Skein *et al*, 2012), ice jackets (Hornery *et al*, 2007, Quod *et al*, 2008), ice vests (Duffield and Marino, 2007) and ice packs (Duffield *et al*, 2010). Although based upon the limitations of these methods documented in section 2.4.3, only ice packs are practical to be used within a soccer-specific setting and benefit soccer performance (Bongers *et al*, 2014, Castle *et al*, 2006, Taylor and Rollo, 2014). Furthermore, section 2.4.4 identifies that the use of ice slurry ingestion has a greater pre-cooling effect prior to exercise compared with cold water as a larger reductions occur to body temperatures during the pre-cooling and exercise period. Therefore, following this critical analysis in section 2.4, it is identified that the use of an internal-external cooling strategy via ice packs and ice slurry ingestion was seen to be the most viable and practical pre-/half-time-cooling strategy to be utilised within soccer match-play.

2.4.5.1. Internal and external mixed-methods cooling

The combination of external and internal mixed-method cooling strategies has been shown to achieve large reductions in core temperature and to improve thermal sensation prior to the commencement of exercise, resulting in improvements in physical performance (Clarke *et al*, 2011, Ross *et al*, 2011) for a long period and persist even after the cooling effect has disappeared. For example, the combination of ice slurry ingestion and the application of iced towels to both upper and lower body for 30 min prior to exercise produced an overall mean increase of 3% (8 W) in power output during the subsequent 46-km time trial (Ross *et al*, 2011).

The improvement in pacing was most evident in the second half of the time trial, despite a lack of difference in core temperatures between trials over this period. Similarly, the combination of wearing a cooling vest for 60 min followed by ingesting fluids prior to a 90 min NMT based soccer-specific simulation created a sustained benefit that allowed subjects to then run 1.2 km/h (10.6%) faster in a self-selected speed test and 28 s (40%) longer during a running capacity test compared with a control condition (Clarke *et al*, 2011). However, the improvements in physical performance was detected following the combination of wearing an ice vest and consuming a carbohydrate-electrolyte beverage meaning that the experimental effect maybe masked somewhat by the carbohydrate supplementation. Utilising a mixed method cooling strategy that combines both internal and external methods is advantageous in soccer as the heat induced fatigue in soccer match-play is specifically caused by the onset of both central and peripheral factors (Taylor and Rollo, 2014). A combination of external and internal mixed-method cooling can target both the central and peripheral factors, respectively (Ross *et al*, 2011). Therefore, both feedback and feedforward pacing factors are enhanced by an increased heat transfer (peripheral) and central drive (central), respectively (Ross *et al*, 2013, Ross *et al*, 2011).

Despite the positive findings from Clarke *et al* (2011), the pre-cooling strategy would lack applicability within elite soccer as of 60 min of pre-cooling fails to fit within the time constraints practitioners have with players prior to soccer match-play (Towlson *et al*, 2013). Subsequently, a recent study by Duffield *et al* (2013) who utilised 20 min of mixed-method (ice vests, ice towels and ice slurry ingestion) and 5 min half-time cooling, found that the combination of both internal and external cooling strategies had no ergogenic effect upon soccer performance at 30°C. However, no ergogenic effect was likely found in this study as the players were residents of a hot climate and were more than likely acclimated to the hot environment. Brade *et al* (2012) identified that pre-cooling has been shown to have no benefit upon heat-acclimated athletes during repeated sprint exercise, as the heat strain the athletes must contend with was not large enough for the pre-cooling to have an ergogenic effect upon performance, showing synergy with earlier research (Castle *et al*, 2011). Furthermore, the use of a soccer match-play design also makes the findings from this study difficult to interpret, due to the high variability of soccer match-play (Gregson *et al*, 2010). Therefore, a soccer-specific simulation would provide reliable empirical data on mixed-method cooling utilising both internal and external cooling strategies.

2.5. Soccer-specific simulations

Due to the prevalence of match factors during soccer match-play, quantifying inferences regarding the aforementioned environmentally mediated decrements (Section 2.3.1-3) and the potential use of pre-/half-time-cooling (Section 2.4) to negate any heat-induced decrements on soccer performance is problematic. One solution to quantify the effect environmental stress has on soccer performance and plausible interventions relative to these decrements, is to increase experimental control (specifically internally validity) via utilising a field or laboratory based simulations (Taylor and Rollo, 2014). Therefore, during the final section of this literature review, the development of soccer-specific simulations will be discussed and how these can be utilised to quantify soccer performance in extreme environments, as well as assessing the efficacy of pre-/half-time-cooling.

2.51. Field based simulations

A field based soccer-specific simulation is argued to be most suited to assess both physical performance and the physiological responses due to its increased ecological validity, as alongside these measures technical skills (e.g. ball skills) and changes of direction can be quantified, without the aforementioned match factors outlined in section 2.1.2 (Williams *et al*, 2010). A seminal field based soccer-specific simulation was the Loughborough intermittent shuttle test (LIST) (Nicholas *et al*, 2000), which consists of two different parts. Part A: A fixed period of variable intensity of shuttle running over 20m and Part B: Continuous 20m shuttle running alternating between 55% and 95% of $\dot{V}O_{2max}$ until exhaustion. The LIST reported high validity for total distance covered (12.4 km), sprint duration (2.4 s), HR (169 b·min^{-1}) and Bla (7 mmol^{-1}) compared with match-play data (Rienzi *et al*, 2000). However, a limitation of the LIST was the increased duration of Part A (75 min) compared to the first half of soccer match-play (45 min), alongside the run to exhaustion during part B as both do not represent soccer match-play (Russell *et al*, 2011). This is important as a plethora of studies have shown a reduction in physical performance during the second half (Bradley *et al*, 2009, Di Mascio and Bradley, 2013, Mohr *et al*, 2003), highlighting the importance of utilising two 45 min duration halves within a well formulated soccer-specific simulation (Russell *et al*, 2011).

Following the limitations of the LIST, two further soccer-specific simulations called the soccer-specific aerobic field test (SAFT$_{90}$) (Small *et al*, 2010) and the Soccer Match Simulation (SMS) (Russell *et al*, 2011), have been developed. Both the SAFT$_{90}$ and SMS are 90 min in duration utilising two 45 min halves interspersed with a 15 min rest period, similar to soccer match-play

(Stolen *et al*, 2005). Furthermore, the physical performance and physiological response reported from both the SMS and SAFT$_{90}$ had good validity compared to soccer match-play. For example, mean (159 ± 4 b·min^{-1}) and peak (197 ± 4 b·min^{-1}) HR values during the SMS were representative of the intensities previously observed in match-play (Mean HR: 161 ± 8 b·min^{-1}; Peak HR: 198 ± 6 b·min^{-1}) data (Bangsbo, 1993, Bangsbo, 1994). However, a limitation of both these simulations is that the physical demands were fixed not allowing true physical performance capacity to be examined, but instead only allows for changes to the physiological response to be ascertained between subjects (Small *et al*, 2010). Therefore, fixed distance soccer-specific simulations do not allow for changes in physical performance to be measured, which can be important when quantifying physical performance within differing extreme environments and the inferences of potential interventions. For example, Mohr *et al* (2010) reported that in a hot environment (31°C), total distance and high-speed distance covered were significantly reduced in the final 15 min of match-play, which may contribute to an increase in the number of goals both scored and conceded during that period (Armatas *et al*, 2007). Therefore, in order to quantify the physical demands of soccer players at different extreme environments and the validity of potential interventions, a variable distance soccer-specific simulation should be utilised to allow players to express their maximal, or not, performance capacity (Bendiksen *et al*, 2012, Williams *et al*, 2010).

Two field based soccer-specific simulations that utilise a variable distance design have been developed, showing high reliability and validity in both amateur (Williams *et al*, 2010) and sub-elite (Bendiksen *et al*, 2012) players. The Ball-Sport Endurance and Sprint Test (BEAST$_{90}$) developed by Williams *et al* (2010) reported comprehensive reliability to assess the physical performance (total distance – CV%: 2.4%) and physiological responses (HR –CV%: 2.8%) in amateur soccer players, however, poor reliability was seen when assessing the technical capacity (Shooting Accuracy – CV%:19.6%) of players. Furthermore, good validity was reported for total distance (8,097 ± 458 m) and high-speed distance (3,271 ± 242 m) covered during the BEAST$_{90}$, showing synergy with amateur soccer match-play data (Van Gool *et al*, 1983). The BEAST$_{90}$ was later modified by Akubat *et al* (2014) who removed the technical capacities from the simulation which in turn increased the reliability of the simulation (total distance – CV%: 1.7%; HR – CV%: 2.4%). Furthermore, the use of a variable distance simulation has been supported further by Bendiksen *et al* (2012) who developed the Copenhagen Soccer Test (CST). The CST showed good validity for performance (total distance covered: 11.29 km; high-speed distance covered 3.28km) variables. However, the simulation

lacked the aforementioned reliability measures, previously outlined. Thus, reliability measures are important to confidently ascertain whether any changes in physical performance and physiological responses can be attributed to an intervention and not the variability of the measure (Currell and Jeukendrup, 2008)

An important limitation with all field based simulations (BEAST$_{90}$ and CST) is that they require a large space to be performed successfully, making it difficult to perform within an environment where temperature or altitude can be controlled. For example, the CST by Bendiksen et al (2012) requires the full field of play to be utilised and performed within, making it difficult to be performed inside a laboratory environment·. An outside environment, is susceptible to changes to location (e.g. altitude) and temperature (e.g. ambient temperature), which are both match factors that can exacerbate the variability between trials for a plethora of physical performance measures and physiological responses (Lago-Peñas, 2012). Therefore, utilising a field based simulation would not be appropriate to assess environmentally mediated changes upon soccer-specific performance. Thus, to reliably ascertain any environmental mediated decrements and inferences from a potential intervention, a laboratory based soccer-specific simulation where both temperature and altitude can be controlled should be utilised. This may impact on external/ecological validity but will enhance internal validity.

2.5.2. Laboratory based simulations

Alongside the development of field based simulations, laboratory based soccer-specific simulations have also been developed, by utilising a treadmill (Drust et al, 2000b, Page et al, 2015). One example of a laboratory based soccer-specific simulation is the soccer-specific protocol developed by Drust et al (2000b). The soccer-specific protocol approached the physical performance and physiological responses associated with soccer match-play, suggesting a laboratory based soccer-specific simulation is a valid tool to assess changes in both physical performance and physiological responses. However, limitations include the short duration (45 min) and the fixed distance which are similar to earlier field based simulations (Russell et al, 2011, Williams et al, 2010). The soccer-specific protocol was modified recently by Taylor et al (2014a), who increased the duration of the simulation to 90 min, in order to investigate the decision making ability of soccer referees in hot, cold and temperate environments. Although the simulation approached the activity profiles of soccer referees, no change was found in decision making in a hot environment compared to a temperate environment, unlike previous research (Maughan et al, 2007, Simmons et al, 2008). Taylor et

al (2014a) attributed this to the motorised treadmill not allowing a player (or in their case referee) to express their true maximal capacity within a laboratory based simulation, due to the use of fixed running speeds, therefore, players may run at speeds slower than their true capacity (Thatcher and Batterham, 2004). One method in which this limitation can be overcome is by utilising a non-motorised treadmill (NMT). Therefore, a simulation can be specified (made relative) for each athlete (Abt *et al*, 2003), which means a NMT based soccer-specific simulation can allow for players to express their near-maximal physical performance capacity.

2.5.3. Non-motorised treadmill based simulations

Early studies with reliability measures have reported good reliability for power output (CV%: <9.3%) and peak sprint speed over two (Tong *et al*, 2001) and three (Hughes *et al*, 2006) NMT based tests. Repeated sprint exercise is an important facet during soccer match-ay, as players have been reported to complete up to 35 sprints over a short duration of match-play (Bradley *et al*, 2009, Di Mascio and Bradley, 2013, Mohr *et al*, 2003). However, previous repeated sprint exercise tests on a NMT have been a shorter duration [7 min - (Tong *et al*, 2001)] and lack external validity, compared to soccer match-play. This is due to the rest to low speed to high speed ratios being dissimilar in NMT based repeated sprint tests [1:1:1 - (Hughes *et al*, 2006)] compared to that seen in elite soccer match-play (2:1:1) (Arrones *et al*, 2014). Therefore, any well formulated soccer-specific simulation must approach the work to rest ratios of soccer match-play in order to be an externally valid tool to measure physical performance and physiological responses specific to soccer.

The reliability of a NMT based soccer-specific simulation is also imperative to allow the accurate quantification any environmentally-induced decrements upon physical performance. A study by Sirotic and Coutts (2008) assessed the reliability of a 30 min team sport simulation over three controlled tests. A significant difference and poor reliability measures [Intraclass Correlation (ICC): 0.182-0.836] were reported between trials 1-2 and 1-3 for high-speed distance covered (CV = 15.5%) and Bla (CV = ~24.45%). These differences in performance were likely due to the lack of familiarisation (Lakomy, 1987). Sirotic and Coutts (2008) suggested that thorough familiarisation (>2 sessions) to the NMT is important when performing high-speed exercise, due to the greater forward lean required to overcome the intrinsic resistance of the NMT (Lakomy, 1987). Therefore, it was suggested that a minimum of two familiarisation sessions should be utilised to optimise high speed movements upon the NMT (Sirotic and Coutts, 2008).

A NMT based soccer-specific simulation must approach the physiological responses (e.g. HR) and physical performance measures (e.g. total distance covered) to be an externally valid compared with soccer match-play (Abt *et al*, 2003, Oliver *et al*, 2007b). The soccer-specific exercise protocol (SSEP) created by Thatcher and Batterham (2004) was based upon the activity pattern of 12 elite and 12 youth-elite players during a 90 min soccer match. The simulation was developed with 5 pre-determined speed thresholds (Standing, Walking, Jogging, Running and sprinting) replicating both the physiological responses (HR: 166 ± 12 b·min-[1]) and physical (total distance covered: 10, 274 ± 609 m) performance compared with soccer match-play. One limitation of the SSEP, was that no reliability measures were quantified. The reliability of a simulation is important as it gives an indication of the biological and technical variation (Currell and Jeukendrup, 2008). Therefore, it is vital that a soccer-specific simulation has good reliability, in order to accurately quantify environmentally mediated changes and potential inferences from an intervention (Currell and Jeukendrup, 2008).

A further NMT based soccer-specific simulation by Oliver *et al* (2007b) created the soccer-specific intermittent exercise test (SSIET), which displayed good reliability measures (CV: <7.9%) well within the accepted level of variance (CV:<10%) (Atkinson *et al*, 1999). Furthermore, total distance (4804 ± 251 m) and sprint distance (551 ± 36 m) covered during SSIET were valid compared with one half of soccer match-play (Mohr *et al*, 2003). Previous NMT based soccer-specific simulations utilised 3 s sprints (Abt *et al*, 2003, Drust *et al*, 2000a), however, observations from previous research suggest that participants take 3 to 5 s to achieve peak sprint speed, a finding that is supported by the work of Lakomy (1987). However, a limitation to the SSIET was that although there was no set speed threshold for sprinting all walking, jogging and cruising were set and were not individualised between participants. Although, it is necessary to note that this is a development from the SSEP by Thatcher and Batterham (2004) who set all speed threshold for sprinting (23 kmh[-1]) and set the simulation as the work rate of the "average" player. Using an NMT is unique as the simulation can be specified to each athlete as it allows for free running to occur (Lakomy, 1987). Specifying (individualising) the speed thresholds of a simulation to each player is important when measuring physical performance in extreme environments and inferences from an intervention to understand how each player's performance may change. Therefore, a true reflection of a player's maximal output would be difficult to quantify without the use of individualised speed

thresholds within a well formulated NMT based soccer-specific simulation (Abt, 2002, Abt *et al*, 2003).

The use of individualised speed thresholds to quantify physical performance during soccer match-play has been increasingly researched in recent years (Abt and Lovell, 2009, Abt *et al*, 2003, Hunter *et al*, 2014, Lovell and Abt, 2013). In particular, research has focused upon the use of these individualised thresholds to assess an individuals high-speed running capacity during soccer match-play due to its association with game defining moments (Gregson *et al*, 2010, Wragg *et al*, 2000) and changes between playing standard (Mohr *et al*, 2003). One method of quantifying this variable is via utilising the VT_{2speed} from each individual, assessed via a $\dot{V}O_{2max}$ test (Abt and Lovell, 2009). Abt and Lovell (2009) utilised this method comparing the arbitrary speed threshold of 19.8 km·h^{-1}, which is typically used by semi-automated camera systems. It was reported that in elite soccer players the high-speed distance reported by semi-automated -camera systems (845 $\pm$ 296 m), was considerably less than the value reported by the new individualised speed threshold (2258 $\pm$ 707 m), suggesting that high-speed distance is often underestimated. This approach has been supported by Lovell and Abt (2013) who reported a 41% difference in the high-speed distance covered between players when utilising an individualised approach unlike the 5-7% difference found when utilising absolute differences. This approach provides information that using individualised speed thresholds would be beneficial to quantify soccer-specific exercise performance. Specifically, for this thesis, it would enable high-speed exercise capacities to be reliability ascertained, whilst adjusting for fitness levels. This would reduce the opportunity for fit individuals to complete without maximal output and individuals with lower fitness terminating exercise prematurely.

However, to individualise a NMT based soccer-specific simulation via VT_{2speed} from each individual can be problematic. To reliably ascertain a player's VT_{2speed} a $\dot{V}O_{2max}$ test would need to be utilised, which are most commonly performed on a motorised treadmill (Tonnessen *et al*, 2013). However, as previously mentioned, the running mechanics differ between both the motorised and NMT, meaning the VT_{2speed} may differ between the two methods (Lakomy, 1987). Not until 2013 was a $\dot{V}O_{2max}$ test created for the NMT simulation (Mauger *et al*, 2013), although it was unclear if the VT_{2speed} could be identified from this study due to exercise not always being steady state. Therefore, for this thesis further methods to individualise speed

thresholds upon a NMT soccer-specific simulation have been developed; in line with Abt *et al* (2003) and Sirotic and Coutts (2008).

The individualisation of a NMT based soccer-specific simulation has been successfully utilised in several recent studies (Abt *et al*, 2003, Sear *et al*, 2010, Sirotic and Coutts, 2008). Individualisation via the peak sprint speed of an athlete would allow a NMT based soccer-specific simulation to be somewhat individualised to the capability of each athlete, avoiding limitations seen in some earlier NMT simulations (Oliver *et al*, 2007b, Thatcher and Batterham, 2004). Therefore, a true reflection of the maximal capacity of an athlete can be ascertained. However, it must be made aware that a limitation to individualising a simulation by peak sprint speed could disadvantage athletes who are faster but have a lower $\dot{V}O_{2max}$ compared with slower players with the same $\dot{V}O_{2max}$ (Hunter *et al*, 2014). This may result in key measures such as high-speed distance covered for a faster player being under-interpreted as players maximum aerobic speed and peak sprint speed may differ creating a different anaerobic speed reserve for each player (Buchheit and Mendez-Villanueva, 2014, Mendez-Villanueva *et al*, 2013). Furthermore, it has also been reported that the peak sprint speed of an athlete on the NMT is approximately 80% of their free-sprinting speed during non-treadmill running (Lakomy, 1987). However, previous NMT based simulations have accounted for this by increasing the speed thresholds which in turn improves the validity of the simulation (Abt *et al*, 2003, Oliver *et al*, 2007b). Furthermore, a more thorough familiarisation, where participants can adapt to the unique running style required when running on a NMT could minimise this effect (Lakomy, 1987). Therefore, the use of individualised speed thresholds based on peak sprint speed is appropriate when formulating an NMT based soccer-specific simulation, however, the aforementioned limitations should be considered.

One study that successfully utilised individualised speed thresholds based upon peak sprint speed was by Abt *et al* (2003), who created a 90 min soccer-specific simulation containing 5 individualised speed thresholds (Stand, Walk, Jog, Run, Fast Run and Sprint) based on an individual's peak sprint speed within three fixed 15 min blocks each 45 min half. The simulation had good validity for the total distance covered (10,196 ± 403 m) and mean peak sprint speed (24.2 ± 0.8 kmh^{-1}) compared to match-play data (Di Salvo *et al*, 2007, Mohr *et al*, 2003). Abt *et al* (2003) was able to quantify fatigue in peak sprint speed, reporting a significant decrement by 7.9% in the 6th 15 min block compared with the 1st block. Therefore, utilising an NMT based soccer-specific simulation with both individualised speed thresholds within fixed

15 min blocks has good validity by reporting similar decrements to key physical performance measures (e.g. high-speed distance) as seen during match-play data.

It has been well documented within this thesis literature review that high-speed distance covered is a key determinant of successful soccer-specific performance (Gregson *et al*, 2010), as it is associated with game defining moments (Gregson *et al*, 2010, Wragg *et al*, 2000) and changes in level of performance (Mohr *et al*, 2003). All soccer-specific simulations to date usually set all speed categories and therefore players are not free to vary their running speed, even if they are capable of running at a faster-speed. Therefore, to further improve the validity of a NMT soccer-specific simulation, it would be beneficial to include measures of both sprinting and the "self-paced" ability to run at 'high-speed' as this would allow for a player to express their true maximal activity profile with and without externally governed targets influencing their performance.

One method to measure a players 'self-paced' ability to run at 'high-speed' is via a "variable run" component which has been utilised previously by Sear *et al* (2010) within the previous NMT based soccer-specific simulation created by Abt *et al* (2003). The speed component is named the 'variable run' and is designed to quantify the distance covered at a self-selected speed above the second ventilatory threshold, which has previously been used to delimit a 'high-speed' threshold as reported in previous match-play research (Abt and Lovell, 2009, Lovell and Abt, 2013). However, the protocol used by Sear *et al* (2010) was only 45 min in duration made up of three 15 min blocks. It was reported that there was no significant difference ($p < 0.05$) between the variable run in the first and last 15 min block of the simulation and therefore, the self-paced speed threshold was unable to quantify changes in high-speed distance. Therefore, potentially increasing the soccer-specific simulation's duration to 90 min, as opposed to the 45 min used by Sear *et al* (2010), may improve the simulations validity allow for a more appropriate analysis of the efficacy of the variable run, in quantifying high-speed distance covered, particularly in extreme environments where fatigue has been reported to be exacerbated (Mohr *et al,* 2010; Mohr *et al,* 2012).

2.6. Summary

The critical analysis in section 2.3 outlines that extreme temperatures of ~30°C, low altitudes of ~1,000m and a combination of these two conditions (Hot-Hypoxic) are the most prevalent extreme environments elite soccer teams would encounter during a season (Taylor and Rollo, 2014). It is also evident that hot, hypoxic and hot-hypoxic environments during elite soccer

match-play accelerate the development of fatigue due to a number of contributing factors, consequently reducing physical performance and physiological responses (Billaut and Aughey, 2013, Garvican *et al*, 2013, Mohr *et al*, 2012, Taylor and Rollo, 2014). Heat and low altitude mediated performance decrements stem principally from elevated body temperatures amongst other multi-factorial mechanisms [Section – 2.3.2. - (Nybo *et al*, 2014)] and reductions in PO_2 [Section 2.3.1. - (Garvican *et al*, 2013)], respectively. Therefore, an intervention which positively influences the physical performance decline mediated by extreme environments would be useful to practitioners in elite soccer (Taylor and Rollo, 2014).

Figure 2.4 outlines that extremes of heat are more prominent in elite soccer compared to hypoxic. Therefore, a plausible ergogenic strategy specific to a hot environment, is to increase an individual's heat storage capacity via pre- and half-time-cooling, thus reducing a player's body temperatures before the match and at half time (Taylor and Rollo, 2014). Section 2.4 identified that the most commonly employed cooling methods include cold water immersion, ingestion of a cold fluid/ice slurry, application of ice packs onto skin, the wearing of ice-cooling garments or a combination (mixed-methods) of these approaches (Tyler *et al*, 2013). Practical cooling methods including ice packs, ice slurry and mixed-methods pre-cooling can elicit a large ergogenic effect on physical performance and physiological responses during hot environments (Bongers *et al*, 2014, Castle *et al*, 2006, Minett *et al*, 2012a, Minett *et al*, 2011), although, their effects relative to soccer-specific exercise in the heat have not been explored securely.

Despite the well-organised and often novel experimental designs utilised by previous pre-/half-time-cooling soccer match-play research (Taylor and Rollo, 2014), section 2.12 outlines that high variability has been reported between matches for key outcome measures [(high-speed distance covered - coefficient of variation (CV); 36%)] (Gregson *et al*, 2010). Due to the large variability in game demands, meaningful inferences from interventions are difficult to ascertain as match performance measures show poor reliability (Gregson *et al*, 2010). One solution to quantify the effect environmental stress has on soccer performance and plausible interventions relative to these decrements, is to increase experimental control (specifically internally validity) via utilising a field or laboratory based simulations (Taylor and Rollo, 2014). Section 2.5 outlined that NMT soccer-specific simulation that incorporate individualised speed thresholds by an athlete's peak sprint speed provides specificity of the simulation to each athlete facilitating a truer expression of their near maximal physical performance (Abt *et al*, 2003). However, the nature of simulations usually means that speed thresholds are set and,

players are not free to vary their running speed even if they are capable of running at a faster-speed. A "variable-run" speed component could quantify the distance covered at a self-selected speed above the VT_{2speed}, by delimiting the 'high-intensity' threshold (Abt and Lovell, 2009, Lovell and Abt, 2013). The successful utilisation of the variable run speed component may provide some additional information on the development of fatigue and help display a player's ability or 'willingness' to run above the VT_{2speed} without an external cue.

2.7. Aims and hypothesis

Experiment 1

To investigate the reliability and validity of a NMT based soccer-specific simulation named the intermittent Soccer Performance Test (iSPT), incorporating a novel speed threshold called the variable run whilst utilising individualised speed thresholds based upon familiarised peak sprint speed (Experiment 1).

- There will be no significant difference for all physical performance and physiological responses between two experimental trials of iSPT.
- In two experimental trials of iSPT, there will be a significant reduction in variable run distance covered in the second half and the final 15 min block, compared to the first half and first 15 min block, respectively.

Experiment 2

To investigate the changes in simulated soccer performance during hot ($30^{o}C$; 50% RH), hypoxic (1,000m; $18^{o}C$ 50% RH), and hot-hypoxic (1,000m; $30^{o}C$ 50% RH) environments compared to a normoxic-temperate environment (0m; $18^{o}C$ 50% RH) utilising the iSPT.

- In the hot, hypoxic, and hot-hypoxic environment, simulated soccer performance will be significantly reduced compared with a normoxic-temperate environment during iSPT.
- In the hot-hypoxic environment, simulated soccer performance will be significantly reduced compared with the hot, hypoxic and normoxic-temperate environments during iSPT.

Experiment 3

To investigate the impact of three different pre- and half-time-cooling methods; 1) external (ice packs upon the quadriceps and hamstrings); 2) internal (ice slurry ingestion); and 3) mixed methods (internal and external) compared with a control (i.e. no-cooling) as a solution to acquiesce any heat-induced-decrements that were present in experiment 2.

- Ice packs, ice slurry and mixed-method pre-cooling will significantly improve simulated soccer performance during the first half of iSPT compared with no-cooling (control) during iSPT at 30°C.

- Ice packs, ice slurry and mixed-method half-time-cooling will significantly improve simulated soccer performance during the second half of iSPT compared with no-cooling (control) during iSPT at 30°C.

- The mixed-method pre- and half-time-cooling will significantly increase simulated soccer performance when compared with ice packs, ice slurry ingestion and control during iSPT at 30°C.

CHAPTER 3: GENERAL METHODOLOGIES

The general methodologies employed during experiment 1-3 are explained comprehensively within the present chapter. Furthermore, within the methods section of each experimental chapter, any chapter specific procedures are explained and the reader will be referred back to the appropriate section within this general methodologies chapter where necessary.

3.1. Ethical approval and location of testing

Ethical approval was granted by the University of Bedfordshire, Department of Sport Science and Physical Activity for studies 1-3. Approval numbers for each experimental chapter are reported below.

Experiment 1: - *Approval number: 2011ASEP006*

Experiment 2: – *Approval number: 2012ASEP019*

Experiment 3: – *Approval number 2013SPA008*

All experimental testing and related procedures were conducted within the Sport and Exercise Science Laboratories at the University of Bedfordshire.

3.2. Participant recruitment and control measures
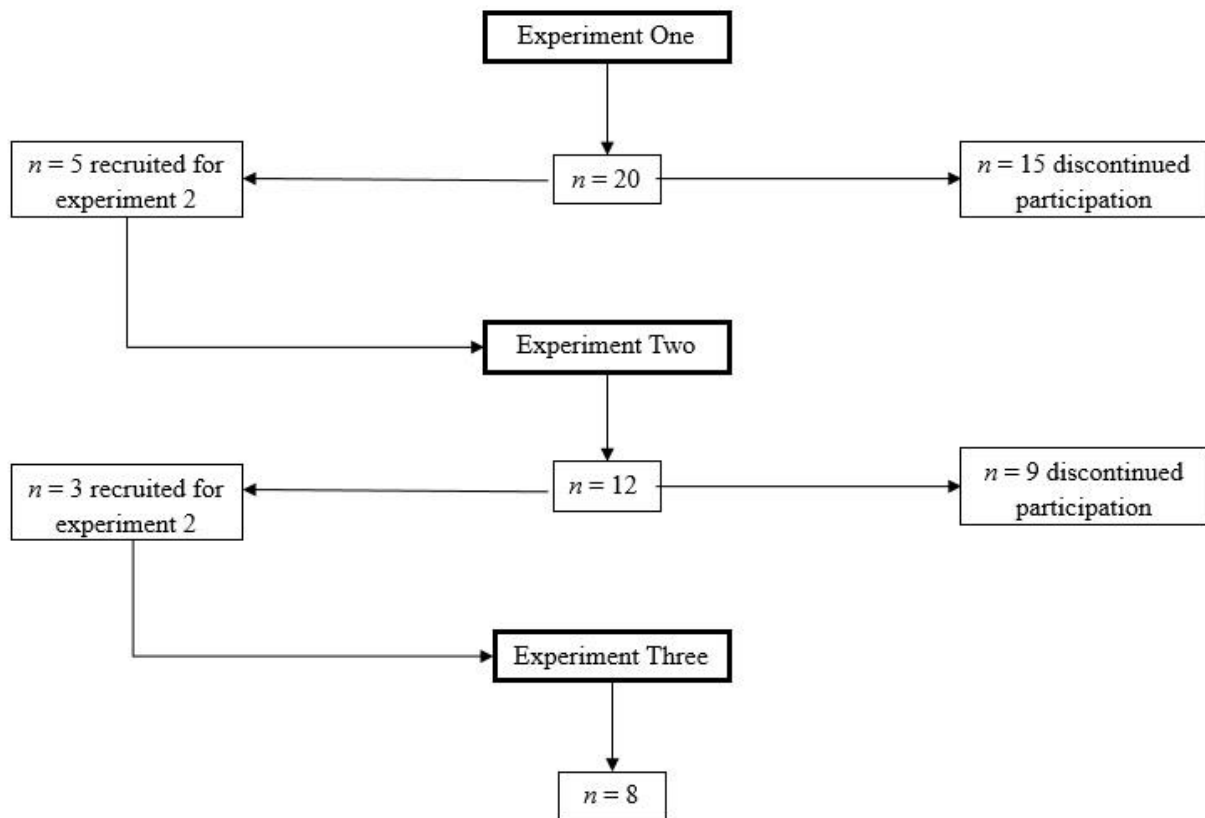
Figure 3.1: A trial profile outlining the flow of all participants between all three experiments conducted within this thesis.

Figure 3.1 reveals that the number of participants who volunteered for experiments 1, 2 and 3 was 20, 12 and 8 participants, respectively. A total of 5 participants who volunteered for experiment one were also recruited in experiment two (Figure 3.1). Furthermore, 3 participants who volunteered in experiment two also participated in experiment three (Figure 3.1). No participants took part in all three experiments. Therefore, a total of 33 participants who were healthy male's, free from musculoskeletal injuries in the last 6 months and aged 18-33 y were recruited for the three experiments within this thesis. All participants were members of the University of Bedfordshire Soccer team who trained at least two times per week and played at least one full 90 min match per week. These controls were adhered to by participants in all experiments and were monitored via a pre-test procedure checklist, which was completed and signed by participant. Those participants who did not adhere to this criteria were excluded from all experiments.

All participants completed and signed a pre-exercise medical questionnaire and PAR-Q (Appendix A) and gave written and verbal informed consent (Appendix B) which provided all

potential risks and discomforts of all testing procedures for each experiment. To minimise potential risks to themselves and the researcher, participants were also medically screened via a blood analysis questionnaire (See Appendix C). All participants were free to withdraw from testing at any point. It was stipulated within the participant information sheet (Appendix D) that participants did not engage in any unaccustomed high-intensity physical activity or consume any supplements or stimulants within 4 h of each test as they may alter fatigue occurring across the simulation (Seifert *et al*, 2010, Wolfe, 2000). Furthermore, it was stipulated that the same clothing and footwear was worn for each test. These were adhered to by participants in all studies and were monitored via a pre-test procedure checklist, which was completed and signed by participants at the commencement of each study; with adherence 100% for all subjects across all conditions.

All participants recruited for this study were non-smokers, as smoking is reported to significantly reduce in $\dot{V}O_{2max}$ performance (20%) and S_aO_2 (7%) during maximal exercise in a temperate-normoxic environment when compared to non-smokers (Klausen *et al*, 1983). Therefore, it is likely that maximal exercise in hot and hypoxic environments will exacerbate these decrements, compromising the findings from this thesis. No alcohol consumption was allowed 24 hr prior to exercise as it has been reported to increase heat loss during rest and exercise (Graham and Dalton, 1980). Furthermore, any caffeine supplementation (Lu *et al*, 2008) was barred from all meals and beverages taken 48 h prior to any testing session, as it is shown to improve repeated straight sprint performance (Glaister *et al*, 2008). Participants also refrained from acetaminophen (Paracetamol) supplementation 24 h prior to all trials, as it can attenuate the onset of pain and the increase in rectal temperature (T_{re}) during exercise bouts in both temperate and hot environments (Foster *et al*, 2014, Mauger *et al*, 2014). Testing times were held constant for individuals due to the effects of circadian variation upon T_{re} (Drust *et al*, 2005b). Participants were excluded from all studies if they had visited or lived at altitudes of 1000m or greater, as well as climates with ambient temperatures in excess of 30°C three months prior to completing an experimental trial as either partial or full heat and/or hypoxia acclimation may have occurred (Taylor, 2014). Individuals were also excluded if they had exposure to any prolonged thermal exposures (baths, saunas, steam rooms etc.) seven days prior to and during all experimental conditions (Taylor *et al*, 2010a). Furthermore, participants who had experienced high pressure environments (e.g. hyperbaria) three months prior to any experiment were excluded from the study. Additionally, all test instructions and verbal encouragement were written down, and therefore, standardised for all trials, to ensure the

investigator did not influence the results (Taylor *et al*, 2010a). Compliance to these measures was monitored via a questionnaire (See Appendix E) administered before each testing session with apparent adherence 100% for all participants across all conditions.

3.3. Anthropometry measurements

Body Mass (kg) and height (cm) were measured using digital scales (Tanita, BWB0800, Allied Weighing) and Holtain Stationmaster (Stadiometer, Harpenden, HAR 98.602, Holtain), respectively. For height measurements, participants stood with their feet together in contact with the Holtain Stationmaster looking straight ahead. Participants were instructed to inhale deeply whilst keeping their heals in contact with the floor. During this time the researcher would then lower the sliding scale upon the top of the head of the participant. Body mass was measured to the nearest 0.1 kg with minimal clothing and no footwear. Prior to each use of the digital scales, they were set for zero.

3.4. Maximum oxygen uptake test

During all experiments, all participants were initially assessed for maximal oxygen uptake ($\dot{V}O_{2max}$) using an incremental exercise test performed on a motorised treadmill (Woodway, PPS51 Med-i, Cranlea, Dudley). The treadmill belt speed was regularly checked by the technician to ensure the desired and the actual speed of the treadmill was within the recommended limits provided by the manufacturer (Maximum tolerance 1%). A ramp protocol was used whereby, the test began at 6 km·h^{-1}, and increased by 0.1 km·h^{-1} every 6 s until volitional exhaustion, with, strong verbal encouragement given throughout (Lucia *et al*, 2000). The test was performed at a gradient of 1% in order to reproduce the energetic cost of outdoor running on a flat surface (Jones and Doust, 1996). Pulmonary gas exchange and minute ventilation were measured continuously during the test using an online gas analysis system (Cortex, Metalyser 3B, Cranlea, Dudley), which was calibrated according to manufacturer instructions prior to each use. The $\dot{V}O_{2max}$ was considered as the highest $\dot{V}O_2$ obtained in any 10 s period and in line with the end-point criteria guidelines of the British Association of Sport and Exercise Sciences (Winter *et al*, 2007).

3.5. Non-motorised treadmill specifications and safety

A commercially available NMT (Woodway, Force 3, Cranlea, Dudley) was utilised for all three experiments (Figure 3.2). The belt speed of the NMT was regularly checked by the technician

to ensure the desired and the actual speed of the treadmill was within the recommended limits provided by the manufacturer (Maximum tolerance 1%).The optimum angle and position the belt (Schiek, Shiek Sports inc., USA) was raised to against the base of the NMT is 8° and between the top of the participant's pelvic bone and the bottom of the participant's lowest rib bone (Figure 3.3), respectively (Lakomy, 1987).

Figure 3.2: An illustration of the NMT utilised during all experiments of this thesis.

Figure 3.3: An illustration of how the precise angle measurement was recorded and the NMT belt position.

3.6. Activity Profile Design of the intermittent Soccer Performance Test (iSPT)

From this point forward, the NMT soccer-specific simulation utilised during all three experiments will be identified as the intermittent soccer performance test (iSPT).
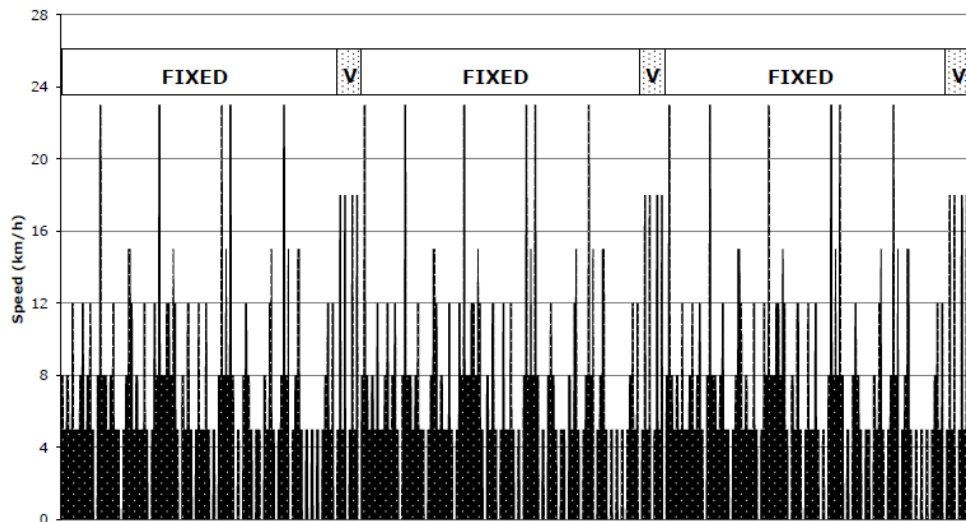
Figure 3.4: The 45 min activity profile of iSPT for a participant with a peak sprint speed of 23 km·h^{-1}

The activity profile of iSPT is based upon several soccer match-play studies (Bangsbo *et al*, 1991, Mayhew and Wenger, 1985, Reilly and Thomas, 1976, Withers, 1982). The duration of iSPT was 90 min consisting of two 45 min halves separated by a 15 min rest period, simulating open aged (>16 y) soccer match-play (Bloomfield *et al*, 2007). In order to reliably quantify changes for both the physical performance and physiological responses throughout iSPT, a 15 min activity profile was developed which was replicated six times throughout the 90 min duration (Figure 3.4). This would allow any variable to be compared between any 15 min block during iSPT, an approach utilised in other NMT based soccer-specific simulations (Abt, 2002, Abt *et al*, 2003, Abt *et al*, 1998, Drust *et al*, 2000a).

Whilst running iSPT, participants interacted with a computer programme (Innervation, Pacer Performance System Software) by following a red line on the screen, which displayed their target speed and their current speed. Subjects were instructed to match their current speed with the target speed as closely as possible throughout the full protocol. Audio cues specific to each movement category (e.g. Jog) were also presented. Before each change in speed, three audible tones were played, which were followed by an audible command to inform the subject of the upcoming activity (e.g. ''beep''.''beep''.''beep''. ''run'')
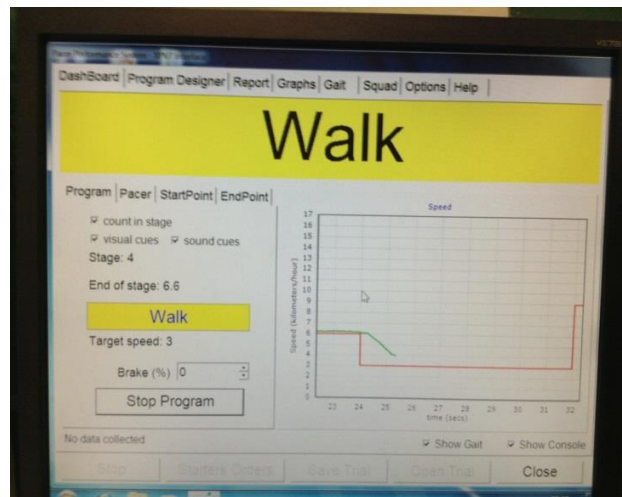
Figure 3.5: The computer programme participants interacted with during iSPT. All participants would follow the commands (e.g. walk) by trying to match their current speed (green line) with the instructed speed (red line).

The data identified from match-play data (Bangsbo *et al*, 1991, Mayhew and Wenger, 1985, Reilly and Thomas, 1976, Withers, 1982) was randomised by a dedicated software program (Innervation, Pacer Performance System Software, Australia) in order to "spread the individual movements across 15 min of activity", thereby simulating the intermittent nature of soccer-specific match-play. This software was programmed with certain rules, to delimit the activity profile around pre-defined parameters.

- A sprint would always be preceded and followed by a jog. This was to ensure that peak sprint speed could be compared between each sprint.
- A maximum of two individual movements could be grouped consecutively (e.g. two walks in a row). This was to avoid having excessively long periods without a change in speed.
- There would be one period where two sprints were performed within a short time period (< 1 min) and a second period where no sprints were performed within a longer duration (> 3 min). This would allow the effects of short and long recovery periods upon peak sprint speed and distance covered to be assessed during all three experiments.
- Furthermore, the formation of one long recovery period consisting of standing and walking on the 12th min of every 15 min of iSPT allowed for the safe collection of Bla.

Table 3.1: The percentage of intensity, frequency and total time spent at each movement category during iSPT.

Movement Category	% of PSS	Frequency	Total Time (s)	% Total Time
Stand	0	240	1920	17.8
Walk	20	456	3936	36.4
Jog	35	300	2592	24.0
Run	50	192	1248	11.6
Fast run	60	72	384	3.6
Variable run	Unset	48	288	2.7
Sprint	100	72	432	4.0
Total	---	690	5400	100

PSS = Peak Sprint Speed; s = seconds; % = Percentage

The iSPT was developed from a previous NMT soccer-specific simulation (Abt, 2002), which in brief was 90 min in duration containing 6 different speed thresholds. Furthermore, the original simulation was individualised by peak sprint speed, however the following alterations were made to develop iSPT to modernise the simulation due to recent research (e.g. association between high-speed distance covered and game defining moments (Gregson *et al*, 2010)) and previous simulations lacking specificity to soccer.

A total of seven different speed thresholds were utilised during iSPT (Table 3.1), dissimilar to the earlier simulation which contained 6 speed thresholds (Abt, 2002). The mean duration and frequency of an individual movement was divided into the total time spent in each movement category during actual match-play (Bangsbo *et al*, 1991, Reilly and Thomas, 1976, Withers, 1982) to determine the frequency in changes. The total frequency in changes for 15, 45 and 90 min are displayed in table 3.1.

The percentage of peak sprint speed of the stand, walk, jog, run, fast run and sprint speed thresholds of iSPT (Table 3.1) was based upon the findings of previous match-play data (Fallowfield *et al*, 1998, Wilkinson *et al*, 1997). However, the percentage of peak sprint speed were adjusted upwards by 5% to account for the inability of the NMT to allow different movement styles (e.g. backwards and sideways running) and due to the unorthodox running style of the NMT (Lakomy, 1987).

3.6.1. Variable run

In addition to the movement categories by Abt (2002) a seventh movement category called the variable run was included in iSPT. The variable run is a self-selected speed above the second ventilatory threshold (Abt and Lovell, 2009, Lovell and Abt, 2013). Match-play data has reported that speeds below sprinting but above the second ventilatory threshold are important

to game defining movements (Gregson *et al*, 2010) and also differ across standards of play (Mohr *et al*, 2003) during soccer match-play (Abt and Lovell, 2009, Lovell and Abt, 2013), hence the development of the variable run.

The iSPT contains a novel speed category referred to as a "variable run", designed to quantify the distance covered at a self-selected speed above VT_{2speed}, which has previously been used to delimit a "high-intensity" threshold (Sear et al, 2010). The variable run consisted of four self-selected high-speed runs situated in the 13th–14th min of each 15-min block. The variable run was 6 s in duration based on the practical realities of changing speed on the NMT (Lakomy, 1987) and the poor reliability of 3 s high speed movements (Hughes et al, 2006). For example, a participant completing the variable run during the iSPT, was asked to cover as much distance as possible at high-speed without sprinting. This then enabled quantification of the distance covered of each participant above VT_{2speed} without an external cue.

3.6.2. 3 s *vs* 6 s Sprints

The original protocol by Abt (2002) included 3 s sprints, which were excluded due to the poor reliability (ICC = 0.49) of these movement types (Abt, 2002, Hughes *et al*, 2006, Sirotic and Coutts, 2008). Therefore, only 6 s sprints were included as high reliability has been quantified for these movement types upon the NMT (Hughes *et al*, 2006).

3.7. Familiarisation with the non-motorised treadmill

As running on a NMT was a novel skill for all participants, familiarisation (FAM) sessions were organised. Three FAM sessions related to iSPT were completed and introduced to:

- Maintain balance whilst changing speed.
- Quantify changes in peak sprint speed
- To complete 90 min of iSPT

Table 3.2: Specific movements and duration of each familiarisation session.

Familiarisation	Time (min)	Speed categories involved
1	9	Stand, walk, jog, run, fast run
	13	Stand, walk, jog, run, fast run, sprint
	15	Stand, walk, jog, run, fast run, sprint
2	45	Stand, walk, jog, run, fast run, sprint
3	90	Stand, walk, jog, run, fast run, sprint

min = Minute

Table 3.2 reveals the duration and the speed categories involved in FAM_1, FAM_2 and FAM_3 prior to all participants completing iSPT. Between each FAM session participants were rested for 7 d Prior to completing FAM2 and FAM3 a peak speed assessment (PSA) was conducted. FAM1-3 robustly familiarised participants to iSPT and the running mechanics of NMT locomotion, which compared to 'free' running and motorised treadmill running has notable differences (Lakomy, 1987). Familiarised participants (i.e. post FAM1-3) subsequently (1 h post-FAM) completed a PSA, which identified each participant's familiarised peak sprint speed. The PSA derived of four 6 s maximal sprints over a 4 min period with equal rest (1 min) between sprints to allow adequate recovery time. For each participant, the peak sprint speed was defined as the fastest speed recorded during the PSA. The participants peak sprint speed was analysed from the PSA by a bespoke spreadsheet (Microsoft Excel 2010, Windows) which was then utilised to set the percentage of all speed thresholds as mentioned in section 3.6. So for example, a participant with a peak sprint speed of 24 km·h-1, would have the following speeds to achieve for each movement category across iSPT; stand (0 km·h-1), walk (5 km·h-1), jog (8 km·h-1), run (12 km·h-1), fast run (14 km·h-1) and sprint (24 km·h-1). The percentage of peak sprint, ascertained from the PSA, and how this determines the required speed for each movement category across iSPT is detailed in Table 1. These speed thresholds determined the speed (target speed/threshold) participants had to obtain for each movement type (stand, walk, jog, run, fast run and sprint).

The PSA was conducted twice to make comparisons to demonstrate no change had occurred and thus familiarisation had occurred. The peak sprint speed was then utilised to individualise all speed thresholds during iSPT. This approach was utilised as the NMT allows for free running ability to be quantified, allowing iSPT to be specified to each participant, as utilised previously (Abt *et al*, 2003, Sirotic and Coutts, 2008).

All performance, physiological and subjective measures were observed during all familiarisation sessions and were assessed for learning effects. For 20 participants a paired sample t-test revealed no significant difference ($p < 0.05$) between the final familiarisation session and all bouts of iSPT, for all physical, physiological and subjective measures examined in experiment 1, revealing that all participants were fully familiarised prior to testing.

3.8. Physical performance measures

All performance variables were recorded from the NMT at a sampling rate of 100 Hz using the software provided by the manufacturer (Innervation, Pacer Performance System Software).

The data were exported to a spreadsheet (Microsoft Excel 2010, Windows) for analysis.3.8.1. The walking, jogging, running and fast-run distance covered were all quantified as the distance covered up to 20, 35, 50, 60% of the athlete's peak sprint speed during iSPT, respectively. The variable run distance covered was quantified as the amount of distance covered during the 6 s period. Finally, the sprint distance covered was recorded as the distance covered at 100% of the athletes peak sprint speed. The high-speed distance covered during iSPT included the distance covered at the fast run, variable run and sprinting speed thresholds. The variable run distance covered was included as a high-speed distance threshold as the component was included within the simulation as it is a marker of distances covered above the second ventilatory threshold. This is also supported as participants were instructed to cover as much distance as possible at high-speed without sprinting. This approach has been previously utilised elsewhere (Sear *et al*, 2010). The low speed distance covered during iSPT included the distance covered at the walking, jogging and running speed thresholds. The total distance covered during iSPT involved all physical performance measures mentioned previously. The peak sprint speed of the participant was ascertained as fastest recorded speed in the 6 s sprint period. This approach has been previously utilised elsewhere.

3.9. Physiological responses

3.9.1. Heart rate monitoring

The HR of each participant was recorded beat-by-beat and averaged every 1 min using a telemetric HR monitor (Polar, FS1, Polar Electro, Cranlea, Dudley). An electro-conductive gel (Other-Sonic, Pharmaceutical Innovations, inc., Newark) coated the inside of the HR monitor to enhance signal detection and fitted around the participant's chest.

3.9.2. Oxygen saturation within the arterial blood

The S_aO_2 was measured every 15 min via a finger pulse oximeter (Onyx® II 9550, Nonin Medical, USA) fixed upon the index finger of the right hand of each participant.

3.9.3. Hydration status assessment

Hydration status was assessed via urine osmolality (Antago Vitech Scientific, Pocket PAL-OSMO, HaB Direct, Southam) prior to all experimental trials. Prior to reporting to the laboratory, subjects were instructed to drink 500ml of water 2 h prior to all (including preliminary testing) exercise bouts which is in accordance with the ACSM position stand

(Sawka *et al*, 2007), unless otherwise stated. If subjects' urine osmolarity was 600 mOsm/l, it was deemed that subjects were at a hydrated state (Hillman *et al*, 2013, Hillman *et al*, 2011), however, if the participant was not deemed hydrated, then the experimental trial would be abandoned. This experimental control was not violated for any subjects for any experimental procedure or intervention.

3.10. Subjective measures

3.10.1. Perceived exertion

The rating of perceived exertion (RPE) was measured using the Borg 6-20 scale (Borg, 1998). This scale is considered to be a valid and reliable marker of exercise intensity during a number of exercise tests (Eston, 2012), and has been widely utilised during previous soccer-specific simulation studies (Oliver *et al*, 2007a, Oliver *et al*, 2007b, Thatcher and Batterham, 2004). The RPE measurements were recorded every 15 min during each testing session as this allowed for an equal number of responses to be recorded each half of ISPT.

3.10.2. Thermal sensation scale

The thermal sensation (TS) was measured using a 0-8 scale (Young *et al*, 1987). This scale is suggested to be a valid and reliable marker of assessing thermal comfort (Gagge, Stolwijk and Hardy, 1967), however, no previous soccer-specific simulation studies have utilised this scale previously. The TS measurements were recorded every 15 min during each testing session as this allowed for an equal number of responses to be recorded each half of ISPT.

3.10.3. Readiness to invest physical and mental effort

Readiness to invest physical effort (RTIPE) and mental effort (RTIME) was measured using a 0-10 scale (Duncan et al, 2012). This measure has been suggested to be a reliable and valid marker how physically and mentally ready a participant is to invest effort prior to exercise (Duncan et al, 2012). The RTIPE and RTIME was recorded pre and post-iSPT during each condition in experimental chapter 3 as previously utilised during research within a hot environment (Coull et al, 2015).

3.11. Body temperature measurement

All body temperature measurements were recorded as instructed by the University of Bedfordshire's ethical guidelines

3.11.1. Rectal temperature

A rectal thermistor (Henleys, 400H, Henleys Medical, Welwyn Garden City) was used to measure rectal temperature (T_{re}) during all experimental trials. The rectal thermistor was measured from a depth of 10cm past the anal sphincter which was recorded to a data logger (Measurement, 4600, Henley medical, Welwyn Garden City). All participants inserted the thermistor in a private room where the door was left unlocked in case the subject suffered an anaphylactic reaction. Fortunately, no anaphylactic reactions occurred during any experimental trial. All T_{re} measurements were recorded every 15 min during each testing session as this allowed for an equal number of responses to be recorded each half of ISPT.

3.11.2. Skin temperature

The skin temperature (T_{sk}) probes (Grant, EUS-U-VS5-0, Wessex Power, Dorset) were attached on the right side of the body at the centre of the muscle on four different sites (Ramanathan, 1964). Where T_{chest}, T_{arm} T_{thigh} and T_{calf} are the temperatures recorded from the chest, arm, thigh and calf respectively. Each thermistor was recorded separately to a data logger (Eltek/Squirrel, Squirrel Series/model 451, Wessex Powe, Dorset) where it was saved and analysed upon the completion of testing. The following formula was then used to calculate T_{sk} (Ramanathan, 1964).

$$T_{sk} = 0.3 \, (T_{chest} + T_{arm}) + 0.2 \, (T_{thigh} + T_{calf})$$

All T_{sk} measurements were recorded every 15 min during each testing session as this allowed for an equal number of responses to be recorded each half of ISPT.

3.11.3. Mean body temperature

The total body temperature (T_{body}) was calculated from T_{sk} and T_{re} using the following equation

$$T_{body} = 0.79(T_{re}) + 0.21(T_{sk})$$

3.11.4. Muscle temperature

The muscle temperature (T_{mu}) was calculated from T_{sk} using the following equation (Racinais *et al*, 2005):

$$T_{mu} = 1.02 \; x \; T_{sk} + 0.89$$

This formula shows a very good ($r = 0.98$) correlation with T_{mu} (de Ruiter *et al*, 1999).

All estimated body temperature measurements reported in section 3.10 were recorded every 15 min during each testing session as this allowed for an equal number of responses to be recorded each half of ISPT.

3.12. Blood collection

Fingertip capillary blood samples were collected using standard techniques. Firstly, the finger was cleaned using a sterile alcohol wipe (Cutisoft Wipes, ESPO, Leicester,). The area was then punctured using a lancet (Haemolance+, HTL-STREFA, S. A., Poland). The first initial droplet of blood was wiped away and then the second drop was used before analysis. All blood sampling was taken within the ethical guidelines set by the University of Bedfordshire.

3.12.1. Blood lactate

Fingertip blood samples were collected into tubes (Microvette, CB 300 LH, Sarsedt) where they were analysed for Bla concentration. The blood sample was then placed under a Bla analyser (YSI, 2500 stat plus, YSI, Hampshire). The Bla was recorded every 0, 12, 27 and 45 min during one half of iSPT due to the irregularity of the simulation.

3.12.2. Blood plasma volume

Blood plasma volume was collected into heparinsed capillary tubes (Hawksley & Sons Ltd, UK), they were then centrifuged at 5,000 RPM for 3 min (Hawksely, Micro Haematocrit centrifuge, Hawksley & Sons Ltd, UK) and haematocrit (Hct) values were read from the Haematocrit reader (Hawksley, UK). Haemoglobin (hB) concentration was then collected via microcurvettes (Hemocue, hB 201, Hemocue Ltd, Sweden). It was then measured using a B-Haemoglobin photometer (Hemocue, Hb 201^{+}, Hemocue Ltd, Sweden).

Changes in blood plasma volume (%ΔPV) both within/ between tests was estimated from Hb and Hct using the following equation (Dill and Costill, 1974):

$$\%\Delta PV = [(Hb_{preex}/Hb_{postex}) \times [(100 - Hct_{postex})/(100 - Hct_{preex})] - 1] \times 100.$$

Where ΔPV is percent change of PV, subscript b, is prior to exercise; and subscript a, is post exercise. Blood plasma volumes were recorded pre and post each half of iSPT.

The Dill and Costill method was used to examine the plasma volume change in each experiment reported within this thesis, as it can estimate these changes from both hB and Hct (Alis et al,

3.13. Generation of hot, hypoxic and hot-hypoxic exposures

All hot, hypoxic and hot-hypoxic exposures were generated and administered within a controlled laboratory environment. The laboratory environment used for all testing conditions was custom designed environmental chamber (Flower House, Farm House, Two Wests and Elliot, Chesterfield). The temperature of each environmental condition was measured every 5 min during each experimental trial via a thermometer (Ellab, DM852, Testo Ltd, Germany). All hot exposures were administered using a portable heater (Bio Green, Arkansas 3000, Hampshire) and the hypoxic exposures were administered via an adjustable hypoxicator (Everest Summit II, The Altitude Centre, UK). The heater would utilise air taken from the room which was heated to increase the temperature of the environmental chamber. The hypoxicator utilises O_2 filteration to generate the necessary hypoxic load (Taylor *et al*, 2012, Taylor *et al*, 2011, Taylor *et al*, 2010a, Taylor *et al*, 2010b). The normobaric pressures for each testing session was measured pre and post each half of the simulation via an O_2 meter (Kane International LTD, KANE 250, Kane International LTD, Hertfordshire).

Table 3.3: The environmental conditions utilised during all three studies of this thesis

Experiment No.	Environmental Condition	Temperature (^oC)	rH (%)	Altitude (m)	Normobaric Pressure (%)
1	Normoxic-Temperate	18 ± 1.2	31 ± 2.2	85	20.93
2	Normoxic-Temperate	18 ± 1.9	34 ± 1.5	85	20.93
	Hot	30 ± 1.4	32 ± 1.4	85	20.93
	Hypoxic	18 ± 0.9	31 ± 0.5	$1,000 \pm 33$	18.4 ± 1.2
	Hot-Hypoxic	30 ± 1.1	33 ± 1.4	$1,000 \pm 29$	18.4 ± 1.2
3	Hot	30 ± 1.8	31 ± 1.1	85	20.93

rH = Relative Humidity

Table 3.3 details the environmental conditions utilised during all three experiments of this thesis. The normoxic-temperate environments utilised in experiment 1 (18 ± 1.2 ^oC; $31 \pm 2.2\%$ RH, 85 m; 20.93%) and 2 (18 ± 1.9 ^oC; $34 \pm 1.5\%$ RH, 85 m; 20.93%) represents a thermo-

neutral environment that has been utilised previously within soccer-specific simulation research (Thatcher and Batterham, 2004). The hot environment utilised in experiment 2 (30 ± 1.8 ^{o}C, 31 ± 1.1% RH, 85 m, 20.93%) and 3 (30 ± 1.4 oC, 32 ± 1.4% RH, 85m, 20.93%) represented an average temperature (30°C, 30% RH) of Southern European countries (e.g. Spain, Greece etc.) during critical points of the season (August-September; April-June). The hypoxic environment utilised in experiment 2 (18 ± 0.9^{o}C, 31 ± 0.5%, 1,000 ± 33m, 18.4 ± 1.2%) was chosen as it was most elevated point in Europe where a UEFA Champions and Europa League team played in the 2013-14 season (1,000m, Molde FC, Norway). Finally, the hot-hypoxic environment utilised in experiment 2 (30 ± 1.1^{o}C, 33 ± 1.4%, 1,000 ± 29m, 18.4 ± 1.2%) represents an extreme temperature and altitude teams could experience between June and August within the UEFA Champions and Europa League (Molde FC, Norway).

3.14. Pre-cooling and half-time-cooling methods

In experiment 3, participants completed 30 min of pre-cooling prior to iSPT and 15 min of per-cooling at half time. The exact procedures are explained below

3.14.1. Cooling environment

All pre-cooling, per-cooling and no-cooling methods were completed whilst participants were seated in a temperate environment (18°C, 30% RH). This environment was chosen to replicate a player using each cooling method in an elite soccer setting within an air-conditioned changing room.

3.14.2. No cooling (control) and water consumption

During the no-cooling (control) trial in experiment 3, participants consumed room temperature water (21 ± 0.6 ^{o}C) which was relative to the amount ice slurry consumed. It was quantified that 100g of ice slurry would melt into 150ml of water. Therefore, the amount of water that participants would consume during the ice no-cooling and mixed-method cooling strategy was quantified from the following equation:

$$Water = Mass\ (kg)\ x\ 7.5g\ \ x\ 1.5ml$$

The total amount of water to be consumed by the participant was divided into 3 portions to be ingested every 10 min to ensure it was ingested evenly throughout the 30 min period. At half-time during the control experimental trial the amount of water consumed was halved and was divided into 3 portions to be ingested every 5 min to ensure it was ingested evenly throughout

the 15 min period. This approach was also utilised during the ice packs trial however, no water was consumed in the ice slurry and mixed methods cooling trials.

3.14.3. Ice packs

Figure 3.6: An illustration of the ice packs placed in a cotton cover to the anterior, lateral and posterior aspects of the quadriceps and hamstring muscle groups

Ice packs (Aeroplast, hot/cold pack, Hertfordshire Suppliers, Hertfordshire) were removed from a freezer at a temperature of -20°C (Lec-medical, IST 47, Fisher Scientific, Loughborough) with a surface temperature of -14 ± 4.6°C. They were then wrapped in a cotton cloth (Compression Cuff Holder, Lewis-Plast, Lewis Medical Supplies, Stockport) to avoid damage to the skin before being placed upon the anterior, lateral and posterior aspects of the quadriceps and hamstring muscle groups (Figure 3.6). As a pre-cooling method the ice packs were placed on the quadriceps for 30 min prior to iSPT. As a half-time cooling method the ice packs were utilised for the full 15 min interval. The use of ice packs via this methodology has been previously advocated as a pre-cooling (Castle *et al*, 2006) and pre-/half-time-cooling strategy (Minett *et al*, 2012a) on intermittent sprint exercise, however, no studies has utilised this method within a soccer-specific setting.

3.14.4. Ice slurry

Figure 3.7: An illustration of the ice slurry being consumed.

Ice cubes were used to create the ice slurry via an ice machine (Ice Maker, think Gizmos, Amazon Ltd, Alton). Once an adequate amount of ice had been created, these were then crushed using an ice slurry machine (Snow Cone Maker, JM Posner, Amazon Ltd, Alton) to make an ingestible ice slurry drink. As a pre-cooling method all participants ingested 7.5g/kg of body mass of ice slurry over a 30 min period min prior to iSPT. For the half-time-cooling method the amount of ice slurry consumed was halved compared to the pre-cooling method and was consumed for the full 15 min interval. For both the pre-cooling and half-time-cooling method the ice slurry was divided equally into 3 portions to be ingested every 10 and 5 min to ensure it was ingested evenly throughout the 30 and 15 min period, respectively.

3.14.5. Mixed-methods

Figure 3.8: An illustration of the mixed-method cooling strategy

Mixed-method pre-cooling and half-time-cooling utilised both ice packs and slurry concurrently (Sections 3.14.2-3.14.3 for specific methods). The use of mixed-methods cooling has been previously utilised both as a pre-/per-cooling strategy by Duffield *et al* (2013), however, the study had a host of limitations as discussed in section 2.3.3.

3.15. Statistical analysis

Analyses were completed using the statistical software package IBM SPSS Statistics version 19.0 (SPSS Inc, Chicago, IL, USA). Standard graphical methods were preferred over null hypothesis significance testing to check statistical assumptions (Grafen and Hails, 2002). Prior to any inferential statistical analyses, descriptive statistics tables were generated to check the central tendency (mean, median), and dispersion (standard deviation (SD), minimum, maximum) of the data. Quantile-quantile (Q – Q) plots were used to check the normality assumption of the results obtained from each experimental condition. Where normality was deemed plausible, central tendency and dispersion were reported as mean ± SD, otherwise they were reported as the medium, minimum and maximum values. The two-tailed alpha level for significance testing was set as $p < 0.05$. All graphs were produced using SigmaPlot version 13.1 (Systat Software Inc, CA, USA). The specific statistical procedures employed in each individual experiment are presented in their respective chapters.

Chapter 4. Experiment 1: The Reliability and Validity of a Soccer-Specific Non-Motorised Treadmill Simulation

This experimental chapter has formed the basis of the publication detailed below:

Aldous, J. W. F., Akubat, I., Chrismas, B. C. R., Watkins, S. L., Mauger, A. R., Midgley, A. W., Abt, G. & Taylor, L. (2013). 'The reliability and validity of a soccer-specific non-motorised treadmill simulation (iSPT)', *The Journal of Strength & Conditioning Research,* 28, (**7**), pp 1971-1980, doi: 10.1519/JSC.0000000000000310.

4.1. Introduction

Section 2.1.2, details that some soccer-specific physical performance measures can vary considerably between matches (Rampinini *et al*, 2007). Indeed large variability between matches for a variety of high speed running measures are seen across three elite soccer seasons (Gregson *et al*, 2010); rendering meaningful inferences on physical performance problematic from soccer match-play without very large and likely unrealistic sample sizes (Gregson *et al*, 2010). Similarly, the efficacy of interventions (e.g. ergogenic aids) and the impact of environmental stress on soccer performance are therefore, also difficult to ascertain when relying on soccer match play data.

The development of laboratory or field based soccer-specific simulations, to replicate soccer performance, have sought to address the outlined issues above (Russell *et al*, 2011). The conceptual frameworks, including advantages and disadvantages of such simulations are reviewed within section 2.5. Such critical review identified that an NMT soccer-specific simulation that incorporates individualised speed thresholds by an athlete's peak sprint speed provides specificity of the simulation to each athlete, facilitating a truer expression of their near maximal physical performance than any other plausible alternative for the purposes of this thesis. Importantly, such a simulation will allow a player to vary their running speed, above or below an individualised threshold (e.g. 'jog, run and fast run'). Such a design facilitates quantification of repeated individualised sprint capacity (central to match outcome - (Gregson *et al*, 2010), in addition to their ability ('variable run') to run at 'high-speed' without an external informed objective (i.e. a specific speed threshold). This 'variable run' allows the distance covered at a self-selected speed (i.e. self-paced) above the second ventilatory threshold to be assessed, an important delimiter of 'high-speed' physical performance in soccer (Abt and Lovell, 2009, Lovell and Abt, 2013).

Admittedly there is a loss of ecological validity due to the lack of changes to direction (Small *et al*, 2010) and measurement of technical skills (Williams *et al*, 2010) by using a NMT based soccer specific simulation (Section 2.5.). However, laboratory environmental control is required when reliably quantifying the effect of environmental extremes and cooling interventions on soccer performance in experiment 2 and 3 respectively. Therefore, the application of a NMT based soccer-specific simulation represents the best compromise to answer the research questions of this thesis.

4.1.1. Experimental aims and hypothesis

Experiment 1

To investigate the reliability and validity of a NMT based soccer-specific simulation named the intermittent Soccer Performance Test (iSPT), incorporating a novel speed threshold called the variable run whilst utilising individualised speed thresholds based upon familiarised peak sprint speed.

- There will be no significant difference for all physical performance and physiological responses between two experimental trials of iSPT.
- In two experimental trials of iSPT, there will be a significant reduction in variable run distance covered in the second half and the final 15 min block, compared to the first half and first 15 min block, respectively.

4.2. Methods

4.2.1. Participants

The twenty male, apparently healthy participants who volunteered for this study had the following characteristics: [median (min-max) age = 21 (18-27) y; mass = 73 (65 - 90) kg; height = 185 (165 - 191) cm; mean $\pm$ SD $\dot{V}O_{2max}$ = 50 $\pm$ 8 ml$\cdot$kg$^{-1}\cdot$min^{-1}]. All participants conformed to the pre-test guidelines and procedures outlined in section 3.2.

4.2.2. General experimental controls

All FAM and testing sessions were completed on the same NMT. For a detailed description of the NMT specifications and safety measures please see section 3.5.

4.2.3. Experimental design

Participants visited the laboratory on six separate occasions to complete one $\dot{V}O_{2max}$ test, three FAM sessions and two experimental trials (Figure 4.1). All participants completed a $\dot{V}O_{2max}$ prior to completing all FAM and iSPT testing sessions. For a detailed description of the $\dot{V}O_{2max}$ test and please see section 3.4. *Visit 2-4 (FAM$_{1-3}$)*: All FAM and PSA sessions were completed as outlined in section 3.7. *Visits 5 and 6 (iSPT$_1$ and iSPT$_2$)*: Participants were then rested for 7 – 9 d and 6 – 10 d before taking part in both iSPT$_1$ and iSPT$_2$, respectively. For a detailed description of the design and the methods utilised to form iSPT, please see section 3.6.

4.2.4. Experimental procedures

Upon arrival to the laboratory on visit 1 all anthropometric measurements were recorded as described in section 3.3. Following the completion of these methods participants were then seated and rested for 5 min before resting physiological [HR (Section 3.9.1) and hydration status (Section 3.9.3.)], subjective [RPE (Section 3.10.1.) and TS (Section 3.10.2)], body temperature [T_{re} (Section 3.11.1.), T_{sk} (Section 3.11.2.) and T_{mu} (Section 3.11.5.)] and blood [Bla (Section 3.12.1.)] measurements were all obtained. Immediately before iSPT, participants performed a 5 min warm up on the NMT at a speed of 8 km·h^{-1}, which included two brief sprints (<4 s); 5 minutes were then allowed to perform individual stretching exercises (Oliver *et al*, 2007b). During the 15 min interval participants were seated and given 500 mL of water to drink.

4.2.5. Soccer performance measurements

During iSPT, HR (Section 3.9.1.), Bla (Section 3.12.1.) and RPE (Section 3.10.1.) were recorded in line with Figure 4.1. All physical performance measures were ascertained as shown in section 3.8. All physical performance and physiological response data from iSPT$_1$ and iSPT$_2$ were used to ascertain measures of validity compared to match-play data (Barnes *et al*, 2014, Bradley *et al*, 2009, Di Mascio and Bradley, 2013, Di Salvo *et al*, 2007, Di Salvo *et al*, 2012, Mohr *et al*, 2003) alongside the calculation of all reliability measures.

4.2.6. Statistical analysis

No power calculation was completed prior to this experiment. However, it has been recommended for a reliability study that a sample of twenty participants should be used (Atkinson and Nevill, 1998). Therefore, a sample size of twenty participants was recruited. Normality of the observed data was assessed using Q - Q plots and was deemed plausible in all instances. Subsequently for descriptive purposes the mean and SD have been used to report the central tendency and dispersion of the observed data. Reliability of all variables from consecutive pairs of trials was assessed using data from iSPT$_1$ and iSPT$_2$. The following reliability measures [change in the mean (CIM), CV, ICC and the typical error of measurement (TE) along with ninety-five percent confidence intervals (95% CI)] were all used. The CV, which is expressed as a percentage, was calculated by dividing the standard deviation of the difference by the square root of two (1.414) and dividing the answer by the grand mean

(Hopkins, 2000). ICC was used to determine the between trial reliability (Brughelli and Van Leemputte, 2013) and was calculated by the following calculation (Vincent and Weir, 2012):

$$ICC = \frac{F - 1}{F + (k - 1)}$$

F = F ratio from LMM analysis; k = (observations – tests)/subjects – 1)

An ICC close to 1 indicates 'excellent' reproducibility, therefore, an ICC of above 0.90 is deemed as 'high' reliability, values between 0.80 and 0.89 is considered 'good' reliability and values lower than 0.80 is deemed 'questionable' reliability (Vincent and Weir, 2012). However, ICC is a limited measure of reliability due to the influence of the magnitude of the between-subject variation and it does not indicate possible systematic bias (Ayala *et al*, 2012). Therefore, systematic Bias was examined graphically with Bland-Altman Plots (Bland and Altman, 1986). The TE was estimated by the sample estimate of the population standard deviation divided by the square root of the sample size (Atkinson and Nevill, 1998). Statistical analysis was completed using linear mixed models (IBM SPSS statistics for Macintosh, Version 20, Armonk, NY) to analyse the main effect for condition and time in all variables between the two conditions ($iSPT_1$ and $iSPT_2$). Furthermore, linear mixed models were also performed to analyse the difference between all physical, physiological and subjective measures between the first and second half and each 15 min block of iSPT. This type of analysis was preferred as it i) allows for missing data, ii) can accurately model different covariate structures for repeated measures data, and iii) can model between-subject variability (Vandenbogaerde and Hopkins, 2010, West *et al*, 2014). Step down Hommel adjusted post-hoc pairwise comparisons were calculated if a significant main effect and/or interaction effect was present (Hommel, 1988). Where significance was obtained, Sidak post-hoc tests were used to locate significant pairs. The 95% CI were also presented where necessary. The percentage change between the first and second half of $iSPT_1$ and $iSPT_2$ are also reported. Two-tailed statistical significance was accepted at the $p < 0.05$ level.
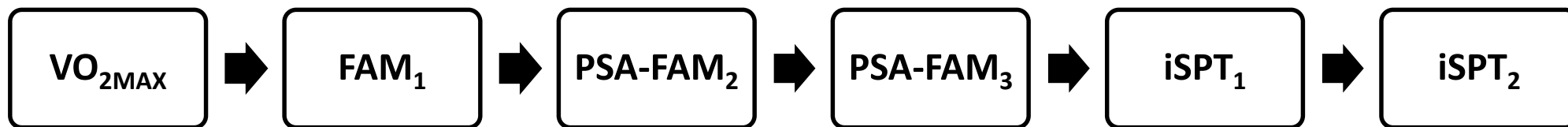
Figure 4.1. Experimental Design
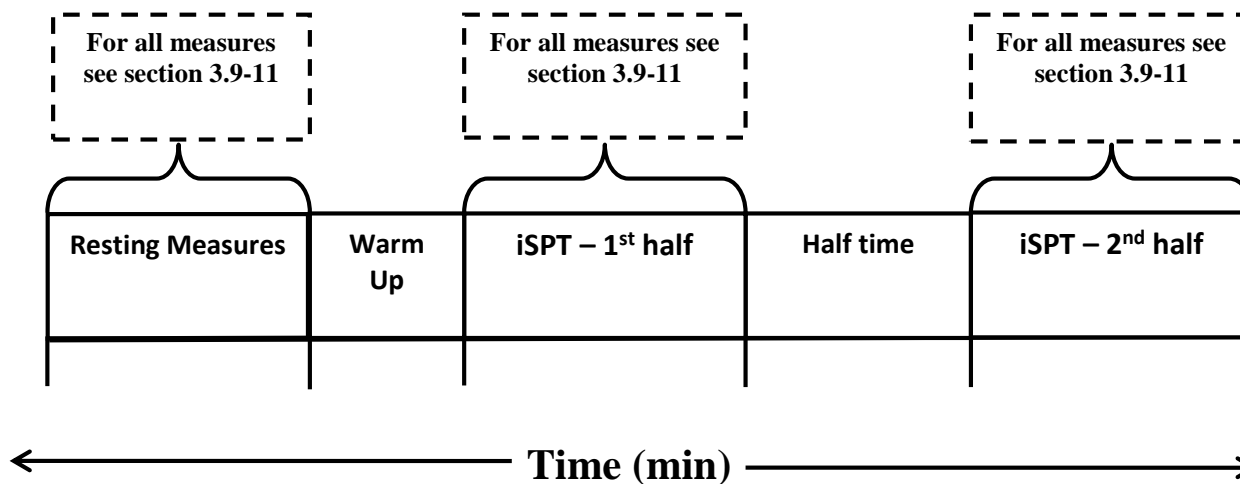
Figure 4.2. A schematic detailing the experimental procedures utilised during experiment 1. All measurements were recorded as documented within the relevant sections of the general methodologies. All physical performance measures were ascertained post iSPT as documented in section 3.8. Blood lactate = lactate; Familiarisation = FAM; heart rate = HR; Intermittent Soccer Performance Test = iSPT; Peak Speed Assessment = PSA;Rating of Perceived Exertion = RPE.

Table 4.1: Mean ± SD, significance values, change in mean (CIM), typical error (TE), intraclass correlation (ICC) and coefficient of variation (CV) with 95%CI for all soccer performance measures from $iSPT_1$ and $iSPT_2$. Any discrepancies between the means and the CIM are due to rounding errors.

Measure	$iSPT_1$	$iSPT_2$	CIM	p value	ICC	CV	TE
TD (m)	8960± 482	8945 ± 471	-15 (-90 to 60)	0.684	0.95 (0.86 to 0.98)	1.3 (1 to 1.9)	114 (86 to 166)
WD (m)	2405 ± 100	2398 ± 97	-7 (-26 to 11)	0.424	0.93 (0.81 to 0.97)	1.2 (0.9 to 1.7)	28 (21 to 41)
JD (m)	2575 ± 186	2577 ± 189	2 (-36 to 40)	0.912	0.91 (0.77 to 0.96)	2.3 (1.8 to 3.4)	58 (44 to 84)
RD (m)	1623 ± 102	1617 ± 96	-6 (-19 to 7)	0.347	0.96 (0.90 to 0.98)	1.2 (0.9 to 1.8)	20 (15 to 29)
FRD (m)	622 ± 30	623 ± 35	1 (-6 to 7)	0.965	0.92 (0.81 to 0.97)	1.6 (1.2 to 2.4)	10 (7 to 14)
VRD (m)	534 ± 46	531 ± 47	-3 (-12 to 7)	0.622	0.91 (0.77 to 0.96)	2.7 (2.1 to 4.0)	14 (11 to 21)
SD (m)	1002 ± 50	997 ± 77	-5 (-18 to 9)	0.490	0.93 (0.83 to 0.97)	2.1 (1.6 to 3.1)	21 (16 to 30)
HSD (m)	2158 ± 137	2153 ± 142	-5 (-27 to 17)	0.635	0.95 (0.88 to 0.98)	1.6 (1.2 to 2.3)	33 (25 to 49)
LSD (m)	6841 ± 351	6847 ± 310	6 (-77 to 62)	0.822	0.93 (0.84 to 0.97)	1.6 (1.2 to 2.3)	104 (79 to 152)
PSS (km·h^{-1})	19.9 ± 1.7	19.2 ± 2.1	-0.7 (-0.8 to 0.43)	0.924	0.92 (0.81 to 0.97)	3.0 (2.3 to 4.5)	0.6 (0.5 to 0.9)
HR (b·min-^{1})	165 ± 8	164 ± 8	-1 (0 to 3)	0.124	0.92 (0.80 to 0.97)	1.5 (1.2 to 2.2)	3 (2 to 4)
HR_{MAX} (%)	83.3 ± 4	82.7 ± 3	-0.6 (-0.9 to 0.3)	0.185	0.90 (0.76 to 0.96)	1.6 (1.2 to 2.3)	0.01 (-0.01 to 0.03)
Lactate (mmol)	4.7 ± 1.3	4.6 ± 1.3	-0.1 (-0.3 to 0.07)	0.434	0.97 (0.92 to 0.99)	5.0 (3.9 to 8.0)	0.3 (0.2 to 0.4)
RPE (mode)	15.1 ± 1.3	15.2 ± 1.5	-0.1 (-0.3 to 0.3)	0.371	0.90 (0.76 to 0.96)	3.3 (2.5 to 4.9)	0.4 (0.3 to 0.7)
T_{re} (ºC)	38.3 ± 0.3	38.2 ± 0.3	-0.1 (-0.2 to 0)	0.456	0.94 (0.83 to 0.95)	0.4 (0.3 to 0.8)	0.1 (0.1 to 0.2)
T_{sk} (ºC)	31.2 ± 1.0	31.1 ± 1.0	-0.1 (-0.3 to 0.4)	0.875	0.89 (0.80 to 0.95)	2.0 (1.5 to 3.0)	0.2 (0.0 to 0.4)
T_{mu} (ºC)	33.8 ± 1.2	33.7 ± 1.1	0.1 (-0.4 to 0.3)	0.755	0.89 (0.82 to 0.92)	2.3 (1.3 to 3.2)	0.2 (0.0 to 0.4)
TS (mode)	5.4 ± 0.7	5.3 ± 0.7	0.1 (-0.4 to 0.1)	0.654	0.90 (0.77 to 0.97)	4.5 (3.2 to 8.0)	0.3 (0.2 to 0.4)

Total Distance = TD; Walk Distance = WD; Jog Distance = JD; Run Distance = RD; Fast Run Distance = FRD; Variable Run Distance = VRD; Sprint Distance = SD; High Speed Distance = HSD; Low Speed Distance = LSD; Peak Sprint Speed = PSS; Heart Rate = HR; Blood Lactate Concentration = Lactate; Rating of Perceived Exertion = RPE; Rectal Temperature = T_{re}; Skin Temperature = T_{sk}; Muscle Temperature = T_{mu} Thermal Sensation = TS Change in Mean = CIM; Intraclass coefficient = ICC; Coefficient of Variation = CV; and Typical Error = TE

Table 4.2: Mean values for the first half and second half, CIM significance values with 95%CI values and percentage changes for all performance and physiological measures from $iSPT_1$ and $iSPT_2$. Any discrepancies between the means and the CIM are due to rounding errors.

Measure	iSPT₁						iSPT₂					
	1st half	2nd half	CIM	95% CI	*p* value	% change	1st half	2nd half	CIM	95% CI	*p* value	% change
TD (m)	4508 ± 236	4452 ± 248	-56 ± 47	-34 to -78	0.001	1.2	4518 ± 253	4427 ± 225	-142 ± 84	-131 to -51	0.001	2.0
WD (m)	1208 ± 52	1197 ± 51	-11 ± 18	-3 to -20	0.01	1.0	1209 ± 53	1189 ± 47	-20 ± 26	-32 to -8	0.003	1.6
JD (m)	1299 ± 91	1276 ± 98	-23 ± 33	-7 to -38	0.006	1.7	1300 ± 100	1277 ± 93	-23 ± 39	-41 to -4	0.02	1.8
RD (m)	814 ± 52	809 ±50	-5 ± 8	-1 to -9	0.01	0.6	815 ± 51	802 ± 47	-13 ± 21	-3 to -22	0.01	1.6
FRD(m)	313 ± 15	310 ± 17	-3 ± 5	-1 to -5	0.04	1.0	314 ± 18	308 ± 17	-6 ± 5	-8 to -4	0.001	1.9
VRD (m)	269 ± 23	265 ± 23	-4 ± 5	-1 to -6	0.003	1.5	270 ± 22	262 ± 26	-8 ± 11	-13 to -3	0.003	3.0
SD (m)	507 ± 36	496 ± 36	-11 ± 17	-3 to -19	0.008	2.2	507 ± 39	491 ± 40	-16 ± 13	-10 to -22	0.001	3.2
HSD (m)	1086 ± 69	1071 ± 69	-14 ± 19	-5 to -23	0.002	1.4	1091 ± 71	1062 ± 72	-29 ± 16	-36 to -21	0.001	2.7
LSD (m)	3442 ± 190	3399 ± 187	-43 ± 54	-21 to -62	0.001	1.2	3428 ± 205	3366 ± 180	-62 ± 83	-101 to -24	0.003	1.8
PSS (km·h⁻¹)	20.2 ± 1.8	19.6 ± 1.8	-0.6 ± 0.7	-0.9 to -0.2	0.002	3.0	20.3 ± 2.1	19.5 ± 2.3	-0.8 ± 0.7	-1 to -0.4	0.001	3.9
HR (b·min⁻¹)	166 ± 8	164 ± 8	-0.6 ± 5	-2 to -1	0.01	1.2	165 ± 8	164 ± 8	-4 ± 6	-3 to -1	0.03	0.6
HR_MAX (%)	83.4 ± 4	83.2 ± 4	-0.1 ± 3	-2 to 0.5	0.001	0.2	83 ± 8	82.4 ± 4	-0.6 ± 3	-2 to -1	0.001	0.7
Lactate (mmol)	5.1 ± 1.4	4.1 ± 2.0	-1.0 ± 0.8	-1.3 to -0.5	0.001	19.6	5.0 ± 1.6	4.1 ± 1.7	-0.9 ± 0.8	-1.4 to -0.6	0.001	18.0
RPE (mode)	14.8 ± 1.3	15.4 ± 1.3	-0.6 ± 0.7	-1 to -0.3	0.001	4.1	14.7 ± 1.2	15.4 ± 1.4	-0.8 ± 0.7	-1.2 to -0.5	0.001	4.8
T_re (ºC)	38 ± 0.3	38.4 ± 0.3	0.4 ± 0.3	-0.5 to -0.2	0.01	1.0	38.1 ± 0.4	38.4 ± 0.2	0.3 ± 0.6	0.1 to 0.4	0.03	0.7
T_sk (ºC)	31.1 ± 0.9	31.2 ± 0.9	0.1 ± 1.3	-0.4 to 0.63	0.66	0.3	31.1 ± 1.0	31.0 ± 1.1	-0.1 ± 1.3	-1.2 to 0.5	0.33	0.3
T_mu (ºC)	33.8 ± 1.0	33.7 ± 1.3	0.1 ± 1.3	-1.1 to 0.9	0.84	0.3	33.7 ± 1.2	33.6 ± 1.1	-0.1 ± 1.3	-0.4 to 1.0	0.52	0.3
TS (mode)	5.2 ± 0.6	5.5 ± 0.7	0.3 ± 0.8	0.1 to 0.4	0.04	5.7	5.1 ± 0.6	5.4 ± 0.7	0.3 ± 0.9	0.2 to 0.6	0.001	5.8

95% Confidence Intervals = 95% CI; see footnote of Table 4.1 for descriptions of abbreviations not listed.
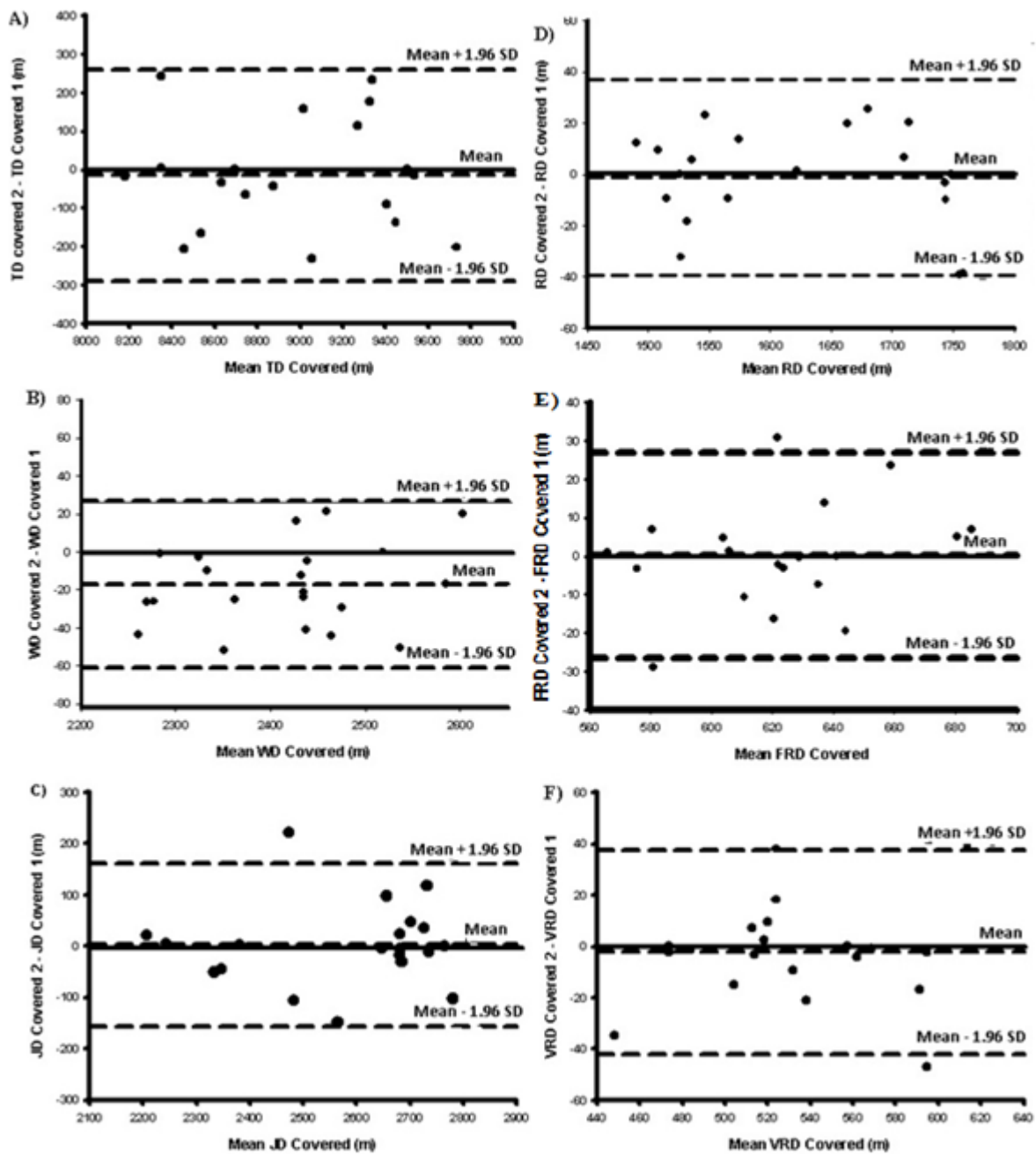
Figure 4.2: Bland-Altman plots showing individual differences between the mean values of iSPT plotted against their individual means (n = 20) for A) TD covered, B) WD Covered, C) JD Covered, D) RD Covered, E) FRD Covered, F) VRD Covered. Horizontal dashed line = 95% limits of agreement; solid line = zero reference line; S_d = within-subject standard deviation.
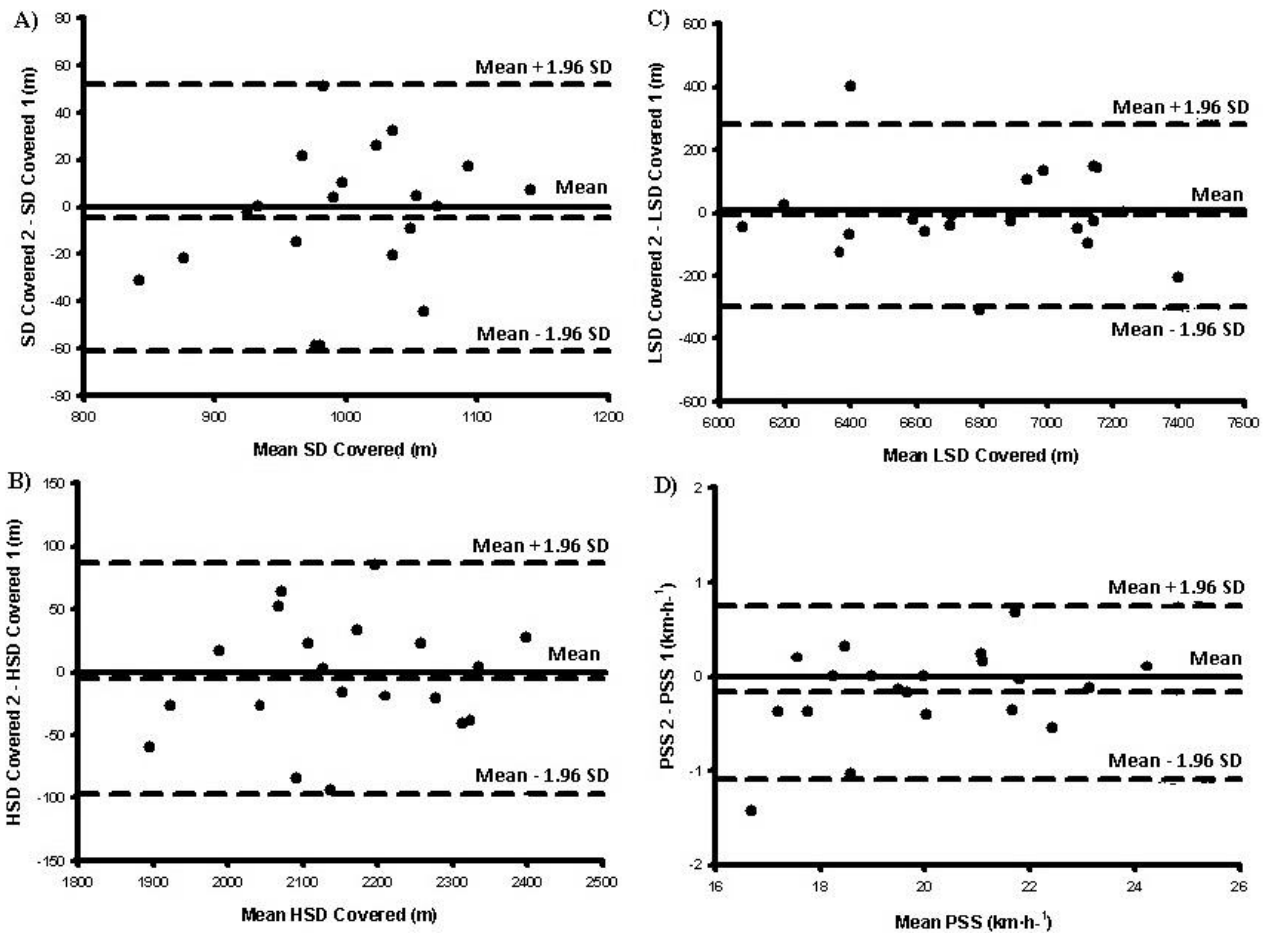
Figure 4.3: Bland Altman plots showing individual differences between the mean values of iSPT plotted against their individual means (n = 20) for A) SD Covered, B) HSD Covered, C) LSD Covered, D) PSS. See footnote of Figure 4.3 for an explanation of the lines.
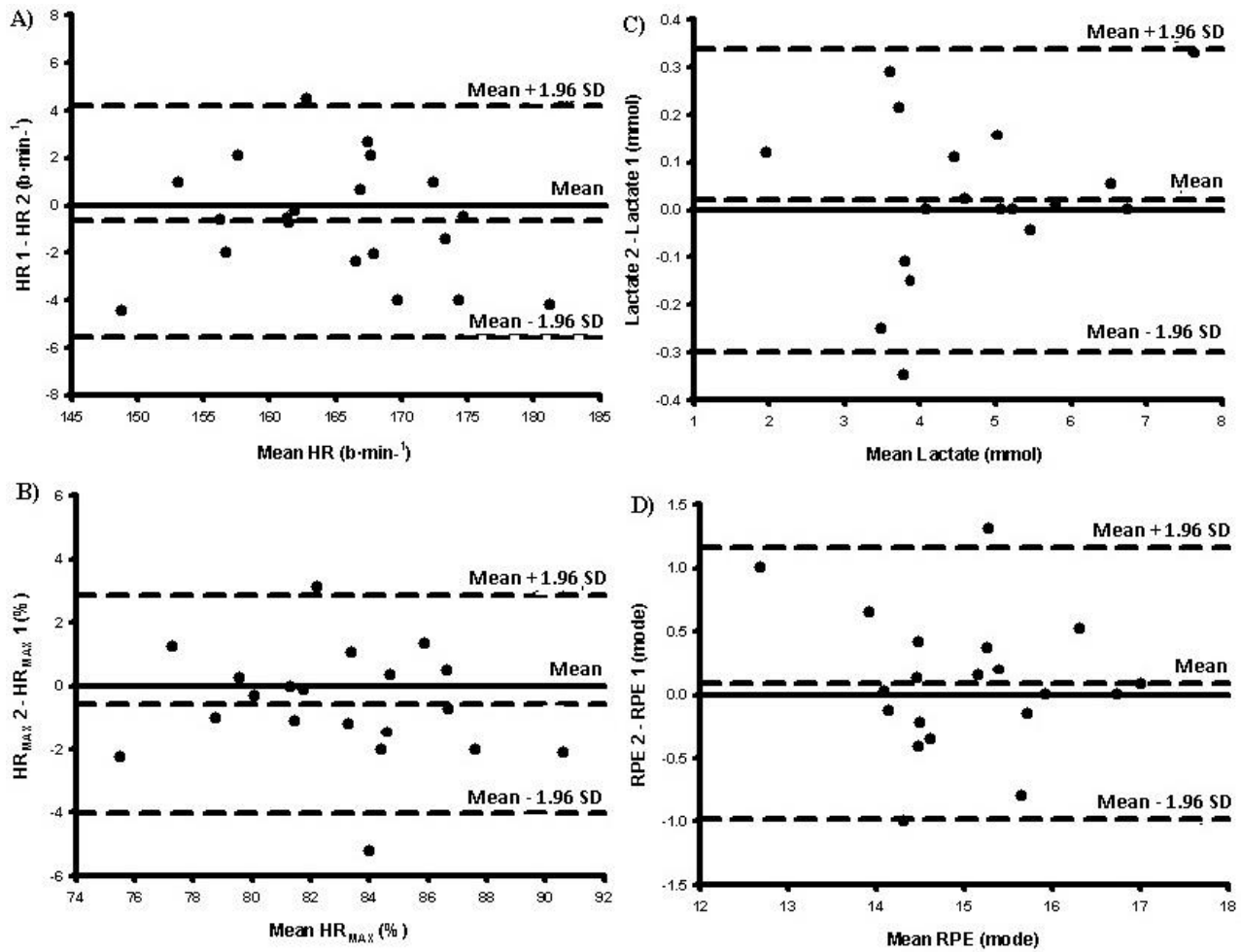
Figure 4.4: Bland Altman plots showing individual differences between the mean values of iSPT plotted against their individual means (n = 20) for A) HR, B) HR$_{MAX}$ C) Lactate, D) RPE. See footnote of Figure 4.3 for an explanation of the lines.

4.3. Results

4.3.1. Overall and between halves

Table 4.1 reveals that there was no significant difference ($p \geq 0.05$) between $iSPT_1$ and $iSPT_2$ in all performance, physiological, body temperature, blood and subjective measures. The reliability statistics for all performance, physiological, body temperature and subjective measures during $iSPT_1$ and $iSPT_2$, including the ICC (>0.89) and CV ($\leq$5%) shown in Table 4.1 demonstrate good reproducibility. Table 4.2 shows there was a significant difference ($p \leq$ 0.05) between halves in both $iSPT_1$ and $iSPT_2$ for all variables except for both T_{mu} and T_{sk} which showed no significant difference ($p \leq 0.05$) between halves in $iSPT_1$ and $iSPT_2$ (Table 4.2). Table 4.3 reveals that there was no significant difference ($p \geq 0.05$) for the fatigue indexes for all physical performance measures in $iSPT_1$ and $iSPT_2$. The reliability statistics for all performance, physiological, body temperature and subjective measures during $iSPT_1$ and $iSPT_2$, including the ICC (>0.81) and CV ($\leq$9%) shown in Table 4.3 demonstrate good reproducibility.

Table 4.3: The intraclass correlation (ICC), coefficient of variation (CV) with 95%CI for the soccer performance decrement measures from $iSPT_1$ and $iSPT_2$.

	$iSPT_1$		$iSPT_2$	
Measure	CV (%)	ICC	CV (%)	ICC
TD covered	9 (5 to 11)	0.81 (0.7 to 0.9)	8 (5 to 11)	0.83 (0.75 to 0.91)
HSD Covered	8 (4 to 10)	0.84 (0.76 to 0.98)	7 (5 to 10)	0.83 (0.75 to 0.94)
SD covered	7 (3 to 9)	0.85 (0.77 to 0.94)	8 (5 to 9)	0.87 (0.76 to 0.91)
VRD covered	9 (5 to 11)	0.82 (0.77 to 0.91)	7 (4 to 10)	0.81 (0.74 to 0.92)
PSS	5 (2 to 9)	0.83 (0.7 to 0.9)	5 (3 to 7)	0.84 (0.73 to 0.93)

See footnote of Table 4.1 for descriptions of abbreviations not listed.

4.3.2. Between 15 min
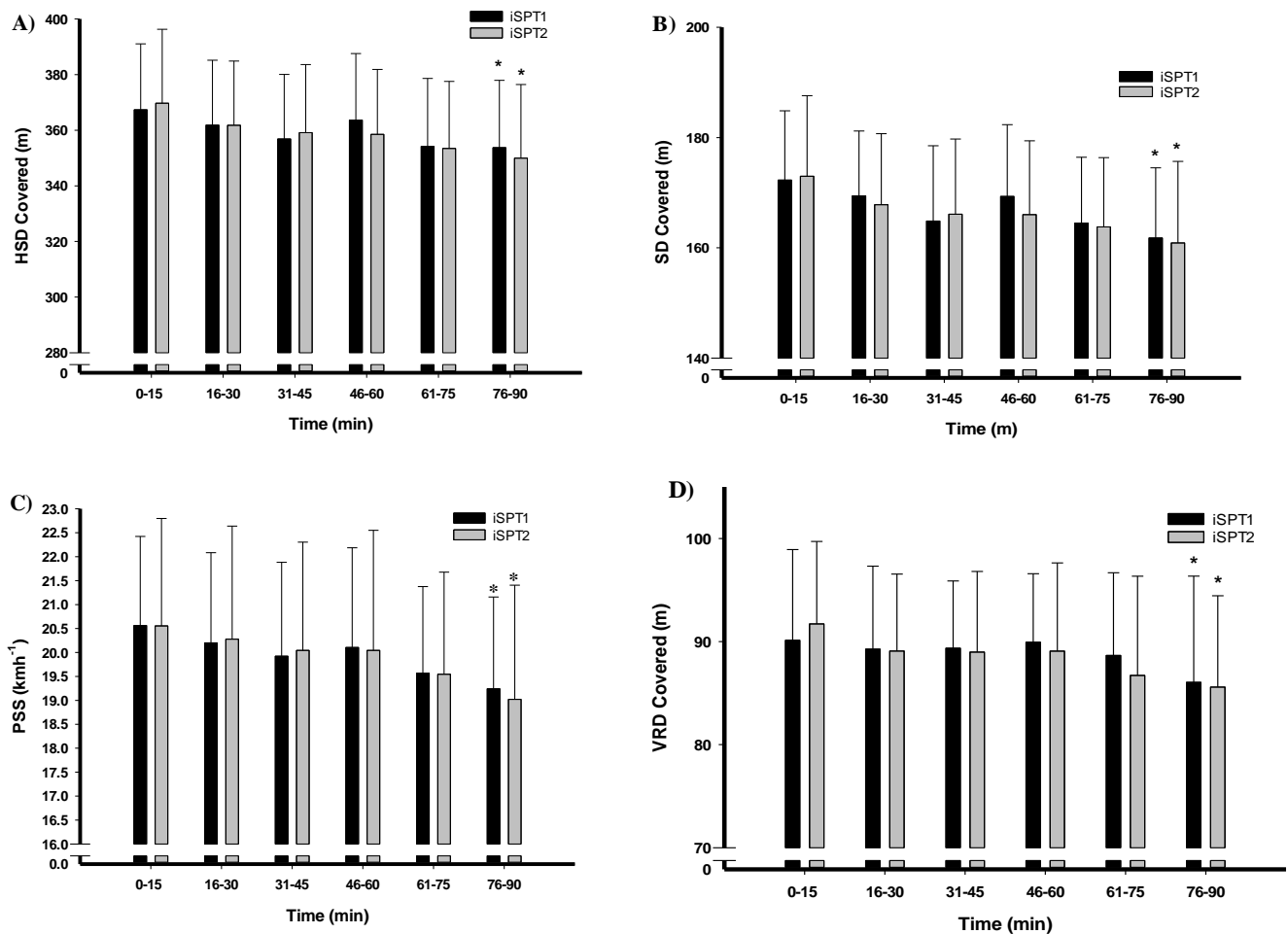
Figure 4.5: The HSD, SD, PSS and VRD covered in each 15 min period throughout iSPT$_1$ and iSPT$_2$. HSD, SD, PSS and VRD were significantly less ($p < 0.05$) in the last 15 min of the second half of iSPT$_1$ and iSPT$_2$* compared to the first 15 min of the first half.

Figure 4.5 shows that high-speed distance (A) covered was significantly decreased in the 76-90 min period compared to the 0-15 min period in iSPT$_1$ ($p = 0.02$, 95% CI: 8 – 20 m, CIM: 9 ± 17 m) and iSPT$_2$ ($p = 0.04$, 95% CI: 14 – 26 m, CIM: 13 ± 10 m). It is also revealed in Figure 4.5 that sprint distance (B) covered was significantly decreased in the 76-90 min period compared to the 0-15 min period in iSPT$_1$ ($p = 0.02$, 95% CI: 6 – 15 m, CIM: 9 ± 7 m) and iSPT$_2$ ($p = 0.02$, 95% CI: 4 – 12 m, CIM: 13 ± 10 m). Furthermore, Figure 4.6 also shows that both peak sprint speed (C) and variable run distance (D) was significantly decreased in the 76-90 min period compared to the 0-15 min period in iSPT$_1$ (peak sprint speed: $p = 0.01$, 95% CI: 0.5 – 2.0 km·h^{-1}, CIM: 0.8 ± 0.8 km·h^{-1}; variable run distance: $p < 0.01$, 95% CI: 4 – 10 m, CIM: 2 ± 13 m), and iSPT$_2$ (peak sprint speed: $p = 0.02$, 95% CI: 1 – 2 km·h^{-1} , CIM: 1.5 ± 0.8 km·h^{-1}; variable run distance: $p < 0.01$, 95% CI: 6 – 11 m, CIM: 6 ± 4 m).
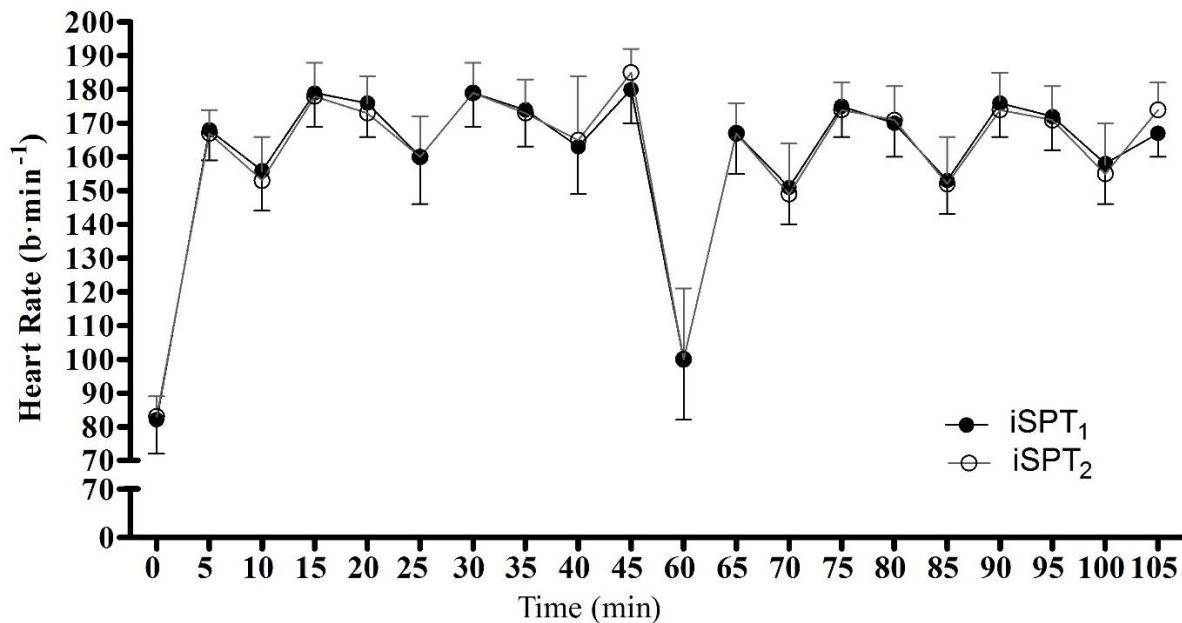
Figure 4.6: The HR at each 5 min period throughout $iSPT_1$ and $iSPT_2$. No significant difference ($p > 0.05$) was seen between each time point in $iSPT_1$ and $iSPT_2$

4.4. Discussion

The primary findings are that all physical performance measures and physiological responses recorded during iSPT have comparability to match-play data with excellent reproducibility between trials also evident (Table 4.1). Furthermore, the "variable run" speed component was able to differentiate between self-paced distances covered at high speed and sprinting by reporting decrements in variable run distance covered during iSPT both between (Table 4.2) and within halves (Figure 4.5). These findings are mirrored by the high-speed distance variable, showing a similar response compared to previous match-play data.

4.4.1. Reliability

An exercise performance test with high reliability is an acceptable method to measure the physical performance and physiological responses of an athlete (Atkinson and Nevill, 1998). Therefore, when utilising a measurement tool within sport science it is appropriate and beneficial that the tool has high test-retest reliability (Sunderland *et al*, 2006). During this experiment, two commonly employed methods of reliability measures were employed; ICC and CV. However, one issue that is rarely mentioned during sport science is how the measurement error relates to the magnitude of the measured variable (Atkinson and Nevill, 1998).

After data analysis, if the random error increases as the measured value increases (e.g. positive skewness), the data is considered heteroscedastic. However, if there is no relationship between the error and size of the measured value (e.g. normally distributed) then the data is homoscedastic. The way these two different types of data should be analysed is different. Heteroscedastic data should be analysed upon a ratio scale (e.g. CV (%) or TE), whereas, homoscedastic data should be expressed as actual units (e.g. correlation or ICC) (Atkinson and Nevill, 1998). However, previous NMT based soccer-specific simulation research, ignores these statistical laws, so during experiment 1 both CV and ICC have been used to calculate the reliability of iSPT (Sirotic and Coutts, 2008). Therefore, all measures of soccer performance obtained during iSPT were averaged from the full 90 min period and were compared between iSPT$_1$ and iSPT$_2$.

4.4.1.1. Physical performance (Reliability)

Previously published data for total distance [CV: 2.2% - (Abt, 2002); CV: 1.9% - (Sirotic and Coutts, 2008)], when compared to iSPT (CV: 1.3%), shows comparable reliability to previous NMT soccer-specific simulations. Both these previously validated NMT soccer-specific (Abt, 2002) and team-sport simulations (Sirotic and Coutts, 2008) utilised individualised speed thresholds, hence why such low reliability was reported.

There was also high reliability between two experimental trials for other physical performance measures such as sprint distance, peak sprint speed and high-speed distance covered (Table 4.1). For example, the sprint distance covered during iSPT (CV: 2.1%) compares favourably to a 42 min duration NMT based soccer-specific simulation [CV: 3.8% - (Oliver *et al*, 2007b)]. Aside from soccer-specific simulations, some reliability measures have been reported for a generic TSS (Sirotic and Coutts, 2008), which iSPT compares favourably with. Sirotic and Coutts (2008) demonstrated that the reliability of high-speed distance covered in TSS (CV: 1.5%; ICC: 0.87) was comparable to the values reported for iSPT (CV: 1.6%; ICC: 0.95). Furthermore, similar variables, such as FRD covered [CV: 1.7%; ICC: 0.90 - (Sirotic and Coutts, 2008)] demonstrate parity with the iSPT specific data (CV: 1.6%; ICC: 0.92). However, some reliability measures specific to these generic TSS are superior to those achieved in iSPT. For example, during iSPT, peak sprint speed demonstrated a CV of 3.0%, which is inferior to the CV reported (1.3%) for some generic TSS (Hughes *et al*, 2006, Sirotic and Coutts, 2008). However, despite these generic TSS having superior CV values they lack specificity due to their shorter duration with regard to movement patterns in soccer (Hughes *et al*, 2006, Sirotic

and Coutts, 2008).This inferiority regarding peak sprint speed may have occurred due to the sampling rate from the NMT used to record the data. During this experiment, the sampling rate was 200Hz unlike the 10Hz used by Sirotic and Coutts (2008). Therefore, utilising such a low sampling rate would likely "miss" data throughout each sprint; resulting in the data from iSPT showing a truer reflection of each sprint and the inherent variability of sprinting *per se*.

It is also important for the changes between halves and 15 min blocks to be consistent between tests. A number of NMT (Oliver et al, 2007a, Oliver et al, 2007b) and cycle ergometer (Fitzsimons et al, 1993, McGawley and Bishop, 2006) based studies have investigated the reliability of fatigue during prolonged repeated tests similar to iSPT. However, several authors have concluded that any fatigue index or percentage decrement score should be viewed with caution as a large amount of variability (11-50%) is present (Oliver, 2009). However, during this experimental chapter, the reliability of the performance decrements was < 10% for all key physical performance measures [total distance (9%), high-speed (8%) and sprint distance (7%) covered], which is favourable compared with previous NMT based repeated sprint data (Hughes et al, 2006). Furthermore, these CV values for each performance decrement measure was acceptable compared to the statistical recommendations [CV: <10%: (Atkinson et al, 1999)]. The greater reliability in performance decrements was likely due to the rigorous familiarisation process (Lakomy, 1987) and the use of individualised speed thresholds (Abt et al, 2003). Therefore, the iSPT is a reliable test to quantify the performance decrements between halves which is necessary to allow the successful completion of experimental chapters 2 and 3 within this thesis.

4.4.1.2. Physiological responses (Reliability)

It would also be an advantage for a NMT soccer-specific simulation to show high reliability when quantifying the physiological responses of an athlete, as several physiological responses during match-play will work in tandem to allow the physical demands of soccer to be successfully met (Bangsbo, 2014). This is especially important when excellent reliability measures are reported for the physical performance measures during iSPT. A thorough literature search has revealed only one NMT soccer-specific simulation study, has assessed the reliability of physiological responses, however, this was for a generic TSS (Sirotic and Coutts, 2008). Sirotic and Coutts (2008) assessed the reliability of a Bla concentration post simulation (CV: 17.6%; ICC: 0.65), however, the iSPT compares favourably in this regard (CV: 5.0%; ICC: 0.97). Improved reliability for Bla concentration in the iSPT may be due to the increased

sampling frequency employed in iSPT. During iSPT, Bla was recorded four times per half, allowing for changes in Bla to be more reliably quantified compared to other simulations. This is important as soccer player's will complete a plethora of high-speed and maximal exercise bouts throughout match-play in an intermittent 'semi-chaotic' fashion, which is likely to impact upon the accumulation of Bla (Buchheit, 2010). However, the TSS by Sirotic and Coutts (2008) only recorded Bla post simulation, so it is difficult to capture how Bla concentration changes throughout a simulation. During iSPT all Bla concentrations were ascertianed 1 min post sprint, so the values reported during iSPT may represent the maximal concentration by a particpant. As outlined in section 2.2.2. this would not be possible within soccer match-play, therefore, iSPT allows a reliable quantification of Bla concentration compared to a previous TSS.

The CIM value reported for HR was -1 $b \cdot min^{-1}$, similar to other values reported in the literature, such as the -3.1 $b \cdot min^{-1}$ reported elsewhere (Williams *et al*, 2010). This demonstrates iSPT's reliability is comparable to other NMT and field based simulations. With regard to iSPT, no significant differences between the two trials of iSPT were evident for any physiological measures. Therefore, it can be noted that iSPT is a reliable simulation to quantify the physical performance and physiological responses of soccer players by utilising its individualised approach. Therefore, the iSPT provides information that can be utilised to reliably assess changes in player performance

In this experiment the reliability of specific body temperature (T_{re}, T_{mu} and T_{sk}) and subjective (TS) measures were ascertained to aid the reliable quantification of the influence of extreme environments and cooling interventions upon soccer performance in a hot environment, as per experiment 2 and 3, respectively. All body temperature and subjective measures showed good agreement (ICC: >0.89; CV%: <4.55%) compared with statistical guidelines (Atkinson *et al*, 1999, Vincent and Weir, 2012). However, no other soccer-specific simulation has gained reliability statistics for such specific measures. Therefore, these findings outline that iSPT has good reliability to quantify changes in body temperature and subjective measures between two experimental trials of iSPT.

4.4.1.3. Nonogram

The reliability of individual variables can have an influence upon the sample size required to detect an adequate change (Batterham and Atkinson, 2005). Batterham and Atkinson (2005), provided a nomogram using a variable's CV% to estimate the sample size required to detect

any change associated with interventions, as also used in recent soccer-related research (Gregson *et al*, 2010, Sirotic and Coutts, 2008). Applying this nomogram to the primary physical performance measures (total distance: 1.3%, variable run distance: 2.7%, sprint distance: 2.1%, peak sprint speed: 3%) suggests that a minimum sample size of between 5–10 or 11–20 is adequate to detect a 10% or 5% change in these aforementioned physical measures, respectively (Batterham and Atkinson, 2005), in-line with previous findings (Sirotic and Coutts, 2008). These findings highlight the advantages of utilising iSPT compared to match-play data when ascertaining a change in soccer performance. Utilising a similar nonogram, Gregson *et al* (2010) detailed that based on the high CV's (>20%) found for all high speed measures between matches in elite soccer players', a sample of at least 80 players would be needed to make meaningful inferences from soccer match-play. However, previous match-play studies that have assessed the impact of extreme environments (Aughey *et al*, 2013, Buchheit *et al*, 2015, Garvican *et al*, 2013, Mohr *et al*, 2010, Mohr *et al*, 2012) and pre-cooling (Duffield *et al*, 2013) on soccer performance, were identified by the use of >20 players which renders these studies as substantially underpowered. Therefore, as iSPT demonstrates considerably lower CV values for key physical performance measures (Table 4.1) compared to match-play data (Gregson *et al*, 2010), meaningful inferences upon soccer performance from environmental stress and pre-/half-time-cooling could be made using more realistic sample sizes. These findings from this aforementioned nonogram then facilitated the successful completion of experiments 2 and 3.

4.4.1.4. Summary of reliability

The iSPT shows high test-retest reliability to measure soccer performance. All the within-subject CV values were below 10%, which is regarded as an acceptable level of reliability (Atkinson and Nevill, 1998). Additionally, all ICC values were between 0.90-0.97, which is considered highly reliable (Currell and Jeukendrup, 2008). Therefore, as the iSPT demonstrated low test-retest error compared with the statistical guidelines and previous NMT based soccer-specific simulations, any changes to soccer performance can be attributed to an intervention and not the variability of the measure (Currell and Jeukendrup, 2008), unlike in soccer match-play designs (Gregson *et al*, 2010).

It is also important that iSPT has high external validity to see whether synergy is shown compared to the soccer performance measures captured during soccer match-play. In the present experiment, all validity data were determined from an average of $iSPT_1$ and $iSPT_2$. Assessing the validity of a soccer simulation is difficult without utilising the same subjects within a match-play situation using expensive specialist equipment (*e.g.* GPS). Furthermore, within a match the physical performance will vary due to the impact of game factors (Gregson *et al*, 2010). Therefore, iSPT was devised to approach soccer performance by balancing the control of a laboratory and the generalised, yet individualised, activity pattern of soccer match-play – specific to a central midfielder.

4.4.2.1. Total distance Covered

Mean total distance was 8953 ± 477 m in iSPT, similar to previous elite match-play [(Rienzi *et al*, 2000) - 8638 ± 1158 m] observations. Evidently, when comparing total distance within university standard soccer players (8968 ± 430 m) with elite European league players [10,860 ± 260 m - (Mohr *et al*, 2003)] a difference of ~1900 m is observed. Such differences are likely underpinned by $\dot{V}O_{2max}$, with a difference of ~9 ml$\cdot$kg$^{-1}\cdot$min^{-1} between university players (51 ± 9 ml$\cdot$kg$^{-1}\cdot$min^{-1}) and highly trained (58 to 62 ml$\cdot$kg$^{-1}\cdot$min^{-1}) 'elite' players (Mohr *et al*, 2003), as discussed elsewhere (Tonnessen *et al*, 2013).

4.4.2.2. High-speed distance and sprint distance Covered

The mean high-speed distance covered in iSPT (2157 ± 140 m) is in line with match-play data detailed elsewhere (2116 ± 369 m - (Di Salvo *et al*, 2007)), and supports the definition of high-speed distance running as >65% of peak sprint speed, as employed in iSPT. sprint distance covered in iSPT (1000 ± 74 m) was greater than values (650-771 m) reported previously (Bangsbo *et al*, 1991, Mohr *et al*, 2003). Mean sprint duration in soccer match-play has been reported to be ~3.5 s shorter than the 6 s sprint duration in iSPT (Barros *et al*, 1999, Mohr *et al*, 2003). However, a shorter sprint duration is less reliable when performed on a NMT (Abt, 2002), due to the different running mechanics when running on a NMT and poor reliability of 3 s sprint efforts (Hughes *et al*, 2006, Lakomy, 1987). Therefore, 6 s sprints were used as earlier research has shown good reliability of 6 s sprints upon the NMT (Hughes *et al*, 2006, Tong *et al*, 2001). However, it is important that iSPT can approach distances covered at different speed

thresholds, particularly those at high speed as these are associated with game defining moments during soccer match-play (Stolen *et al*, 2005).

Furthermore, due to the nature of a NMT, iSPT allows thorough quantification of straight sprinting. This is an important measure to ascertain relative to soccer performance as a straight sprint often precedes a goal being scored and/or assist being executed in elite soccer match-play (Faude *et al*, 2012). However, changes to sprint performance have been detected in a plethora of soccer match-play studies due to the prevalence of hot (Mohr *et al*, 2010) and hypoxic (Garvican *et al*, 2013) environments, which could have a detrimental effect upon the number of goal scoring opportunities in match-play. Thus, it is important that iSPT can assess these in line with the aims of experimental chapter 2.

It is recognised that the amount of sprint distance, high-speed distance and total distance covered are less in the second half compared to the first half of a soccer match (Mohr *et al*, 2003, Mohr *et al*, 2005); with such decrements evident between the first and second halves of iSPT$_1$ and iSPT$_2$, demonstrating external validity compared with match-play data. The significantly lower total distance covered in the second half compared to the first half (56-142 m) is consistently reported within the literature, (Di Salvo *et al*, 2007, Mohr *et al*, 2003) which is comparable with iSPT$_1$ (56 ± 47) and iSPT$_2$ (142 ± 84). Conversely, some research has suggested that total distance is not significantly less in the second half, demonstrated within elite European soccer players (Di Salvo *et al*, 2007), suggesting that despite there being a decline in physical performance in some cases, this may not be a systematic change. However, it was suggested by Di Salvo *et al* (2007) that players adopted a pacing strategy whereby during the second half players will cover a greater sprint distance making up for the decrement in high-speed distance. However, iSPT was made up of two 45 min halves requiring the same physical demands for both halves, so decrements in physical performance were more likely to occur, due to removal of self-pacing in iSPT due to externally framed demands (e.g. individualised speed thresholds). Therefore, unlike within soccer match-play, an athlete's ability to adopt an adaptive pacing strategy due to the externally framed demands via the individualised speed thresholds is minimised, meaning that maximum capability of a player's physical performance can be gained.

4.4.2.3. Peak sprint speed

A significant reduction (5%) in peak sprint speed was reported during iSPT$_2$ in the second half (19.5 ± 2.0 km·h^{-1}) of the simulation compared with the first half (20.3 ± 2.1 km·h^{-1}), similar to reductions reported previously (8% - (Abt *et al*, 2003)). Other soccer simulations have failed to find such a substantial decrease in physical performance, where it was reported by Williams *et al* (2010) that peak sprint speed decreased by 2%. One reason why such a substantial decrease in peak sprint speed may occur is due to the 6 s duration of the sprint effort used in iSPT (Williams *et al*, 2010). It was reported in previous studies (Hughes *et al*, 2006, Sirotic and Coutts, 2008) that a 3 s sprint produced less reliable sprints compared to using a 6 s sprint. Hughes *et al* (2006) identified that participants take 3 to 5 seconds to achieve peak speed, a finding that is supported by previous research (Lakomy, 1987). This is due to the practical realities of running on a NMT which makes a 6 s sprint more appropriate to assess physical performance (Lakomy, 1987). Therefore, this rationalises the 6 s sprint duration chosen for iSPT.

4.4.2.4. Variable run distance Covered

The iSPT contains three 15 min blocks per half enabling measurement of decrements in physical performance between and within halves. The variable run element is novel to iSPT as participants were asked to run at a self-selected speed (no set speed threshold) to allow quantification of self-paced decrements in high speed running separate to sprint performance. Furthermore, utilising the variable run allowed for the quantification of the self-paced decrements within the simulation. high-speed distance significantly decreases in the last 15 min of match-play (Mohr *et al*, 2005), and it was found in iSPT$_1$ and iSPT$_2$ that there was a significant decrease in variable run distance in the last 15 min of the second half compared to the first 15 min of match-play (Figure 4.5). The iSPT provides evidence that variable run distance can quantify, to some degree, the self-paced decrements demonstrated by other "classic" variables of high-speed distance, such as FR, in line with the experimental objectives. Sear *et al* (2010) reported no reduction in variable run distance within a similar NMT based simulation, however, this used a 45 min simulation, which was seemingly not long enough to induce a decline in variable run distance. Furthermore, Sear *et al* (2010) only used one familiarisation session prior to completing the main trials. It has been reported that utilising a thorough familiarisation before a main trial is beneficial due to the unorthodox running style that is adopted (Lakomy, 1987). Therefore, when utilising the variable run during a 90 min

soccer-specific simulation, it is important that a thorough familiarisation is utilised, as this will allow for a decline in high-speed distance similar to match-play data to be reported (Mohr *et al*, 2005).

4.4.2.5. HR

The mean HR during $iSPT_1$ and $iSPT_2$ was 165 ± 8 b·min^{-1} which is similar to findings (166 ± 15 b·min^{-1}) from match-play data (Ali and Farrally, 1991). Furthermore, a significant decrease in HR between first and second half in both $iSPT_1$ (166 ± 8 b·min^{-1} vs 164 ± 8 b·min^{-1}) and $iSPT_2$ (165 ± 8 b·min^{-1} vs 164 ± 8 b·min^{-1}) was evident, which is in synergy with previous match-play studies (Stroyer et al, 2004). A decline in HR in the second half of iSPT is likely to be underpinned by a significant reduction in physical performance (e.g. total distance covered) during the second half of match-play (Stroyer et al, 2004). Although, during iSPT participants were asked to follow a line for each individualised speed threshold, towards the end of the simulation players found it more difficult to follow each line (i.e. fatigue was present), resulting in a reduction in exercise intensity which manifests as a decrease in HR (Bangsbo, 2014). In hot and hypoxic environments during prolonged high-speed exercise an increase in HR is likely to occur due to a plethora of physiological mechanisms, for example cardiovascular drift (Stembridge et al, 2015a, Stembridge et al, 2015b, Wingo, 2015, Wingo et al, 2012). However, previous soccer match-play data revealed that HR was unchanged at 43°C compared with 21°C (Mohr et al, 2012), likely due to the players adopting an altered pacing strategy, outlined by the reduction in physical performance seen at 43°C. Therefore, as experimental chapter 1 reveals that iSPT can reliably quantify changes in HR between two tests (Figure 4.7) and is valid when compared with soccer match-play data, thus it facilitated the measurement of this key physiological response in experimental chapter 2 and 3 within this thesis.

4.4.2.6. Blood Lactate

As previously outlined, Bla concentration was recorded four times in each half, once every 15 min during iSPT. This approach has also been adopted by the Copenhagen Soccer Test (Bendiksen *et al*, 2012). The findings from iSPT showed that the mean Bla concentration in the first half (5.34 ± 1.54 mmol^{-1}) during both $iSPT_1$ and $iSPT_2$ was significantly less in the second half (4.77 ± 1.71 mmol^{-1}), showing synergy with previous recent match-play data in semi-professional soccer players [(Krustrup *et al*, 2006) – (1st half: 6.00 ± 0.4 mmol^{-1} vs 2nd

half: $5.00 \pm 0.4\text{mmol}^{-1}$)]. Ascertaining a player's Bla concentration is difficult to obtain during match-play as this would interfere with a player's physical performance during match-play (Stolen *et al*, 2005). However, a well formulated soccer-specific simulation will allow for changes in Bla concentration to be ascertained as close to a high speed exercise bout as possible meaning that a true change in an athlete's anaerobic energy provision can be ascertained. Furthermore, another methodological advantage of iSPT is that the physical demands before each Bla sample are fixed (e.g. 15 min blocks), therefore, it can be assured that Bla results recorded during iSPT are able to reliably ascertain changes in Bla concentration within and between halves.

A significant reduction in Bla was also present in the second half compared with the first half of both iSPT_1 and iSPT_2 (Table 4.2), showing parity with earlier soccer match-play research (Bangsbo, 1994). These observations are in accordance with the reduced distance covered and lower intensity reported in most other studies (Bangsbo, 1994, Krustrup et al, 2004). The onset of fatigue in soccer match-play is more complex and beyond simply changes in Bla, others have identified that fatigue can be attributed to a plethora of temporally ordered factors (Mohr et al, 2005). These factors include a reduction in T_{mu} at the start of the second half (Mohr et al, 2004), increase in muscle lactate coinciding with a reduction in pH (Krustrup et al, 2004), muscle acidosis (Fitts, 1994) and lower muscle creatine phosphate (Krustrup et al, 2006). Furthermore, central fatigue mechanisms could also account for the fatigue seen during the second half of soccer match-play, due to a reduction in central drive of the central nervous system (Waldron and Highton, 2014), causing a decrease in muscle activation (Rampinini et al, 2011). It is likely that fatigue in soccer match-play is multi-faceted due to central and muscular fatigue mechanisms (Waldron and Highton, 2014). Additionally, these central and peripheral fatigue mechanisms could be exacerbated in hot and/or hypoxic environments (Billaut and Aughey, 2013, Nybo et al, 2014). Therefore, future study designs are needed to further explore these muscular and central fatigue mechanisms.

4.4.2.7. Summary of Validity

The physical performance measures (total distance, high-speed distance and sprint distance etc.) reported in the iSPT show similarities with previously reported match-play and soccer-specific simulation data. Therefore, both the excellent reliability and validity of soccer performance measures during iSPT could provide a novel individualised soccer-specific simulation, where external parameters which are subject to large variance during match-play

could be assessed with accuracy to inform and evaluate both training (Akubat *et al*, 2014), and match-play performance (Thatcher and Batterham, 2004). Furthermore, the iSPT would also assess the efficacy of interventions and the impact of extreme environments upon soccer performance within a controlled environment. However, limitations of iSPT must also be considered.

4.4.3. Experimental limitations

A NMT based soccer simulations can contain certain experimental limitations. Due to the uni-directional nature of NMT based simulations, they are unable to contain soccer-specific movements such as backwards and sideways running (Akubat *et al*, 2014) or the assessment of technical skills such as passing and shooting (Williams *et al*, 2010). Therefore, the use of an NMT simulation (iSPT) should not be utilised to assess these particular facets of soccer performance. This is despite iSPT not containing these certain activities the simulation still approaches the same physical performance (e.g. total distance covered) and physiological responses (e.g. HR) of soccer match play (Thatcher and Batterham, 2004). Moreover, utilising a laboratory based simulation compared with match-play data is more advantageous for understanding soccer performance. This is because a greater experimental control to quantify soccer performance measures is required, as the environmental factors which prevent the expression of maximal soccer performance are minimised (Gregson *et al*, 2010).

4.4.4. Potential applications

Experimental chapter 1 demonstrates that iSPT is a valid and reliable soccer simulation, and the utilisation of the variable run phase was also shown to successfully determine decrements in self-paced high-speed running capability. Therefore, iSPT could be used in a number of ways. For the purpose of this thesis the laboratory based nature of iSPT could be used for quantifying the environmentally mediated decrements in soccer physical performance (Carling *et al*, 2011, Mohr *et al*, 2012, Nassis, 2013), which would be ideal for scientists and coaches within their practice. With this in mind, match-play data has shown that decrements on key game-defining physical performance measures (e.g. high-speed distance) in hot (Mohr *et al*, 2012) and hypoxic (Nassis, 2013) exposures occur due to a greater load placed upon a players physiological capacity. However, due to the high variance shown in match-play data caused by game factors quantifying these decrements reliably from match-play data is unlikely (Gregson *et al*, 2010). This is likely due to a number of soccer match-play studies being substantially

underpowered (< 20 participants) compared to the sample size proposed by Gregson *et al* (2010) to gain meaningful inferences from an intervention from soccer match-play paradigms. Therefore, utilising an NMT based soccer-specific simulation (iSPT) would allow for these decrements (environment mediated) to be reliably quantified in a laboratory based environment, as well as the efficacy of various interventions.

4.5. Conclusion

In conclusion, the present experiment demonstrates that iSPT is a valid and reliable soccer simulation. Furthermore, the utilisation of the variable run phase was shown to successfully determine decrements in self-paced high speed running capability. Therefore, the findings from this experimental chapter directly answer the first aim of this thesis: 'To investigate the reliability and validity of a NMT based soccer-specific simulation named the intermittent Soccer Performance Test (iSPT), incorporating a novel speed threshold called the variable run whilst utilising individualised speed thresholds based upon familiarised peak sprint speed'. Furthermore, all three experimental hypotheses are accepted. Subsequently, iSPT will be utilised within the following experiment to investigate the effects of hot, hypoxic and hot-hypoxic environments upon soccer performance. This will then directly answer the second aim of this thesis: '*To investigate the changes in simulated soccer performance during hot (30ºC; 50% RH), hypoxic (1,000m; 18ºC 50% RH), and hot-hypoxic (1,000m; 30ºC 50% RH) environments compared to a normoxic-temperate environment (0m; 18ºC 50% RH) by utilising the iSPT*'.

Chapter 5. Experiment 2: The Influence of Extreme Environments (Hypoxia, Hot and Hot-Hypoxia) on Soccer-Specific Physical Performance and Physiological Responses.

This experimental chapter has formed the basis of the publication detailed below:

- **Aldous, J. W. F.,** Chrismas, B. C. R., Akubat, I., Dascombe, B., Abt, G., & Taylor, L. (2015). Hot and hypoxic environments inhibit simulated soccer performance and exacerbate performance decrements when combined. *Frontiers in Physiology, 6*. doi:10.3389/fphys.2015.00421.

5.1. Introduction

The critical analysis in section 2.3 outlines that extreme temperatures of ~30°C (Hot), low altitudes of ~1,000m (Hypoxic) and a combination of these two conditions (Hot-Hypoxic) are the most prevalent extreme environments elite soccer teams would encounter during a season (Taylor and Rollo, 2014). Research from soccer match-play, in both heat (30-43°C) (Mohr *et al*, 2010, Mohr *et al*, 2012) and hypoxia; (1,200-1,600m) (Aughey *et al*, 2014, Garvican *et al*, 2013) demonstrates a reduction in physical performance [total distance (3-7%) and high-speed distance (15-26%) covered] compared with temperate and normoxic environments, respectively. Heat and hypoxic-decrements stem principally from elevated body temperatures amongst other multi-factorial mechanisms [Section – 2.3.2. - (Nybo *et al*, 2014)] and reductions in PO$_2$ [Section 2.3.1. - (Garvican *et al*, 2013)], respectively.

Sections 2.5 outlines the advantages of studying soccer performance in a more controlled environment (i.e. iSPT), due to the high match-to-match variation that has been reported previously for key physical performance measures within soccer match play (e.g. high-speed distance covered) (Gregson *et al*, 2010). Indeed a recent review (Taylor and Rollo, 2014), specifically recommends that an individualised, valid and reliable soccer simulation, that facilitates replication of near maximal soccer-specific physical performance and physiological responses (i.e. iSPT) be utilised to quantify such environment mediated decrements. This is due to a number of soccer match-play studies being substantially underpowered (< 20 participants) compared to the sample size proposed by Gregson *et al* (2010) ($n = 80$) to gain meaningful inferences on how environmental stress influences soccer performance via a soccer match-play paradigm. Furthermore, the high variation in soccer performance may be exacerbated in both heat and hypoxia as an altered "pacing strategy" and exercise intensity, individualistic in nature, has been reported to occur during match-play (Garvican *et al*, 2013, Mohr *et al*, 2012). The design of iSPT externally dictates physical performance (i.e. individualised movement speed thresholds), thus precluding an adaptive pacing strategy being adopted (Abt *et al*, 2003). Experiment 1 demonstrated that iSPT is a valid and reliable simulation to ascertain changes in soccer performance compared with match-play. Therefore, utilising iSPT will allow for changes in both physical performance and physiological responses between the identified environments to be ascertained in a controlled environment, where

pacing strategies and match factors would be minimised unlike previous match-play derived data (Garvican *et al*, 2013, Mohr *et al*, 2012).

5.1.1. Experimental aims and hypothesis

To investigate the changes in simulated soccer performance during hot (30°C; 50% RH), hypoxic (1,000m; 18°C 50% RH), and hot-hypoxic (1,000m; 30°C 50% RH) environments compared to a normoxic-temperate environment (0m; 18°C 50% RH) by utilising the iSPT.

- In the hot, hypoxic, and hot-hypoxic environment, simulated soccer performance will be significantly reduced compared with a normoxic-temperate environment during iSPT.
- In the hot-hypoxic environment, simulated soccer performance will be significantly reduced compared with the hot, hypoxic and normoxic-temperate environments during iSPT.

5.2. Method

5.2.1. Participants

The twelve male, apparently healthy university soccer players who volunteered for this experiment had the following characteristics: [median (min-max) age = 23 (18-33) y; mass = 77 (67 - 93) kg; height = 181 (168 - 195) cm; mean ± SD $\dot{V}O_{2max}$ = 57 ± 2 ml$\cdot$kg$^{-1}\cdot$min^{-1}]. Section 3.2 details that five participants who volunteered for experiment one were used in the present experiment (Figure 3.1). All participants conformed to the pre-test guidelines and procedures outlined in section 3.2.

5.2.2. General experimental controls

All FAM, PSA and testing sessions were completed on the same NMT. For a detailed description of the NMT specifications and safety measures please see section 3.5.

5.2.3. Experimental design

This experiment employed a counter-balanced, crossover design in which participants visited the laboratory on eight separate occasions (Figure 5.1). The participants were blinded between both normoxic and hypoxic environments; however, no blinding could occur between hot and temperate environments. *Visit 1*: All participants completed a $\dot{V}O_{2max}$ prior to completing all FAM and iSPT testing sessions. For a detailed description of the $\dot{V}O_{2max}$ test, please see section

3.4. *Visit 2-4 (FAM$_{1-3}$)*: All FAM and PSA sessions were completed as outlined in section 3.7. All participants during this experiment had the following peak sprint speed: [median (min-max) 21 (20-22 km·h^{-1}). *Visits 5-8*: During the four randomised experimental trials, participants completed iSPT in four different environmental conditions: CON - (0m; 18ºC 50% RH), HOT - (30ºC; 50% RH), HYP - (1,000m; 18ºC 50% RH), and HH - (1,000m; 30ºC 50% RH). All visits to the laboratory were separated by at least 7 d to allow for a full recovery to all soccer performance measures, as utilised in previous counter-balanced, crossover design studies quantifying soccer performance in extreme environments (Coull *et al*, 2015, Taylor *et al*, 2014a). For a detailed description of the design and the methods utilised to form iSPT, please see section 3.6.

5.2.4. Experimental procedures

Upon arrival to the laboratory on visit 1 all anthropometric measurements were recorded as described in section 3.3. A warm up was completed prior to all experimental trials, as outlined in section 4.2.4. As referred to in section 3.6, the iSPT consisted of two 45 min halves separated by a 15 min interval (Figure 3.4). During the 15 min interval participants were seated and all consumed 500 mL of water to drink in each condition. The generation of the hot and hypoxic exposures utilised in this experimental chapter are reported in section 3.1.4.

5.2.5. Soccer performance measures

During iSPT physiological [HR (Section 3.9.1.), S$_a$O$_2$ (Section 3.9.2.) and body mass (Section 3.9.4)], subjective [RPE (Section 3.10.1.) and TS (Section 3.10.2)], body temperature [T$_{re}$ (Section 3.11.1.), T$_{sk}$ (Section 3.11.2.), T$_{body}$ (Section 3.11.3), Estimated T$_{mu}$ (Section 3.11.5.)] and blood [Bla (Section 3.12.1.) and plasma volume (Section 3.12.2.)] measurements were all recorded. All physical performance measures were ascertained as detailed in section 3.8.

5.2.6. Statistical analyses

An a *priori* power calculation (G*Power 3) was used to determine the number of participants required for this experiment (n = 12) with an alpha level of 0.05 and a statistical power of 99%, using data [(High-speed distance covered) - minimum worthwhile effect = 5 m; SD = 50] from experimental chapter 1 (Appendix F). As discussed in section 2.1.1. HSD covered was used for this power calculation as it is central to game defining moments (Gregson et al, 2010). Furthermore, as discussed in section 2.3.2.6. decrements in HSD covered are commonly seen in both hot (Ekblom, 1986, Mohr et al, 2012) and hypoxic (Garvican et al, 2014, Nassis, 2013)

environments in soccer match-play. Normality of the observed data was assessed using Q - Q plots and was deemed plausible in all instances. Therefore, values are presented as mean ± SD. Differences between condition, time, and condition x time for all physical performance measures and physiological responses were analysed using linear mixed models (IBM SPSS statistics for Windows, Version 21, Armonk, NY). A linear mixed model was also used to assess the differences between halves, and fifteen minute intervals for all physical performance (total distance, high-speed distance, variable run distance and sprint distance covered) measures between conditions. Please see section 4.2.6 for a justification to why a linear mixed model analysis was chosen for this experimental chapter. Additionally, a stepwise regression analysis was employed to analyse the strength of existing relationships between the dependant (Physiological Responses) and independent (Physical Performance Measures) measures. A backward regression method was adopted for this analysis as it chooses the independent variable with the largest Pearson correlation with the dependant variable. First all the physiological, subjective, body temperature and blood measures were entered at once and then those variables with significance aove the default criterion of 0.05 were removed (Atkinson and Nevill, 1998). A backward regression method was adopted for this analysis as it chooses the independent variable with the largest Pearson correlation with the dependant variable (Field, 2013). First all the physiological, subjective, body temperature and blood measures were entered at once and then those variables with significance above the default criterion of 0.05 were removed (Field, 2013). The model was then performed again with those variables that had a default criterion below 0.05. to adjust for the small sample size within this experiment (n = 12). Therefore, this is a valid approach in line with statistical best practice guidelines (Field, 2013). The percentage changes between all physical performance measures are also reported and 95% CI presented where necessary. Two-tailed statistical significance was accepted at the $p < 0.05$ level.

Experimental Design (A)
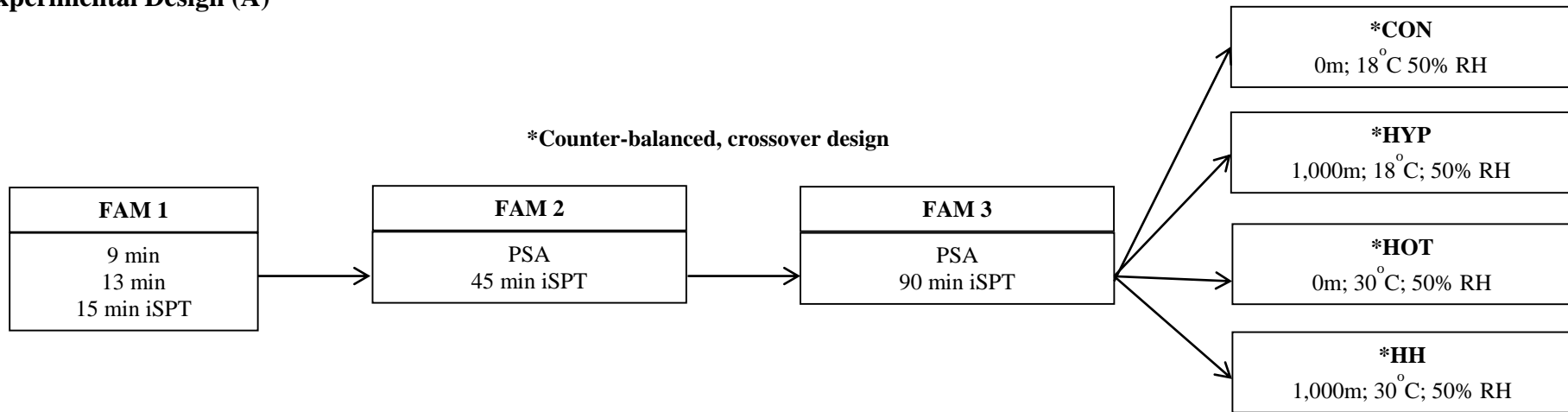
Experimental Procedures (B)
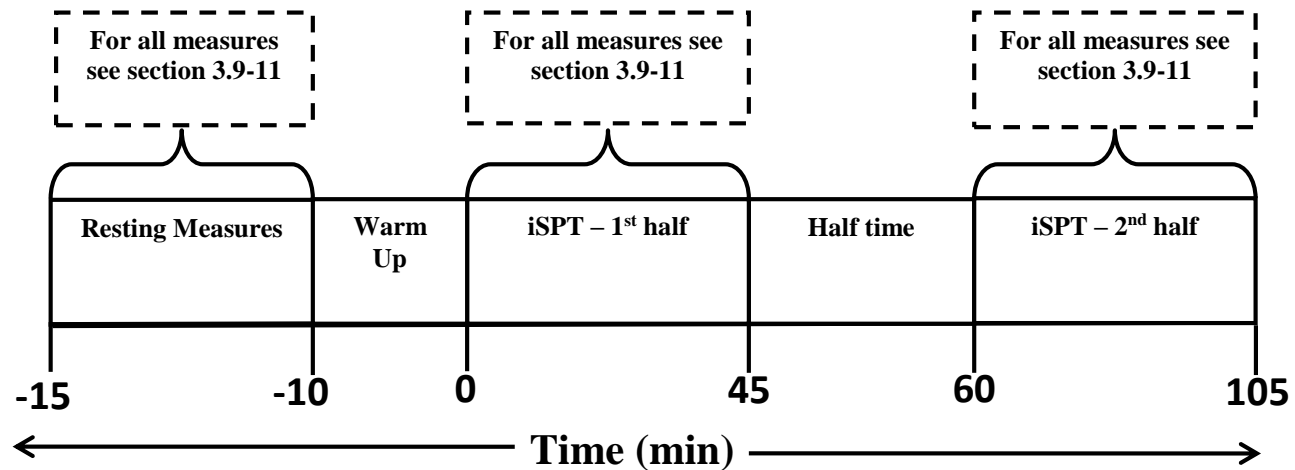
Figure 5.1. A schematic detailing the experimental design (A) and procedures (B) utilised during this experimental chapter. All measurements were recorded as documented within the relevant sections of the general methodologies. All physical performance measures were ascertained post iSPT as documented in section 3.8.

5.3.1. Physical performance measures

5.3.1.1. Between conditions and halves
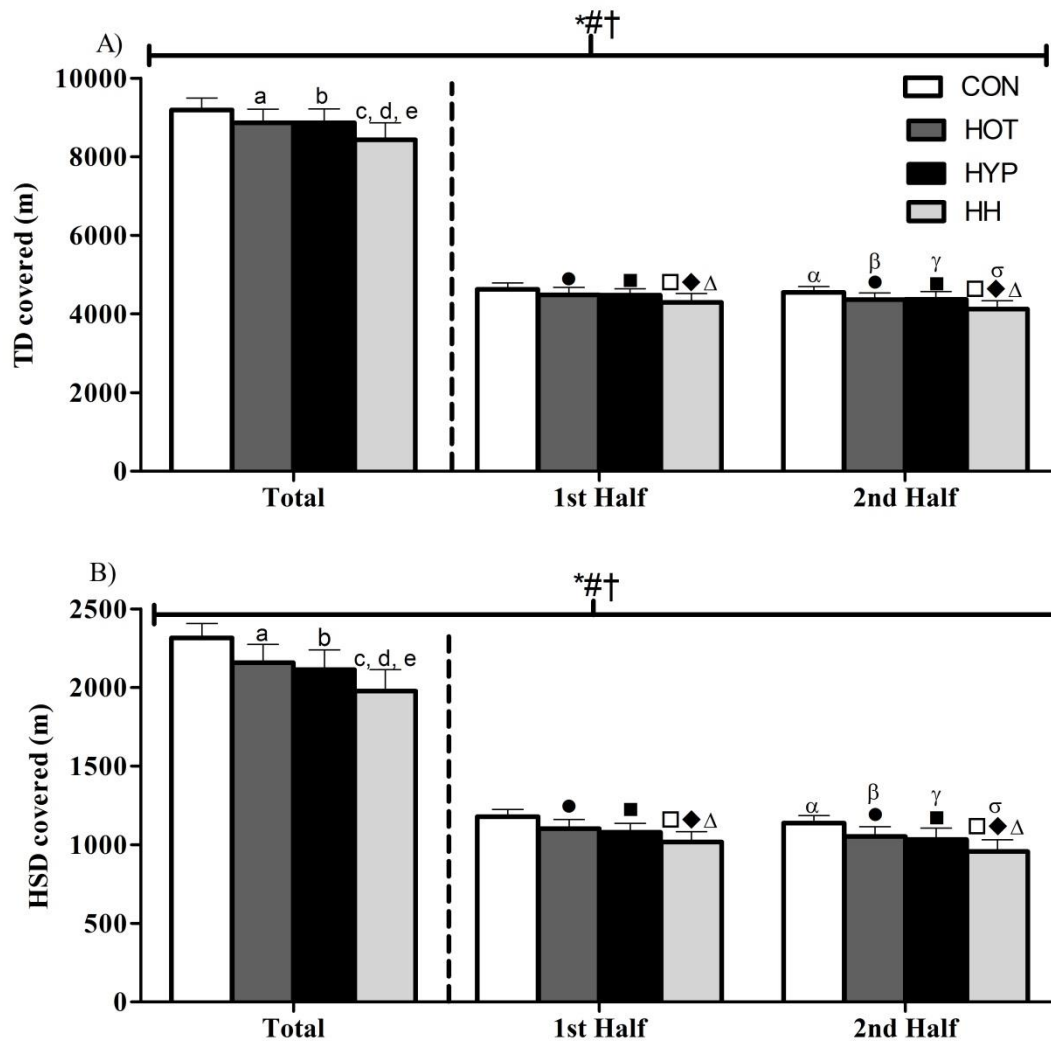
Figure 5.2: The TD covered (A) and HSD covered (B) in total and in each half at CON, HOT, HYP and HH. *Significant main effect for condition; #Significant main effect for time; †Significant interaction effect (condition x time); [a]Significant main effect for condition between CON and HOT ($p < 0.05$); [b]Significant main effect for condition between CON and HYP ($p < 0.05$); [c]Significant main effect for condition between CON and HH ($p < 0.05$); [d]Significant main effect for condition between HOT and HH ($p < 0.05$); [e]Significant main effect for condition between HOT and HH ($p < 0.05$); [α]Significant difference from the first half in CON; [β]Significant difference from the first half in HOT; [γ]Significant difference from the first half in HYP; [δ]Significant difference from the first half in HH; •Significant interaction effect (condition x time) between CON and HOT; ▪Significant interaction effect between CON and

HYP; □Significant interaction effect between CON and HH ♦Significant interaction effect between HOT and HH; ^ΔSignificant interaction effect between HYP and HH.

5.3.1.1.1. Total distance covered

A significant main effect for condition ($F = 16.5$; $p < 0.001$), time ($F = 202.8$; $p < 0.001$) and an interaction effect for condition x time ($F = 3.6$; $p = 0.03$) was observed for total distance covered. When compared to CON, the total distance covered was 4% shorter (321 ± 131 m) in both HOT ($p = 0.001$, 95% CI: 65 – 256 m) and HYP ($p = 0.004$, 95% CI: 44 - 282 m). A 9% (756 ± 142 m) reduction was also observed between HH ($p < 0.001$, 95% CI: 196 – 560 m) and CON. Furthermore, the overall total distance covered was 5% (431 ± 132 m) shorter in HH compared to both HOT ($p = 0.01$, 95% CI: 41 - 395 m) and HYP ($p = 0.01$, 95% CI: 45 - 385 m) (Figure 5.2).

A 1% (81 ± 66 m), 2% (120 ± 45 m), 3% (101 ± 66 m) and 4% (164 ± 60 m) greater total distance covered was seen in the first compared to the second half in CON ($p = 0.001$, 95% CI: 39 - 123 m), HOT ($p < 0.001$, 95% CI: 91 - 148 m), HYP ($p < 0.001$, 95% CI: 59 - 143 m) and HH ($p < 0.001$, 95% CI: 126 - 202 m), respectively. In both halves at CON, the total distance covered was 3% (141 ± 53 m) (first half) and 4% (152 ± 32 m) (second half) greater compared to HOT (1st half: $p = 0.007$, 95% CI: 33 - 249 m, 2nd half: $p = 0.001$, 95% CI: 88 - 272 m) and HYP (1st half: $p = 0.006$, 95% CI: 34 - 271 m; 2nd half: $p = 0.006$, 95% CI: 41 – 305 m). Furthermore, an 8% (336 ± 32 m) and 10% (420 ± 63 m) decrement was seen in HH compared to CON during the first ($p < 0.001$, 95% CI: 144 - 529 m) and second half ($p < 0.001$, 95% CI: 242 - 597 m), respectively. Finally, a 4% (184 ± 43 m) and 6% (240 ± 32 m) reduction in total distance covered was seen in the first and second half at HH compared to HOT (1st half: $p = 0.04$, 95% CI: 10 – 380 m; 2nd half: $p = 0.004$, 95% CI: 68 - 412 m) and HYP (1st half: $p = 0.04$, 95% CI: 10 - 381 m; 2nd half: $p = 0.04$, 95% CI: 73 - 420 m), respectively (Figure 5.2).

5.3.1.1.2. High-speed distance covered

A significant main effect for condition ($F = 39.1$; $p < 0.001$), time ($F = 22.1$; $p < 0.001$) and an interaction effect ($F = 3.1$; $p = 0.04$) was observed for total distance covered. The high-speed distance covered at CON was 7% (160 ± 21 m), 9% (203 ± 32 m) and 15% (340 ± 43 m) shorter in HOT ($p = 0.001$, 95% CI: 16, 78 m), HYP ($p < 0.001$, 95% CI: 62 - 81 m) and HH ($p < 0.001$, 95% CI: 91 - 152 m), respectively. Furthermore, an 8% (180 ± 36 m) decrement in high-speed distance covered was seen in HH compared to HOT ($p < 0.001$, 95% CI: 44 - 105 m),

whereas a 7% (149 ± 36) reduction was seen compared to HYP ($p < 0.001$, 95% CI: 28 - 89 m) (Figure 5.2).

A 3% (39 ± 16 m) and 6% (60 ± 30 m) greater high-speed distance covered was seen in the first compared to the second half in CON ($p < 0.001$, 95% CI: 39 - 123 m) and HH ($p < 0.001$, 95% CI: 126 - 202 m), respectively. Furthermore, a 4% (HOT: 48 ± 22 m; HYP: 46 ± 33 m) greater high-speed distance covered was seen in the first compared to the second half in both HOT ($p < 0.001$, 95% CI: 91 - 148 m) and HYP ($p = 0.003$, 95% CI: 59 - 143 m). Figure 5.2 also shows that compared to CON, high-speed distance covered was significantly reduced during the first half in HOT ($p = 0.002$, 95% CI: 21 - 86 m), HYP ($p < 0.001$, 95% CI: 23 - 110 m) and HH ($p < 0.001$, 95% CI: 78 - 164 m) by 6% (76 ± 36 m), 8% (98 ± 64 m) and 14% (160 ± 58 m), respectively Furthermore, the high-speed distance covered was also significantly reduced in the second half by 7% (84 ± 58 m), 9% (105 ± 48 m) and 16% (180 ± 68 m) in HOT ($p = 0.001$, 95% CI: 7 - 94 m), HYP ($p = 0.001$, 95% CI: 41 - 305 m) and HH ($p < 0.001$, 95% CI: 78 - 165 m) compared to CON (Figure 5.2). Furthermore, an 8% (84 ± 47 m) and 6% (96 ± 54 m) reduction in high-speed distance covered was evident at HH compared to HOT during the first ($p = 0.009$, 95% CI: 35 - 121 m) and second ($p = 0.009$, 95% CI: 28 - 114 m) half, respectively. Finally, a 9% (61 ± 46 m) and 7% (75 ± 54 m) decrement in high-speed distance covered was observed at HH compared to HYP during both the first ($p = 0.007$, 95% CI: 19 - 106 m) and second ($p = 0.007$, 95% CI: 12 - 98 m) half, respectively (Figure 5.2).
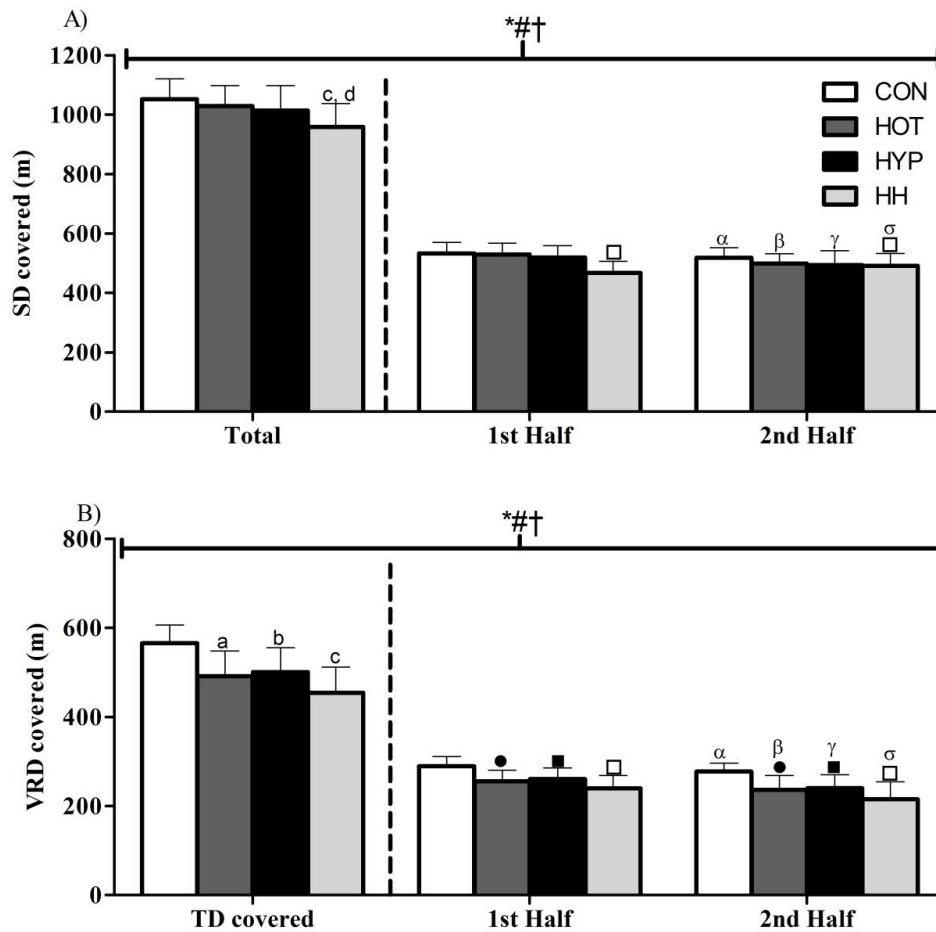
Figure 5.3: The SD covered (A) and VRD covered (B) in total and in each half at CON, HOT, HYP and HH. See Figure 5.2 for explanation of symbols.

5.3.1.1.3. Sprint distance covered

There was a significant main effect for condition ($F = 4.8$; $p = 0.01$), time ($F = 92.6$; $p < 0.001$) and an interaction effect ($F = 3.7$; $p = 0.03$) for sprint distance covered. The sprint distance covered during iSPT was significantly reduced in HH by 8% (93 ± 36 m) and 7% (78 ± 46 m) compared with CON ($p = 0.009$, 95% CI: 9 - 83 m) and HOT ($p = 0.04$, 95% CI: 7 - 69 m). However, there was no significant difference between HYP and HH ($p = 0.08$ 95% CI: -9 - 83 m). Moreover, no significant differences in sprint distance covered ($p > 0.05$) were evident between CON, HOT and HYP. There was a 3% (15 ± 9 m) and 6% (30 ± 17 m) greater sprint distance covered in the first compared to the second half in CON ($p < 0.001$, 95% CI: 9 - 21 m) and HOT ($p < 0.001$, 95% CI: 20 - 41 m), respectively. Furthermore, there was 5% (HYP: 26 ± 24 m; HH: 24 ± 19 m) reduction in sprint distance covered in the second half compared with the first half in both HYP ($p = 0.003$, 95% CI: 11 - 41 m) and HH ($p = 0.001$, 95% CI: 12

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- 36 m). At CON, the sprint distance covered was 8% (38 ± 25 m) and 10% (51 ± 35 m) greater in the first ($p = 0.04$, 95% CI: 1.9, 81.5 m) and second ($p = 0.003$, 95% CI: 14.2, 87.8 m) half in HH, respectively (Figure 5.3).

5.3.1.1.4. Variable run distance covered

There was a significant main effect for condition ($F = 28.9$; $p < 0.001$), time ($F = 229.9$; $p < 0.001$) and interaction effect ($F = 5.8$; $p = 0.008$) for variable run distance covered. The variable run distance covered was 13% (74 ± 24 m), 12% (65 ± 35 m) and 15% (111 ± 37 m) greater in CON compared with HOT ($p < 0.001$, 95% CI: 22 - 53 m), HYP ($p < 0.001$, 95% CI: 17 - 48 m) and HH ($p < 0.001$, 95% CI: 34 - 78 m), respectively. Figure 5.3 also demonstrates that the variable run distance covered was significantly reduced in the second half compared to the first by 4% (12 ± 5 m), 7% (19 ± 7 m), 8% (20 ± 10 m) and 10% (24 ± 10 m) in CON ($p < 0.001$, 95% CI: 9 - 15 m), HOT ($p < 0.001$, 95% CI: 14 - 23 m), HYP ($p < 0.001$, 95% CI: 14 - 27 m) and HH ($p < 0.001$, 95% CI: 18 - 30 m), respectively. The variable run distance covered was also 10% (34 ± 30 m), 9% (29 ± 23 m) and 17% (50 ± 35 m) greater in the 1st half at CON compared with HOT ($p < 0.001$, 95% CI: 20 - 48 m), HYP ($p < 0.001$, 95% CI: 14 - 43 m) and HH ($p < 0.001$, 95% CI: 28 - 72 m), respectively. Finally, in CON, a 15% (41 ± 38 m), 13% (37 ± 25 m) and 22% (62 ± 31 m) reduction in variable run distance covered was seen in 2nd half at HOT ($p < 0.001$, 95% CI: 22 59 m), HYP ($p < 0.001$, 95% CI: 18 - 55 m) and HH ($p < 0.001$, 95% CI: 38 - 89 m), respectively (Figure 5.3).

5.3.1.2. Between 15 min blocks

5.3.1.2.1. High-speed distance covered

In CON and HOT, more high-speed distance was covered ($p < 0.001$) in the first four 15 min blocks than in the final 15 min. However, in HYP and HH, the high-speed distance covered was significantly reduced in the final 15 min compared to all other time points. Thus, the fatigue index between the first and last 15 min block for CON, HOT, HYP and HH was 7% (27 ± 6 m), 8% (31 ± 2 m), 10% (35 ± 7 m) and 14% (49 ± 5 m), respectively. Furthermore, table 5.1 also reveals that high-speed distance covered was significantly reduced ($p < 0.05$) in all 15 min blocks in HOT [Range (%, m): 6-8%, 26 – 40 m], HYP [Range (%, m): 9-11%, 43 – 51 m] and HH [Range (%, m): 16-18%, 45 – 67 m] compared to CON, respectively (Table 5.1).

5.3.1.2.2. Sprint distance covered

The sprint distance covered in CON ($p = 0.007$, 95% CI: 2 - 23 m) and HOT ($p = 0.005$, 95% CI: 1 - 13 m) was only significantly reduced in the final 15 min block compared with the first 15 min. However, in HYP and HH, the sprint distance covered was significantly reduced in the final 15 min compared to all other time points. Thus, the fatigue index between the first and 15 min block for CON, HOT, HYP and HH was 7% (12 ± 14 m), 11% (18 ± 12 m), 10% (18 ± 12 m) and 13% (22 ± 11 m), respectively. A 6% (HOT: m; 10 ± 13 m; HYP: 12 ± 21 m) significant decrease in sprint distance covered was seen in the final 15 min of iSPT in CON compared with the identical time point in HOT ($p = 0.03$, 95% CI: 1 - 20 m) and HYP ($p = 0.03$, 95% CI: 1 - 24 m). Furthermore, at CON compared with HH, the sprint distance covered was also significantly increased by 9% (18 ± 12 m) ($p = 0.002$, 95% CI: 6 - 30 m) and 12% (25 ± 11 m) ($p < 0.001$, 95% CI: 9 - 33 m) in the final two 15 min blocks, respectively (Table 5.1).

5.3.1.2.3. Variable run distance covered

The variable run distance covered in CON ($p = 0.04$, 95% CI: 1 - 7 m), HOT ($p = 0.001$, 95% CI: 4 - 18 m) HYP ($p = 0.04$, 95% CI: 1 - 21 m) and HH ($p = 0.04$, 95% CI: 1 - 17 m) was significantly reduced in the final 15 min block compared with the first 15 min. The fatigue index between the first and last 15 min block for CON, HOT, HYP and HH was 7% (10 ± 8 m), 8% (14 ± 9 m), 10% (15 ± 21 m) and 14% (17 ± 21 m), respectively. Furthermore, table 5.1 also reveals that variable run distance covered was significantly reduced ($p < 0.05$) in all 15 min blocks by ~18% [Range (%, m): 16-18%, 16 – 23 m] in HH compared to CON. Finally, a 8% (HOT: 16 ± 12 m; HYP: 16 ± 13 m) significant decrease in variable run distance covered was seen in the final 15 min of iSPT in CON compared with the identical time points in HOT ($p = 0.009$, 95% CI: 2 - 17 m) and HYP ($p = 0.01$, 95% CI: 2 - 17 m) (Table 5.1).

5.3.1.2.4. Peak sprint speed

Table 5.1 reveals a significant main effect for condition ($F = 26.3$; $p < 0.001$), time ($F = 262.2$; $p < 0.001$) and an interaction effect ($F = 3.9$; $p = 0.001$) for peak sprint speed. The peak sprint speed reached in iSPT was 4% (3 ± 1 km·h^{-1}), 4% (4 ± 1 km·h^{-1}) and 7% (5 ± 1 km·h^{-1}) faster in all 15 min blocks HOT than in CON ($p = 0.03$, 95% CI: 1 - 2 km·h^{-1}), HYP ($p = 0.03$, 95% CI: 1 - 3 km·h^{-1}) and HH ($p = 0.03$, 95% CI: 1 - 3 km·h^{-1}), respectively. Furthermore, there was no significant difference ($p > 0.05$) in peak sprint speed between CON, HYP and HH (Table 5.1).

Table 5.1: The HSD, SD, VRD covered and PSS in 15 min blocks during CON, HOT, HYP and HH. The HSD, SD and VRD covered are presented as an overall distance covered during each 15 min period. The PSS is presented as the fastest speed recorded in each 15 min period.

	0-15 min	15-30 min	30-45 min	45-60 min	60-75 min	75-90 min
HSD covered (m)						
CON	400 ± 15ε●■□	393 ± 17 ε●■□	384 ± 17 ε●■□	388 ± 14 ε●■□	378 ± 18 ●■□	373 ± 21 ●■□
HOT	374 ± 22ζ	366 ± 20ζ	362 ± 19ζ	359 ± 22ζ	352 ± 23	343 ± 24
HYP	367 ± 20η	362 ± 19	351 ± 24	355 ± 23	347 ± 24	332 ± 27
HH	355 ± 24θ	339 ± 26	324 ± 30	333 ± 19	319 ± 20	306 ± 29
SD covered (m)						
CON	182 ± 13ε	177 ± 12	174 ± 12	174 ± 13	175 ± 11	170 ± 12●■□
HOT	178 ± 14ζ	177 ± 13	174 ± 12	170 ± 11	169 ± 13	160 ± 11
HYP	176 ± 13η	173 ± 13η	171 ± 17η	169 ± 17η	167 ± 17η	158 ± 15
HH	171 ± 16θ	164 ± 17θ	157 ± 14θ	162 ± 12θ	157 ± 12θ	149 ± 16
VRD covered (m)						
CON	100 ± 8 ε□	95 ± 7□	94 ± 9□	95 ± 7□	92 ± 7□	90 ± 7●■□
HOT	88 ± 8ζ	85 ± 9	83 ± 11	84 ± 11	79 ± 10	74 ± 11
HYP	89 ± 8 η	86 ± 9	85 ± 9	86 ± 9	80 ± 11	74 ± 12
HH	84 ± 10θ	80 ± 11	76 ± 10	77 ± 11	71 ± 11	67 ± 10
PSS (km·h^{-1})						
CON	21.5 ± 1.2	21.1 ± 1.7	20.2 ± 1.6	19.8 ± 1.3	21.1 ± 1.7	21.5 ± 1.8
HOT	22.1 ± 1.5●♦★	23.2 ± 1.4●♦★	23.2 ± 1.5●♦★	21.1 ± 1.2●♦★	22.1 ± 1.3●♦★	22.6 ± 1.4●♦★
HYP	21.1 ± 1.3	22.1 ± 1.2	22.6 ± 1.8	21.7 ± 1.5	22.2 ± 1.5	21.5 ± 1.9
HH	20.4 ± 1.1	20.0 ± 1.6	19.8 ± 1.2	18.1 ± 1.1	19.2 ± 1.5	19.1 ± 2.0

CON: Normoxic-Temperate; HH – Hot-Hypoxic; HOT – Hot; HSD – High Speed Distance; Hyp – Hypoxic; PSS – Peak Sprint Speed SD – Sprint Distance; VRD Variable Run Distance; εSignificant difference from the last 15 min in CON; ζSignificant difference from the last 15 min in HOT; ηSignificant difference from the last 15 min in HYP; θSignificant difference from the last 15 min half in HH; ★Significant interaction effect (condition x time) between HOT and HYP. See Figure 5.2 for an explanation of all other symbols

5.3.2. Body temperature measures
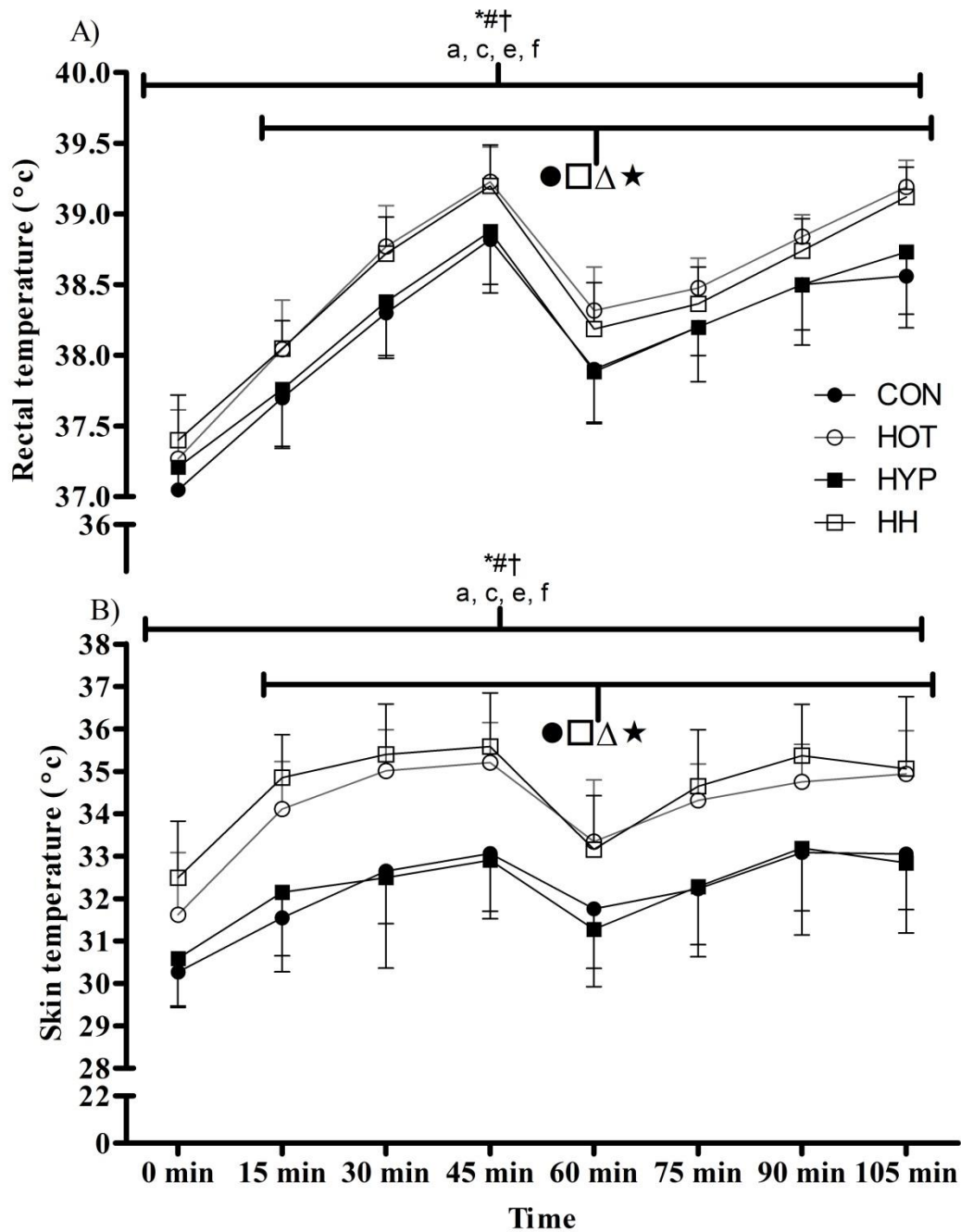
Figure 5.4: The T_{re} (A) and T_{sk} (B) during the first (0-45 min) and second (60-105 min) half in CON, HOT, HYP and HYP. [f]Significant interaction effect for condition between HOT and HYP ($p < 0.05$). See Figure 5.3 and Table 5.1 for an explanation of all symbols.
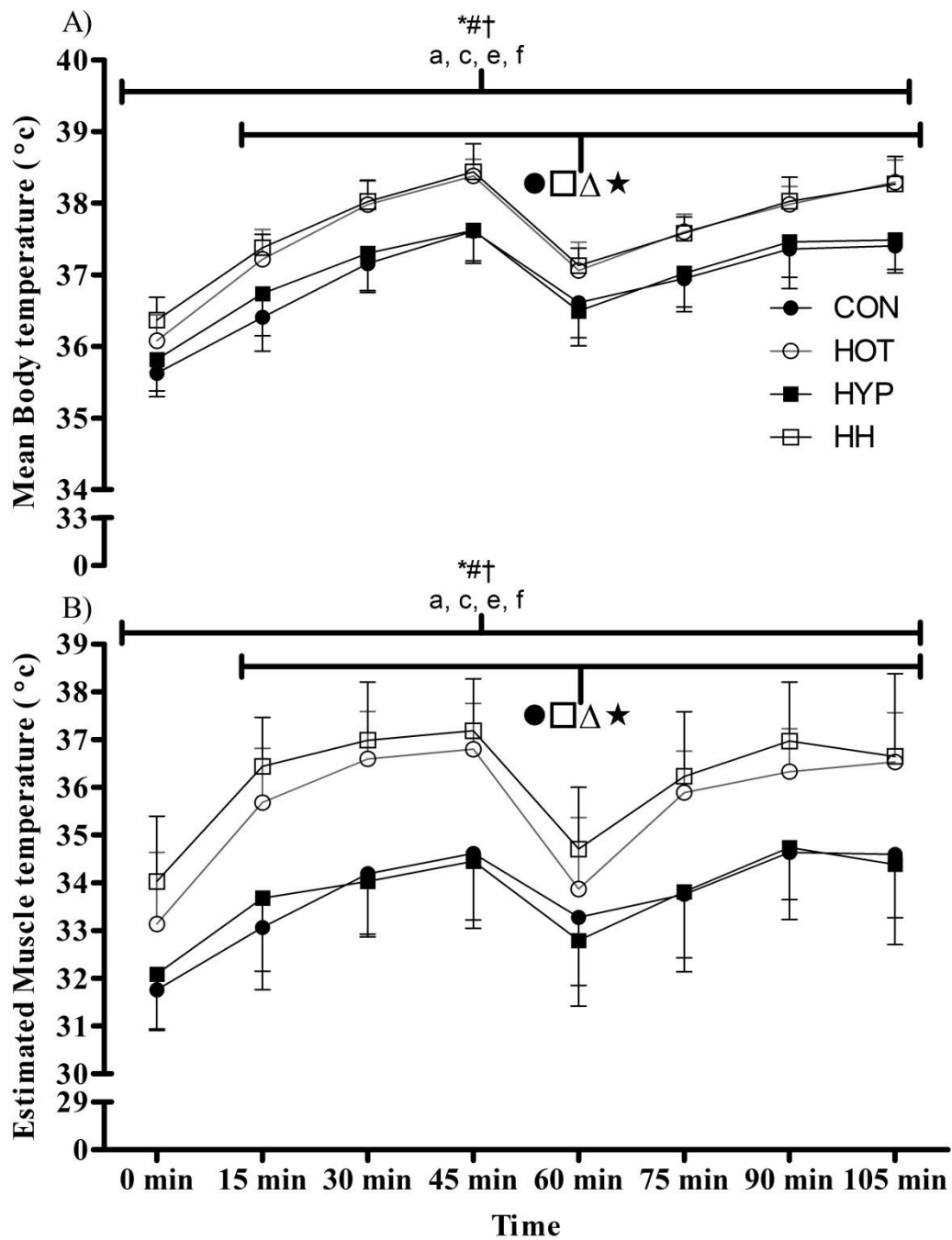
Figure 5.5: The T_{body} (A) and T_{mu} (B) during the first (0-45 min) and second (60-105 min) half in CON, HOT, HYP and HYP. See figures 5.2 and 5.4 and Table 5.1 for an explanation of all symbols.

Figure 5.4 demonstrates a significant main effect for condition ($F = 4576.7$; $p < 0.001$), time ($F = 12.9$; $p < 0.001$) and an interaction effect ($F = 2.2$; $p = 0.007$) for T_{re}. The T_{re} was significantly elevated in HOT (38.7 ± 0.2 °C) and HH (38.6 ± 0.2 °C) compared with CON [38.2 ± 0.3 °C (HOT: $p < 0.001$, 95% CI: $0.2 - 0.5$ °C; HH: $p = 0.001$, 95% CI: $0.1 - 0.6$ °C)] and HYP [38.4 ± 0.4 °C (HOT: $p = 0.001$, 95% CI: $0.1 - 0.4$ °C, HH: $p = 0.009$, 95% CI: $0.1 - 0.4$ °C)]. There was a significant main effect for condition ($F = 2163.7$; $p < 0.001$), time ($F = 40.9$; $p < 0.001$) and interaction effect for condition x time ($F = 28.9$; $p < 0.001$) for T_{sk}. There was a significant main effect for condition ($F = 2163.7$; $p < 0.001$), time ($F = 40.9$; $p < 0.001$) and main effect for condition x time ($F = 28.9$; $p < 0.001$) for T_{sk}. The T_{sk} was significantly elevated in HOT (37.1 ± 1.0 °C) and HH (37.5 ± 1.2 °C) compared with CON [34.1 ± 1.0 °C (HOT: $p < 0.001$, 95% CI: $1 - 3$ °C; HH: $p < 0.001$, 95% CI: $1 - 3$ °C)] and HYP [34.5 ± 1.2 °C (HOT: $p < 0.001$, 95% CI: $1 - 2$ °C; HH: $p < 0.001$, 95% CI: $1 - 3$ °C)]. The HOT and HH exposure displayed a significant increase ($p < 0.05$) to T_{re} and T_{sk} compared to CON and HYP at all-time points after 15 min but not before (Figure 5.4).

There was a significant main effect for condition ($F = 2163.7$; $p < 0.001$), time ($F = 40.9$; $p < 0.001$) and an interaction effect ($F = 28.9$; $p < 0.001$) for T_{mu}. The T_{mu} was significantly elevated in HOT (35.6 ± 1.0 °C) and HH (36.1 ± 1.2 °C) compared with CON [34.1 ± 1.3 °C (HOT: $p < 0.001$, 95% CI: $1 - 2$ °C; HH: $p < 0.001$, 95% CI: $1 - 3$ °C)] and HYP [33.9 ± 1.6 °C (HOT: $p < 0.001$, 95% CI: $1 - 2$ °C; HH: $p < 0.001$, 95% CI: $1 - 3$ °C)]. Figure 5.5 reveals a significant main effect for condition ($F = 44.2$; $p < 0.001$), time ($F = 178.0$; $p < 0.001$) and main effect for condition x time ($F = 3.0$; $p = 0.002$) for T_{body}. The T_{body} was significantly elevated in HOT (37.7 ± 0.3 °C) and HH (37.8 ± 0.2 °C) compared with CON [37.1 ± 0.3 °C (HOT: $p < 0.001$, 95% CI: $0.4 - 0.9$ °C; HH: $p < 0.001$, 95% CI: $0.4 - 1.0$ °C)] and HYP [37.1 ± 0.5 °C (HOT: $p < 0.001$, 95% CI: $0.3 - 0.8$ °C; HH: $p < 0.001$, 95% CI: $0.4 - 0.9$ °C)]. Figure 5.5 also demonstrates that T_{body} and T_{mu} was also significantly increased ($p < 0.05$) at all-time points after 15 min during HH and HOT compared to HYP and CON (Figure 5.5).

5.3.3. Subjective measures
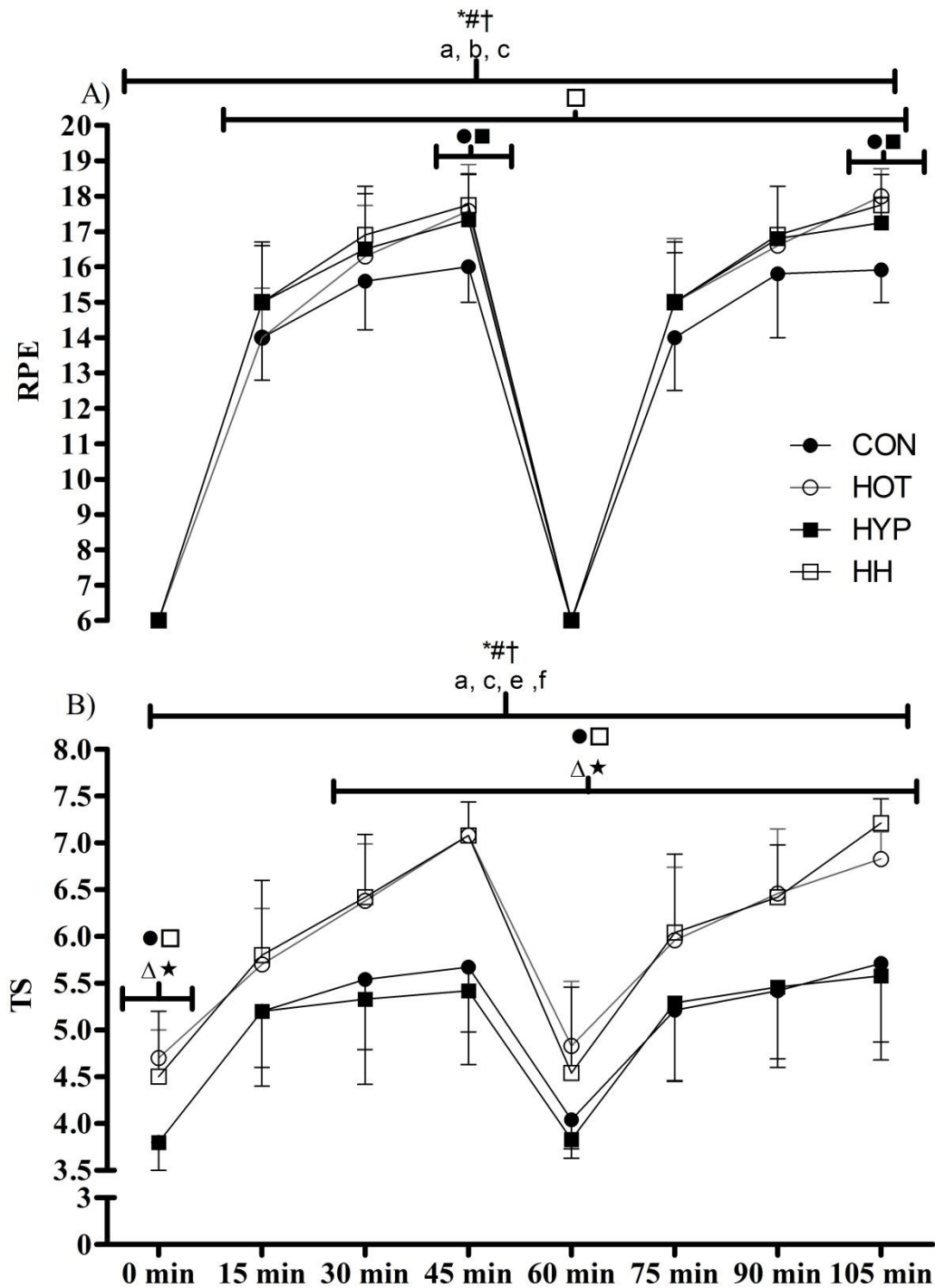
Figure 5.6**: The RPE (A) and TS (B) during the first (0-45 min) and second (60-105 min) half in CON, HOT, HYP and HYP.** See figures 5.2 and 5.4 and Table 5.1 for an explanation of all symbols.

5.3.3.1. Perceived Exertion

There was a significant main effect for condition ($F = 20.8$; $p < 0.001$), time ($F = 1140.3$; $p < 0.001$) and an interaction effect ($F = 1.8$; $p = 0.02$) for RPE. The RPE during iSPT was significantly lower during CON (15 ± 2) compared with HOT (16 ± 1, $p < 0.001$, 95% CI: 0 - 1), HYP (16 ± 1, $p < 0.001$, 95% CI: 0 - 1) and HH (17 ± 1, $p < 0.001$, 95% CI: 1 - 2). The RPE was significantly greater ($p < 0.05$) in HH compared to CON from all-time points after 15 min and not before. Furthermore, RPE was significantly increased at 45 and 105 min in both HOT (*45 min*: $p < 0.001$, 95% CI: 1 - 3; *105 min*: $p < 0.001$. 95% CI: 1 - 3) and HYP (*45 min*: $p = 0.001$. 95% CI: 1 - 3; *105 min*: $p = 0.006$, 95% CI: 1 - 3) compared to CON (Figure 5.6).

5.3.3.2. TS

Figure 5.6 reveals a significant main effect for condition ($F = 96.5$; $p < 0.001$), time ($F = 106.2$; $p < 0.001$) and an interaction effect ($F = 1.8$; $p = 0.01$) for TS. The TS was significantly lower during CON (5 ± 1) and HYP (5 ± 1) compared with HOT [6 ± 1 - (CON: $p < 0.001$, 95% CI: 1 - 2; HYP: $p < 0.001$, 95% CI: 1 - 2)] and HH [6.0 ± 0.4 - (CON: $p < 0.001$, 95% CI: 1 - 2; HYP: $p < 0.001$, 95% CI: 1 - 2)]. A significant increase ($p < 0.05$) in TS during HOT and HH at 0 and 30-105 min compared with CON and HYP was also revealed during iSPT.

5.3.4. Physiological measures

5.3.4.1. S_aO_2
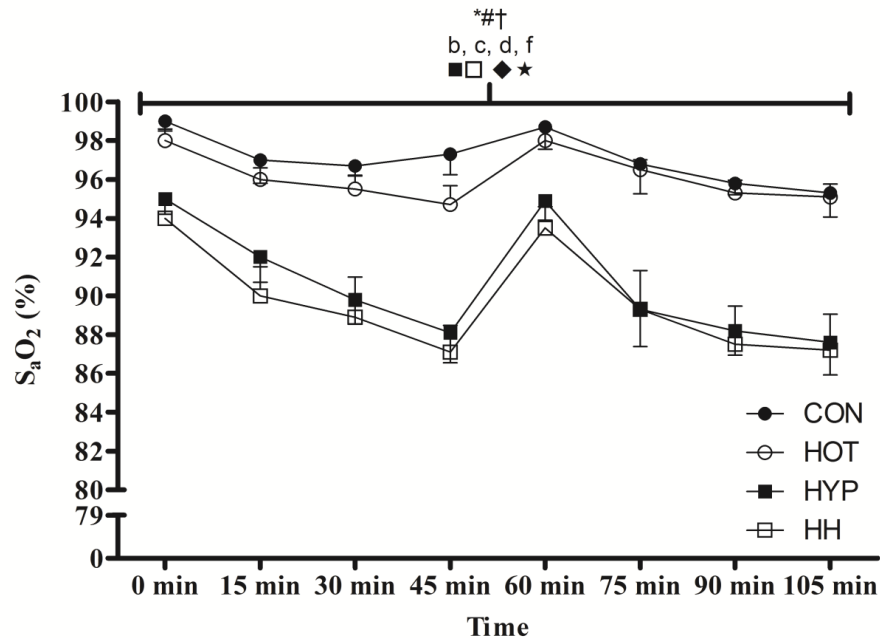
Figure 5.7: The S_aO_2 during the first (0-45 min) and second (60-105 min) half in CON, HOT, HYP and HYP. See figures 5.2 and 5.4 and Table 5.1 for an explanation of all symbols.

There was a significant main effect for condition ($F = 453.8$; $p < 0.001$), time ($F = 133.4$; $p < 0.001$) and an interaction effect ($F = 12.2$; $p < 0.001$) for S_aO_2. The S_aO_2 during iSPT was significantly lower during HYP (91 ± 1 %) and HH (89 ± 1 %) compared with CON [97 ± 1 % - (HYP: $p < 0.001$, 95% CI: 6 - 8 %; HH: $p < 0.001$, 95% CI: 7 - 9 %)] and HOT [96 ± 1 % (HYP: $p < 0.001$, 95% CI: 5 - 6 %; HH: $p < 0.001$, 95% CI: 6 - 7 %). Figure 5.7 also demonstrates a significant reduction ($p < 0.05$) in S_aO_2 during HYP and HH compared with CON and HOT at all-time points of iSPT (Figure 5.7).

5.3.4.2. Body mass

There was a significant main effect for condition (F = 10.8; p < 0.001), time (F = 162.5; p < 0.001) and an interaction effect (F = 2.9; p = 0.04) for body mass. Body mass was significantly reduced post-iSPT by 2% (2 ± 1 kg) in both HOT (HOT vs CON: 75 ± 12 kg, p < 0.001, 95% CI: 1 - 2 kg; HOT vs HYP: p < 0.001, 95% CI: 1 - 2 kg) and HH (HH vs CON: 75.6 ± 11.2 kg,

p = 0.005, 95% CI: 0 - 2 kg; HH vs HYP: p = 0.005, 95% CI: 0 - 2 kg) compared to CON (77 ± 11 kg) and HYP (77 ± 11 kg).

5.3.4.3. HR
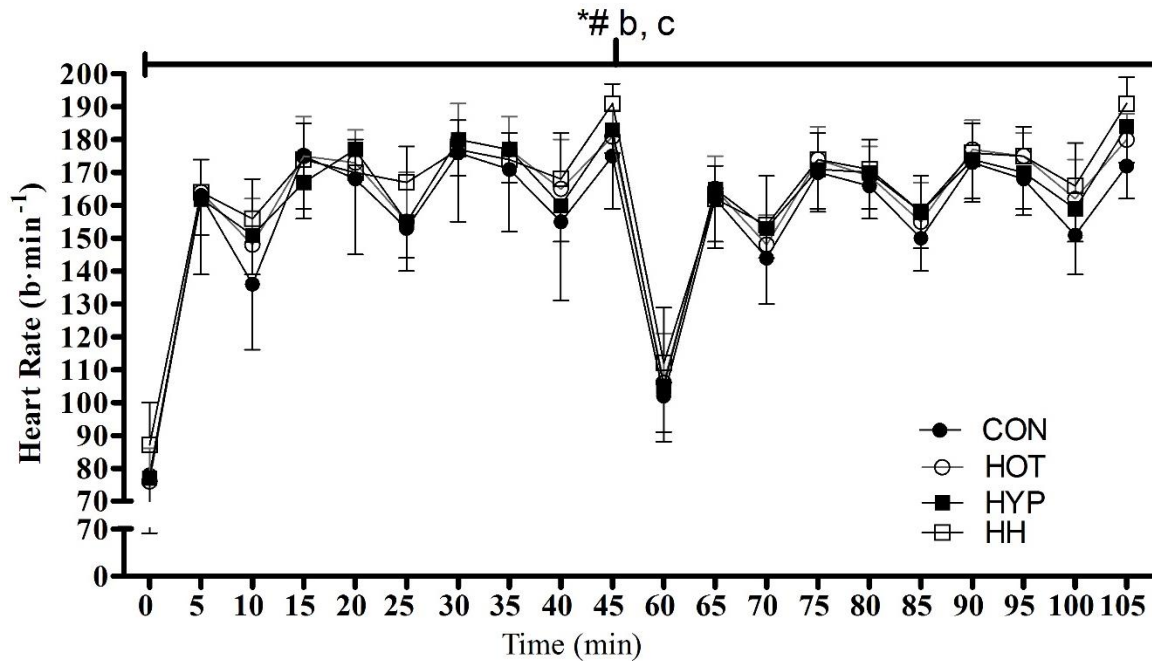
Figure 5.8: The HR during the first (0-45 min) and second (60-105 min) half in CON, HOT, HYP and HYP. See figure 5.2 for an explanation of all symbols.

There was a significant main effect for condition (F = 5.8; p = 0.004), but there was no significant main effect for time (F = 1.3; p = 0.28) and no interaction effect (F = 0.1; p = 0.99) for HR. Mean HR during CON, HOT, HYP and HH was 161 ± 10 b·min-1, 163 ± 3 b·min-1, 165 ± 7 b·min^{-1} and 168 ± 8 b·min^{-1}, respectively. In HH, a significant increase (7 ± 11 b·min^{-1}, $p < 0.001$, 95% CI: 1 - 13 b·min^{-1}) by 4% was seen compared with CON. Furthermore, The HR was also increased (4 ± 9 b·min^{-1}, $p = 0.002$, 95% CI: 2 - 13 b·min^{-1}) by 3% in HYP compared with CON. No significant change (2 ± 9 b·min^{-1}, $p = 0.30$, 95% CI: -2 - 8 b·min^{-1}) in HR was seen between CON and HOT (Figure 5.8).

5.3.5. Blood measures

5.3.5.1 Bla

There was a significant main effect for condition ($F = 18.4$; $p < 0.001$) and time ($F = 90.1$; $p < 0.001$), for Bla. However, no interaction effect ($F = 0.7$; $p = 0.77$) was evident between halves and individual time points for Bla. Between conditions, the Bla concentration at HH was only

significantly increased (1.5 mmol^{-1}, $p < 0.001$, 95% CI: 1-2 mmol^{-1}) compared with CON. No significant difference ($p < 0.05$) in Bla concentration was evident between CON, HOT and HYP (Table 5.2).

5.3.5.2. Plasma volume changes

There was also a significant main effect for condition (F = 20.2; p < 0.001), time (F = 88.6; p < 0.001) and interaction effect (F = 0.9; p = 0.04) for plasma volume change. Between pre- and post-iSPT, there was a significant reduction in plasma volume change in CON (-2%, p = 0.001, 95% CI = -1- -3 %), HOT (-3% p < 0.001, 95% CI = -1 - -5 %), HYP (-3%, p < 0.001, 95% CI = - 1 - -7 %),) and HH (-7%, p < 0.001, 95% CI = -3- -11 %), between pre- and post-iSPT. In HH, a significantly greater reduction (-5%, p < 0.001, 95% CI: - 3- -7%) in plasma volume change was evident compared with CON.

5.3.6. Regression analysis

A stepwise regression analysis identified that absolute TS at the end of HOT was a predictor of the total distance (r = 0.82, *p* = 0.05) and high-speed distance covered (r = 0.82, *p* = 0.05) during the HOT condition. The absolute rise from the start to end of HOT for T$_{mu}$ (r = 0.84, *p* = 0.02) and T$_{sk}$ (r = 0.82, *p* = 0.02) was also a predictor for the total distance and high-speed distance covered at HOT. The absolute TS during HOT was also a predictor of the percentage reduction (5%) for the total distance covered (r = 0.82, *p* = 0.02) from CON to HOT.

Table 5.2: The Bla concentration and plasma volume changes at each individual time point, half and total during CON, HOT, HYP and HH. The Bla concentration is presented in mmol^{-1}. Plasma volume change is presented as a percentage (%) change between pre- and post-iSPT.

	0 min	12 min	27 min	45 min	1st half	57 min	72 min	90 min	2nd half	Total
Bla concentration (mmol^{-1})										
CON	0.9 ± 0.3	4.8 ± 1.0	4.6 ± 1.0	4.7 ± 1.1	4.6 ± 1.0	4.3 ± 1.4	4.0 ± 1.5	3.3 ± 1.3	3.9 ± 1.3	4.3 ± 1.3
HOT	0.9 ± 0.3	5.1 ± 1.8	5.4 ± 1.4	4.1 ± 1.7	5.1 ± 1.5	3.9 ± 1.6	4.7 ± 2.0	3.3 ± 1.5	4.0 ± 1.3	4.4 ± 1.9
HYP	0.8 ± 0.2	5.3 ± 1.0	5.3 ± 1.1	4.4 ± 1.7	5.1 ± 1.1	4.0 ± 1.9	4.3 ± 1.5	3.3 ± 0.9	3.8 ± 1.2	4.5 ± 1.6
HH	0.9 ± 0.3	6.7 ± 1.1	6.0 ± 1.4	5.5 ± 1.5	6.0 ± 1.0	5.7 ± 1.2	5.7 ± 1.3	5.0 ± 1.3	5.6 ± 1.0	5.8 ± 1.8[c]
Plasma volume Change (%)										
CON	0 ± 0	-	-	-	-	-	-	-	-	-2.3 ± 1.2
HOT	0 ± 0	-	-	-	-	-	-	-	-	-3.1 ± 1.5
HYP	0 ± 0	-	-	-	-	-	-	-	-	-3.1 ± 1.7
HH	0 ± 0	-	-	-	-	-	-	-	-	-7.2 ± 2.2[c]

Bla – Blood lactate CON - Normoxic-Temperate; HH – Hot-Hypoxic; HOT – Hot; Hyp – Hypoxic; [c]Significant difference between CON and HH ($p < 0.05$).

5.4. Discussion

The aim of this experimental chapter was to quantify the changes to near-maximal soccer performance in HOT, HYP and HH conditions compared with CON, by utilising the recently validated iSPT (Experimental Chapter 1). The main finding revealed a marked decline in total distance, high-speed distance and variable run distance covered during HOT, HYP and HH conditions when compared to CON (Figure 5.2 and 5.3), supporting the first experimental hypothesis. Furthermore, a greater decline in physical performance was seen in HH even though physiological changes in body mass and temperatures (Figure 5.4 and 5.5), HR, subjective measures (Figure 5.6) and SaO2 (Figure 5.7) were not exacerbated compared to HOT and HYP. This change in physical performance was likely due to alterations in Bla concentration and plasma volume which were only present in HH, supporting the second experimental hypothesis.

5.4.1. Physical performance

It is well acknowledged that during soccer match-play, players will change their exercise intensity and adopt a different pacing strategy when competing in both hot (Mohr *et al*, 2012, Nassis *et al*, 2015) and hypoxic (Buchheit *et al*, 2015, Garvican *et al*, 2013) environments. The data from this experimental chapter reveals a 4% reduction in the total distance and high-speed distance covered in both the HOT and HYP conditions compared with CON, which are in accordance with previous match-play data in hot [30°C - (Ekblom, 1986), 43°C - (Mohr *et al*, 2012)] and low altitude [1,200m - (Nassis, 2013), 1,600m - (Garvican *et al*, 2013)] environments. Furthermore, the data from this experimental chapter revealed that the fatigue index for total distance, high-speed distance, and variable run distance covered between halves was exacerbated in HOT, HYP and HH when compared to CON (Figure 5.2 and 5.3). However, data by Mohr *et al* (2012) identified that the fatigue index between halves was greater during temperate match-play, (21°C) when compared to a hot environment (43°C), dissimilar to the findings from this experimental chapter. This smaller reduction between halves in previous heat-situated match play data, compared to the presented data (Figure 5.2), is likely to have occurred due to the altered pacing strategy mentioned previously. The change in environment is likely to reduce the willingness of an athlete to perform physical exercise during match-play, which assists to preserve technical skills during match-play in hot (Mohr *et al*, 2012, Nassis *et al*, 2015) and low altitude (Nassis, 2013) environments. However, due to the formulation of iSPT (Experimental Chapter 1) the pacing strategies and previously elucidated match factors

(Gregson *et al*, 2010) were minimised as player's completed the same exercise each half – in line with the individualised externally parameterised speed thresholds (Experimental Chapter 1). Therefore, the well formulated approach adopted by iSPT allows for the environmentally induced fatigue to be reliably ascertained between halves and conditions, without a feed forward/back mediated adaptive pacing strategy occurring (Nybo *et al*, 2014).

A player's 'willingness' to perform high-speed exercise at a self-paced speed (which is somewhat indicative of 'pacing strategy') was ascertained during iSPT via the variable run component, designed to quantify high speed running without an external cue (Experimental Chapter 1) (Abt and Lovell, 2009, Lovell and Abt, 2013). This experimental chapter reported a significant reduction ($p < 0.05$) in variable run distance covered in HOT, HYP and HH compared with CON both between halves (Figure 5.3) and in the final 15 min (Table 5.1). Furthermore, the magnitude of decline in the last 15 min during HOT, HYP and HH was significantly exacerbated by ~8%. ~10% and ~13% compared with CON, respectively (Table 5.1). This decline in variable run distance supports the notion that the individualised externally parameterised movement patterns employed by iSPT prevented participants adopting an altered pacing strategy [in line with discussions elsewhere (Mohr *et al*, 2012, Nassis, 2013, Nassis *et al*, 2015)], however, when these external cues/constraints are removed in the variable run, participants choose a lower running speed in HOT, HYP and HH compared to CON (Figure 5.3). Changes in this self-paced high speed running performance in HOT, HYP and HH are important to match-play, as this variable has a good association with game defining moments during soccer (Gregson *et al*, 2010). Therefore, a reduction in high speed running in these environments may reduce the number of game defining moments that occur during soccer (Taylor and Rollo, 2014).

The high-speed distance, sprint distance and variable run distance covered were all significantly reduced across all conditions) in the final 15 min when compared with the first 15 min (Table 5.1). These findings show synergy with the validity data shown in experimental chapter 1 and previous soccer match-play data (Mohr *et al*, 2005, Mohr *et al*, 2010, Mohr *et al*, 2012). However, dissimilar to a previous soccer match-play study at 43°C (Mohr *et al*, 2012), the fatigue index between the first and last 15 min block was exacerbated in HOT, HYP and HH when compared with CON environment (Table 5.1), which is likely due to control of the pacing strategies mentioned previously (Buchheit *et al*, 2015, Mohr *et al*, 2012). It is reasonable to suggest that such time-dependant differences in high-speed distance and sprint distance covered at HOT, HYP and HH may be pivotal to the match outcome. For example, in the 1998

and 2002 FIFA World Cups most goals were scored in the second half (Armatas *et al*, 2007). Furthermore, Armatas *et al* (2007) also revealed that more goals were scored/conceded in the final 15 min of match-play (76-90 min) compared to all other 15 min time phases. This phenomenon in goals scored/conceded is likely due to the inability to maintain repeated sprint exercise or discrete episodes of non-fatigued maximal physical performance, within the final 15 min of match-play [which are often pivotal within game defining moments (Faude *et al*, 2012, Gregson *et al*, 2010)]. Coaches may look to exploit these phenomena, with appropriate offensive positional substitutions potentially taking advantage of the fatigue repeated sprint ability within defensive players. Therefore, the results from this experimental chapter indicate that these decrements seem to be exacerbated in HOT, HYP and HH environments.

5.4.2. Physiological responses

The current experimental chapter reveals there were no differences in any physical performance measures between the HOT and HYP conditions when compared to CON. However, the physiological responses which underpin the observed decrements in physical performance are very different. For example, this experimental chapter demonstrated that an elevation in both T_{re} (Figure 5.4) and estimated T_{mu} (Figure 5.5) in HOT and HH was seen from 15 min onwards compared with CON and HYP. Aughey *et al* (2013) demonstrated that elite Australian rules football players can tolerate a T_c exceeding 40^oC in the final stages of match-play, however, this initiated a marked decline in high speed running during this period. This decline can be loosely associated to the critical 'T_c temperature hypothesis' and the 'central fatigue mechanism', which are both discussed in section 2.3.2.3 (Gonzalez-Alonso *et al*, 1999, Nielsen *et al*, 1993) – though large inter and intra-individual variance is seen regarding the tolerance to high T_c and T_{mu} (Racinais *et al*, 2012). These findings show synergy with previous soccer match-play data (Mohr *et al*, 2010, Mohr *et al*, 2012). Indeed, Mohr *et al* (2012) highlighted that there was no correlation between the distance deficit in high-speed distance covered between match-play in 43^oC and 21^oC compared with the peak or absolute rise in T_c. This may mean that changes in physical performance during match-play is not simply a function of T_{re} but may also be potentially dictated by other peripheral factors and perception of the heat, leading player's to adopt the different pacing strategies seen within soccer match-play (Mohr et al, 2012). Taylor and Rollo (2014) reported that the precise mechanism by which a hot environment reduces soccer performance is not clear, with intricate interplay between peripheral (feedback) and central factors (feed forward) known to occur dependent on exercise modality, intensity and duration. Furthermore, Nybo et al (2014) identified an integrative

model with the potential central and peripheral factors that may influence fatigue during prolonged exercise in the heat. Therefore, it is apparent that the heat induced decrements in soccer is a complex interplay between both peripheral central factors.

Alongside the elevation in both T_{re} and T_{mu} seen in HOT and HH, a rise in T_{sk} was also seen in both these conditions when compared with a CON and HYP environments (Figure 5.4). To the research team's knowledge, this experimental chapter is the first to acknowledge the impact of T_{sk} during individualised near-maximal soccer performance, as this measurement is difficult to ascertain during soccer match-play, due to the equipment required to quantify these changes. Sawka *et al* (2012) identified that a rise in T_{sk} causes an increase in skin blood flow due to a narrowing in the gradient between high T_{sk} and T_{re}, reducing convective heat loss from the active skeletal muscle to the atmosphere. The findings from this experimental chapter show that the gradient between T_{sk} and T_{re} was reduced in HOT (4 ^{o}C) and HH (4 ^{o}C) when compared with CON (6 ^{o}C) and HYP (6 ^{o}C). Therefore, the disruption in heat balance in favour of heat gain is likely to explain the significant increase in TS seen during HOT and HH compared with CON and HYP (Figure 5.6). Therefore, it is likely that a player's thermal comfort may have been significantly reduced in HOT and HH, contributing to the reduced exercise intensity in these aforementioned conditions (HOT and HH) (Sawka *et al*, 2012).

Regression analysis allows for the examination of key measures and their relationship to physical performance, offering a more insightful review of interaction between the physical performance measures and physiological responses (Watkins *et al*, 2014). Multiple regressions reveals that the absolute rise in T_{sk} and T_{mu} were a predictor of both total distance and high-speed distance covered in HOT (Section 3.6). Furthermore, absolute TS at the end of HOT was also a predictor in the decrement in total distance covered seen in HOT compared with CON (Section 5.3.6). Therefore, it is believed that as TS increases the decrement in total distance covered between CON and HOT is also enhanced. As both T_{sk} and TS have been shown previously to have a strong relationship (Nybo *et al*, 2014, Sawka *et al*, 2012, Sawka *et al*, 2011), this could highlight that thermal comfort (T_{sk} x TS) has a large role on the heat-induced decrements on physical performance seen in this experimental chapter (Sawka *et al*, 2012, Sawka *et al*, 2011). Therefore, as the regression analysis revealed that T_{mu}, T_{sk} and TS were the main predictors of the heat-induced decrements between CON and HOT in this experimental chapter, future interventions should look to target these specific factors. A successful intervention would hopefully go some way to maintain 'temperate like' match play soccer performance, or similar.

It is clear that fatigue in soccer within the heat is likely to be multi-factorial and that several multifactorial mechanisms should be considered besides the elevated body temperatures already acknowledged within this experimental chapter (Sawka *et al*, 2012, Sawka *et al*, 2011). For example, maximal intensity exercise in the heat, is said to be limited by changes to the cardiovascular systems which facilitate O_2 delivery to the active skeletal muscle whilst maintaining adequate thermoregulatory outputs (Nybo *et al*, 2014). However, this experimental chapter reveals that the HR and Bla concentration seen during the near-maximal soccer performance in the HOT condition were not different when compared with CON, which is accordance with previous match-play data (Mohr *et al*, 2012). A recent review by Bangsbo (2014) identified that the physical demands of soccer players are dependent upon the complex interaction of the cardiovascular and muscular systems in supporting both aerobic and anaerobic energy provision during match-play. However, it appears that different permutations to the cardiovascular system do not occur during soccer match-play in the heat compared with a temperate environment (Mohr *et al*, 2012). Therefore, it is likely that the muscular (elevated T_{mu}) and other peripheral (elevated T_{sk} and TS) implications highlighted previously are likely to have a predominant role in the heat-induced decrements compared with a temperate environment seen during this experimental chapter and previous match-play studies (Mohr *et al*, 2010, Mohr *et al*, 2012, Özgünen *et al*, 2010).

An increase in HR at HYP when compared with CON, shows synergy with previous soccer match-play data at 1,600m above sea level (Garvican et al., 2014). The rise in HR seen in HYP can be attributed to a hemodynamic response arising from a reduction in S_aO_2 which drives a compensatory increase in cardiac output (Mazzeo, 2008; Stembridge et al., 2015a; Stembridge et al., 2015b). However, during high-speed exercise bouts at altitude a decrease in stroke volume can decrease O_2 delivery to the active muscles as it cannot match the muscle demand, manifesting as a decline to physical performance in HYP (Mazzeo, 2008). A reduction in S_aO_2 by ~8% compared to CON was also apparent by the end of iSPT in both HYP and HH which indicates the onset of exercise induced arterial hypoxemia had occurred causing a plethora of detrimental physiological responses (Billaut and Aughey, 2013), driving the exacerbated performance decrements seen in HYP and HH (Figure 5.2 and 5.3). Indeed, reduced phosphocreatine re-synthesis at altitude is due to sub-optimal re-oxygenation of the active skeletal muscle elongating the recovery time between high-speed exercise bouts (Garvican et al., 2014). Changes in high-speed running are important for maintaining match-play physical performance, due to its association with game defining moments (Gregson et al., 2010),

possibly impacting upon the match result (Taylor and Rollo, 2014). Furthermore, the employed design cannot distinguish precisely between whether the changes in S_aO_2 were apparent due to exercise and/or environmentally-induced-arterial-hypoxemia, highlighting that future work should look to explore these complex phenomena within an appropriate design. Data by Billaut and Smith (2010) indicates that intermittent running based exercise can induce exercise-induced-arterial-hypoxemia in University level soccer players. Therefore, although the employed design cannot distinguish precisely between exercise and environmentally-mediated-arterial-hypoxemia future work should look to explore these complex phenomena within an appropriate design.

In HH, the largest performance decrement both between halves (Figure 5.2 and 5.3) and 15 min blocks was evident (Table 5.1). However, all changes in TS (Figure 5.6), body mass and temperature (Figure 5.4 and 5.5) were similar compared with HOT. Furthermore, all changes to both S_aO_2 (Figure 5.6) and HR were comparable with HYP. This is despite a greater decline in total distance and high-speed distance covered, as well as an additional reduction in sprint distance covered in HH which were not present in HOT and HYP (Figure 5.3). This exacerbated reduction to physical performance in HH may have been due to a significant increase in Bla concentration which may indicate a greater anaerobic energy release compared with CON, HOT and HYP (Amann et al, 2006). Furthermore, a 5% reduction in plasma volume (Table 5.2) which coincided with a 2% change in body mass post-iSPT in HH may have meant that the participants finished iSPT in a hypo-hydrated state, (Cheuvront et al, 2003) causing an increase to the rate of heat storage and sweat output which in turn can impair prolonged high-speed activities in hot environments (Cheuvront and Kenefick, 2014). Additionally, HR was also increased during HH, showing parity with previous research in a hot and low altitude environment [30°C; 1,900m (Buono et al, 2012)]. This augmented HR response in HH likely stemmed from an impaired stroke volume and/or cardiac output, previously seen during prolonged exercise bouts in heat (González-Alonso et al, 2008) and hypoxia (Mazzeo, 2008). Thus, the exacerbated decline in performance was likely caused by a combination of both hot and hypoxic-mediated fatigue mechanisms. It is already acknowledged that both heat and hypoxia induce performance decrements via these mechanisms during soccer match-play, likely influencing match outcome (Taylor and Rollo, 2014). Therefore, the number of game defining moments may be further decreased within HH.

5.4.3. Experimental limitations

Typical of most experimental research, the results from this experimental chapter are somewhat specific to the cohort of participants and experimental protocols employed. The altitudes and temperatures chosen for this experimental chapter are specific to those experienced during elite soccer tournaments such as the UEFA Champions and Europa League; they display good external validity in this regard. Therefore, one obvious issue is the use of University level sub-elite soccer players for the present experiment, rather than elite soccer players. The recruitment of elite soccer players has its obvious challenges (Section 4.4.3.); however, as documented in section 2.3, hot, hypoxic and hot-hypoxic environments can also be problematic to sub-elite players. Furthermore, prior to completing all familiarisation protocols participants were required to have a $\dot{V}O_{2max}$ greater than 55 ml·kg^{-1}·min^{-1} to show some parity with elite soccer players (Tonnessen *et al*, 2013). Therefore, the influence of this issue was likely minimised, however, any generalisation of the results to the wider population should be considered cautiously.

Despite similar mean values for total, high-speed, variable run and sprint distance covered in experimental chapter 1 and 2, there are some discrepancies between halves and 15 min blocks between the two chapters. For example, the variability between halves in high-speed distance covered is smaller in experimental chapter 1 (15 ± 26 m) compared with CON in experimental chapter 2 (39 ± 16 m). Similarly, there was also variability between the decrements in high-speed distance covered between the first and the last 15 min block in experiment 1 (9 ± 17; 13 ± 10) and experiment 2 (27 ± 6 m). It is unlikely that this variability between halves could have been due to a "carry-over bias" as only five participants took part in both experiments. Therefore, these discrepancies between experiments could potentially be due a small training effect, likely attributable to the use of sub-elite soccer players utilised in experimental chapter 2. When inspecting the SD for the decrements in high-speed distance covered between halves and 15 min blocks it is apparent that there is less variability in the present experiment, compared with experiment 1. An enhanced exclusion criteria was implemented (section 5.2.1) in experiment 2, as participants were required to have a $\dot{V}O_{2max}$ greater than 55 ml·kg^{-1}·min^{-1}, compared to experiment 1 (50 ± 8 ml·kg^{-1}·min^{-1}) to go some way in showing parity with elite soccer players (Tonnessen et al, 2013). The mean $\dot{V}O_{2max}$ [57 (55-59) ml·kg^{-1}·min^{-1}] and peak sprint speed [21 (20-24) km·h^{-1}] for the participants in experimental chapter 2 were greater and more homogenous compared with experimental chapter 1 [$\dot{V}O_{2max}$ 50 (42 – 58) ml·kg^{-1}·min^{-1};

A further limitation of this experimental chapter was that as iSPT was utilised, the assessment of technical skills and multi-directional movements were unable to be quantified, which has been acknowledged previously (Experimental Chapter 1). The performance of technical skills in soccer has been shown to be unchanged and in some cases enhanced when competing in soccer match-play in hot and hypoxic environments (Mohr and Krustrup, 2013, Mohr *et al*, 2012, Nassis, 2013, Nassis *et al*, 2015). However, it is acknowledged that players alter their pacing strategy to preserve their technical skills (Mohr *et al*, 2012). Therefore, utilising a soccer-specific simulation which eliminates all pacing strategies and the assessment of a player's technical skills would be advantageous and of interest to practioners.

5.4.4. Potential applications

The exacerbated decrements to key physical performance measures pivotal to the match outcome may benefit coaches during competitive elite soccer match-play. A coach may look to exploit the reduction in repeated sprint ability within defensive players by using an appropriate offensive positional substitution to take advantage of this phenomenon. Due to the high reliability and validity of iSPT (Experimental Chapter 1), the simulation can be utilised to ascertain the efficacy of any ergogenic intervention to offset the environmentally-induced decrements which have been observed in the current experimental chapter. The efficacy of these interventions may be important for governing bodies if the continued use of countries where extremes of heat and hypoxia are apparent.

5.5. Conclusion

The heat-induced-decrements in HOT stem from increasing body temperatures, TS and the 2% reduction in body mass. The hypoxic-induced-decrements in HYP were most likely initiated by a decrease in S_aO_2 and increase in HR. Similar changes in TS, body mass and temperatures were seen in HOT compared with HH, whilst similar changes in HR and S_aO_2 were evident in HH compared to HYP. Furthermore, both Bla and plasma volume change alterations were only seen in HH compared with CON, highlighting that both these measures may play a role in the exacerbated decrements seen in HH. However, a deductive design to assess whether simulated soccer performance would still decrease in HH if plasma volume was maintained is needed to understand the mechanistic cause of these findings. Therefore, these findings directly answer the aim of this experimental chapter: '*To investigate the changes in simulated soccer performance during hot (30°C; 50% RH), hypoxic (1,000m; 18°C 50% RH), and hot-hypoxic (1,000m; 30°C 50% RH) environments compared to a normoxic-temperate environment (0m; 18°C 50% RH) by utilising the iSPT*'.

Subsequently, due to the greater prevalence of hot environments within elite soccer compared with both hypoxia and hot-hypoxic (Section 2.3.2.), a valid intervention to offset the heat induced decrements is needed. Therefore, the iSPT will be utilised within the following experimental chapter to investigate the effects of three different pre-/half-time-cooling strategies to attenuate the heat-induced decrements reported in this experimental chapter. This will then directly answer the third aim of this thesis: '*To investigate the impact of three different pre- and half-time-cooling methods; 1) external (ice packs upon the quadriceps and hamstrings); 2) internal (ice slurry ingestion); and 3) mixed methods (internal and external) compared with a control (i.e. no-cooling) as a solution to acquiesce any heat-induced-decrements that were present in experiment 2*'.

Chapter 6. Experiment 3: The Influence that Pre-Cooling and Half-Time-Cooling Has on Soccer-Specific Physical Performance and Physiological Responses in a Hot Environment, when utilising iSPT.

6.1. Introduction

Relative to extreme environments that elite soccer is played within, Section 2.3 outlines that hot environments (> 30°C), compared to those of a hypoxic nature (Taylor and Rollo, 2014), are most commonly encountered. Hot environments (30°C) mediate a marked decline on near-maximal soccer performance compared with a temperate environment [(18°C) see experimental chapter 2]. Peripheral body temperatures (T_{sk} and T_{mu}) and TS predict this decline in soccer performance between environments (Experimental Chapter 2); pre- and half-time-cooling is therefore a plausible intervention to offset these performance decrements (Section 2.4). Section 2.4 identified that the most commonly employed cooling methods include cold water immersion, ingestion of a cold fluid/ice slurry, application of ice packs onto skin, the wearing of ice-cooling garments or a combination (mixed-methods) of these approaches (Tyler *et al*, 2013). Cooling vests have negligible to small effects on performance whilst cold water immersion has moderate positive effects but lacks practicality. Conversely (Section 2.4), practical cooling methods including ice packs placed upon the upper legs to target the main locomotive muscles, ice slurry ingestion and mixed-methods pre-cooling can elicit a large ergogenic effect on physical performance and physiological responses during exercise-heat stress (Bongers *et al*, 2014, Castle *et al*, 2006, Minett *et al*, 2012a, Minett *et al*, 2011), however, their effects relative to soccer-specific exercise in the heat have not been explored securely.

Previous soccer cooling studies have used a soccer-specific simulation (Section 2.1.2) to ascertain the efficacy of pre- and half-time-cooling (Section 2.4) in soccer (Clarke *et al*, 2011, Drust *et al*, 2000a, Price *et al*, 2009), due to the high variability that is associated with key physical performance variables during soccer match-play. However, these simulations have utilised a fixed distance (Price *et al*, 2009) and non-individualised speed thresholds (Clarke *et al*, 2011, Drust *et al*, 2000a); therefore inferences regarding pre-cooling and half-time-cooling on both physical performance and the physiological responses in soccer are still relatively limited, due to these limitations (fixed distance and generic speed thresholds) within the sparse previous literature. Taylor and Rollo (2014) recommend that an individualised, valid and reliable soccer simulation (i.e. iSPT; as demonstrated in experimental chapter 1) should be utilised to quantify the efficacy of pre- and half-time-cooling in soccer. Therefore, utilising iSPT will allow for the effects of practical pre-cooling and half-time cooling on near maximal soccer performance in the heat to be investigated in a controlled environment, unlike in previous studies (Clarke *et al*, 2011, Drust *et al*, 2000a, Price *et al*, 2009).

6.1.1. Experimental Aims and hypothesis

To investigate the impact of three different pre- and half-time-cooling methods; 1) external (ice packs upon the quadriceps and hamstrings); 2) internal (ice slurry ingestion); and 3) mixed methods (internal and external) compared with a control (i.e. no-cooling) as a solution to acquiesce any heat-induced-decrements that were present in experiment 2.

- In the hot, hypoxic, and hot-hypoxic environment, simulated soccer performance will be significantly reduced compared with a normoxic-temperate environment during iSPT.
- In the hot-hypoxic environment, simulated soccer performance will be significantly reduced compared with the hot, hypoxic and normoxic-temperate environments during iSPT.

6.2. Methodology

6.2.1. Participants

The eight male, apparently healthy participants who volunteered for this experiment had the following characteristics: [median (min-max) age = 22 (18 - 24) y; mass = 76 (66 - 88) kg; height = 186 (165 - 192) cm; mean ± SD $\dot{V}O_{2max}$ = 57 ± 4 ml·kg^{-1}·min^{-1}]. Section 3.2 details that three participants who volunteered for experiment two were used in the present experiment (Figure 3.1). All participants conformed to the pre-test guidelines and procedures outlined in section 3.2.

6.2.2. General experimental controls

All FAM and testing sessions were completed on the same NMT. For a detailed description of the NMT specifications and safety measures please see section 3.5.

6.2.3. Experimental design

This experiment employed a counter-balanced, crossover design in which participants visited the laboratory on eight separate occasions (Figure 6.1). *Visit 1*: All participants completed a $\dot{V}O_{2max}$ test prior to completing all FAM and iSPT testing sessions. For a detailed description of the $\dot{V}O_{2max}$ test, please see section 3.4. *Visit 2-4 (FAM₁₋₃)*: All FAM and PSA sessions were completed as outlined in section 3.7. *Visits 5-8*: During the four experimental trials, participants completed iSPT in a hot environment (30°C; 50% RH). For a detailed description of the design

and the methods utilised to form iSPT, please see section 3.6. Before the participants completed iSPT, all participants underwent a 30-min period of either no [CON (Section 3.15.1.)], external [PACKS (Section 3.15.2.)], internal [SLURRY (Section 3.15.3.)] or mixed-method [MM (Section 3.15.3.)] pre cooling. At half-time, all participants undertook 15 min of half-time-cooling via the same strategy utilised during pre-cooling. All half-time cooling strategies are explained in section 3.15.1-4. Furthermore, all pre- and half-time-cooling strategies were completed in a temperate environment, as explained in full detail within section 3.15.

6.2.4. Experimental procedures

Upon arrival to the laboratory on visit 1 all anthropometric measurements were recorded as described in section 3.3. Furthermore, during all half-time-cooling strategies the same measures were ascertained pre- and post-cooling. A warm up was completed prior to all experimental trials, as outlined in section 4.2.4. The generation of the hot exposures utilised in this experimental chapter are reported in section 3.14. Water consumption during all pre-cooling and half-time cooling strategies is outlined in section 3.15.1.

6.2.5. Soccer performance measures

During iSPT and all pre- and half-time-cooling strategies physiological [HR (Section 3.9.1.), and body mass (Section 3.9.4)], subjective [RPE (Section 3.10.1.), TS (Section 3.10.2), RTIPE and RTIME (Section 3.10.3)], body temperature [T_{re} (Section 3.11.1.), T_{sk} (Section 3.11.2.), T_{body} (Section 3.11.3), Estimated T_{mu} (Section 3.11.5.)] and blood [Bla (Section 3.12.1.) and plasma volume (Section 3.12.2.)] measurements were recorded in 15 min intervals. All physical performance measures were ascertained as detailed in section 3.8.

Experimental Design (A)

FAM 1	FAM 2	FAM 3
9 min 13 min 15 min iSPT	PSA 45 min iSPT	PSA 90 min iSPT

CON*
(30 °C; 50% RH)

PACKS*
(30 °C; 50% RH)

SLURRY*
(30 °C; 50% RH)

MM*
(30 °C; 50% RH)

*Counter-balanced, crossover design

Experimental Procedures (B)

For all measures see section 3.9-11

For all measures see section 3.9-11

For all measures see section 3.9-11

For all measures see section 3.9-11

Resting Measures	Pre-cooling	Warm Up	iSPT – 1st half	Half-time-cooling	iSPT – 2nd half

-45　　**-40**　　**-10**　　**0**　　**45**　　**60**　　**105**

Time (min)

Figure 6.1. A schematic detailing the experimental design (A) and procedures (B) utilised during this experimental chapter. All measurements were recorded as documented within the relevant sections of the general methodologies. All physical performance measures were ascertained post iSPT as documented in section 3.8.

6.2.6. Statistical analysis

An a *priori* power calculation (G*Power 3) was used to determine the number of participants required for this experiment (n = 8) with an alpha level of 0.05 and a statistical power of 99%, using data [(high-speed distance covered) - minimum worthwhile effect = 160 m; SD = 21] from experimental chapter 2 (Appendix G). This measure was chosen as high-speed distance covered is associated with game defining moments in soccer match-play (Gregson *et al*, 2010), a measure that was significantly reduced in HOT during experimental chapter 2. Normality of the observed data was assessed using Q - Q plots and was deemed plausible in all instances. Therefore, values are presented as mean ± SD. Differences between condition, time and condition x time for all physical performance measures and physiological responses were completed using linear mixed models (IBM SPSS statistics for Windows, Version 21, Armonk, NY). A linear mixed model was also used to assess the differences between halves and fifteen minute intervals for all physical performance (total distance, high-speed distance, variable run distance and sprint distance covered) measures between conditions. Please see section 4.2.6 for a justification to why a linear mixed model analysis was chosen.

6.3. Results

6.3.1. Physical performance

6.3.1.1 Between conditions and halves
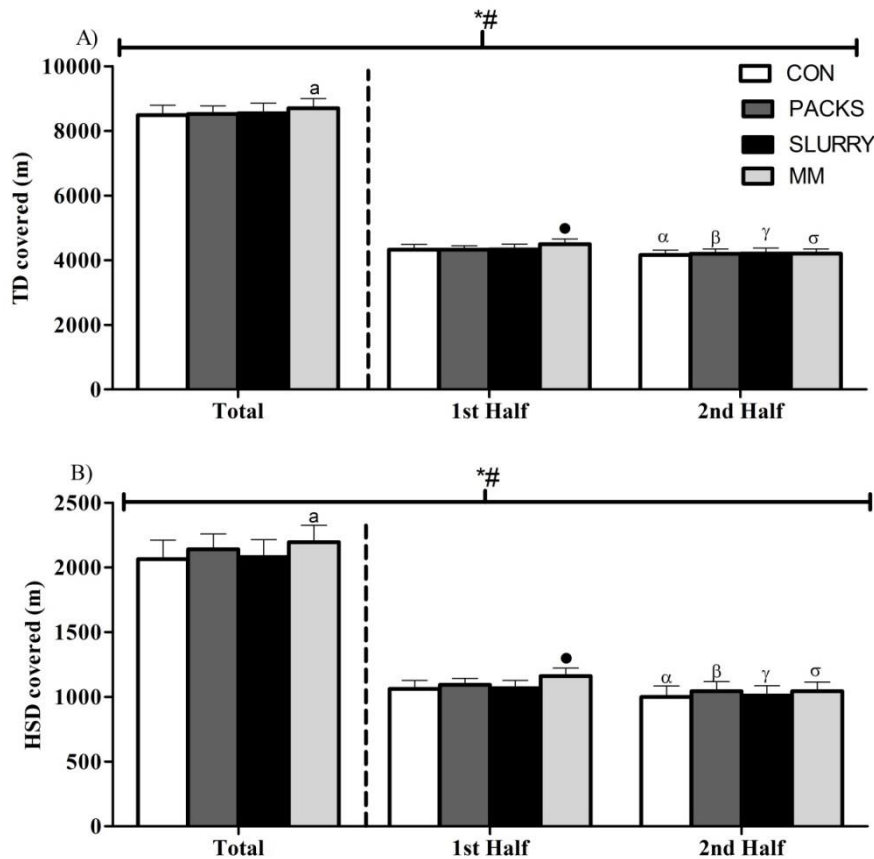
Figure 6.2: The TD covered (A) and HSD covered (B) in total and in each half for CON, PACKS, SLURRY and MM conditions. *Significant main effect for condition; #Significant main effect for time; [a]Significant main effect for condition between CON and MM ($p < 0.05$); [α]Significant difference from the first half in CON; [β]Significant difference from the first half in PACKS; [γ]Significant difference from the first half in SLURRY; [δ]Significant difference from the first half in MM; [•]Significant interaction effect (condition x time) between CON and MM ($p < 0.05$). The individual responses are shown in appendix H

6.3.1.1.1. Total distance covered

There was a significant main effect for condition (F = 3.9; p = 0.01) and time (F = 29.9; p < 0.001) for total distance covered, however, there was no interaction effect (F = 0.4 p = 0.763). When compared to CON, the total distance covered was 3% (258 ± 280) greater in MM (p = 0.01, 95% CI: 16 – 197 m). However, no difference was observed between PACKS (p = 0.251) and SLURRY (p = 0.952) when compared with CON (Figure 6.2).

A 4% (153 ± 82 m) and 2% (101 ± 64 m) greater total distance covered was seen in the first half compared with the second half in CON (p = 0.001, 95% CI: 68 – 256 m) and MM (p = 0.04, 95% CI: 4 – 185 m), respectively. A 3% (PACKS: 128 ± 109 m; SLURRY: 133 ± 86 m) greater total distance covered was seen in the first half compared to the second half in PACKS (p = 0.009, 95% CI: 34 – 223 m) and SLURRY (p = 0.007, 95% CI: 39 – 227 m). Finally, the total distance covered during the first half in MM was 3% (155 ± 174 m) greater compared with CON (p = 0.02, 95% CI: 13 – 271 m) (Figure 6.2).

6.3.1.1.2. High-speed distance covered

There was a significant main effect for condition (F = 8.0; p < 0.001) and time (F = 21.0; p < 0.001), but there was no interaction effect (F = 0.5 p = 0.667) for high-speed distance covered. The high-speed distance covered was 4% (158 ± 132 m) greater in MM when compared with CON (p < 0.001, 95% CI: 25 – 107 m). However, no difference was observed between PACKS (p = 0.08) and SLURRY (p = 0.995) when compared with CON (Figure 6.2).

A 5% (62 ± 32 m) and 3% (31 ± 31 m) greater high-speed distance covered was seen in the first half compared with the second half in CON (p = 0.005, 95% CI: 19 – 104 m) and MM (p = 0.02, 95% CI: 16 – 69 m), respectively. A 4% (PACKS: 49 ± 42 m; SLURRY: 56 ± 25 m) greater high-speed distance covered was seen in the first half compared to the second half in PACKS (p = 0.02, 95% CI: 7 – 92 m) and SLURRY (p = 0.01, 95% CI: 14 – 99 m). Finally, the high-speed distance covered in the first half was 6% (95 ± 83 m) greater in MM compared with CON (p = 0.02, 95% CI: 25 – 141 m) (Figure 6.2).
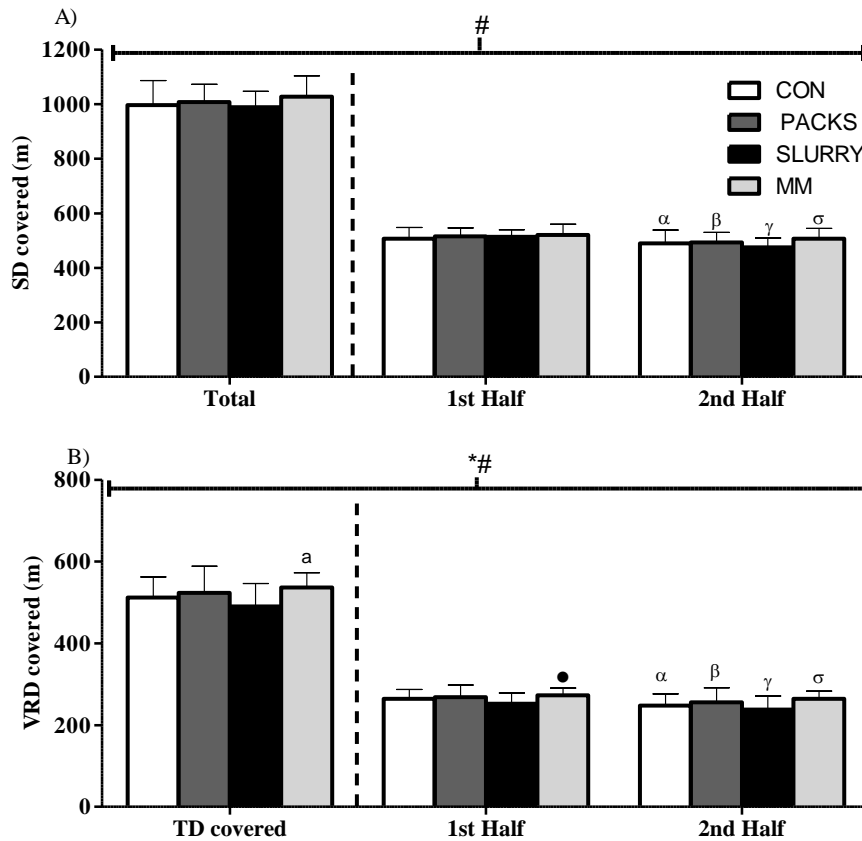
Figure 6.3: The SD covered (A) and VRD covered (B) in total and in each half for CON, PACKS, SLURRY and MM conditions. See figure 6.2 for explanation of symbols.

6.3.1.1.3. Sprint distance covered

There was a significant main effect for time ($F = 15.6$; $p < 0.001$), however there was no significant main effect for condition ($F = 2.1$; $p = 0.112$) and interaction effect ($F = 0.9$; $p = 0.470$) for sprint distance covered. A 2% (15 ± 8 m), 3% (17 ± 13 m), 4% (22 ± 22 m) and 6% (38 ± 15 m) greater sprint distance covered in the first half compared to the second half in MM ($p = 0.001$, 95% CI: $8 - 21$ m), CON ($p = 0.01$, 95% CI: $6 - 28$ m), PACKS ($p = 0.02$, 95% CI: $4 - 40$ m) and SLURRY ($p < 0.001$, 95% CI: $26 - 51$ m) (Figure 6.3).

6.3.1.1.4. Variable run distance covered

There was a significant main effect for condition ($F = 11.4$; $p < 0.001$) and time ($F = 5.3$; $p < 0.001$), but there was no significant interaction effect ($F = 0.5$ $p = 0.938$) for variable run distance covered. The variable run distance covered was 5% (25 ± 47 m) shorter in MM ($p = 0.02$, 95% CI: $1 - 8$ m) when compared with CON. No difference was observed between PACKS ($p = 0.546$) and SLURRY ($p = 0.547$) when compared with CON (Figure 6.3).

A 6% (13 ± 12 m) and 3% (9 ± 9 m) greater variable run distance covered was seen in the first half compared with the second half in CON ($p = 0.004$, 95% CI: 7 – 26 m) and MM ($p = 0.03$, 95% CI: 1 – 16 m) respectively. A 5% (PACKS: 13 ± 12 m; SLURRY: 15 ± 21 m) greater total distance covered was seen in the first half compared to the second half in PACKS ($p = 0.03$, 95% CI: 2 – 23 m) and SLURRY ($p = 0.04$, 95% CI: 2 – 32 m). Finally, the variable run distance covered in in the first half was 6% (16 ± 25 m) greater in MM compared with CON ($p = 0.04$, 95% CI: 2 – 51 m) (Figure 6.3).

6.3.1.2. Between 15 min

6.3.1.2.1. High-speed distance covered

There was no interaction effect ($F = 0.5$ $p = 0.848$) between 15 min periods for high-speed distance covered. In MM, PACKS and SLURRY, more high-speed distance was covered ($p < 0.05$) in the first 15 min interval compared with the final 15 min interval. However, in CON the high-speed distance covered was significantly reduced in the last 15 min block of iSPT compared to the first and second 15 min block. Thus, the fatigue index between the first and last 15 min block for CON, PACKS, SLURRY and MM was 11% (40 ± 15 m), 9% (33 ± 20 m), 10% (37 ± 24 m) and 8% (29 ± 15 m) (Table 6.1).

6.3.1.2.2. Sprint distance covered

There was no interaction effect ($F = 0.3$ $p = 0.458$) between 15 min periods for sprint distance covered. The sprint distance covered in CON ($p = 0.004$, 95% CI: 3 – 30 m), PACKS ($p = 0.008$, 95% CI: 2 – 29 m) and SLURRY ($p < 0.001$, 95% CI: 12 – 38 m) was significantly reduced in the final 15 min block compared with the first 15 min. However, no significant difference ($p > 0.05$) was seen between any 15 min blocks in MM (Table 6.1).

6.3.1.2.3. Variable run distance covered

There was no interaction effect ($F = 0.9$ $p = 0.428$) between 15 min periods for variable run distance covered. The variable run distance covered in CON ($p = 0.01$, 95% CI: 1 – 19 m), PACKS ($p = 0.03$, 95% CI: 3 – 17 m), SLURRY ($p = 0.04$, 95% CI: 1 – 20 m) and MM ($p = 0.02$, 95% CI: 2 – 17 m) was significantly reduced in the final 15 min block compared with the first 15 min by 10% (8 ± 13 m), 9% (7 ± 12 m), 8% (6 ± 13 m) and 7% (5 ± 12 m), respectively (Table 6.1).

Table 6.1: The HSD, SD, VRD covered and PSS in 15 min blocks for CON, PACKS, SLURRY and MM conditions. The HSD, SD and VRD covered are presented as an overall distance covered during each 15 min period.

	0-15 min	15-30 min	30-45 min	45-60 min	60-75 min	75-90 min
HSD covered (m)						
CON	$366 \pm 22\varepsilon$	$352 \pm 20\varepsilon$	345 ± 30	346 ± 14	330 ± 33	324 ± 32
PACKS	$375 \pm 20\zeta$	361 ± 20	357 ± 19	356 ± 24	346 ± 32	343 ± 23
SLURRY	$370 \pm 24\eta$	354 ± 18	344 ± 25	346 ± 24	334 ± 28	331 ± 25
MM	$388 \pm 28\theta$	363 ± 26	359 ± 21	368 ± 22	360 ± 26	355 ± 24
SD covered (m)						
CON	$174 \pm 14\varepsilon$	168 ± 13	164 ± 15	169 ± 17	162 ± 16	160 ± 18
PACKS	$178 \pm 12\zeta$	171 ± 12	166 ± 12	168 ± 13	162 ± 16	162 ± 12
SLURRY	$179 \pm 11\eta$	170 ± 9	164 ± 8	162 ± 14	159 ± 11	160 ± 12
MM	177 ± 13	173 ± 16	173 ± 12	173 ± 12	173 ± 13	172 ± 13
VRD covered (m)						
CON	$89 \pm 9\ \varepsilon$	87 ± 8	86 ± 8	84 ± 8	83 ± 12	78 ± 9
PACKS	$91 \pm 11\zeta$	89 ± 10	88 ± 10	86 ± 12	86 ± 14	84 ± 12
SLURRY	$87 \pm 10\eta$	85 ± 10	85 ± 9	82 ± 10	76 ± 14	79 ± 10
MM	$93 \pm 6\theta$	91 ± 9	88 ± 10	89 ± 8	85 ± 7	83 ± 7

CON: No-cooling; HSD – High Speed Distance; MM – Mixed-methods; PACKS – Ice Packs; SLURRY – Ice Slurry; SD – Sprint Distance; VRD - Variable Run Distance; [ε]Significant difference from the last 15 min in CON; [ζ]Significant difference from the last 15 min in PACKS; [η]Significant difference from the last 15 min in SLURRY; [θ]Significant difference from the last 15 min half in MM. See figure 6.2 for an explanation of all other symbols.

6.3.1.2.4. Peak sprint speed

There was no significant main effect for condition ($F = 0.1$; $p = 0.981$), time ($F = 7.0$; $p = 0.434$) or interaction effect ($F = 1.5$; $p = 0.152$) for peak sprint speed.

6.3.2. Body Temperature Measures
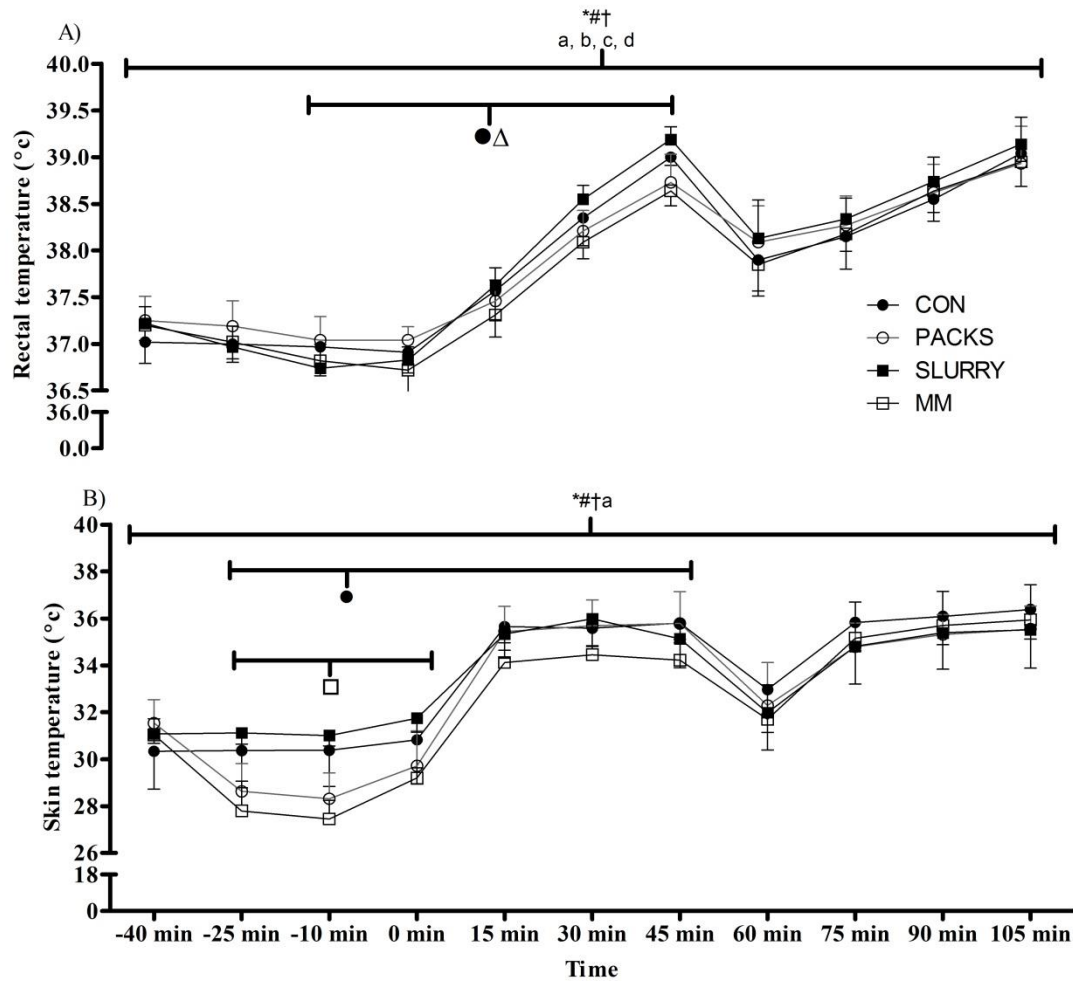
Figure 6.4. The T_{re} (A) and T_{sk} (B) during the pre-cooling (-40 - - 10 min), warm up (-10 – 0 min) first half (0-45 min), half-time-cooling (45-60 min) and second half (60-105 min) for CON, PACKS, SLURRY and MM conditions. [b]Significant interaction effect for condition between PACKS and MM ($p < 0.05$); [c]Significant interaction effect for condition between SLURRY and MM ($p < 0.05$); [d]Significant interaction effect for condition between CON and SLURRY ($p < 0.05$); [Δ]Significant interaction effect (condition x time) between CON and SLURRY ($p < 0.05$); [□]Significant interaction effect (condition x time) between PACKS and CON ($p < 0.05$). See figure 6.2 and table 4.1 for an explanation of all other symbols.

_6.3.2.1. T_{re}_

Figure 6.4 demonstrates a significant main effect for condition ($F = 11.1$; $p < 0.001$), time ($F = 439.2$; $p < 0.001$) and an interaction effect ($F = 2.6$; $p < 0.001$) for T_{re}. The mean T_{re} in MM

(38.0 ± 0.2 °C) was significantly lower compared with CON [38. 2 ± 0.2 °C ($p = 0.04$, 95% CI: 0.1 – 0.3 °C)], PACKS [38.1 ± 0.3 °C ($p = 0.001$, 95% CI: 0.1 – 0.3 °C)] and SLURRY [38. 3 ± 0.2 °C ($p = 0.03$, 95% CI: 0.1 – 0.3 °C)]. Furthermore, the mean T_{re} was significantly increased in SLURRY compared with CON ($p = 0.03$, 95% CI: 0.1 – 0.3 °C) (Figure 6.4).

Pre-cooling: No change ($p > 0.05$) in T_{re} was seen in CON ($p = 1.000$) and PACKS ($p = 1.000$) throughout the 30 min pre-cooling period. At the end of the 30 min pre-cooling period and at the start of iSPT, T_{re} was significantly reduced in both SLURRY ($p = 0.04$, 95% CI: 0.1 – 0.6 °C) and MM ($p = 0.04$, 95% CI: 0.1 – 0.3 °C) by 0.4 ± 0.6 °C and 0.5 ± 0.6 °C compared with CON, respectively. Furthermore, 30 min of SLURRY ($p = 0.001$, 95% CI: 0.2 – 0.6 °C) and MM ($p = 0.04$, 95% CI: 0.1 – 0.7 °C) pre-cooling significantly reduced T_{re} by 0.5± 0.8 °C and 0.6 ± 0.7 °C from the resting values respectively (Figure 6.4).

First half: From 0-45 min T_{re} was significantly reduced ($p < 0.05$) in MM compared with CON. Furthermore, T_{re} was significantly increased in SLURRY compared with CON ($p < 0.05$) from 0-45 min. *Half-time-cooling and Second half*: Although T_{re} was significantly reduced in CON ($p < 0.001$, 95% CI: 0.7 – 1.4 °C), PACKS ($p < 0.001$, 95% CI: 0.2 – 1.0 °C), SLURRY ($p < 0.001$, 95% CI: 0.7 – 1.4 °C) and MM ($p < 0.001$, 95% CI: 0.4 – 1.1 °C) at the end of the first half compared with the start of the second half, the half-time-cooling period had no interaction effect on any conditions. Finally, there was no significant difference in T_{re} in all conditions throughout the second half (60-105 min) (Figure 6.4).

6.3.2.2. T_{sk}

There was a significant main effect for condition ($F = 8.9$; $p < 0.001$), time ($F = 143.0$; $p < 0.001$) and an interaction effect ($F = 4.3$; $p < 0.001$) for T_{sk}. The mean T_{sk} during iSPT was significantly reduced in MM [33.7 ± 1.1 °C ($p < 0.001$, 95% CI: 0.3 – 1.0 °C)] compared with CON (34.0 ± 0.9 °C). No significant differences were evident between PACKS [33.8 ± 1.2 °C ($p = 0.997$)] and SLURRY [33.8 ± 1.2 °C ($p = 0.121$)] compared with CON (Figure 6.5).

Pre-cooling: No change ($p > 0.05$) in T_{sk} was seen in CON ($p = 1.000$) and SLURRY ($p = 1.000$) throughout the 30 min pre-cooling period. The T_{sk} was significantly reduced ($p < 0.05$) by PACKS pre-cooling at -25 min ($p = 0.01$, 95% CI: 0.2 – 3.2 °C) and -10 min ($p = 0.001$, 95% CI: 0.6 – 3.5 °C) compared with CON by 1.7 ± 1.4 °C and 2.0 ± 1.0 °C, respectively. The MM pre-cooling significantly reduced ($p < 0.05$) T_{sk} -25 min ($p < 0.001$, 95% CI: 1.1 – 4.0 °C) and -10 min ($p < 0.001$, 95% CI: 1.4 – 4.3 °C) compared with CON by 2.6 ± 1.4 °C and 2.7 ±

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1.0 ºC, respectively (Figure 6.4). Furthermore, 30 min of PACKS ($p < 0.001$, 95% CI: 1.3 – 5.0 ºC) and MM ($p < 0.001$, 95% CI: 1.7 – 5.4 ºC) cooling reduced T_{sk} by 3.2 ± 4.1 ºC and 3.1 ± 4.6 ºC compared with basal T_{sk}, respectively (Figure 6.4).

first half: During CON, T_{sk} was significantly higher compared with MM from 0-45 min during iSPT. No statistical changes ($p > 0.05$) were seen between SLURRY and PACKS compared with CON (Figure 6.4).

Half-time-cooling and second half: Although T_{sk} was significantly reduced in in CON ($p = 0.03$, 95% CI: 0.1 – 3.7 ºC), PACKS ($p = 0.001$, 95% CI: 0.6 – 4.3 ºC), SLURRY ($p < 0.001$, 95% CI: 1.3 – 5.0 ºC) and MM ($p < 0.001$, 95% CI: 2.6 – 6.3 ºC) at the end of the first half compared with the start of the second half, the half-time-cooling period had no interaction effect on any conditions. Finally, there was no significant difference in T_{sk} in all conditions throughout the second half (Figure 6.4).
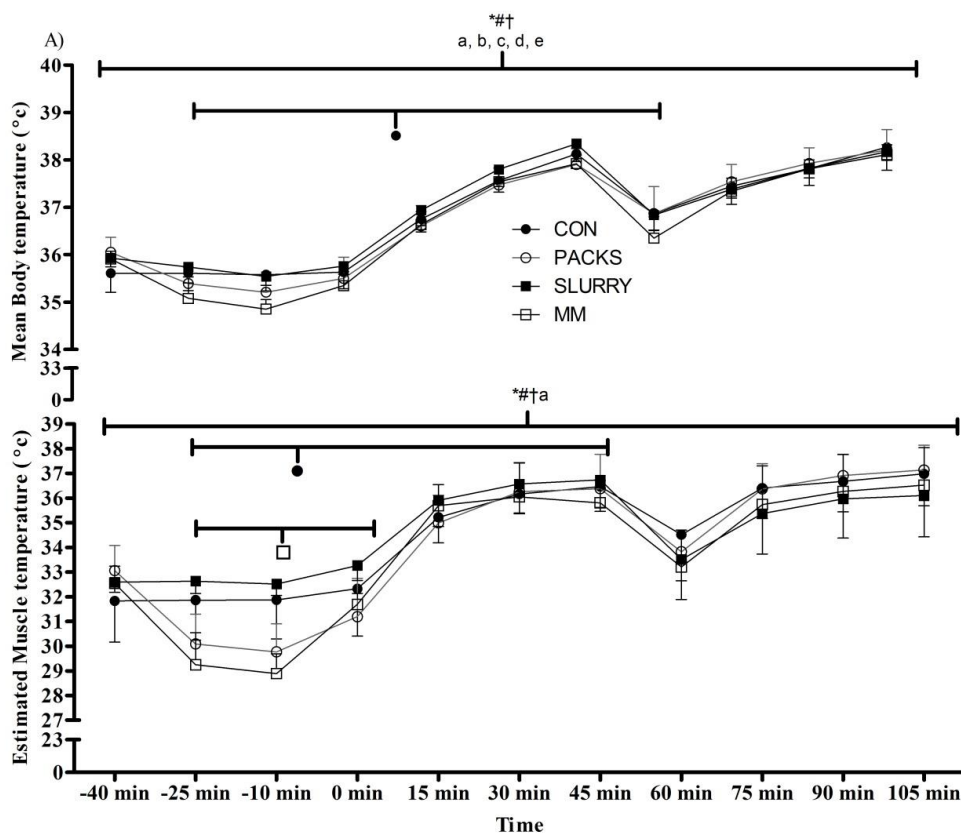
Figure 6.5. The T$_{body}$ (A) and Estimated T$_{mu}$ (B) during the pre-cooling (-40 - - 10 min), warm up (-10 – 0 min) first half (0-45 min), half-time-cooling (45-60 min) and second half (60-105 min) for CON, PACKS, SLURRY and MM conditions. [e]Significant interaction effect for condition between PACKS and SLURRY ($p < 0.05$). See figures 6.2 and 6.4 as well as table 6.1 for an explanation of all other symbols.

There was a significant main effect for condition ($F = 8.9$; $p < 0.001$), time ($F = 143.0$; $p < 0.001$) and interaction effect ($F = 4.3$; $p < 0.001$) for T_{mu}. The mean estimated T_{mu} during iSPT was significantly reduced in MM [35.2 ± 1.0 °C ($p < 0.001$, 95% CI: 0.2 – 1.1 °C)] compared with CON (35.6 ± 1.0 °C). No significant differences were evident between PACKS [35.4 ± 1.2 °C ($p = 0.998$)] and SLURRY [35.4 ± 1.2 °C ($p = 0.120$)] compared with CON (Figure 6.5).

Pre-cooling: No change ($p > 0.05$) in estimated T_{mu} was seen in CON ($p = 1.000$) and SLURRY ($p = 1.000$) throughout the 30 min pre-cooling period. The estimated T_{mu} was significantly reduced ($p < 0.05$) by PACKS pre-cooling at -25 min ($p = 0.01$, 95% CI: 0.2 – 3.2 °C) and -10 min ($p = 0.001$, 95% CI: 0.6 – 3.5 °C) compared with CON by 1.7 ± 1.4 °C and 2.0 ± 1.0 °C, respectively. The MM pre-cooling significantly reduced ($p < 0.05$) estimated T_{mu} -25 min ($p < 0.001$, 95% CI: 1.1 – 4.0 °C) and -10 min ($p < 0.001$, 95% CI: 1.4 – 4.3 °C) compared with CON by 2.6 ± 1.4 °C and 2.7 ± 1.0 °C, respectively. Furthermore, 30 min of PACKS ($p < 0.001$, 95% CI: 1.3 – 5.0 °C) and MM ($p < 0.001$, 95% CI: 1.7 – 5.4 °C) cooling reduced estimated T_{mu} by 3.2 ± 4.1 °C and 3.1 ± 4.6 °C compared with basal estimated T_{mu}, respectively (Figure 6.5).

First half: During CON, estimated T_{mu} was significantly higher ($p < 0.05$) compared with MM from 0-45 min during iSPT (Figure 6.5).

Half-time-cooling and second half: Although estimated T_{mu} was significantly reduced in CON ($p = 0.03$, 95% CI: 0.1 – 3.7 °C), PACKS ($p = 0.001$, 95% CI: 0.6 – 4.3 °C), SLURRY ($p < 0.001$, 95% CI: 1.3 – 5.0 °C) and MM ($p < 0.001$, 95% CI: 2.6 – 6.3 °C) at the end of the first half compared with the start of the second half, the half-time-cooling period had no interaction effect on any conditions. Finally, there was no significant difference in estimated T_{mu} in all conditions throughout the second half (Figure 6.5).

6.3.2.4. T_{body}

Figure 6.5 demonstrates a significant main effect for condition ($F = 16.7$; $p < 0.001$), time ($F = 445.2$; $p < 0.001$) and interaction effect ($F = 2.2$; $p < 0.001$) for T_{body}. The mean T_{body} was significantly reduced in MM (37.1 ± 0.2 °C) compared with CON [37. 3 ± 0.1 °C ($p < 0.001$, 95% CI: 0.1 – 0.3 °C)], PACKS [37. 3 ± 0.2 °C ($p = 0.002$, 95% CI: 0.1 – 0.3 °C)] and SLURRY [37. 4 ± 0.3 °C ($p < 0.001$, 95% CI: 0.1 – 0.4 °C)]. Furthermore, mean T_{body} was significantly

increased in SLURRY compared with CON ($p = 0.04$, 95% CI: $0.1 - 0.3$ °C) and PACKS ($p = 0.008$, 95% CI: $0.1 - 0.3$ °C) (Figure 6.5).

Pre-cooling: No change ($p > 0.05$) in T_{body} was seen in CON ($p = 1.000$) and SLURRY ($p = 1.000$) throughout the 30 min pre-cooling period. The MM cooling significantly reduced ($p < 0.05$) the T_{body} at -25 min ($p = 0.008$, 95% CI: $0.1 - 1.3$ °C) and -10 min ($p = 0.008$, 95% CI: $0.9 - 2.4$ °C) of the pre-cooling period compared with CON by 0.6 ± 1.3 °C and 1.1 ± 2.3 °C, respectively. Furthermore, 30 min of MM pre-cooling significantly reduced ($p < 0.001$, 95% CI: $0.5 - 2.1$ °C) T_{body} by 1.5°C compared to basal T_{body} (Figure 6.5).

First half: Also, T_{body} was significantly reduced from 0-45 min of iSPT in the MM condition compared with CON (Figure 6.5).

Half-time-cooling and second half: Although T_{body} was significantly reduced in CON ($p = 0.03$, 95% CI: $0.5 - 5.2$ °C), PACKS ($p = 0.001$, 95% CI: $0.3 - 4.2$ °C), SLURRY ($p < 0.001$, 95% CI: $1.8 - 6.0$ °C) and MM ($p < 0.001$, 95% CI: $2.6 - 6.3$ °C) at the end of the first half compared with the start of the second half, the half-time-cooling period had no interaction effect on any conditions (Figure 6.5).

6.3.3. Subjective Measures

6.3.3.1. Perceived exertion
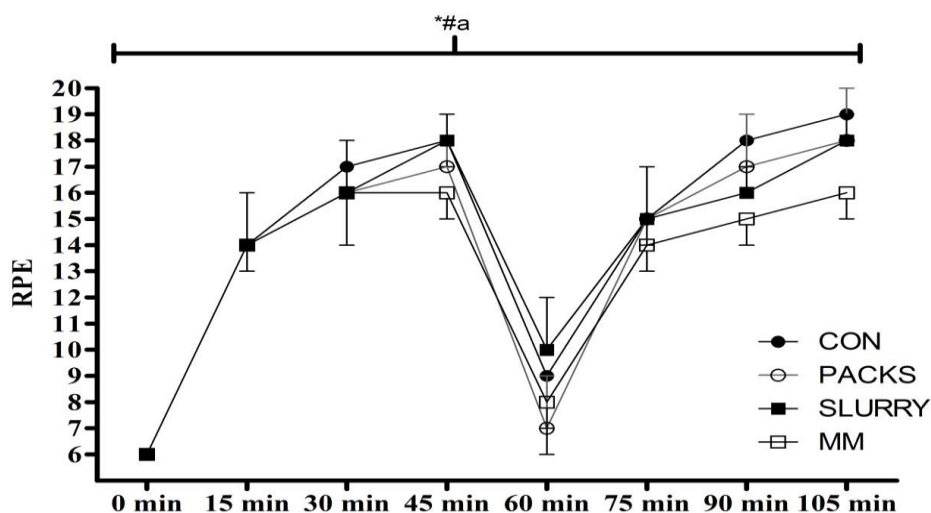
Figure 6.6: The RPE during the first (0-45 min) and second (60-105 min) half for CON, PACKS, SLURRY and MM conditions. See figures 6.2 and 6.4 as well as table 6.1 for an explanation of all other symbols.

There was a significant main effect for condition ($F = 10.7$; $p < 0.001$) and time ($F = 266.7$; $p < 0.001$), but there was no interaction effect ($F = 1.2$ $p = 0.249$) for RPE. The mean RPE during iSPT was significantly reduced in MM [13 ± 0.8 ($p < 0.001$, 95% CI: $1 - 2$)] compared with CON. No significant difference was seen between PACKS [13.8 ± 1.8 ($p = 0.536$)] and SLURRY [14.3 ± 1.2 ($p = 1.000$)] compared with CON (14.3 ± 0.5) (Figure 6.6).

6.3.3.2. TS
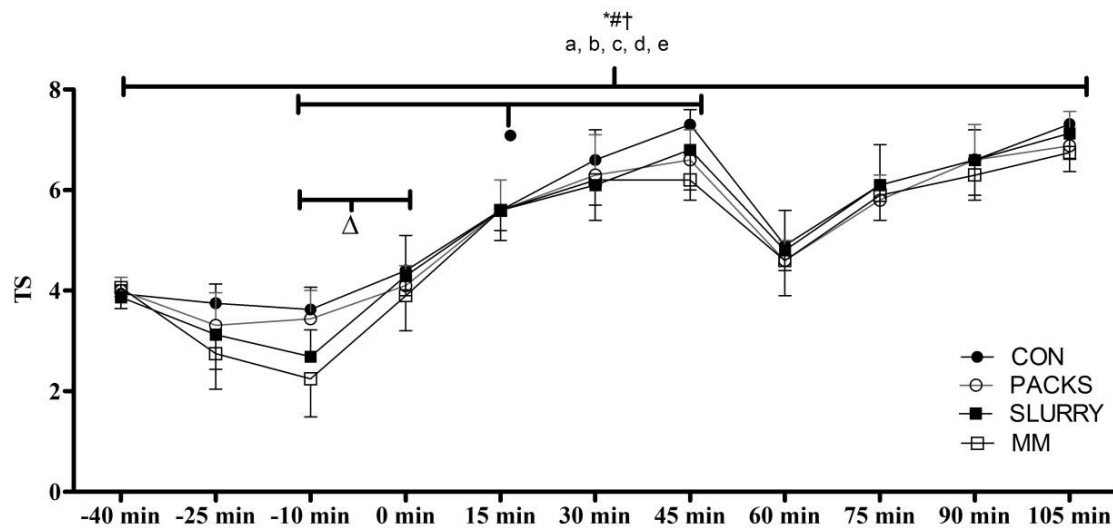
Figure 6.7: The TS during the pre-cooling (-40 - - 10 min), warm up (-10 – 0 min) first half (0-45 min), half-time-cooling (45-60 min) and second half (60-105 min) for CON, PACKS, SLURRY and MM conditions. See figures 6.2, 6.4, 6.5 and table 6.1 for an explanation of all other symbols.

Figure 6.6 reveals a significant main effect for condition ($F = 15.8$; $p < 0.001$), time ($F = 254.8$; $p < 0.001$) and an interaction effect ($F = 1.6$; $p = 0.01$) for TS. The mean TS was significantly lower during MM [4.9 ± 0.3 ($p < 0.001$, 95% CI: $0.3 – 0.7$)], PACKS [5.2 ± 0.4 ($p = 0.002$, 95% CI: $0.1 – 0.4$) and SLURRY [5.2 ± 0.4 ($p = 0.001$, 95% CI: $0.3 – 0.7$) compared with CON (5.5 ± 0.2). Furthermore, a significant reduction for mean TS was also seen in MM compared with PACKS ($p = 0.008$, 95% CI: $0.1 – 0.4$) and SLURRY ($p = 0.01$, 95% CI: $0.1 – 0.4$) (Figure 6.7).

Pre-cooling: No change ($p > 0.05$) in T_{re} was seen in CON ($p = 1.000$) and PACKS ($p = 1.000$) throughout the 30 min pre-cooling period. The SLURRY ($p = 0.002$, 95% CI: $1 – 2$) and MM ($p < 0.001$, 95% CI: $1 – 2$) pre-cooling significantly reduced TS at -10 min and at the start of

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iSPT (0 min) compared with CON. Both SLURRY ($p < 0.001$, 95% CI: $1 - 2$) and MM ($p < 0.001$, 95% CI: $1 - 2$) pre-cooling significantly reduced TS at the end of the 30 min period compared to the resting TS values (Figure 6.7).

First half: A significant difference ($p < 0.05$) in TS was evident from 0-45 min in MM compared with CON ($p < 0.05$) (Figure 6.7).

Half-time-cooling and second half: Although TS was significantly reduced in CON ($p < 0.001$, 95% CI: $1 - 3$), PACKS ($p < 0.001$, 95% CI: $1 - 3$), SLURRY ($p < 0.001$, 95% CI: $1 - 3$) and MM ($p < 0.001$, 95% CI: $1 - 3$) at the end of the first half compared with the start of the second half, the half-time-cooling period had no interaction effect on any conditions (Figure 6.7). Finally, there was no significant difference in TS in all conditions throughout the second half (Figure 6.7).

6.3.3.3. RTIPE and RTIME

For both RTIPE and RTIME, there was a significant main effect for time (RTIPE: $F = 143.8$; $p < 0.001$, RTIME: $F = 124.5$; $p < 0.001$), however, there was no significant main effect for condition (RTIPE: $F = 0.3$; $p = 0.807$, RTIME: $F = 0.6$; $p = 0.588$) and interaction effect (RTIPE: $F = 0.9$; $p = 0.580$, RTIME: $F = 0.8$; $p = 0.664$). The RTIPE and RTIME were significantly reduced over time across conditions with a lower RTIPE and RTIME after 90 (RTIPE: 2.8 ± 1.8; RTIME: 2.7 ± 1.2) min than 0 min (RTIPE: 8.3 ± 2.5; RTIME: 8.4 ± 2.5).

6.3.4. Physiological Responses

6.3.4.1. HR

There was a significant main effect for time ($F = 29.9$; $p < 0.001$), however, there was no significant main effect for condition ($F = 3.9$; $p = 0.502$) and no interaction effect ($F = 0.7$ $p = 0.974$) for HR.
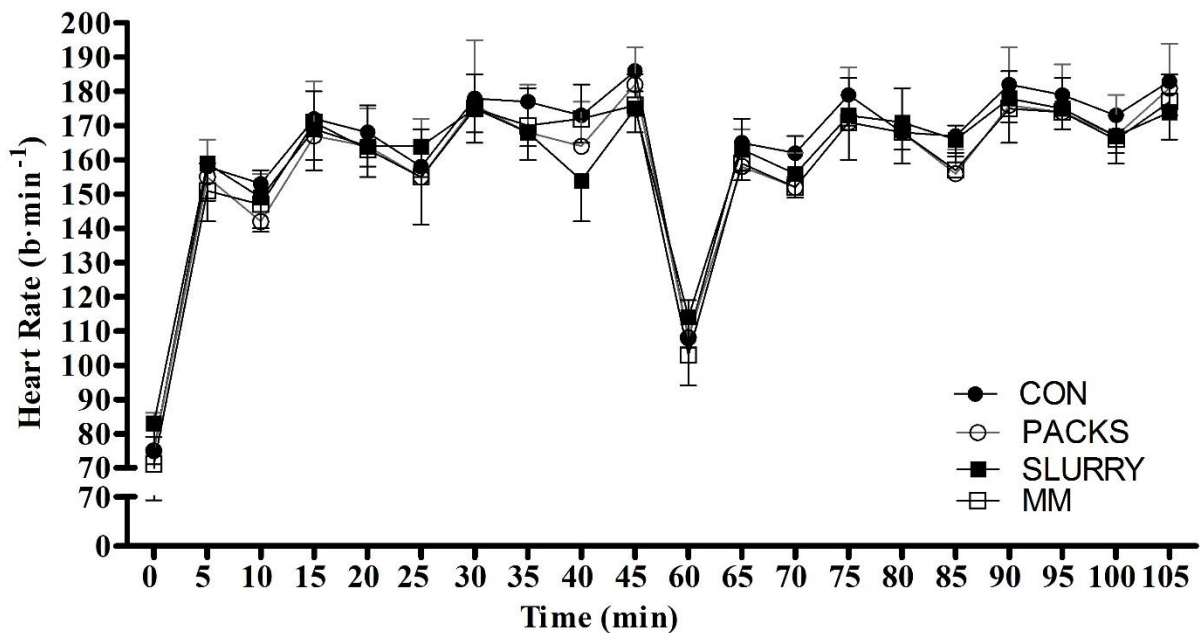
Figure 6.8: The TS during the pre-cooling (-40 - - 10 min), warm up (-10 – 0 min) first half (0-45 min), half-time-cooling (45-60 min) and second half (60-105 min) for CON, PACKS, SLURRY and MM conditions.

6.3.4.2. Body mass

There was a significant main effect for time ($F = 24.3$; $p < 0.001$), however, there was no significant main effect for condition ($F = 1.1$; $p = 0.352$) and no interaction effect ($F = 0.2$; $p = 0.907$) for body mass.

6.3.5. Blood measures

6.3.5.1. Bla and plasma volume changes

There was a significant main effect for time ($F = 69.1$; $p < 0.001$), however, there was no significant main effect for condition ($F = 8.0$; $p = 0.281$) and no interaction effect ($F = 0.8$; $p = 0.693$) for Bla. Furthermore, there was no significant main effect for condition ($F = 0.1$; $p = 0.981$), time ($F = 7.0$; $p = 0.434$) and interaction effect ($F = 1.5$; $p = 0.152$) for plasma volume.

Table 6.2: The percentage (%) change in plasma volume and body mass pre- and post-iSPT during CON, PACKS, SLURRY and MM.

	Body Mass change (%)		Plasma Volume change (%)	
	0 min	105 min	0 min	105 min
CON	-	2.1	-	3.1
PACKS	-	2.1	-	3.1
SLURRY	-	2	-	2.9
MM	-	1.9	-	2.8

6.4. Discussion

The aim of this experimental chapter was to utilise iSPT (Experimental Chapter 1), to investigate the impact of 30 min of pre-cooling and 15 min of half-time-cooling via PACKS, SLURRY and MM (PACKS and SLURRY) compared with CON, to acquiesce the heat induced decrements ascertained in experimental chapter 2. The main finding from this experimental chapter identified that after 30 min of MM pre-cooling compared with CON, a statistically significant improvement ($p < 0.05$) was seen by 3%, 4% and 5% in total distance, high-speed distance and variable run distance covered during the 1st half of iSPT, respectively (Figures 6.2 and 6.3), supporting the first experimental hypothesis. The significant improvement in physical performance during the first half in MM was observed alongside a significant decrease ($p < 0.05$) in T_{re}, T_{sk}, estimated T_{mu}, T_{body} and TS compared to the same time points in CON (Figures 6.4-6.5 and 6.7). However, no significant improvement ($p > 0.05$) to any physical performance measure was seen in the second half of iSPT in PACKS, SLURRY and MM, rejecting the second experimental hypothesis. Finally, no change in physical performance (Figures 6.2 and 6.3) and physiological responses (Figures 6.4-6.7) was seen in both PACKS and SLURRY compared with CON, whereas changes in physical performance and physiological responses were seen in the first half in MM, supporting the third experimental hypothesis.

6.4.1. Physical performance

The significant increase ($p < 0.05$) upon total distance (3%), high-speed distance (4%) and variable run distance (5%) covered during the first half (45 min) of iSPT in MM compared with CON (Figures 6.2-6.3), shows synergy with previous mixed-methods pre-cooling laboratory based research that have combined both internal and external pre-cooling methods (Clarke *et al*, 2011, Ross *et al*, 2011). Indeed, Clarke *et al* (2011) reported that 60 min of ice vest pre-cooling in combination with the ingestion of a carbohydrate drink solution (temperature not reported) had a 10% improvement upon total distance covered during

simulated soccer performance at 30°C. However, the ergogenic effect could have been altered in this study (Clarke *et al*, 2011) by the combined carbohydrate supplementation. Conversely, the findings from this experiment are dissimilar to a previous soccer match-play study in 29°C (Duffield *et al*, 2013). Duffield *et al* (2013) identified that 20 min of mixed method pre-cooling via an ice vest and ice slurry ingestion had no statistical improvement ($p > 0.05$) upon the physical performance of elite soccer players. However, as a soccer match-play design was utilised (Duffield *et al*, 2013), ascertaining the efficacy of an intervention can be problematic (Experimental Chapter 1); due to a plethora of match-factors (Section 2.1.2.) and the adaptive pacing strategies elite soccer players adopt when playing soccer in the heat (Section 2.3.2.). Furthermore, the study by Duffield *et al* (2013) was also likely to be underpowered (< 20 participants) compared to the sample size proposed by Gregson *et al* (2010) to gain meaningful inferences from an intervention from soccer match-play paradigms. The formulation of iSPT means that both the pacing strategies of players and previously elucidated match factors (Section 2.1.2.) are minimised as participants will complete the same individualised externally parameterised speed thresholds in each half (Experimental Chapter 1). Therefore, the well formulated approach adopted by iSPT allows for the efficacy of pre-cooling and half-time-cooling to be reliably ascertained, without an adaptive pacing strategy (Nassis *et al*, 2015) occurring and the plethora of match factors that have been previously recognised (Gregson *et al*, 2010).

To the 'authors' knowledge this is the first experiment to quantify the use of pre-cooling on soccer performance in 30°C via an individualised, validated and reliable soccer-specific simulation (Experimental Chapter 1). A previous soccer-specific simulation by Price *et al* (2009) identified that 20 min of pre-cooling and 15 min of half-time cooling via an ice vest significantly reduced the elevated body temperatures compared with no cooling prior to soccer-specific exercise at 30°C. However, as the simulation was a fixed distance, changes to all physical performance measures were not quantified. A seminal NMT based soccer-specific-simulation by Drust *et al* (2000a) identified that 60 min of cold shower induced cooling had no benefit upon physical performance, although, as the simulation was performed in a temperate environment (20.5 ± 0.88 °C; $71.6 \pm 8.4\%$ RH), this was not surprising. Furthermore, a recent NMT based soccer-specific simulation undertaken at 30°C utilised 60 min of ice vest pre-cooling reporting ergogenic response on high-speed distance covered (Clarke *et al*, 2011). However, this ergogenic effect was only witnessed when the pre-cooling was combined with carbohydrate supplementation, thus the experimental effect is masked somewhat by multiple

treatment interference. Alongside the aforementioned limitations to the previous NMT based soccer-specific simulations, these simulations (Clarke *et al*, 2011, Drust *et al*, 2000a, Price *et al*, 2009) lacked the individualised externally parameterised speed thresholds used in iSPT (Experimental Chapter 1). Therefore, due to the individualised approach adopted within iSPT (Experimental Chapter 1), the ergogenic response of both pre- and half-time-cooling for key physical performance measures (total distance, high-speed distance and variable run distance covered) were able to be ascertained with greater experimental control.

Due to the formulation of iSPT, a player's 'willingness' to perform high-speed exercise at a self-paced speed was ascertained via the variable run component, designed to quantify high speed running without an external cue (Abt and Lovell, 2009, Lovell and Abt, 2013). During this experiment, a 5% improvement across both halves in variable run distance covered was seen in MM when compared with CON (Figure 6.3.). This significant increase in variable run distance covered supports the notion that the individualised externally parameterised movement pattern employed by iSPT prevents participants from adopting an altered pacing strategy. Participants chose a faster running speed after MM pre- and half-time-cooling when compared with CON (Figure 6.3). This increase to variable run distance in the first half for MM is likely due to a strong sensory effect, as recent evidence has revealed that reductions in T_{sk} and TS can on occasions be beneficial to an athlete if no change in core body temperature occurs (Schulze et al, 2015). A sensory effect from menthol mouth rinse cooling, in the absence of T_{re} changes, has been shown to improve NMT based running performance over 5km whilst significantly, and favourably altering blood derived bio-chemical indices [prolactin (Stevens *et al*, 2015a, Stevens *et al*, 2015c)]. The ergogenic effect was attributed to activation of thermoreceptors in the mouth (Stevens et al, 2015a). Within MM, it is likely that the ice slurry ingestion induced activation of the thermoreceptors located within the mouth, whilst the ice packs reduced T_{sk} of the quadriceps, causing a similar ergogenic effect to Stevens et al (2015a) upon the variable run distance covered during the first half in MM. In turn, this ergogenic effect could have some impact upon the match outcome as high-speed movements are associated with game defining moments during soccer match-play (Gregson et al, 2010). Therefore, this strong sensory effect from MM may play a role in the increase in variable run distance covered in MM and may then go some way in maintaining a more 'temperate like' match play dynamic [i.e. more movements associated with game defining moments (Faude et al, 2012, Gregson et al, 2010)].

It was also identified that both sprint distance covered and peak sprint speed were unchanged between conditions during this experiment (Figure 6.3.). Experimental chapter 2 identified that sprint distance covered is unchanged and peak sprint speed is enhanced in a hot environment when compared with temperate conditions (Section 5.4.). A change to T_{mu} prior to and during sprint based exercise in the heat can be seen to alter its effect upon physical performance (Drust *et al*, 2005a, Gonzalez-Alonso *et al*, 1999). For example, previous soccer match-play research has identified that an elevated T_{mu} increases both the sprint distance covered and peak sprint speed at $43^{o}C$ compared to $21^{o}C$ (Mohr *et al*, 2012). Whereas, the cooling of the active muscles by up to $8^{o}C$ can reduce the peak power output of cyclists by up to 32% (Davies and Young, 1983, Yamane *et al*, 2015) via the Q_{10} effect on ATP re-synthesis (Ferretti *et al*, 1992). During this experiment T_{mu} was only reduced by ~2-3 ^{o}C after PACKS and MM pre-cooling, showing that the duration and volume of the pre-cooling strategy had no adverse effect upon sprint performance in near maximal soccer performance (Figure 6.3.). Moreover, there was no significant ($p > 0.05$) decline in sprint distance covered between the first (0 – 15 min) and last 15 min (76 – 90 min) blocks of iSPT in MM, however, a significant decline was seen between the same time points for, PACKS, SLURRY and CON (Table 4.1). Armatas *et al* (2007) revealed that more goals were scored/conceded in the final 15 min of match-play (76-90 min) compared to all other 15 min blocks, due to an inability to maintain sprinting performance which is associated with goals and assists in soccer (Faude *et al*, 2012). Furthermore, experimental chapter 2 revealed that this phenomenon is exacerbated at $30^{o}C$ compared with $18^{o}C$. Coaches may then be able to utilise these findings when compiling tactics prior to match-play by adopting a more offensive strategy during the last 15 min of the match, to take advantage of the exacerbated fatigue in repeated sprint ability within defensive players. Therefore, the appropriate use of MM half-time cooling may preserve the number of goal scoring opportunities in the last 15 min by negating some of the decrements observed in experimental chapter 2.

6.4.2. Physiological responses

The 30 min of mixed method pre-cooling in MM was the most effective pre-cooling method showing a significant reduction to T_{re}, T_{sk}, T_{mu} and T_{body} compared with CON (Figures 6.4. and 6.5.), showing synergy with earlier research (Clarke *et al*, 2011). However, the 30 min of pre-exercise ice slurry ingestion in SLURRY only significantly reduced T_{re} by 0.5 ± 0.3 ^{o}C from rest (no change to T_{sk}, T_{mu} and T_{body}) (Figures 6.4. and 6.5.), which also agrees with a previous study (Siegel *et al*, 2010). Previous studies have identified smaller [0.4 ± 0.1 ^{o}C - (Siegel *et al*,

2012)] and larger changes in T_{re} [1.1°C - (Ihsan *et al*, 2010)] when compared with SLURRY. However, limitations to these studies are the pre-cooling being performed in a hot environment which reduced convective heat loss (Siegel *et al*, 2012), and core temperature being inaccurately measured via a gastrointestinal pill (Wilkinson *et al*, 2008). The use of gastrointestinal pills to measure core temperature after the ingestion of cold fluids, could lead to distorted measurements as a result of the direct effect of the ice slurry on the pill (Wilkinson *et al*, 2008). The 30 min of pre-cooling via ice packs on the upper legs in PACKS reduced both T_{mu} and T_{sk} by 3°C compared to rest (Figures 6.4 and 6.5.), which due to the extended duration of ice packs pre-cooling from 20 min to 30 min, was a larger change than that seen within previous research [~1°C (Castle *et al*, 2006)]. No change in T_{re} was seen during the ice packs pre-cooling in PACKS (Figure 6.4.), which is dissimilar to previous research (Castle *et al*, 2006). However, part-body pre-cooling via ice garments over a 15-65 min period can still be beneficial without the reduction in T_{re} as convective heat loss from the periphery can be improved via changes to skin blood flow (Bogerd *et al*, 2010). However, although 65 min of part body ice garment pre-cooling had the greatest ergogenic effect, this lacks external validity within a soccer paradigm as practitioner only have up to 30 min with players prior to a match (Towlson *et al*, 2013). The heat induced decrements seen in soccer are caused via the intricate interplay between peripheral (feedback) and central (feed forward) pacing factors (Nybo *et al*, 2014, Taylor and Rollo, 2014, Taylor, 2014), yet the pre-cooling in PACKS and SLURRY only targeted the peripheral (T_{sk} and T_{mu}) and central (T_{re}) body temperature measures, respectively. Therefore, the singular use of ice slurry ingestion and ice packs pre-cooling is unlikely to attenuate the heat induced decrements quantified in experimental chapter 2. As the mixed-method pre-cooling was the only pre-cooling strategy to target peripheral (e.g. T_{sk} and T_{mu}) and central (e.g. T_{re}) body temperature factors, it is likely to be the most ergogenic upon soccer performance and could be recommended to practitioners.

A critical finding from this experimental chapter was that no significant changes in physical performance were evident in SLURRY compared with CON (Figure 6.2 and 6.3). The rate of rise in T_{re} and ending T_{re} at the conclusion of the first half were significantly greater in SLURRY, compared with CON (Figure 6.4.). The increase to the rate of rise in T_{re} in SLURRY could be from a pre-exercise widening to the T_{re}-T_{sk} gradient (Siegel *et al*, 2012), as ice slurry ingestion reduced T_{re}, without a concurrent decrease in T_{sk} (Figure 6.4.). However, peripheral body temperatures (T_{sk} and T_{mu}) predict the decline in soccer performance in hot environments (Experimental Chapter 2), yet in SLURRY, only T_{re} was reduced pre-exercise causing the T_{re}-

T_{sk} gradient to be unchanged compared to CON (Figure 6.4.). The pre-exercise reduction to the peripheral body temperatures in PACKS, contributed to the decreased rate of rise in T_{re} once exercise had commenced, as heat was stored in both the periphery and the body's core (Siegel *et al*, 2012). However, no change in physical performance was evident between PACKS and CON, dissimilar to previous research (Castle *et al*, 2006). Castle *et al* (2006) revealed that a 4% increase in peak power output after 20 min ice packs pre-cooling was centrally activated by a change in T_{re} causing a feed forward pacing strategy to occur by increasing muscle activation during each sprint (Drust *et al*, 2005a). However, as the ice packs pre-cooling period failed to reduce T_{re} (Figure 6.4.), no change in T_{re} was evident during the first half in PACKS compared with CON (Figure 6.4.). Therefore, as an intricate interplay between peripheral (feedback) and central factors (feed forward) is the cause for the heat induced decrements seen during soccer match-play (Nybo *et al*, 2014, Taylor and Rollo, 2014), it is unsurprising that no change to soccer performance was seen in both SLURRY and PACKS compared with CON. As the mixed-method cooling in MM targeted both the central (e.g. T_{re}) and peripheral (e.g. T_{sk}) body temperatures via a pre-cooling strategy, it would have a greater chance to attenuate the heat-induced fatigue recognised in experimental chapter 2. This may explain why no ergogenic effect upon soccer performance was seen in PACKS and SLURRY compared with CON.

In MM when compared with both PACKS, SLURRY and CON, there was a significant reduction in T_{re}, T_{sk}, T_{mu} and T_{body} throughout the first half when compared with CON (Figure 6.4. and 6.5.). Clarke et al (2011) supported these findings by identifying a reduction in T_{re} throughout the first half of simulated soccer performance at 30°C after mixed methods pre-cooling, however, no change in T_{mu} was seen throughout the first half. This is likely to be due to the ice vest only targeting T_{sk} as no contact is made to the active muscles responsible for the physical performance in soccer match-play (Ross et al, 2013). During this experiment ice packs were used as the external pre-cooling method as they targeted these active muscles, explaining why a change in T_{mu} was seen in this experiment. Furthermore, a reduction in T_{re} was seen throughout the first half in MM compared with CON, despite the single use of ice slurry ingestion in SLURRY having the opposite effect upon T_{re}. A reduction in T_{re} in the first half in MM was explained by Ross et al (2011) due to a lowering in the T_{re}-T_{sk} gradient meaning that more heat was able to be lost from the core to the skin. Therefore, these findings reveal that targeting both the peripheral (T_{sk} and T_{mu}) and central measures (T_{re}) via a mixed-method pre-cooling strategy, contributed to an increased physical performance during the first half in

MM compared with CON (Figures 6.2. and 6.3.). A greater ergogenic response was likely seen in MM compared to CON, PACKS and SLURRY due to both the feedback and feedforward pacing factors being enhanced by an increased heat transfer (peripheral) and central drive (central), respectively.

The data for the present experiment recommends that the MM pre-cooling strategy is utilised prior to the first half (e.g. all body temperatures and thermal sensation were reduced in MM compared with CON). Furthermore, the individual response graphs shown in appendix H and I support this assumption as these detail the changes more clearly, revealing an improvement in total, high-speed and variable run distance covered for all 8 participants in MM compared with CON. However, the last point of interest was that the 15 min of half-time-cooling offered no significant change to both the physical performance and physiological responses in the second half between all conditions (Figures 6.2-5 and 6.7.). Thus, utilising the same mixed-method pre-match-cooling strategy investigated in this experiment, as a 15 min half-time-cooling intervention, was proven ineffective to cool the body prior to the second half, showing parity with previous soccer-specific research (Clarke et al, 2011, Duffield et al, 2013).

The lack of change in physical performance during the second half could have been due to both plasma volume and body mass changes pre- and post-iSPT being similar in MM compared with CON (Table 6.2). Experimental chapter 2 outlined that a 2% body mass change may have played a role in the heat-induced-decrements during simulated soccer performance at $30^{o}C$, showing parity with the data for CON (-~2%) and MM (-~2%) in the present experiment (Table 6.2). This may have meant that the participants finished both CON and MM in a hypo-hydrated state, (Cheuvront et al, 2003). Previous research by Siegel et al (2012) reported that 7.5 $g \cdot kg^{-1}$ of ice slurry ingestion prior to a run to exhaustion test, significantly reduced body mass changes post-exercise, whilst also having a moderate to very large effect upon exercise capacity compared to cold water immersion. However, the mean duration of the exercise period utilised by Siegel et al (2012) was 30 min which is shorter compared with one half of soccer match-play (~45 min). This may mean that the volume of ice slurry the participants ingested prior to the second half was not sufficient (>7.5 $g \cdot kg^{-1}$) to initiate changes in hydration (e.g. plasma volume and body mass unchanged) by the end of the second half in MM compared with CON. Nevertheless, an increase to the volume of ice slurry that is ingested at half-time could have detrimental consequences such as increasing gastrointestinal stress (e.g. bloating, vomiting, etc.) reducing its practicality to be used prior to soccer-specific exercise (Siegel and Laursen, 2012, Taylor and Rollo, 2014). Pre-cooling via the ingestion of 14 $g \cdot kg^{-1}$ of ice slurry, has been

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shown to improve 150 min cycling time trial performance by 3% and reduce the change in body mass pre to post-exercise compared with no-cooling (Ross et al, 2011). Therefore, further research is required to identify an optimum strategy (e.g. ice slurry is ingested during match-play at natural stoppages such as injuries, substitutions, etc.) and volume (7.5-14 g·kg^{-1}) of ice slurry to be ingested at half-time to improve physical performance and hydration status (e.g. body mass and plasma volume changes) during soccer match-play in hot environments.

The minimal effect that the 15 min half-time-cooling had upon all physical performance measures and physiological responses during this experiment could be explained due an appropriate volume of cooling not being used (Minett et al, 2011). Experimental chapter 2 identified that T_{re} and T_{mu} exceeded ~39°C and 37°C, respectively, at the end of the first half of iSPT at 30°C. However, the PACKS, SLURRY and MM half-time-cooling interventions did not significantly reduce these prior to commencement of the 2nd half of iSPT. The increase in body temperatures at the end of the first half indicate that a large proportion of the heat produced during the first half of iSPT is likely to be situated within the body's periphery (Nybo et al, 2014), within the active muscles and skin. Therefore, an effective method of half-time-cooling should look to cover as much of the body's periphery as possible (Minett *et al*, 2012a, Minett *et al*, 2011). A recent study by Minett et al (2011) used 5 min of whole body-cooling at half-time and found it to be sufficient to improve total distance covered by 5% and significantly reduce T_{re} and T_{mu} during the second half of a self-paced intermittent sprint exercise protocol (2 x 35 min) at 33°C. This approach has good applicability with soccer as coaches and scientists only have 2-6 min of time available to utilise an intervention at half time, as revealed in a recent review (Russell et al, 2015). Therefore, half-time-cooling strategies should cover a greater volume of the body compared with a pre-cooling strategy, due to the greater amount of heat gained in the first half (Minett et al, 2011); i.e. half-time-cooling needs to be more aggressive than pre-match-cooling. Furthermore, these strategies must also be practical and abide by the limited time (2-6 min) practitioners have with players during the half-time interval (Russell et al, 2015).

6.4.3. Experimental limitations

Several limitations which have been documented in sections 4.4.3 and 5.4.3 including the use of sub-elite soccer players and the limited assessment of soccer-specific technical skills are also relevant to this experiment. A further limitation of this experiment was that a temperate environment trial was not utilised, to help ascertain whether the PACKS, SLURRY and MM

cooling interventions were able to maintain a 'temperate like' soccer performance. This approach has been utilised previously by Taylor *et al* (2014b), who reported no significant difference in 2000m rowing time trial performance in female rowers, between the pre-cooling trial in 35°C and the control/temperate trial performed at 20°C, after a 20 min cold shower pre-cooling. Experimental chapter 2 identified that an intervention should be able to maintain a 'temperate like' match-play soccer performance, or similar, therefore, utilising the approach (control/temperate trial) used by Taylor *et al* (2014b) would allow for this response to be reliably ascertained. A further limitation of this experimental chapter was the use of a 15 min half-time-cooling strategy, as a recent review paper conducted after the completion of this experiment has outlined that practitioners only have 2-6 min with players at half-time (Russell *et al*, 2015). In order to counteract this issue, future research should look to adopt the approach by Minett *et al* (2011) who used 5 min of pre-cooling, via a more aggressive approach by covering ice packs all over the body. Therefore, future research should look to assess this approach within a reliable laboratory based setting (e.g. iSPT).

Relative to the large variability for key physical performance measures between experiment 1 and 2 discussed within section 5.4.3, experiment 3 demonstrates variance when compared to experiment 1 but not 2. For example, in the present experimental chapter in CON, the decrement in HSD covered between halves and first and last 15 min block (Halves: 36 ± 19 m; 15 min blocks: 13 ± 10 m) differed compared to the experimental chapter 1 (Halves: 62 ± 32 m; 15 min blocks: 40 ± 20 m). However, these values are aligned to experiment 2 (Section 5.4.3) due to the same inclusion/exclusion criteria being utilised within experiment 2 and 3; as discussed within section 5.4.3. Consequently, the similar variance between 2 and 3 supports the postulations provided within experimental chapter 2, postulating the experimental chapter 2 compared to experimental chapter 1 variance is due to the enhanced inclusion criteria adopted in experiments 2 and 3.

6.4.4. Potential applications

The practical applications from the findings presented within this experimental chapter is that 30 min mixed method pre-cooling via ice packs upon the upper legs and ice slurry ingestion (MM) helps to attenuate some of the heat induced decrements seen during the first half in experimental chapter 2. As there is currently no governing body dictated restriction upon the use of pre- and half-time-cooling prior to soccer performance, the findings from this

experiment would be useful for scientists and coaches to utilise within their practice (Taylor and Rollo, 2014).

These findings from this experimental chapter may also be of interest to coaches from elite soccer teams that are competing in a hot environment, without sufficient time to complete an appropriate acclimation protocol. In MM, an increase in high-speed distance and variable run distance covered was seen during the first half of iSPT, which could manifest itself as more game defining moments occurring during match-play. Therefore, a coach could take advantage of this elevated exercise intensity by orchestrating a team to play at a higher intensity during the first half (e.g. Play a higher pressing/tempo match) which could increase the number of goals as the number of game defining moments is increased.

Furthermore, the findings from MM have good practical application to be utilised within the field. The use of cold water immersion (Castle *et al*, 2006, Siegel *et al*, 2012) and ice vests (Duffield and Marino, 2007, Price *et al*, 2009) has been shown to have a positive effect upon both physical performance and physiological responses during exercise. However, despite the large statistically significant results seen by these methods, they lack practicality and ecological validity to be utilised within an elite soccer setting (McNeely, 2015, Taylor *et al*, 2014b). The use of ice packs and ice slurry ingestion during pre- and half-time-cooling are widely available methods that can improve soccer performance in the heat and should be considered by players and coaches to be used before playing soccer in the heat (McNeely, 2015).However, as mentioned previously future work is required to optimise a more aggressive half-time-cooling strategy to complement the positive experimental effects seen from MM relative to the 1st half. This despite the positive influence on sprint distance covered seen in the second half of MM (Table 6.1.).

6.5. Conclusion

In conclusion, 30 min of mixed-methods pre-cooling, which utilised ice packs upon the quadriceps and hamstrings in conjunction with ice slurry ingestion, prior to simulated soccer performance in a hot environment (30°C) was able to attenuate the heat-induced-decrements in the first half of iSPT for total distance, high-speed distance and variable run distance covered compared to when no-cooling was administered (Figure 6.2 and 6.3.), supporting the first experimental hypothesis. The 15 min of mixed-methods half-time-cooling did not significantly improve any physical performance measures in the second half (Figures 6.2 and 6.3.), rejecting the second experimental hypothesis. The significant improvement in all physical performance

measures during the first half after mixed-methods pre-cooling was accompanied by a significant reduction in T_{re}, T_{sk}, estimated T_{mu} and TS compared with the no pre-cooling prior to near-maximal soccer performance at 30°C (Figures 6.4-6.5 and 6.7.). However, this experimental chapter also reveals that individual use of ice packs and ice slurry ingestion as a pre- and half-time-cooling strategy had no significant benefit upon any physical performance measure during this experiment (Figures 6.2 and 6.3), whereas a combination of both cooling methods had an ergogenic effect in the first half, meaning the third experimental hypothesis is accepted. Therefore, the third experimental aim of this thesis is directly answered within this experiment: '*To investigate the impact of three different pre- and half-time-cooling methods; 1) external (ice packs upon the quadriceps and hamstrings); 2) internal (ice slurry ingestion); and 3) mixed methods (internal and external) compared with a control (i.e. no-cooling) as a solution to acquiesce any heat-induced-decrements that were present in experiment 2*'.

CHAPTER 7: SYNTHESIS OF EXPERIMENTAL FINDINGS AND GENERAL DISCUSSION

7.1. General discussion

This thesis aimed to assess the reliability and validity of the iSPT (Experiment 1), to enable the reliable investigation of environmental stress on soccer performance (Experiment 2) and the efficacy of pre- and half-time-cooling to attenuate any heat-induced decrements (Experiment 3). Experiment 1 identified that the iSPT showed good validity with previous soccer match-play data (Bradley *et al*, 2009, Di Mascio and Bradley, 2013, Di Salvo *et al*, 2012). Furthermore, strong reproducibility was reported between two experimental trials of iSPT, as all soccer-specific physical performance measures and physiological responses showed good agreement (ICC: >0.9; CV%: <5%) compared with statistical guidelines (Atkinson *et al*, 1999, Vincent and Weir, 2012). These reliability statistics also compared favourably with previous NMT based soccer-specific simulations, as iSPT incorporated individualised speed thresholds based on peak sprint speed (Thatcher and Batterham, 2004), a 90 min duration (Oliver *et al*, 2007b), three familiarisation sessions (Abt *et al*, 2003) and 6 s sprint periods (Sirotic and Coutts, 2008), which are all recommendations from previous research. Therefore, as the iSPT demonstrated low test-retest error compared with the statistical guidelines (Atkinson *et al*, 1999, Vincent and Weir, 2012) and previous NMT based soccer-specific simulations, any changes to soccer performance can be attributed to an intervention and not the variability of the measure (Currell and Jeukendrup, 2008), unlike in soccer match-play designs (Gregson *et al*, 2010). The findings within experimental chapter 1 then facilitated the reliable and valid quantification of the aims and objectives investigated in experimental chapters 2 and 3.

A further finding from experimental chapter 1 was that as hypothesised, the novel speed component in iSPT called the variable run differentiated between self-paced distances covered at high speed and sprinting by reporting that decrements had occurred in the second half and final 15 min. The self-paced decrements for variable run distance covered are in agreement with classic variables of high-speed distance covered quantified in experimental chapter 1, such as FRD covered, and also show synergy with previous soccer match-play data (Bradley *et al*, 2009, Mohr *et al*, 2005). A previous study by Sear *et al* (2010) utilised the variable run speed

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component within a 45 min NMT soccer-specific simulation and identified that the variable run distance covered was not able to induce the self-paced decrements seen within experimental chapter 1. However, as most fatigue in soccer match-play is seen in the second half (Mohr *et al*, 2003), the simulation may not have been a sufficient duration to induce a decline in variable run distance covered. The variable run can then be used to ascertain a player's 'willingness' to perform high-speed exercise at a self-paced speed, which could be pivotal to the match outcome as high-speed distance covered is associated with game defining moments in soccer (Gregson *et al*, 2010). For example, a reduction to a players 'willingness' to perform high speed exercise may be why more goals are scored/conceded in the second half and final 15 min of match-play compared to any other 15 min block (Armatas *et al*, 2007). This 'willingness' to perform high speed exercise has been shown to be reduced during soccer match-play in hot (Mohr *et al*, 2003) and hypoxic (Garvican *et al*, 2013) environments, meaning this phenomenon could be exacerbated (Taylor and Rollo, 2014). Therefore, the variable run speed component within iSPT will facilitate precise quantification of this factor within experimental chapters 2 and 3 of this thesis.

Experimental chapter 2 identified that the physical performance of soccer players is reduced at 30°C (HOT), 1,000m (HYP) above sea level and in a combination of these two conditions (HH) when compared to a normoxic-temperate (CON) environment. Although no difference in decline was seen between the HOT and HYP conditions, the decline is exacerbated within the HH condition. The heat-induced decrements from experimental chapter 2 stemmed from increasing body temperatures which manifests as changes to both TS and RPE. Furthermore, the hypoxic-induced decrements demonstrated within experimental chapter 2 were initiated by changes to both S_aO_2 and HR causing changes to RPE. Therefore, the exacerbated reduction in physical performance in HH is likely due to a combination of both heat and hypoxia physiological responses.

Large decrements to the key physical performance measures including high-speed distance and variable run distance covered are seen in HOT, HYP and HH, compared to CON, which may manifest as a reduction in the number of game defining moments in soccer match-play (Gregson *et al*, 2010). Furthermore, high-speed distance, variable run distance and sprint distance covered were significantly reduced in the final 15 min of match-play compared with CON, which may have a pivotal impact upon the match outcome (Faude *et al*, 2012) as more goals have been reported to be scored/conceded in the final 15 min of match-play (76-90 min) compared to all other 15 min time phases. This indicates that these decrements seem to be

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exacerbated in HOT, HYP and HH environments. These findings would be particular interest for scientists and coaches within their practice to maintain a 'temperate like' running performance and match characteristics during soccer match-play. This is particularly likely to occur within hot environments as these are the most prevalent for future elite soccer tournaments over the next 10 years (Taylor and Rollo, 2014). Regression analysis highlighted that TS, T_{sk} and T_{mu} were a predictor of the heat induced decrements of both total distance and high-speed distance covered in experimental chapter 2. An ecologically valid strategy to target these peripheral measures is via pre- and half-time-cooling by utilising mixed methods, ice packs and ice slurry ingestion strategies (Bongers $et\ al$, 2014). Therefore, as governing bodies choose to continue hosting elite soccer competitions within hot environments the efficacy of pre- and half-time-cooling to attenuate any heat-induced decrements was considered an important research question to answer in experimental chapter 3 following the findings from experimental chapter 2.

Experimental chapter 3 identified that no significant changes in physical performance and physiological responses were evident after the singular use of ice packs (PACKS) and ice slurry ingestion pre-and half-time-cooling due to the 30 min pre-cooling period only targeting the peripheral and central body temperatures, respectively. Taylor and Rollo (2014) suggested that an intricate interplay between peripheral (feedback) and central factors (feed forward) is the cause for the heat induced decrements seen during soccer match-play, so it is unsurprising that no change to soccer performance was seen in both SLURRY and PACKS compared with CON. The 30 min of mixed-method (MM) pre-cooling, which utilised ice packs upon the quadriceps and hamstrings, and ice slurry ingestion prior to near-maximal soccer performance in a hot environment (30ºC) was able to attenuate the heat induced decrements in the first half of iSPT for total distance, high-speed distance and variable run distance covered compared to CON. These findings show synergy with previous mixed-methods pre-cooling laboratory based research that have combined both internal and external pre-cooling methods (Clarke $et\ al$, 2011, Ross $et\ al$, 2011). The significant improvement in all physical performance measures during the first half after mixed-methods pre-cooling was accompanied by a significant reduction in T_{re}, T_{sk}, estimated T_{mu} and TS compared with the no pre-cooling prior to near-maximal soccer performance at 30ºC. As both high-speed distance and variable run distance covered were significantly improved in the first half, this may increase the number of game defining specific movements occurring during a match, by continuing 'temperate like' match play dynamics (Gregson $et\ al$, 2010). These findings may also be beneficial for practitioners as coaches would

not have to differ their tactics in the first half of match-play between temperate and hot environments as players are able to continue a 'temperate like' soccer performance.

There was also no significant decline in sprint distance covered between the first (0 – 15 min) and last 15 min (76 – 90 min) blocks of iSPT in MM, unlike in PACKS, SLURRY and CON. As experimental chapter 2 revealed that this phenomenon is exacerbated at 30°C compared with 18°C, the appropriate use of MM half-time cooling may preserve the number of goal scoring opportunities in the last 15 min by negating some of the decrements observed in experimental chapter 2. The 15 min of mixed-methods half-time-cooling did not significantly improve any physical performance measures or attenuate the rise in physiological stress in the second half of MM. As previously explained in section 6.4.2, the minimal effect that the 15 min half-time-cooling had upon all physical and physiological performance measures is likely due to the greater T_{re} and T_{mu} seen at the end of the first half, meaning that the cooling technique was not aggressive enough to increase the amount of heat loss needed within the periphery of the body (Minett *et al*, 2011, Nybo *et al*, 2014). Therefore, future half-time-cooling research should look to cover as much of the body's periphery as possible by increasing the volume and aggression of the cooling strategy (Minett *et al*, 2011).

7.2. Limitations and future research recommendations

Several limitations have been highlighted in relation to the investigations of experimental chapters 1-3 (Sections 4.4.3, 5.4.3 and 6.4.3.), which should be considered for future research. Due to iSPT being completed upon a NMT, individualising the simulation was easily facilitated by peak sprint speed after a PSA, as used in previous NMT based soccer-specific simulations (Abt *et al*, 2003, Sirotic and Coutts, 2008). Few studies have applied this approach within the field with relative success, based upon the player's peak sprint speed (Buchheit *et al*, 2010, Cahill *et al*, 2013). However, individualising via peak sprint speed means that a player with a greater peak speed will have higher speed thresholds compared to a player who is slower, even if there $\dot{V}O_{2max}$ is equivalent (Hunter *et al*, 2014). This has resulted in key measures such as high-speed distance covered for a faster player being under-interpreted by up to 61% compared to a slower player (Hunter *et al*, 2014). Physiological responses should be considered when individualising speed thresholds due to their importance to the adaptive response seen to physical performance in soccer (Impellizzeri *et al*, 2004, Weston, 2013). Physiological responses such as VT (Abt and Lovell, 2009, Lovell and Abt, 2013), HR deflection points (Vigne *et al*, 2010), maximum aerobic speed via the anaerobic speed reserve (Buchheit and

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Mendez-Villanueva, 2014, Mendez-Villanueva *et al*, 2013) or in combination (Hunter *et al*, 2014) to individualise the speed thresholds in soccer performance. However, to individualise a NMT based soccer-specific simulation via these approaches is problematic as a $\dot{V}O_{2max}$ test would need to be utilised, which are most commonly performed on a motorised treadmill (Tonnessen *et al*, 2013). However, as the running mechanics differ between both the motorised and NMT, speed thresholds are likely to differ between methods (Lakomy, 1987). A validated NMT based $\dot{V}O_{2max}$ test was not created until after the commencement of testing for this thesis (Mauger *et al*, 2013), therefore, future research should look to compare the physical performance (peak sprint speed and MSS), physiological responses (maximum aerobic speed, VT) or combination methods to individualise speed thresholds via iSPT. However, it was unclear if the VT_{2speed} can be identified from this protocol due to exercise not always being at a steady state. This approach has been completed previously by Hunter *et al* (2014) however, these findings must be taken with caution as a soccer match-play design was used in a small sample size ($n = 18$) so inferences regarding the appropriate method to individualise soccer performance may be difficult to ascertain.

All experimental trials during this thesis were performed upon a NMT Woodway Force 3 (Dudley, Birmingham) treadmill, as previously utilised by a plethora of soccer-specific (Abt *et al*, 2003, Clarke *et al*, 2005, Drust *et al*, 2000a, Oliver *et al*, 2007b, Thatcher and Batterham, 2004), generic team-sport simulations (Sirotic and Coutts, 2008), and repeated sprint exercise tests (Hughes *et al*, 2006, Oliver *et al*, 2007a, Tong *et al*, 2001). However, the flat design of the NMT Woodway Force treadmills has been reported to impede natural running style dynamics due to the use of a harness and its instrumentation (Gonzalez *et al*, 2013, Lakomy, 1987). Furthermore, participants must overcome a resistance prior to completing sprint demands, that requires an unorthodox and different running style compared to running during soccer match-play (Lakomy, 1987, Ross *et al*, 2009). Recently a NMT Woodway curve treadmill has been increasingly examined by researchers (Gonzalez *et al*, 2013, Stevens *et al*, 2015b, Tofari *et al*, 2015) to facilitate soccer-specific exercise as due to its curved design, a harness is not needed so sprinting is unrestricted. Furthermore, this has also been showed to have good reliability and validity to assess aerobic (Stevens *et al*, 2015b), self-paced and repeated sprint exercise (Gonzalez *et al*, 2013, Tofari *et al*, 2015), all facets which are necessary during soccer performance. In particular, Tofari *et al* (2015) has shown a greater reliability when quantifying sprinting characteristics (sprint distance: 1.8% ; peak sprint speed: 1.9%) compared with the findings from experimental chapter 1 (sprint distance: 2.1% ; peak sprint

speed: 4.5%), which is likely due to unrestricted design and minimal change between ground and treadmill running styles (Gonzalez *et al*, 2013). This is important as the precise quantification of straight sprinting amongst intermittent exercise is important for soccer-specific exercise, due its association with goals scored and assists making it pivotal to the match outcome (Faude *et al*, 2012). As a Woodway Curve (Dudley, Birmingham) would allow more reliable quantification of this measure compared to the findings from experimental chapter 1, the reliability and validity of iSPT upon a Woodway Curve treadmill should be ascertained.

It is also identified within experimental chapter 1 that due to iSPT being performed upon a NMT, limitations including the assessment of technical skills and multi-directional movements were unable to be quantified (Small *et al*, 2010). The performance of technical skills in soccer has been shown to be unchanged and in some cases enhanced when competing in soccer match-play in hot and hypoxic environments (Mohr and Krustrup, 2013, Mohr *et al*, 2012, Nassis, 2013, Nassis *et al*, 2015). However, it is acknowledged that players alter their pacing strategy to preserve their technical skills (Mohr *et al*, 2012). It is obvious that due to the treadmill nature of iSPT, technical performance is not able to be quantified during the simulation. However, the use of battery tests could be utilised both prior to and at the end of each half of iSPT. Coull *et al* (2015) utilised a battery of computer based cognitive tests to quantify how tyrosine supplementation would benefit cognitive performance, however, the use of these tests lacks applicability to technical performance in soccer (Stolen *et al*, 2005). A recent study by Russell *et al* (2010) created simulated tests to assess the passing, shooting and dribbling skills of elite players which showed excellent validity and reliability compared with previous match-play data and statistical guidelines, respectively. Despite the reliability of these simulated technical performance tests (Passing – CV: 5.2%; Shooting – CV: 3.5%), they took up to 47 min complete which would not be appropriate to use at the end of each half of iSPT. Therefore, future work should look to incorporate appropriate skill based tests into iSPT which would be advantageous for the assessment of a player's technical skills between and within trials and conditions.

Typical of most experimental research, the results from this project are somewhat specific to the cohort of participants and experimental protocols employed. The altitudes and temperatures chosen for this project are specific to those experienced during elite soccer tournaments such as the UEFA Champions and Europa League. Therefore, one obvious issue is the recruitment of recreationally active male volunteers for the present experiment, rather than elite soccer players. The recruitment of elite soccer players has its obvious challenges; however, prior to

completing all familiarisation protocols participants were required to have a $\dot{V}O_{2max}$ greater than 55 ml$^.$kg^{-1}$^.$min^{-1} to show some parity with elite soccer players (Tonnessen *et al*, 2013). Therefore, the influence of this issue was likely minimised, however, any generalisation of the results from all experimental chapters to the wider population should be considered cautiously.

During this thesis, only an intervention to attenuate the heat induced decrements upon performance was investigated due to its greater prevalence in elite soccer (Taylor and Rollo, 2014). However, as hypoxic environments are still apparent in elite soccer, specific nutritional interventions may be of significance to attenuate the hypoxia induced decrements seen during experimental chapter 2 (Taylor and Rollo, 2014). Dietary nitrate has been shown to improve muscle oxygenation during sub-maximal and maximal exercise in acute severe hypoxia (Masschelein *et al*, 2012), reduce negative muscle metabolic perturbation during high-intensity exercise within hypoxia (Vanhatalo *et al*, 2011) and improve intense intermittent exercise performance at sea level (Thompson *et al*, 2015, Wylie *et al*, 2013). However, the practical recommendations to optimise nitrate supplementation (dose, nitrate source, acute or chronic supplementation) specific to soccer within hypoxia is difficult. This is due to the high variability in supplementation strategies seen across studies which have demonstrated worthwhile effects of supplementation on exercise and the lack of nitrate and exercise based studies within hypoxia (Hoon *et al*, 2014, Masschelein *et al*, 2012, Vanhatalo *et al*, 2011). Nevertheless, an acute dose of inorganic nitrate (300 mg to 600 mg, delivered by ingesting nitrate-rich vegetable products such as beetroot juice) 75-150 min before exercise appears to improve exercise performance and/or efficiency – though chronic supplementation of at least several days increases the likelihood of exercise performance benefits being observed (Hoon *et al*, 2014, Jones, 2013). Therefore, future research utilising iSPT should look to ascertain the loading phase required for dietary nitrate to be successfully utilised to attenuate the hypoxia-induced decrements seen within experimental chapter 2.

Following the minimal effect that the 15 min half-time-cooling had upon all physical performance measures and physiological responses in experimental chapter 3, future research should look to investigate the efficacy of further half-time-cooling strategies. As previously mentioned, this adverse effect was likely due to an appropriate volume of cooling not being used at half-time (Minett *et al*, 2011). During this thesis, the duration of the cooling was increased at half-time due to the prolonged nature of one half of soccer match-play, showing synergy with a previous study (Minett *et al*, 2012a). However, a recent study by Minett *et al* (2011) used only 5 min of half-time-cooling but increased the volume of cooling by covering

the whole body and found it to be sufficient to improve total distance covered in the second half during a self-paced intermittent sprint exercise protocol (2 x 35 min) at 33°C by 5%. This approach has good applicability with soccer as coaches and scientists only have 2-6 min of time available to utilise an intervention at half time, as revealed in a recent review (Russell *et al*, 2015). Therefore, future research should look at an effective method of half-time-cooling covering as much of the body's periphery as possible, by increasing the volume of cooling due to the greater amount of heat gained in the first half (Minett *et al*, 2012a, Minett *et al*, 2011). Furthermore, these strategies must also be practical and abide by the limited time (2-6 min) practioners have with players during the half-time interval (Russell *et al*, 2015).

The iSPT would not be appropriate to assess the changes in soccer performance during women's soccer. This is due to the soccer-specific physical performance and physiological responses differing between men and women (Bradley *et al*, 2013). The simulation was based upon the physical performance and physiological responses of a male central midfielder, however, work by Bradley *et al* (2013) identified that elite male soccer players will cover a greater number of high speed movements compared with elite female players in the UEFA Champions Leagues. Therefore, a female-specific iSPT should be designed and assessed for its reliability and validity before being utilised as a tool to benefit female soccer.

The iSPT could also be used in a number of ways for a plethora of other research questions away from the aims of this thesis. For example, iSPT could be utilised as a training tool to provide objective feedback to both players and coaches by quantifying both a player's physical performance and physiological responses. The iSPT could also be used to assess players who are returning from injury. Additionally, iSPT could be completed by the player post-injury and compared against the pre-injury soccer-specific capacity. This approach would be useful for a coach to understand if a player is ready to return to competitive soccer. Furthermore, iSPT could be used for player rehabilitation, as the simulation does not contain any multi-directional movements (e.g. twisting and turning) or the contact involved in a soccer match. The iSPT may also be used when evidence is required with regard to the efficacy of a nutritional intervention (Clarke *et al*, 2012), particularly those ergogenic aids which are reputed to delay fatigue (Mujika, 2000).

7.3. Conclusions

The major conclusions from this thesis are:

1. All physical performance measures and physiological responses recorded during the iSPT were comparable to previous soccer match-play data. Furthermore, the iSPT showed excellent reproducibility compared with previous NMT based soccer-specific simulations and statistical guidelines. The variable run speed component showed good efficiency to differentiate between self-paced distances covered at high speed and sprinting by reporting that fatigue had occurred in iSPT both between and within halves. However, technical skills and multi-directional movements were not able to be measured in iSPT.

2. Individualised near-maximal soccer performance is significantly impaired in HOT, HYP and HH when compared to CON. No difference is seen for all physical performance measures in both HOT and HYP, however, the heat and hypoxic induced decrements stem from increasing body temperatures and changes to both S_aO_2 and HR, respectively. Regression Analysis identified that TS, T_{sk} and T_{mu} were a predictor of the heat induced decrements seen in experimental chapter 2, thus, any intervention should look to target these measures.

3. The heat induced decrements in CON were attenuated in the first half of MM after 30 min of mixed-methods pre-cooling, prior to near-maximal soccer performance in a hot environment (30ºC). These were accompanied by a significant reduction in T_{re}, T_{sk}, estimated T_{mu} and TS compared with CON. However, the 15 min of mixed-methods half-time-cooling in MM did not significantly improve any physical performance measure in the second half. No significant benefit upon any physical performance measure was seen in PACKS and SLURRY. The MM pre-cooling goes someway in maintaining a 'temperate like' match play soccer performance.

4. Future research should look to apply the findings from pre-cooling within an elite soccer setting, whereas, more laboratory based research within a controlled environment should look to identify a more aggressive half-time-cooling strategy, which is suited to the time constraints (Russell *et al*, 2015, Taylor and Rollo, 2014) associated with elite soccer.

CHAPTER 8: REFERENCES

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APPENDICES

Appendix A

PAR-Q

1. Have you ever been told by your doctor that you have a heart condition and advised only to participate in physical activity approved by your doctor?

2. Do you experience any chest pains when you participate in physical activity?

3. Have you recently experienced any chest pains whilst not participating in physical activity?

4. Do you ever lose consciousness?

5. Do you ever lose your balance as a result of dizziness?

6. Do you have any problems with your bones and joints that could cause further problems if you participate in physical activity?

7. Are you aware of any reasons as to why you should not participate in physical activity?

8. Have you used a non-motorised treadmill in the last 6 months?

Name: .... Signature: .... Date: ....

Appendix B

CONSENT FORM

TO BE COMPLETED BY PARTICIPANT

NAME:………………………………………………(Participant)

I have read the Information Sheet concerning this project and understand what it is about. All my further questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

- My participation in the project is entirely voluntary and I am free to withdraw from the project at any time without disadvantage or prejudice.

- I will be required to attend 6 sessions to complete the project.

- As part of the study I will have to:
  • Perform a Vo2max and a lactate threshold test
  • Perform an Intermittent Yo-Yo test
  • Perform 4 familiarisation sessions to understand how to use a non-motorised treadmill
  • Perform a Peak Speed Assessment
  • Perform 2 soccer simulation protocol tests lasting 2 x 45 minutes separated with a 15 minute break.
  • Have core temperature measured using a rectal thermomete
  • Give a sample of urine prior to each test so that hydration can be measured.
  • Have skin temperature measured in four areas of the body (Chest, upper arm, Thigh and Calf)
  • Have blood lactate and plasma levels measured during testing by using a blood lactate analyser

- I am aware of any risks that may be involved with the project.

- All information and data collected will be held securely at the University indefinitely. The results of the study may be published but my anonymity will be preserved.

Signed:……………………………… (Participant)   Date: ………………

CONSENT FORM

TO BE COMPLETED BY PARTICIPANT

NAME:…………………………………………….(Participant)

I have read the Information Sheet concerning this project and understand what it is about. All my further questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

-        My participation in the project is entirely voluntary and I am free to
         withdraw from the project at any time without disadvantage, prejudice or reason.
-        I will be required to attend 9 sessions to complete the project.

As part of the study I will have to:
   • Perform a Maximal oxygen uptake (VO_{2MAX}) test.
   • Have body composition assessed using the Bod Pod
   • Perform 3 familiarisation sessions to understand how to use a non-motorised treadmill.
   • Perform a Peak Speed Assessment
   • Perform a newly validated soccer-specific simulation upon the non-motorised treadmill called the intermittent soccer performance test (iSPT) (2 x 45 min halves; 15 min break) 5 times in 30°C and 50% humidity after the following interventions:
   • Perform 1 control condition of iSPT in 18°C and 30% relative humidity.
   • Perform iSPT in three different types of extreme environments
        ○ Hot (30°C and 30% relative humidity)
        ○ Hypoxic (18°C and 30% relative humidity, 1,000m above sea level)
        ○ Hot-Hypoxic (30°C and 30% relative humidity, 1,000m above sea level)
   • Have rectal temperature measured using a rectal thermometer.
   • Give a sample of urine prior to each test so that hydration can be measured.
   • Have skin temperature measured in four areas of the body (Chest, upper arm, Thigh and Calf).
   • Have blood lactate and plasma levels measured via a finger prick blood sample
-        I am aware of any risks that may be involved with the project.

-        All information and data collected will be held securely at the University indefinitely. The results of the study may be published but my anonymity will be preserved.

Signed:………………………………. (Participant)   Date: ………………

CONSENT FORM

TO BE COMPLETED BY PARTICIPANT

NAME:……………………………………………….(Participant)

I have read the Information Sheet concerning this project and understand what it is about. All my further questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

- My participation in the project is entirely voluntary and I am free to withdraw from the project at any time without disadvantage, prejudice or reason.
- I will be required to attend 9 sessions to complete the project.

As part of the study I will have to:
- Perform a Maximal oxygen uptake (VO$_{2MAX}$) test.
- Have body composition assessed using the Bod Pod
- Perform 3 familiarisation sessions to understand how to use a non-motorised treadmill.
- Perform a Peak Speed Assessment
- Perform a newly validated soccer-specific simulation upon the non-motorised treadmill called the intermittent soccer performance test (iSPT) (2 x 45 min halves; 15 min break) 5 times in 30°C and 50% humidity after the following interventions:
- Perform 1 control condition of iSPT in 30°C and 50% humidity with no pre-cooling.
- Perform 4 different types of pre-cooling 30 min prior to iSPT and for 15 min during half time.
  - Ice Slurry (7.5 g/kg of body mass)
  - Ice Packs
  - Combination (Ice Slurry and Ice Packs)
- Have rectal temperature measured using a rectal thermometer.
- Give a sample of urine prior to each test so that hydration can be measured.
- Have skin temperature measured in four areas of the body (Chest, upper arm, Thigh and Calf).
- Have blood lactate and plasma levels measured via a finger prick blood sample
- I am aware of any risks that may be involved with the project.

- All information and data collected will be held securely at the University indefinitely. The results of the study may be published but my anonymity will be preserved.

Signed:………………………………… (Participant)   Date: ………………

Appendix C

Please read the following:

    a. Are you suffering from any known active, serious infection?
    b. Have you had jaundice within the previous year?
    c. Have you ever had any form of hepatitis?
    d. Have you any reason to think you may be HIV positive?
    e. Have you ever been involved in intravenous drug use?
    f. Are you a haemophiliac?
    g. Is there any other reason you are aware of why taking blood might be hazardous to your health?
    h. Is there any other reason you are aware of why taking your blood might be hazardous to the health of the technician?

Can you answer Yes to any of questions a-g? Please tick your response in the box below:

Yes ☐   No ☐

Small samples of your blood (from finger or earlobe) will be taken in the manner outlined to you by the qualified laboratory technician. All relevant safety procedures will be strictly adhered to during all testing procedures (as specified in the Risk Assessment document available for inspection in the laboratory).

I declare that this information is correct, and is for the sole purpose of giving the researcher guidance as to my suitability for the test.   Name   ……………………………………………   Signed   ……………………………………………   Date   ……………………………………………   If there is any change in the circumstances outlined above, it is your responsibility to tell the person administering the test immediately.

This Blood Sampling Form will be held in a locked filing cabinet in the Department of Sport and Exercise Science laboratories at the University for a period of one-three years. After that time all documentation will be destroyed by shredding.

Appendix D

INFORMATION SHEET

Research Title:

The Validity & Reliability of a non motorised treadmill based soccer simulation

Dear Participant,

Thank you for showing an interest in participating in the study. Please read this information sheet carefully before deciding whether to participate. If you decide to volunteer we thank you for your participation. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the aim of the project?

The purpose of the study is to test the validity and reliability on a new (yet to be named) soccer simulation protocol. The development of this protocol will then allow for an improvement into how soccer performance is assessed accurately. The research could also develop into further studies researching into possible interventions at how performance can be improved. This study is being undertaken as part of the requirements of a MSc degree.

What type of participant is needed?

Participants will need to be between 18-40 years of age to take part in the study. Each participant must take part in a team sport (preferably soccer) or other team sorts that require an intermittent activity profile. Participant must also be injury free before taking part in the study so they can perform maximally in each condition.

What will participants be asked to do?

The participant will be asked to complete testing on 6 separate days. The first day will consist of a VO2max and lactate threshold test. Participants will complete these tests until they have reached exhaustion. The second testing day will be 3-4 days later which will consist of an intermittent Yo-Yo test. The participant will again be asked to run until they have reached exhaustion. 3-4 days later on the third testing date the participant will be asked to complete three familiarisation sessions so the participant can get used to running on the non-motorised treadmill (NMT). 3-4 days later a peak speed assessment test will be used so the top speed of each participant on the NMT can be found. This will be used to work out the individual speed thresholds for each principle (e.g. walk, jog, run etc). A 4th familiarization will also be completed on this day consisting of 45 minutes of the testing protocol. 7 days later the participant will complete the first of the soccer simulation testing protocols. The testing protocol will consist of 2x45 minute halves separated by a fifteen minute break. 7 days later a second simulation test will be completed for reliability and validity purposes.

What are the possible risks of taking part in the study?

During testing several physiological and performance variables will be measured. Core temperature will be measured using a rectal thermometer. The rectal thermometer method will be placed 10cm into the rectum however when placing this, the participant will be asked to leave the toilet door unlocked in case of an anaphylactic shock.

Blood samples to measure blood lactate and plasma levels will also be taken during the testing process. Before any blood samples are taken each participant will be required to fill in a blood analysis consent form. All blood will be taken in a clean and sterile environment to avoid the chance of infection. A first aider will also be near in case the participant feels faint or dizzy

during the collection. After the collection of blood, all wounds will be treated until bleeding has stopped. The area will then be covered to minimize the chance of infection at the area.

A urine sample will also be taken before testing to test hydration levels of the participant. The urine will be collected in a sterile container and will then be removed in a safe manner.

Participants will also be asked to exercise maximally at times during the testing period which can cause a reaction from the participant. To help with this each participant will be supplied with water before and after exercise to help restore hydration levels. A warm up and cool down will be used before and after testing to help reduce the chances of muscular and joint injury.

What if you decide you want to withdraw from the project?
If, at any stage you wish to leave the project, then you can. There is no problem should you wish to stop taking part and it is entirely up to you. There will be no disadvantage to yourself should you wish to withdraw.

What will happen to the data and information collected?
Everyone that takes part in the study will receive their own results for the tests that they complete. All information and results collected will be held securely at the University of Bedfordshire and will only be accessible to related University staff. Results of this project may be published, but any data included will in no way be linked to any specific participant. Your anonymity will be preserved.

What if I have any questions?
Questions are always welcome and you should feel free to ask myself Jeff Aldous or Lee Taylor (Director of Study) any questions at anytime. See details below for specific contact details.

Should you want to participate in this study then please complete the attached consent form, which needs to be returned before commencing the study.

This project has been reviewed and approved by the Ethics Committee of the Department of Sport and Exercise Sciences.

Many Thanks,

Jeff Aldous (BSc) Hons   Dr Lee Taylor
MSc Research Student   Email lee.taylor@beds.ac.uk
Institute of Sport and Physical Activity (ISPAR)   ,
University of Bedfordshire
Bedford Campus,
Polhill Avenue,
Bedford
Tel: 07749030047
Email: Jeffrey.aldous@beds.ac.uk

INFORMATION SHEET

Research Title:
Quantify the influence of extreme environments (hypoxia, hypo- and hyper-thermic) on soccer specific physiological and performance capacity utilising a validated soccer NMT protocol

Dear Participant,

Thank you for showing an interest in participating in the study. Please read this information sheet carefully before deciding whether to participate. If you decide to volunteer we thank you for your participation. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the aim of the project?
The purpose of the study is to test the effects of different environmental conditions (Hot, cold and hypoxic exposure) on soccer specific physiological and performance capacity utilising a newly validated soccer NMT protocol This study is being undertaken as part of the requirements of a PhD.

What type of participant is needed?
Participants will need to be between 18-40 years of age to take part in the study. Each participant must take part in a team sport (preferably soccer) or other team sorts that require an intermittent activity profile. Participant must also be injury free before taking part in the study so they can perform maximally in each condition.

What will participants be asked to do?
The participant will visit the sport science laboratory at University of Bedfordshire on 7 occasions. The first visit will consist of a VO_{2max}. Participants will exercise until they have reached exhaustion.

Participants will make there second visit to the laboratory 3-4 days later. Participant will complete three familiarisation sessions to familiarize themselves with running on the non-motorised treadmill (NMT).

The participants will then visit the Laboratory 3-4 days later for their third visit where a peak speed assessment test will be used so the top speed of each participant on the NMT. This will be used to assess the individual speed thresholds for each principle (e.g. walk, jog, run etc). A 4th familiarization will also be completed on this day consisting of 45 minutes of the testing protocol.

Seven days later the participant will complete the final familiarisation session. This consists of 2x45 minute halves separated by a fifteen minute half time break.

Participants will then complete three separate tests each a minimum of 7 days between tests. Testing will be completed in a hot condition at 30ºC and 50% humidity, in a cold condition at 0ºC and 50% humidity and in a hypoxic condition at an altitude of 2,980m equating to an oxygen concentration of 14.5% at barometric pressure of 775 mmHg. at 18ºC and 50% humidity.

What are the possible risks of taking part in the study?

When completing a VO2max test it will require participants to exercise maximally creating some discomfort. A Defibrillator trained person (Jeff Aldous) will be present during all testing especially maximal testing. Water will be supplied before and after exercise to help restore hydration levels. A warm up and cool down will be used before and after testing to help reduce the chances of muscular and joint injury.

Core body temperature will also be analysed throughout the testing protocol. A heart rate monitor must be attached prior to using a rectal thermometer. This can be helpful for the experimenter to diagnose and assess the severity of anaphylactoid shock should it occur. If the participant's core temperature reaches 39.7°C or increases by 2°C or decreases by 1.5°C from their resting value then testing will be terminated.

Blood samples to measure blood lactate and plasma levels will be taken. Before blood samples can be taken each participant will be required to fill in a blood analysis consent form. All blood will be taken in a clean and sterile environment to avoid the chance of infection. A first aider will also be near in case the participant feels faint or dizzy during the collection of blood however all blood collection will happen whilst the participant is seated. After the collection of blood, all wounds will be treated until bleeding has stopped. They will then be covered to stop infection of the area.

When performing in the heat several heat illnesses could be apparent (e.g. Heat Cramps, Heat Syncope, Heat Exhaustion, Heat Stroke). If one of these illnesses becomes apparent then further measures will be taken to treat the illness. A urine refractometer will be used to measure the hydration levels.

If core temperature decreases to the level suggested above the participant will be removed from the environmental chamber and will be given warm clothing to wear and a hot beverage to drink. They will be kept under supervision until they have reached their resting core temperature.

Participants will exercise in an altitude During hypoxic exposure there is potential risk of altitude sickness. If these symptoms involved (headache, dehydration, drowsiness, vomiting) appear, testing will be terminated and the participant will be removed from the environmental chamber immediately.

What if you decide you want to withdraw from the project?
Participants are able to withdraw from the study at any time without a reason. There will be no disadvantage or prejudice to yourself should you wish to withdraw.

What will happen to the data and information collected?
Everyone that takes part in the study will receive their own results for the tests that they complete. All information and results collected will be held securely at the University of Bedfordshire and will only be accessible to related University staff. Results of this project may be published, but any data included will in no way be linked to any specific participant. Your anonymity will be preserved.

What if I have any questions?
Questions are always welcome and you should feel free to ask myself Jeff Aldous or Lee Taylor (Director of Study) any questions at anytime. See details below for specific contact details.

Should you want to participate in this study then please complete the attached consent form, which needs to be returned before commencing the study.

This project has been reviewed and approved by the Ethics Committee of the Department of Sport and Exercise Sciences.

Many Thanks,

Jeff Aldous (BSc) Hons   Dr Lee Taylor
MPhil Research Student   Email lee.taylor@beds.ac.uk
Institute of Sport and Physical Activity (ISPAR)   ,
University of Bedfordshire
Bedford Campus,
Polhill Avenue,
Bedford
Tel: 07749030047
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INFORMATION SHEET

Research Title:
To quantify the influence that Pre-cooling has in hot, humid conditions prior to and at half time on soccer-specific physiological and performance capacity whilst utilising a validated soccer non-motorised treadmill protocol (intermittent soccer performance test - (iSPT))

Dear Participant,

Thank you for showing an interest in participating in the study. Please read this information sheet carefully before deciding whether to participate. If you decide to volunteer we thank you for your participation. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the aim of the project?
The purpose of the study is to quantify the influence that three methods of pre-cooling (Internal: Ice Slurry; External: Ice packs and Combination: Ice Slurry and Ice Packs and Paracetamol ingestion) will have upon soccer-specific physiological and performance capacity whilst utilising a newly validated soccer non-motorised treadmill protocol (intermittent soccer performance test (iSPT)). This study is being undertaken as part of the requirements of a PhD.

What type of participant is needed?
You will need to be aged between 18-40 years to volunteer in the study and must take part in a team sports which require an intermittent activity profile (e.g. soccer, rugby, hockey etc.). You must also be injury free before taking part in the study so they can perform maximally in each condition.

What will participants be asked to do?
You will visit the sport science laboratory at University of Bedfordshire on nine separate occasions. The first visit will consist of a body composition test and then a Maximal Oxygen Uptake (VO_{2max}) test on a motorized treadmill where you will be asked to exercise to exhaustion. Before completing a BodPod the participant must come to labs fasted over night to get an accurate assessment of the participant's lean body mass.

On the second, third and fourth visits to the laboratory, participants will complete three familiarisation sessions to familiarise themselves with running on the non-motorised treadmill (NMT). On the second visit to laboratory participants will complete three separate protocols on the NMT 9, 13 and 15 min in duration 4 days after completing the VO_{2MAX} test. Seven days later on the third visit you will complete a peak speed assessment test will be used so the top speed of each participant on the NMT. This will be used to assess the individual speed thresholds for each principle (e.g. walk, jog, run etc). Following this you will then also complete a 45 minute protocol for familiarisation.

The fourth visit, seven days later the you will complete the final familiarisation session. This consists of the iSPT protocol 90 minutes in duration consisting of two 45 minute halves separated by a fifteen minute half time break.

During the fifth, sixth, seventh, eighth and ninth visits to the laboratory, you will then complete the iSPT protocol in a hot and humid condition at 30°C and 50% humidity. Prior to each visit you will complete different types of pre-cooling for 30 min before the protocol and during the 15 min period of half time.

The four different types of pre-cooling will be

1) Control (no pre-cooling)
2) Flavoured Ice Slurry
3) Ice Packs
4) Combination (Ice Slurry and Ice Packs)
5) Paracetamol ingestion

What are the possible risks of taking part in the study?

When completing a VO2max test it will require participants to exercise maximally creating some discomfort. A Defibrillator trained person (Jeff Aldous) will be present during all testing especially maximal testing. Water will be supplied before and after exercise to help restore hydration levels. A warm up and cool down will be used before and after testing to help reduce the chances of muscular and joint injury.

Rectal temperature will also be analysed throughout the testing protocol. A heart rate monitor must be attached prior to using a rectal thermometer. This can be helpful for the experimenter to diagnose and assess the severity of anaphylactoid shock should it occur. If your rectal temperature reaches 39.7°C or increases by 2°C then testing will be terminated. During all pre-cooling rectal temperature will also be measured for the participant's safety. If the rectal temperature of the participant decreases by 1.5°C or falls below 35°C from their resting value then testing will be terminated.

Blood samples to measure blood lactate and plasma levels will be taken via finger prick collected into capillary tubes. All bloods will be taken in a clean and sterile environment to avoid the chance of infection. A first aider will also be near in case you feel faint or dizzy during the collection of blood; however, all blood collection will happen whilst you are seated. After the collection of blood, all wounds will be treated until bleeding has stopped and then covered to stop infection of the area.

When exercising in hot and humid conditions there are risks of heat illnesses being apparent (e.g. Heat Cramps, Heat Syncope, Heat Exhaustion, Heat Stroke). These illnesses will be prevented by ensuring you are hydrated for all testing measures, however, if one of these illnesses becomes apparent then further measures will be taken to treat the illness.

Paracetamol is the most commonly used non-prescription pain killer in the UK and is considered one of the safest pain killers at standard doses. Very rarely, side-effects do occur, with allergic reactions ranging from minor rashes to anaphylactic shock (a severe allergic reaction). The risks of using paracetamol do not increase with exercise and are unlikely to occur if paracetamol has safely been used in the past. In the interests of your safety, you will be asked to complete a paracetamol risk assessment questionnaire accompanying this information sheet and will be asked to not drink alcohol or ingest caffeine 48 hours before testing. You will also be asked not to take paracetamol or any paracetamol containing product 24 hours before testing.

The coldness of the ice slurry could potentially cause sphenopalatine ganglioneuralgia (brain freeze) or gastrointestinal discomfort during or after ingestion, if you experience either of these symptoms then you need to alert the researcher so they can monitor these symptoms, if gastrointestinal discomfort continues; ingestion of the ice slurry is stopped.

The coldness of the ice packs could also potentially cause frost nip. If signs of frost nip begin to show then the researcher will terminate the testing quickly and this will be treated by a first aider.

What if you decide you want to withdraw from the project?
Participants are able to withdraw from the study at any time without a reason. There will be no disadvantage or prejudice to yourself should you wish to withdraw.

What will happen to the data and information collected?
Everyone that takes part in the study will receive their own results for the tests that they complete. All information and results collected will be held securely at the University of Bedfordshire and will only be accessible to related University staff. Results of this project may be published, but any data included will in no way be linked to any specific participant. Your anonymity will be preserved.

What if I have any questions?
Questions are always welcome and you should feel free to ask myself Jeff Aldous or Lee Taylor (Director of Study) at any time. See details below for specific contact details.

Should you want to participate in this study, please complete the attached consent form, which needs to be returned before commencing the study.

This project has been reviewed and approved by the Ethics Committee of the Department of Sport and Exercise Sciences.

Many Thanks,

Jeff Aldous (BSc) Hons   Dr Lee Taylor
MPhil Research Student   Email lee.taylor@beds.ac.uk
Institute of Sport and Physical Activity (ISPAR)   ,
University of Bedfordshire
Bedford Campus,
Polhill Avenue,
Bedford
Tel: 07749030047
Email: Jeffrey.aldous@beds.ac.uk

Appendix E

Honestly answer these few questions before performing the experimental tests.

Name: ………………………….

- Have you consumed alcohol or caffeine in the last 24 hours?

  YES   NO

- Have you taken part in exhaustive exercise in the last 24 hours?
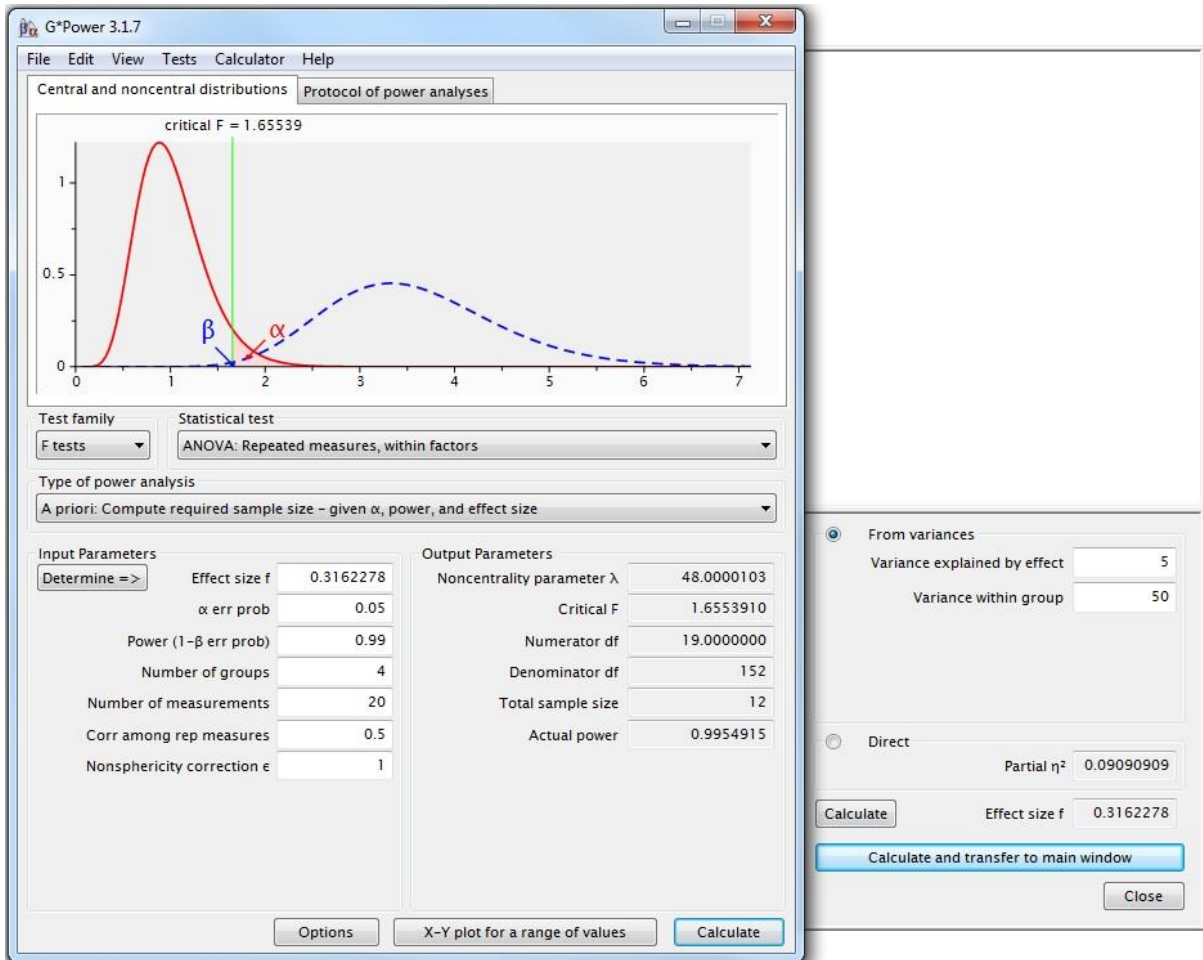
  YES   NO

- Have you had any prolonged thermal exposures (baths, saunas, steam rooms etc.) seven days prior to the testing date
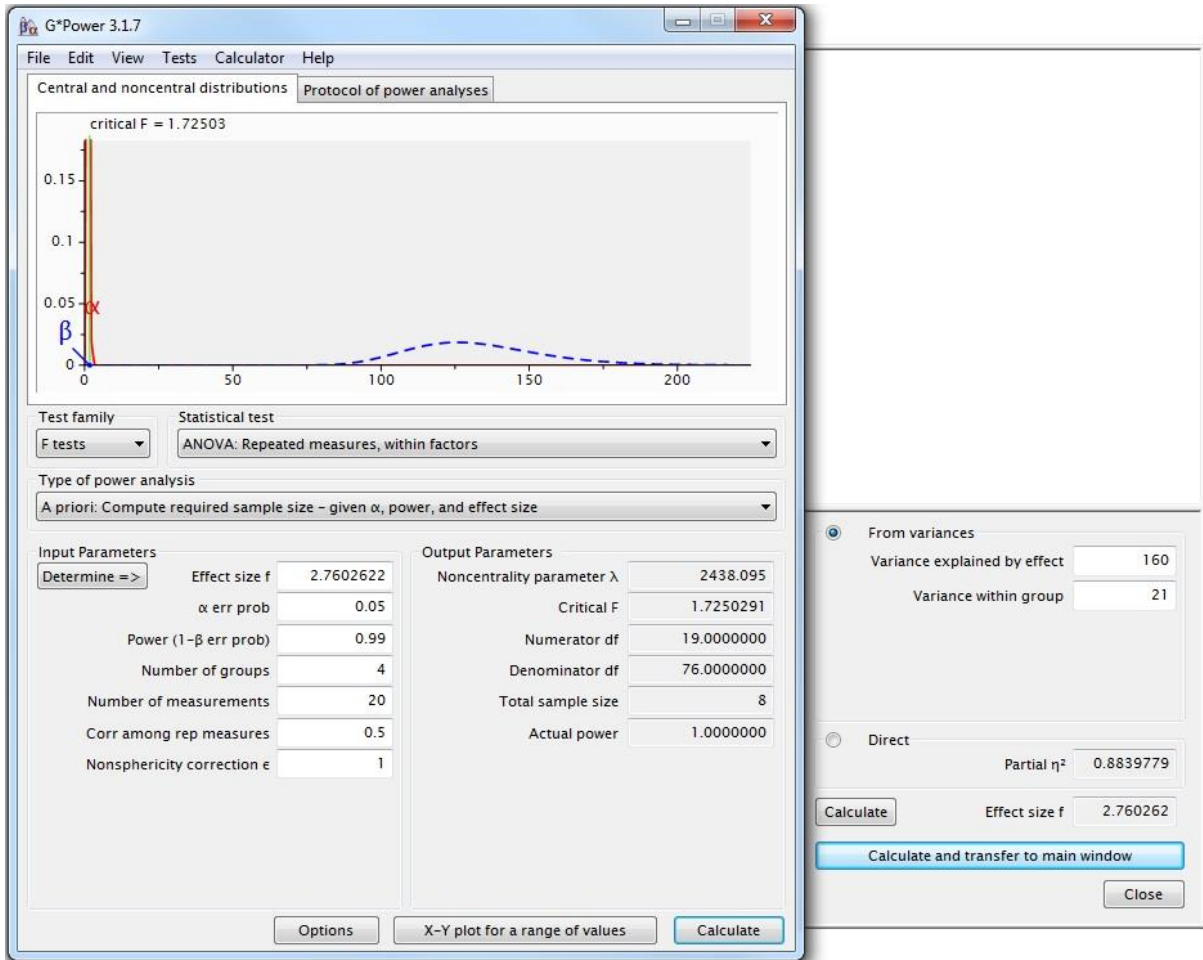
  YES   NO

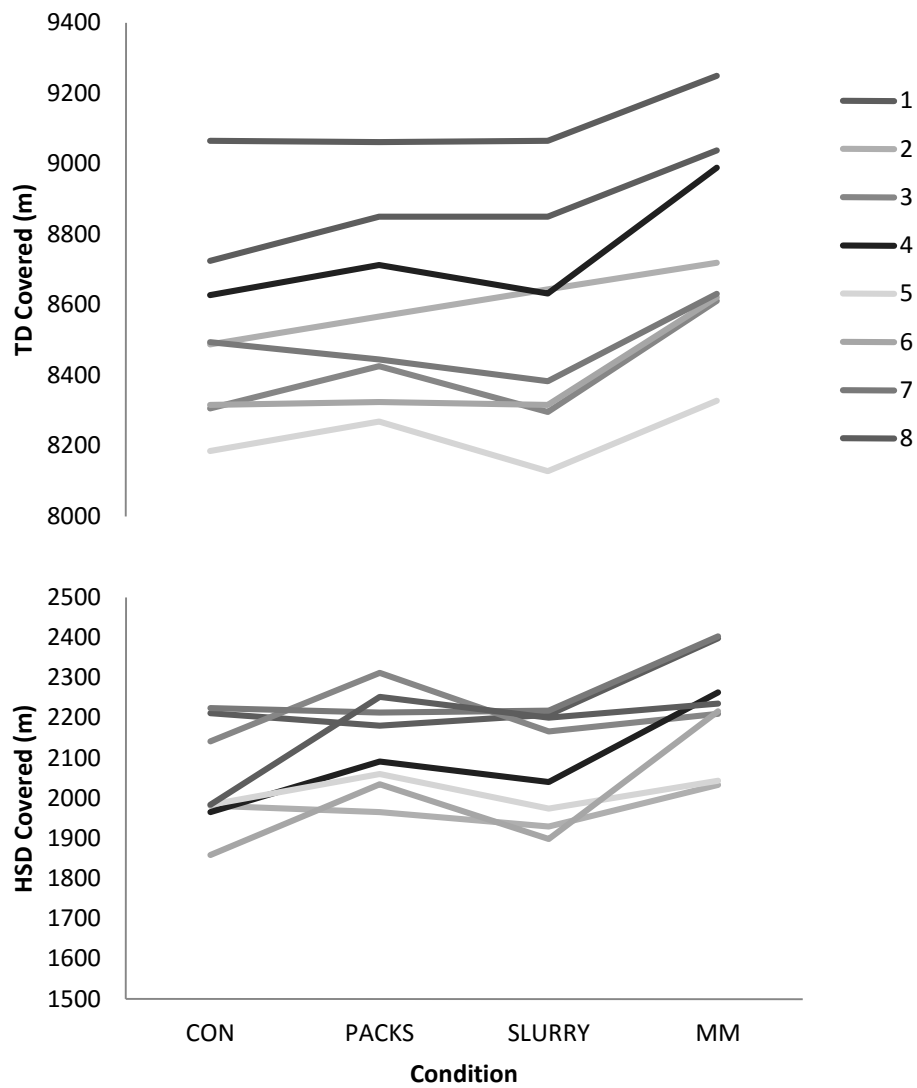- Have you experienced high pressure environments (e.g. hyperbaria) three months prior to the testing date

  YES   NO

Appendix F

Appendix G

Appendix H – The Individual responses for Total Distance (TD) and high-speed distance (HSD) covered in CON, PACKS, SLURRY and MM in experimental chapter 3

Appendix I – The Individual responses for variable run distance (VRD) and sprint distance (SD) covered in CON, PACKS, SLURRY and MM in experimental chapter 3