Does ice slurry ingestion influence performance and mucosal immunity following intermittent exercise in a hot environment?

Ryan J. Morrow

A thesis submitted to Auckland University of Technology in partial fulfillment of the requirements for the degree of Master of Sport and Exercise (MSpEx)

School of Sport and Recreation, Faculty of Health and Environmental Sciences

Abstract

Mucosal immunity provides initial defence against upper respiratory tract infections (URTI). Recent literature suggests that salivary secretory immunoglobulin–A (S-IgA) could be used to help indicate an athlete's risk of URTI. Football, a physically demanding intermittent sport often played in hot environments, has shown to place strain on the salivary immune system during heavy competition periods. Performing exercise in hot conditions can result in reductions in salivary S-IgA concentrations and provides a further stress on immune response. Pre-cooling an athlete prior to exercise reduces the level of stress, and helps delay fatigue. The effect pre-cooling prior to exercise in heat has on mucosal immunity is currently unknown. The purpose of this study was to determine the effect pre-cooling ice slurry ingestion had on mucosal immunity and intermittent exercise performance in hot environments.

In a randomised crossover design, 8 semi-professional football players completed two trials of the intermittent soccer performance test (ISPT) in a heat chamber, set to 30°C and 50% relative humidity. Participants consumed, at a standardised rate, 7.5g·kg^{-1} of ice slurry beverage or control fluid, with a further 2g·kg^{-1} provided at half-time. Unstimulated whole saliva samples were collected at baseline, pre-exercise, half-time, post-exercise, and 1 h post-exercise. Ice slurry ingestion significantly lowered core temperature prior to exercise and showed a *possibly beneficial* effect in end of exercise core temperature, perceived comfort and exertion scores, mean sprint speed, and distance covered in the last 15 min of exercise. Ice slurry ingestion showed *possibly harmful* effects on salivary S-IgA concentration and secretion rate prior to exercise. The salivary S-IgA concentration and secretion rate decreases were short lived as post-exercise values returned to baseline levels. Ice slurry ingestion may cause a transient drop in salivary immune response after consumption which as sport scientists is important to note, however, it doesn't appear to influence S-IgA following exercise when the open window period is most apparent.

In conclusion, the findings of this thesis show that ingestion of ice slurry may be beneficial for intermittent performance, and aid an athlete's perceived difficultly and thermal comfort. Ice slurry ingestion does not influence post-exercise salivary immune response, however, provides an effective method of pre-cooling an athlete prior to exercise without impairing performance or inducing gastric discomfort.

Key words: Pre-cooling, Ice Slurry, Salivary S-IgA, Football, Intermittent performance

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ANOVA	analysis of variance
BM	body mass
CHO	carbohydrate
CV	coefficient of variation
g	gram
h	hour
HR	heart rate
ISPT	intermittent soccer performance test
kg	kilogram
kJ	kilojoule
km	kilometer
L	litre
LIST	loughborough intermittent shuttle test
m	meter
mg	milligram
min	minute
ml	milliliter
mmol	millimole
N	newtons
nmol	nanomole
NMT	non-motorised treadmill
pIgR	polymeric immunoglobulin receptor
PSA	peak speed assessment
RER	respiratory exchange ratio
RPE	ratings of perceived exertion
s	second
SAFT90	soccer specific aerobic field test
SD	standard deviation
S-IgA	salivary secretory immunoglobulin A
SMS	soccer match simulation
SWC	smallest worthwhile change
T-bicep	bicep skin temperature
Tbody	body temperature
TC	thermal comfort
T-calf	calf skin temperature
T-chest	chest skin temperature
T-mu	muscle temperature
T-quad	quadriceps skin temperature
Tsk	skin temperature

URTI	upper respiratory tract infection
VO_{2peak}	peak oxygen uptake
y	years
°C	degrees Celsius
µg	microgram
µl	microliter
µmol	micromole

Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgments), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Signed: 

Date: 26/10/17

Acknowledgements

To my primary supervisor, Dr Deborah Dulson, thank you for your advice and guidance, especially appreciated in such a busy time of your life. Thank you for showing me the immunology side of physiology, your knowledge and help in this area was crucial through this journey. I wish you and your family all the best and a healthy future.

To my secondary supervisor, Associate Professor Andrew Kilding, your help and guidance are also hugely appreciated. Thank you for helping me figure out what academic path I want to pursue and for getting me there.

To my amazing participants, I can't explain how grateful I am for your efforts during this study. Thank you so much for donating your time, for being a great group of guys, and drooling into a container for me on multiple occasions. I appreciate how hard the protocol was and that some parts of the testing were not enjoyable but your willingness to stick in there, has meant I am where I am today. Thank you.

To all of the academics and support staff that helped me through this time. To Fabrice Merien, thank you for assistance and expertise in immunology. I really appreciate the time you took out your busy schedule to do the cortisol testing for this thesis and thank you for your advice around future projects. To Jono Neville, thank you for creating the program that processed my raw performance data. The program saved me hours of tedious work and potentially my sanity. To Allan Carman, thank you for all of your troubleshooting abilities. I also really appreciate your time and help in moving the Woodway treadmill into the heat chamber, a much bigger task than I expected.

To the Auckland University of Technology Ethics Committee for granting ethical approval -17th May 2011; 11/73.

To my immediate family; Darren, Marilyn, and Bayley, thank you so much for your endless support. I wouldn't have got through this process without you or your encouragement at Sunday dinners. To my extended family and friends, wish I could mention you all. Thank you all so much for your support and for keeping me sane through this process.

To my partner, Reane, I can't thank you enough for your support through this thesis. You kept me focused, listened, and supported through the difficult patches and frustrations. You also cooked meals and did life admin so I could write, and kept me laughing through the whole process. Words can't explain how appreciative I am. I will continually strive to support you in the same manner you have supported me.

Intellectual Property Rights

Ethical Approval

Ethical approval for the studies undertaken in this thesis was granted from Auckland University of Technology's Ethics Committee (AUTEC) on 1 June 2016; Ethics Application Number: 16/148.

Thesis overview

This thesis adheres to pathway 1, as classified by AUT University post-graduate thesis structure guidelines (AUT Post Graduate handbook 2017). This thesis consists of five chapters. Chapter One provides an introduction to the thesis topic. Chapter Two (Review of Literature), introduces the reader to mucosal immunity and how different exercise modes affect these measures. Football demands and team sport simulations are discussed in this section. Internal pre-cooling methods are discussed and the application to sports performance. Finally the application of pre-cooling on mucosal immunity is examined. Chapter Three includes the study design and methodology. Chapter Four consists of the main results and statistical analysis of the thesis, with relevant tables and figures provided. Chapter Five provides a discussion around the study findings, alludes to the limitations, potential future research in the area, and applications to the sport physiology field.

Chapter 1: Introduction

To be successful in endurance and team-based sports, athletes are required to expose themselves to high training loads (intensity and volume) (Foster, Daines, Hector, Snyder, & Welsh, 1996; Stewart & Hopkins, 2000; Stølen, Chamari, Castagna, & Wisløff, 2005). However, high levels of intense training or competition load can negatively affect an athlete's health and ability to compete at the top level. Depending on the amount of exercise completed, physical activity can have both positive and negative effects on immune function and susceptibility to minor illnesses (Gleeson, 2007). The relationship between exercise and likelihood of infection has been described as a "J"- shaped curve (Nieman, 1994). This "J" shaped model suggests that, although partaking in moderate amounts of exercise may enhance immune function above sedentary levels, impaired immune function may occur with prolonged, highly intense exercise bouts (Gleeson, 2007). Examining an athlete's immunity provides an insight into the health of the athlete in response to acute bouts of exercise and prolonged training periods.

Mucosal immunity provides the first line of defence against upper respiratory tract infections (URTI) (Brandtzaeg et al., 1999); the most commonly reported illness among athletes (Fricker et al., 2000; Peters, 1997; Pyne & Gleeson, 1998). It has been suggested that over 90% of all infections occur in the mucosal system, being a portal of entry for microbial colonisation (Brandtzaeg, 2003). Mucosal secretions play an important role in innate immunity and provide a mechanical washing effect against foreign pathogens (Bishop, Blannin, Armstrong, Rickman, & Gleeson, 2000). These defence systems are mediated by secretory immunoglobulins, particularly IgA and IgM (Gleeson, Cripps, & Clancy, 1995; Mackinnon, 1996). Salivary S-IgA provides immediate protection on mucosal surfaces by the exclusion, neutralisation, and elimination of infectious viral pathogens (Gleeson, 2000). Salivary S-IgA prevents viral and bacterial material to replicate and attach itself to mucosal epithelium of the mouth, throat, and upper respiratory tract (Cunniffe et al., 2011). Salivary S-IgA has been proposed as the most promising immune marker for identifying those athletes at risk of upper respiratory illness (Neville, Gleeson, & Folland, 2008; Pyne & Gleeson, 1998; Shephard & Shek, 1998). Salivary S-IgA concentration changes can occur in response to both acute exercise sessions (Blannin et al., 1998; Gleeson et al., 1999; Mackinnon, Ginn, & Seymour, 1993) and through prolonged periods of intense training (Gleeson, Ginn, & Francis, 2000; Mackinnon, Chick, Van As, & Tomasi, 1989). Strenuous exercise in both moderately and highly trained athletes is commonly followed by reports of depressed mucosal immune response and upper respiratory symptoms (Gleeson et al., 2000).

Football is a physically demanding intermittent team sport. During match-play, players are highly taxed with mean and peak heart rates around 85% and 98% of maximum heart rate, respectively (Ali & Farrally, 1991; Bangsbo, 1993; Krustrup, Mohr, Ellingsgaard, & Bangsbo, 2005; Reilly & Thomas, 1976). Typical distance covered by a top-class outfield player during a match is 10 – 13 km, with midfield players covering greater distances than other outfield players

(Bangsbo, Mohr, & Krustrup, 2006). Elite football players perform 150 – 250 brief intense actions during a game (Mohr, Krustrup, & Bangsbo, 2003) indicating the high rate of anaerobic energy turnover at certain times (Bangsbo et al., 2006). Competitive football players are involved in long seasons which consist of weeklong microcycles with multiple trainings, tapering, recovery, and a competitive game. Football clubs can frequently have additional fixtures within a macrocycle due to local or international tournaments (Ispirlidis et al., 2008). The additional fixtures increase the injury risk, reduce performance due to fatigue, and increase muscle damage and/or inflammation (Parry-Billings & Newsholme, 1992).

Competitive football matches are commonly played in thermally challenging environments. Major football tournaments can be located in countries with high ambient temperatures, including the upcoming 2022 FIFA World Cup in Qatar, with temperatures recorded up to 45°C (Mohr, Nybo, Grantham, & Racinais, 2012). Mohr et al. (2010) reported high intensity running decreased markedly in the last 15 min of a football match played in moderately high environmental temperatures (>30°C). In this study, muscle temperatures of some players rose to an excess of 41°C at the end of the match. The findings of Mohr et al. (2010) indicate that the ability to perform high intensity running in the final stages of match-play may be heavily impacted by thermally challenging environments compared to cooler conditions. This period of play (last 15 min) is known to be a crucial period for game success, with more goals score in this period than in any other (Njororai, 2014). A reduced performance during the last 15 min of play could prevent a goal scoring opportunity or result in conceding a goal, game/tournament defining events for competitions performed in hot climates.

Performing exercise in thermally stressful environments produces greater disturbances on the immune system compared to heat exposure alone (Lim & Mackinnon, 2006; Shephard, Castellani, & Shek, 1998; Walsh & Whitham, 2006). Heat exposure is a further stress for athletes during exercise, elevating body core temperature with coinciding alterations in immune responses (Sari-Sarraf, Doran, Clarke, Atkinson, & Reilly, 2011). Performing prolonged exercise in the heat, with the concomitant increase in fluid loss, appears to result in large reductions in saliva flow rate (Horswill, Stofan, Horn, Eddy, & Murray, 2006). The reduction in salivary flow has been noted in other performance studies (Davison, 2011; Engels, Fahlman, Morgan, & Formolo, 2004; Sari-Sarraf et al., 2011) and is believed to be due to a drying of oral surfaces caused by intermittent exercise protocols (Sari-Sarraf et al., 2011). Sari-Sarraf et al. (2011) examined a football specific protocol in the heat on salivary S-IgA, finding that salivary total protein increased by 51% after the football specific intermittent exercise. The authors' results indicate that intermittent football specific exercise in thermally challenging environments may further strain the immune system and make athletes more susceptible to URTI. For the purpose of this thesis, URTI will be used when an infection was reported and clinically diagnosed, while upper respiratory symptoms (URS) will be used when individuals self-report an infection. Contracting an URTI can be hugely detrimental to an athlete's training regime and if acquired around major events, the illness can greatly reduce performance and may prevent an athlete from competing.

Sport scientists have looked at combating the extra strain caused by heat through acclimation and cooling the body in the lead up to competition in hot environments. Various pre-cooling methods have been examined, such as; ice baths, cool water immersion, placement of ice packs and ice slurries (as reviewed in Siegel and Laursen (2012)). Price, Boyd, and Goosey-Tolfrey (2009) examined the effect of ice vests worn pre-exercise and during half-time on 8 female soccer players, finding that it is an effective way to reduce thermal strain and offset heat storage during intermittent exercise in the heat (30.6°C, 63.4% RH). Zhang et al. (2014) found improvements in intermittent performance (Yo-Yo Intermittent Recovery Level 1), thermal comfort, and sweat rate via utilising two methods of partial skin cooling (forearm and hand cooling, and neck cooling) performed at a half-time interval.

Recently, the use of internal cooling strategies, such as ice slurries, have been investigated due to their practical nature, ability to lower core temperature, and have shown to increase athletic performance in hot environments. Ice slurries consist of millions of small ice particles within a liquid, which has a greater surface area than cool beverages, encouraging conductive heat transfer via creation of a 'heat sink'. The heat sink allows for greater heat storage, delaying the onset of critically high core temperatures that reduce or stop exercise performance. The ingestion of the ice is also believed to cool thermoreceptors in the mouth, oesophagus, and the abdominal region, allowing the body to feel cooler by afferent feedback. Siegel, Maté, Watson, Nosaka, and Laursen (2012) completed a cross over study examining ice slurry ingestion, cold water immersion and warm fluid ingestion on running time. Eight participants ran to exhaustion on three separate occasions following a pre-cooling method or control trial. The results indicated that participants ran significantly longer in the cold water immersion ($P = 0.008$) and ice slurry ingestion ($P = 0.005$) compared to the warm fluid trial. Rectal temperature at exhaustion was significantly higher following the ice slurry ingestion protocol compared to control and higher than cold water immersion. Siegel et al. (2012) concluded that ice slurry ingestion of 7.5 grams per kilogram of body mass ($g \cdot kg^{-1}$) can be used as an alternative to other pre-cooling protocols in prolonging submaximal running in the heat. The effect ice slurry ingestion has on intermittent performance has not been thoroughly examined in the literature. Aldous (2016) completed one of the only studies in this area, examining the effect of an internal cooling (ice slurry), external cooling (ice packs), and a combination pre-cooling method (ice slurry and ice packs) on stimulated intermittent soccer performance. The ice slurry ingestion method showed significant improvements in rectal temperature and thermal sensation prior to exercise. Aldous (2016) also found that the combination of ice slurry ingestion with placed ice packs (mixed method) significantly improved ($p<0.05$) total distance, high-speed distance and variable run distance covered by 3%, 4% and 5% during the 1st half of intermittent exercise.

To this authors knowledge, no research has examined the effect pre-cooling has on the mucosal system following exercise in the heat. Janský et al. (1996) examined the effect of single cold water immersions on the immune system of 10 young athletic males while at rest in a thermoneutral environment. The results indicated minimal effect on immunity after single water immersions. The continuation of water immersions (three times per week for six weeks)

resulted in small changes in immune response, with no significant changes found in salivary S-IgA concentrations. Janský et al. (1996) concluded that repeated cold water immersion caused shivering which increased metabolic rate and increased blood concentrations of catecholamines, activating the immune system to a slight extent. Although the effect internal precooling has on exercise performance has been investigated, it is unknown what the resulting changes in core temperature and increased performance does to the immune system. As intermittent exercise has previously been shown to decrease mucosal immunity markers, it is undetermined whether the improvements noted from ice slurry ingestion to intermittent exercise will provide subsequent improvements or further strain the mucosal immune system.

In conclusion, mucosal immunity provides the first line of defence against foreign pathogens and infections. Mucosal immunity can be negatively affected by prolonged highly intense bouts of exercise, further exacerbated by competing in hot and humid environments. Pre-cooling provides a method to reduce the heat stress on an athlete, however, further research needs to examine the effect that pre-cooling has on mucosal immunity.

Study aims

Therefore, the aim of this thesis was to determine the effect of ice slurry ingestion on mucosal immunity and intermittent football performance in the heat.

Study hypotheses

Established from the surrounding academic literature it was hypothesised that:

- Ice slurry ingestion could help reduce depression in salivary immune response caused by the exercise protocol and heated environment

- Ice slurry ingestion would result in a lower core temperature at the start of exercise and improve perceived thermal comfort

- Ice slurry ingestion would aid performance during intermittent soccer exercise.

- Exercise alone could be strenuous enough to cause a depression in saliva S-IgA secretion rate.

Significance of Thesis

The current thesis aimed to examine the effect of ice slurry ingestion on performance and mucosal immune response. These findings could provide insight on the performance effects of ice slurry ingestion prior to intermittent exercise in the heat. Additionally, this thesis could inform sports professionals/scientists of any positive or negative effects resulting from ice slurry ingestion on the salivary immune system and likelihood of contracting upper respiratory tract infections. Overall, this thesis aimed to provide further insights into the application of ice slurry ingestion, potential mechanisms involved, and the possible benefit to the athlete.

Section 2: Literature review

2.1 Demands of Football (soccer)

Football is a physically demanding sport, which is best described as intermittent in nature. Players are highly taxed with mean and peak heart rates around 85% and 98%, respectively (Ali & Farrally, 1991; Bangsbo, 1993; Krustrup et al., 2005; Reilly & Thomas, 1976). The average work rate for elite football players is approximately 70% of VO_{2peak} (Bangsbo, 1993), while performing 150 – 250 brief intense actions during a game (Mohr et al., 2003) indicating the high rate of anaerobic energy turnover at certain times (Bangsbo et al., 2006). Mean blood lactate concentrations of 2 – 10 mM have been observed during football games, with individual values above 12 mM (Bangsbo, 1993; Ekblom, 1986; Krustrup et al., 2005). These findings indicate that the rate of muscle lactate production is high during match-play, but muscle lactate is rarely measured in the literature (Bangsbo, 1993).

Competitive football seasons consist of weeklong microcycles with multiple trainings, tapering, recovery, and a competitive game. Clubs can frequently have additional competitive fixtures within a microcycle due to participation in local or international tournaments (Ispirlidis et al., 2008). The inclusion of 2 to 3 games per microcycle elevates the stress imposed on the players, thereby increasing potential risk of upper respiratory tract infections (URTI), injuries, performance decline due to fatigue, muscle damage and/or inflammation (Parry-Billings & Newsholme, 1992). Players must fully recover and be ready to compete for a full 90 min plus stoppage time in the subsequent game within 3 to 6 days (Ispirlidis et al., 2008).

The demands of football change with different positions in the team and roles in the tactical plan of the coach. Generally, the midfielders (2.23 ± 0.15 km), fullbacks (2.46 ± 0.13 km) and attackers (2.28 ± 0.14 km) cover a significantly greater ($P < 0.05$) distance in high intensity running than the defenders (1.69 ± 0.10 km) (Mohr et al., 2003), with midfielders engaged in significantly less time standing still and shuffling (Bloomfield, Polman, & O'Donoghue, 2007). A motion analysis of football players found that attackers and fullbacks cover greater distances when sprinting than midfield players and defenders (Mohr et al., 2003). Bloomfield et al. (2007) found that defenders performed the highest amount of jogging, skipping and shuffling movements and spent significantly less amount of time sprinting and running than of other positions. Midfielders and strikers also engaged in significantly more of the 'other' type of movements (jumping, landing, diving, sliding, slowing down, falling and getting up) with strikers performing the most of the three positions (Bloomfield et al., 2007). Strikers were also observed to have higher levels of stopping at high intensity as well as swerving and slowing more rapidly (Bloomfield et al., 2007). It has been observed in time motion analysis studies that the amount of high intensity running increases as the standard of competition rises, with high level professional players completing significantly more than those at a lower standard (Bangsbo, Nørregaard, & Thorsoe, 1991; Ekblom, 1986). Mohr et al. (2003) found that total distance covered by top class players was significantly more (10.86 ± 0.18 km) than players of moderate

ability (10.33 ± 0.26 km). Typical distance covered by a top-class outfield player during a match is between 10 to 13 km, with midfield players covering greater distances than other outfield players (Bangsbo et al., 2006). Mohr et al. (2003) found that top-class players complete 28% more high intensity running than moderate players, as well as 58% greater sprint distances compared to the same group. A motion analysis study found that the quality of opposition had an effect on the amount of high intensity running and distance covered during a match. Players cover greater distances against higher quality opponents compared to lower quality opponents (Rampinini, Coutts, Castagna, Sassi, & Impellizzeri, 2007). High intensity running distances change over the course of a football match with players reducing distance covered in the last 15 min, with distances 14 – 45% lower ($P < 0.05$) than in the first four 15 min periods (Mohr et al., 2003). Sprinting distance covered is also significantly reduced (43%) in the final 15 min of a football match compared to the initial 15 min (Mohr et al., 2003). Variations in total distance and high intensity distance covered by top-class players occur throughout a season, with peak values seen at the end of the season (Mohr et al., 2003). The physical load elite football players are exposed to during a match is important to understand. This knowledge allows sport scientists to develop strategies and provide interventions to help reduce fatigue, ensure full recovery, and improve performance during games.

In summary, football is a physically demanding sport, including various high intensity activities that are crucial for performance success. The demands of the sport vary according to playing position, level of competition, and elite athletes can compete in multiple games within a week. Success in the sport has a range of benefits, including being financially rewarding for players, clubs, and national sporting organisations. Academic literature has utilised various methods and interventions to examine how we might better understand and improve football performance.

2.2 Football performance exercise simulations

Due to the variations in match conditions and factors during football games, football match simulations are frequently used in the surrounding literature (Abt, Reaburn, Holmes, & Gear, 2003; Aldous et al., 2014; Ali, Foskett, & Gant, 2014; Russell, Benton, & Kingsley, 2011; Sirotic & Coutts, 2008; Slattery, Wallace, Bentley, & Coutts, 2012; Small, McNaughton, Greig, & Lovell, 2010). As no football game is exactly the same, it is difficult to establish whether an intervention has provided a benefit to the athlete. To examine any affects that different interventions may have on increasing physical performance in football, specific simulations are often utilised. Simulations can be either field or laboratory based.

The Loughborough Intermittent Shuttle Test (LIST) was designed as an indoor simulation representing the physical demands during a football game (Nicholas, Nuttall, & Williams, 2000). The first part of the simulation requires completing five-15 min exercise periods with recovery periods of 3 min. Each 15 min period consists of walking, sprinting, recovery, and running speeds corresponding to 55 and 95% of the individuals VO_{2peak}. Part two requires the participant to run at speeds corresponding to 55% and 95% of predicted VO_{2peak}, the speed alternating

every 20 m. The LIST protocol and match play data are similar in total distance covered, sprint duration, blood lactate, and heart rate values (Bangsbo, 1993; Nicholas et al., 2000; Rienzi, Drust, Reilly, Carter, & Martin, 2000). Several studies have utilised a modified LIST protocol including a ball passing component before and after the exercise blocks (McGregor, Nicholas, Lakomy, & Williams, 1999) and between blocks (Ali & Williams, 2009). Utilising a 90 min protocol is of importance as a plethora of studies have shown a reduction in physical performance during the second half (Bradley et al., 2009; Di Mascio & Bradley, 2013; Mohr et al., 2003), highlighting the importance of utilising two 45 min duration halves within a well formulated football specific simulation (Russell et al., 2011).

The soccer specific aerobic field test (SAFT90) (Small et al., 2010) is a multidirectional field-based football simulation involving backwards, sideward, and sidestepping movements while moving through field poles. Two 45 min halves are completed; each containing a 15 min activity profile repeated three times to match the physical demands of a football game. Verbal instructions are provided throughout the protocol to ensure each speed category is achieved. A limitation of this simulation is that the physical demands are of fixed distances not allowing performance differences between participants to be examined, only physiological responses. A similar limitation is found in the Soccer Match Simulation (SMS) designed by Russell et al. (2011). The simulation is a modified version of the LIST protocol, including football specific ball actions throughout the test. Participants in the SMS are required to complete passing, dribbling, and shooting skills throughout the two 45 min halves. Russell et al. (2011) found good validity in the time spent walking, sprinting, jogging, and striding between the SMS and match play.

Laboratory based exercise protocols allow for accurate analysis of performance and physiological measures in a controlled environment. Recently, laboratory based protocols have successfully used non-motorised treadmills (NMT) to individualise football specific simulations (Abt et al., 2003; Sear, Hoare, Scanlan, Abt, & Dascombe, 2010; Sirotic & Coutts, 2008). Individualisation from the peak sprint speed of an athlete allows a NMT based football specific simulation to be catered to the capability of each athlete, avoiding limitations seen in some earlier NMT simulations (Oliver, Armstrong, & Williams, 2007; Thatcher & Batterham, 2004). 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).

One study that successfully utilised individualised speed thresholds based upon peak sprint speed was by Abt et al. (2003), who created a 90 min football-specific simulation containing 6 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. Based off the Abt et al. (2003) protocol, Aldous et al. (2014) created a similar football specific protocol called the intermittent soccer performance test (ISPT). The ISPT included a unique self-selected speed category called the "variable run". This self-selected pace was utilised to examine high speed running, a key determinant of success in football performance (Gregson, Drust, Atkinson, & Salvo, 2010). The variable run provides insight to the athlete's willingness to complete high

intensity running which may be vital in key moments in football games. Aldous et al. (2014) stated that the ISPT was a valid and reliable football specific simulation. Variable run speed was also shown to be successful at indicating decrements in high speed running capability (Aldous et al., 2014). The ability of the ISPT to indicate high speed decrements, while being football specific, would provide insight into the effectiveness of intervention studies. The football simulation may also provide insights into fatigue related performance variations and athlete health with relevant monitoring.

2.3 Mucosal immunity

Mucosal immunity provides the first line of defence against upper respiratory tract infections (Brandtzaeg et al., 1999); the most commonly reported illness amongst athletes (Fricker et al., 2000; Peters, 1997; Pyne & Gleeson, 1998). It has been suggested that over 90% of all infections occur in the mucosal system, being a portal of entry for microbial colonisation (Brandtzaeg, 2003). The mucosal immune system is the largest immune network in the body, this network is comprised of many different mucosal associated lymphoid tissues (MALT), containing the highest number of immune cells in the body (Williams, 2011). The sheer magnitude of these defences is easier to comprehend if you consider that the surface area of the human mucosa is around 400 m^2, roughly equivalent to the size of a basketball court. In contrast, the surface area of another important defensive structure, the skin, is only 1.8 m^2 (Gleeson, Bishop, & Walsh, 2013). A MALT is defined by the secretion of mucous across an epithelial layer and the ability to contribute to immune defence (Williams, 2011), which includes gut-associated lymphoid tissue, nasal-associated lymphoid tissue, bronchial/tracheal-associated lymphoid tissue and salivary glands (Bishop & Gleeson, 2009). Mucosal secretions play an important role in innate immunity and provide a mechanical washing effect against foreign pathogens (Bishop et al., 2000). This is achieved via a process starting with the humoral arm of the immune system where circulating B-cells differentiate to become antibody-producing B plasma cells (Gleeson, McFarlin, & Flynn, 2006). These cells are capable of producing 5 antibody isotypes; immunoglobulin (Ig) A, G, M, D and E, depending on which antigen is presented at the cell surface. These defence systems are mediated by secretory immunoglobulins, particularly antibody isotypes IgA and IgM (Gleeson, Cripps, et al., 1995; Mackinnon, 1996). IgA is the predominate immunoglobulin found in mucosa and is considered to provide the 'first line of defence' against pathogens and antigens presented at mucosal surfaces. Such salivary biomarkers have been favoured in literature because of their non-invasive fashion (Lindsay et al., 2015). Peñailillo, Maya, Niño, Torres, and Zbinden-Foncea (2015) state that although blood collection has traditionally been used to detect hormonal and immune response to exercise, recent studies have used saliva samples as they offer many advantages as compared to blood sampling. Saliva is constantly being produced, samples can be obtained over short periods and be acquired in the field without a skilled healthcare professional, eliminating the risk of needle stick injuries and causing only minimal distress to the individual (Peñailillo et al., 2015).

2.3.1 Saliva composition

Saliva is a vital component of the mucosal immune system, with approximately 750-1500 ml secreted each day (Bishop & Gleeson, 2009). Saliva is a transparent fluid, consisting primarily of water (>99%), with a density ranging from 1002 to 1012 $g \cdot L^{-1}$ (Schneyer, Young, & Schneyer, 1972) and a pH of approximately 6.64, which is affected by circulating blood CO_2 levels (Kreusser, Heidland, Hennemann, Wigand, & Knauf, 1972). Saliva is composed of a number of different components; including antibodies, enzymes, acids, peptides, hormones, mucus and antibacterial compounds (Chicharro, Lucía, Pérez, Vaquero, & Ureña, 1998). Typically, concentrations of these compounds are lower in saliva than in the blood (Schneyer et al., 1972), however, specific proteins, such as S-IgA, are synthesised in the salivary glands and tend to have higher levels in saliva than found in blood.

Saliva secretion is produced by the sub-maxillary, parotid, sub-lingual and numerous minor mucous glands found in the oral cavity (Dawes, 1974). Although largest of the major salivary glands, the parotid glands, only contribute to around 25% of secreted saliva. The majority of saliva production occurs through the sub mandibular glands, accounting for 70% and the sublingual glands around 5% (Gleeson et al., 2013). Consistency of saliva depends on origin of production. Parotid saliva tends to be watery, whereas that from the sublingual glands has a higher mucous content (Gleeson et al., 2013). The submandibular secretion is a combination of the two consistencies. Regulation of saliva secretion is primarily controlled by the autonomic nervous system, with salivary glands being innervated by parasympathetic cholinergic and sympathetic adrenergic nerves that, unlike many other physiological systems in the body, act synergistically rather than antagonistically (Baum, 1987; Chicharro et al., 1998; Gleeson et al., 2013). Secretion rate of S-IgA represents the amount of S-IgA available on the mucosal surfaces for protection against pathogens, and is calculated by multiplying the concentration of S-IgA times saliva flow rate, the latter value being calculated by dividing the total of the saliva sample by the time taken to produce each sample.

Innervation of the different salivary glands also varies. Parasympathetic nerve innervation in the parotid and sublingual glands (Baum, 1987), produce a high volume of watery saliva that is low in protein content and this secretion is associated with a pronounced vasodilation of blood vessels supplying the gland, thought to be mediated by local release of vasoactive peptides (Gleeson et al., 2013). In contrast, sympathetic nerve innervation occurs in the sub-maxillary and minor mucous glands (Baum, 1987), is relatively low in volume and high in protein mainly owing to the increased exocytosis of salivary proteins from the salivary gland or associated cells (Proctor & Carpenter, 2007). Sympathetic activity, in contrast to parasympathetic activity, is associated with a vasoconstriction of blood vessels and has been suggested to reduce blood flow to the salivary glands and reduce salivary flow rate as a result (Chicharro et al., 1998). While normal saliva secretion is achieved by a combination of both parasympathetic and sympathetic innervation, parasympathetic activity provides the primary stimulus for increased saliva secretion (Schneyer et al., 1972). Given that intensive exercise is associated with enhanced sympathetic nervous system activation it seems logical to assume that strenuous

physical activity would modify secretion of saliva and its constituent proteins (Gleeson et al., 2013).

2.3.2 Salivary Secretory IgA

Salivary S-IgA is the main class of antibody present in the body secreted fluids such as saliva, tears, and mucus (Trochimiak & Hübner-Woźniak, 2012). It is produced by mature B cells (Macpherson, McCoy, Johansen, & Brandtzaeg, 2008) in the blood and is secreted into bodily fluids (Klentrou, Cieslak, MacNeil, Vintinner, & Plyley, 2002), such as saliva, tears, as well as nasopharyngeal, bronchial, intestinal and urogenital secretions (Gleeson, 2000; McGhee, Mestecky, Elson, & Kiyono, 1989), and penetrates freely through the mucous membrane. There are two subclasses of IgA: IgA1 and IgA2, with IgA2 the predominant subclass in saliva (Gleeson & Pyne, 2000). Secretory IgA is a dimeric molecule (Bishop & Gleeson, 2009), that underlies the mucosal epithelium which is largely secreted as dimers containing two conventional immunoglobulin subunits, each with two heavy and two light polypeptide chains, and an intersubunit J chain (Lamm, 1997). It also contains the epithelial-derived secretory component of the receptor (known as the polymeric immunoglobulin receptor or pIgR) that binds IgA and allows its transport across the mucosal epithelium. The pIgR is synthesised by mucosal epithelial and glandular cells and expressed on the basolateral (inside) membrane, where it is ideally placed to bind to locally produced IgA (and IgM) (Bishop & Gleeson, 2009). Transepithelial transport of the resulting pIgR-IgA complex occurs via endocytosis and vesicular transport to the apical (outside) cell membrane, where the pIgR component is proteolytically cleaved, leaving the external portion, secretory component, bound to IgA (Bishop & Gleeson, 2009; Gleeson et al., 2013; Lamm, 1997). Gleeson et al. (2013) states the remaining secretory component is not just an artefact of previous cellular transport; rather, the covalent binding of secretory component makes secretory IgA more resistant to protease degradation in secretions such as saliva (Gleeson et al., 2013). This secreted form of IgA on the apical surface is known as salivary S-IgA (Williams, 2011).

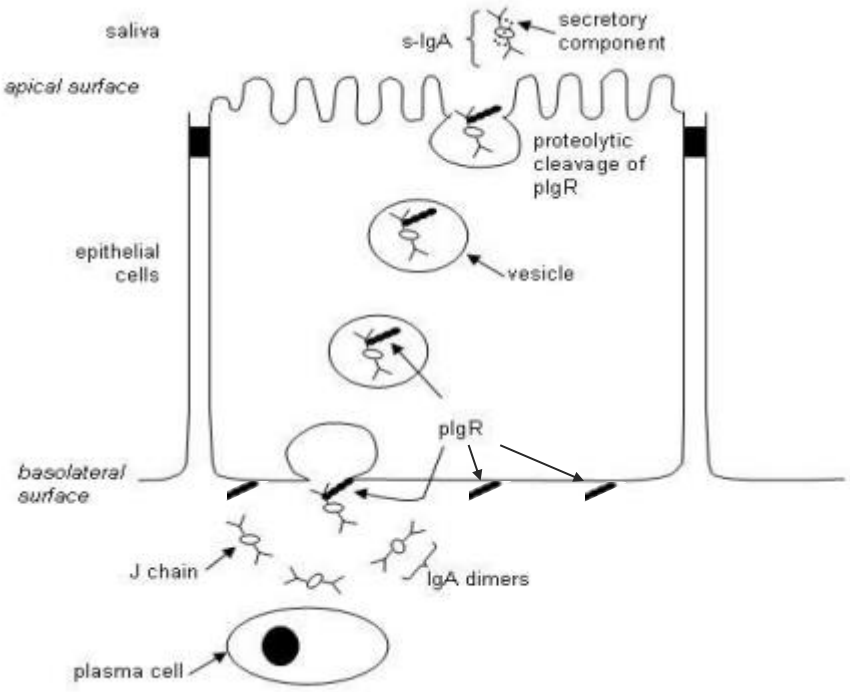

Figure 2.1 Transport of IgA through epithelial cells into saliva. Adapted from Bishop and Gleeson (2009)

Most IgA is produced from conventional B cells that are derived from mucosa associated lymphoid tissue (Williams, 2011). The transport of IgA (figure 2.1) bound to pIgR across the mucosal epithelium essentially provides three aspects of protection by S-IgA: (1) through prevention of pathogen adherence and penetration of the mucosal epithelium; (2) by neutralising viruses within the epithelial cells during transcytosis; and (3) by removal of locally formed immune complexes across mucosal epithelial cells to the luminal surface (Gleeson et al., 2013; Lamm, 1997). Mucous allows neutralisation of bacterial toxins via S-IgA, which prevents some of the pathogenic consequences of infection (Williams, 2011). S-IgA bound to the surface of bacteria is able to induce natural killer cell mediated antibody-dependant cellular cytotoxicity (Williams, 2011).

Salivary S-IgA is generally recognised as the first line of defence, providing immediate protection on mucosal surfaces by the exclusion, neutralisation, and elimination of infectious viral pathogens (Gleeson, 2000). Salivary S-IgA prevents viral and bacterial material to replicate and attach itself to mucosal epithelium of the mouth, throat, and upper respiratory tract (Cunniffe et al., 2011). Reducing absorption of food-related antigens, neutralisation of viruses, toxins and microbial enzymes, inhibition of virus release, and by enhancing the non-specific immune elements like lactoperoxidase or lactoferrin (Brandtzaeg, 2009; Czyzewska-Buczyńska, Lewandowicz-Uszyńska, & Jankowski, 2006; Majkowska-Skrobek & Augustyniak, 2004; Orysiak, Malczewska-Lenczowska, Szyguła, & Pokrywka, 2012). Pathogen adherence is generally considered the most important function of S-IgA as it inhibits bacteria from penetrating mucosal epithelium at the apical surface (Teeuw, Bosch, Veerman, & Nieuw Amerongen, 2004)

and excretes antigens into the lumen (Lamm, 1997). This is achieved when S-IgA binds to antigen making it too large to penetrate cells, or by blocking the ability of antigen to bind to cells (Williams, 2011).

The decreased level of the salivary S-IgA is associated with an increased incidence of URTI (Neville et al., 2008), thus it may be a useful biological marker of clinical predisposition to diseases of the upper respiratory tract (Fahlman & Engels, 2005). Salivary S-IgA has been proposed as the most promising immune marker for identifying those athletes at risk of upper respiratory illness (Neville et al., 2008; Pyne & Gleeson, 1998; Shephard & Shek, 1998). It is believed that the concentration of salivary S-IgA varies depending on physiological state and physical activity (Daly, Seegers, Rubin, Dobridge, & Hackney, 2005; Trochimiak & Hübner-Woźniak, 2012).

2.4 Effects of exercise on mucosal immunity

Exercise can have both positive and negative effects on immune function and susceptibility to minor illnesses (Gleeson, 2007). The relationship between exercise and likelihood of infection has been described as a "J"- shaped curve (Nieman, 1994). This "J" shaped model suggests that, although partaking in moderate amounts of exercise may enhance immune function above sedentary levels, impaired immune function may occur with prolonged, highly intense exercise bouts (Gleeson, 2007). Malm (2006) furthered this by proposing an "S-shaped" curve, including elite athletes. The new curve suggests that elite athletes appear to have a reduced risk of infection compared to non-elite athletes performing high intensity exercise. Gleeson, Pyne, and Callister (2004) state that fitness levels may be responsible for some discrepant findings in S-IgA exercise responses seen in the surrounding literature. The theory being that athletes of a higher fitness level (greater $\dot{V}O_{2max}$) may be better able to cope with strenuous demands of some exercise protocols employed, and may therefore have a dampened stress and/or immune response to exercise compared to less fit individuals (Malm, 2006; Mastorakos, Pavlatou, Diamanti-Kandarakis, & Chrousos, 2005). Research by Kunz et al. (2015) supports this finding, the authors found that fitness was a major determinant to the response of salivary antimicrobial proteins (salivary S-IgA, α-Amylase, lactoferrin, lysozyme) to a single bout of exercise. The exercise consisted of three 30 min cycling trials at −5, +5 and +15 % of the individual blood lactate threshold, finding that fitter individuals produced greater concentrations and/or secretion rates of the salivary antimicrobial proteins. Specifically, the study found that highly fit cyclists increased salivary S-IgA concentration by +181% compared to the less fit participants. These results indicate that there may be training specific adaptations occurring to parasympathetic and sympathetic nervous system activation in highly fit individuals. Secretory immunoglobulins, such as salivary S-IgA, can vary greatly between individuals. Early studies in the field indicated that levels of S-IgA concentrations were lower in endurance athletes compared with sedentary individuals (Tomasi, Trudeau, Czerwinski, & Erredge, 1982), whereas more recent studies indicate that S-IgA levels are generally not lower in athletes compared with non-athletes, except when athletes are engaged in periods of very heavy training or competition periods (Gleeson,

1999). A new way of examining salivary S-IgA response is to look at the concentration relative to their 'healthy' baseline values (Neville et al., 2008), as opposed to only examining absolute concentrations, as seen in the early literature (Tomasi et al., 1982). Although S-IgA levels were not examined in these studies, it has been reported that there is a 100–500% increase in risk of picking up an infection after competitive ultra-endurance running events (Nieman, Johanssen, Lee, & Arabatzis, 1990; Peters, Goetzsche, Grobbelaar, & Noakes, 1993). This finding suggests that the high workloads required for such events challenges mucosal immunity and may hinder the first line of defence against infection.

Several other factors have been shown to influence salivary S-IgA concentration and salivary flow rate. Increased levels of circulating stress hormones, including cortisol, have been previously attributed to reduced production and transport of S-IgA (Hucklebridge, Clow, & Evans, 1998; Sabbadini & Berczi, 1995). Hucklebridge et al. (1998) found that a period of marked activation of the hypothalamic-pituitary-adrenal (HPA) axis, during the first 30 min after awakening, reflected in an elevation of salivary cortisol, which was also associated with rapid changes in S-IgA concentration. The author suggested that the two had an inverse relationship, with the S-IgA decline positively correlated with the cortisol rise (Hucklebridge et al., 1998). Cortisol, a circulating human glucocorticoid, is a key moderator of systemic and psychological stress responses (Cunniffe, 2012). It is highly responsive to various stress states and is therefore used as a global stress marker. These include physiological stress, nutrition and exercise status (Brandenberger & Follenius, 1975; Brandenberger, Follenius, & Hietter, 1982), and sleep (Pietrowsky, Meyrer, Kern, Born, & Fehm, 1994). Cortisol, is also a powerful natural immunosuppressant (Petrovsky, McNair, & Harrison, 1998) making it a hormone of interest in studies evaluating immune function.

Allgrove, Geneen, Latif, and Gleeson (2009) suggested that the increase in circulating cortisol and other stress hormones may have explained in part the reason for why no differences in the S-IgA response were observed in prolonged cycling between fed and fasted participants. The study utilised a 2 h protocol of cycling on a stationary ergometer at 65% of maximal oxygen uptake in a randomized, crossover design, with 16 active adults (Males = 8, Females = 8). The results indicated that a fed or short term fasted state before exercise does not influence resting S-IgA or the response to prolonged cycling. Furthermore, these results showed lower levels of S-IgA and osmolality in women than in men at rest. Although not related to exercise performance, Fan et al. (2009) found a similar salivary S-IgA and cortisol correlation. Twenty five healthy participants completed a stress test (mental arithmetic task), with salivary immune response measured prior to, immediately after, and 20 min after the test. The levels of salivary cortisol and S-IgA both significantly increased after the acute mental arithmetic challenge. However, the increase of S-IgA was transient; the S-IgA fall was significantly correlated ($P < 0.001$) with the cortisol rise during the 20 min after stress, suggesting that cortisol levels influence S-IgA levels.

Salivary S-IgA concentration changes can occur in response to both acute exercise sessions

(Blannin et al., 1998; Gleeson et al., 1999; Mackinnon, Ginn, & Seymour, 1993) and through prolonged periods of intense training sessions (Gleeson, Ginn, & Francis, 2000; Mackinnon, Chick, Van As, & Tomasi, 1989). It could be suggested that some of the potential suppression in immune function and susceptibility to illness may be due to the change in S-IgA levels. Strenuous exercise in both moderately and highly trained athletes is commonly followed by reports of depressed mucosal immune response and upper respiratory symptoms (as reviewed in Gleeson et al., 2000). Gleeson et al. (2000) monitored an elite level kayaker (25 y) over a 14 day period, measuring salivary S-IgA before and immediately after every training session. The athlete had previous experiences with URTI episodes, averaging 5-6 per year, at training periods which were associated with high levels of fatigue. Through analysing whole unstimulated saliva, the authors suggest (although further research is needed) that monitoring decreases in salivary S-IgA during training periods may allow sports scientists to predict an athlete's risk of mucosal infection.

Gleeson et al. (1995) examined the impact of a 7-month training season prior to a national championship on the systemic and mucosal immunity of elite Australian swimmers. The results showed resting serum and salivary immunoglobulin concentrations were significantly depressed in athletes with long-term training at an intensive level. Additionally, an inverse correlation was observed between resting S-IgA concentrations at the start of the training period with infection rates, and the number of infections observed in the swimmers was predicted by the preseason and mean pre-training S-IgA levels (Gleeson, 2007). Neville et al. (2008) completed a study examining relative salivary S-IgA concentrations and the ability to predict URTI illness in athletes (38 elite America's Cup yachtsmen). They found that healthy athletes with low relative salivary IgA values (<40% of an individual's baseline concentration) had an estimated 48% chance of contracting a URTI within 3 weeks, compared to a 28% chance when values were below 70% of the athletes healthy resting rate. Contracting a URTI can be hugely detrimental to an athlete. If an athlete contracts an infection prior to an event, they may not be able to partake and therefore, disregard the prior months or years of training. Likewise, if an athlete picks up a URTI prior to an event, it can stop athletic progression and adaption, meaning athletes may not be able to compete at their peak. As stated previously by Neville et al. (2008), an athlete with low relative saliva (<40%) is at risk of contracting a URTI for the proceeding 3 weeks. Therefore, it is important for practitioners to monitor salivary antimicrobial proteins to help prescribe training load, reducing this load when athletes are showing signs of relatively low salivary S-IgA.

2.4.1 Effects of intermittent exercise on mucosal immunity

At present, there are very few studies that examine the effect intermittent exercise has on mucosal immunity. Generally studies have investigated mucosal immune response to interval exercise protocols (Davison, 2011; Engels, Fahlman, & Wirth, 2003; Fahlman, Engels, Morgan, & Kolokouri, 2001; Robson-Ansley, Blannin, & Gleeson, 2007), games of intermittent team sport (Lindsay et al., 2015; Peñailillo et al., 2015), and monitoring of intermittent sport training periods

(Coad, Gray, & McLellan, 2015; Fahlman & Engels, 2005; Owen et al., 2016; Ueno et al., 2013). Of the research that has been conducted, the effect that intermittent exercise has on salivary S-IgA appears to have inconsistent results in the relevant academic literature.

Davison (2011) conducted a crossover study on 9 recreational active males, acting as their own resting control, analysing innate immunity response to a Wingate interval protocol (4 x 30 s, 4 min recovery). A significant ($P < 0.05$) change in salivary S-IgA concentration (pre-exercise 164 ± 49 mg·L⁻¹, post-exercise 376 ± 49 mg·L⁻¹) and an immunodepression of neutrophil oxidative burst activity (OBA) 30 min post-exercise (formyl-leucylmethionyl-phenylalanine stimulated OBA, $P = 0.008$; phorbol-12-myristate-13-acetate stimulated OBA, $P = 0.002$) was found after completion of the interval protocol (Davison, 2011). Engels et al. (2003) similarly observed that exercise induced reductions in salivary S-IgA levels occurred after completion of Wingate interval training. The study examined 35 recreationally active adults (Females = 19, Males = 16) who performed three successive 30 s Wingate leg cycling tests (0.075 kp·kg), each separated by 3 min of recovery. The results indicated a decrease in post-exercise S-IgA (35.8 ± 21.1 mg·L⁻¹), S-IgA: protein ratio (17.1 ± 11.4 mg IgA·mg protein), and salivary flow rate (320.3 ± 209.5 µg·min^{-1}) compared to pre-exercise values (52.7 ± 28.0 mg·L⁻¹, 28.7 ± 17.9 mg IgA·mg protein, 415.7 ± 197.9 µg·min^{-1}, respectively). The authors found that there was no difference in response patterns between male and female study groups and the exercise-induced suppression of S-IgA was not followed by URTI in 33 of 35 subjects. Utilising the same protocol, Fahlman et al. (2001) examined S-IgA concentration and incidence of URTI in 26 physically active females. The authors found a decrease in saliva flow rates (478.5 ± 50; 345.4 ± 50 µg·min^{-1}), S-IgA (55.8 ± 4.7; 35.4 ± 3.6 mg·L⁻¹), and S-IgA:Protein ratio (30.7 ± 3.0; 17.5 ± 1.8 mg S-IgA·mg protein); however, this exercise induced decrease did not increase the clinical symptoms of URTI in the weeks post exercise protocol. MacKinnon and Jenkins (1993) found that decreased (16%) S-IgA concentrations after repeated bouts of supramaximal exercise may have been one of the contributing factors to URTI incidence in athletes. Their study utilised an 8 week intervention of 12 male active students completing 5 x 60 s cycling bouts, 3 times per week (MacKinnon & Jenkins, 1993). The authors suggested that a decrease in saliva flow rate was a contributing factor to the decreased levels of salivary S-IgA. Similar findings were reported by Robson-Ansley et al. (2007), who showed that increasing training load with interval training on 3 consecutive days for 2 weeks had minimal effect on salivary S-IgA levels.

Sari-Sarraf et al. (2011) found that football-specific intermittent exercise resulted in an increase in salivary S-IgA secretion rate (pre-exercise 402 ± 142, post-exercise 725 ± 250 µg·min⁻¹) in 10 moderately trained males. The football specific protocol consisted of 5 different exercise categories, representing the increasing exercise intensities (standing “0”, walking “4”, jogging “10”, cruising “13” and sprinting “19 Km·h^{-1}”) that are observed in football match-play. The protocol comprised of six 15-min blocks with a 15-min half-time intermission between the third and fourth exercise blocks. The increase in salivary S-IgA secretion rate is of interest as previous literature has reported that cortisol concentrations are elevated above normal during a football match (Carli et al., 1986). Additionally, performing exercise in hot conditions with

elevated salivary cortisol concentration could result in a decrease in salivary S-IgA concentration, via the salivary cortisol and S-IgA inverse relationship examined previously in this review. However, Sari-Sarraf et al. (2011) found a non-significant change of salivary cortisol concentration post-exercise, therefore, theorising that a short-term or delayed effect of salivary cortisol at the concentrations noted may not have an inhibitory effect on the transepithelial transport of salivary S-IgA. Most likely the psychological stress of a real match provides an additional stimulus for cortisol secretion besides the physiological stress of exercise (Sari-Sarraf et al., 2011).

The previous studies are very similar in their intermittent protocols with the majority of them utilising Wingate intermittent tests of short duration. To the authors knowledge only Sari-Sarraf et al. (2011) has conducted a study examining the effect of football specific intermittent exercise on salivary S-IgA measures. Further research is needed in the area to further examine the effects of longer duration intermittent and football specific intermittent exercise on the response of salivary antimicrobial proteins and subsequent incidence of URTI.

2.4.2 Effects of intermittent based sports on mucosal immunity

Due to the lack of literature on salivary S-IgA response to intermittent based exercise, the following section will examine mucosal immunity in intermittent based sports. Peñailillo et al. (2015) completed a study examining salivary hormones of football (soccer) players during and after an international friendly match. The results indicated similar findings to the intermittent exercise protocol studies mentioned in the previous section. Salivary S-IgA levels decreased by 74.5% from pre-exercise levels, the authors suggesting that this decrease put the footballers at risk of contracting URTIs due to the reduction in mucosal immunity (Peñailillo et al., 2015). The authors found no change ($P = 0.66$) between pre-exercise and post-exercise cortisol levels, however, found a significant difference in testosterone ($P = 0.001$). Higher salivary testosterone levels were associated with smaller depression of salivary S-IgA. Similarly, participants with smaller decreases in testosterone were also found to cover higher distances. The authors recommended that football players should implement resistance training exercise into their periodization plan. Resistance training has shown to increase testosterone and therefore, would compensate the catabolic effect of competitive football and result in smaller depression of salivary S-IgA (Peñailillo et al., 2015).

Morgans, Orme, Anderson, Drust, and Morton (2014) had similar findings with English Premier League footballers noting that an intense winter period (5 games in 15 days) was sufficient to decrease S-IgA levels below 40% of baseline values in a significant number (game 1 = 14, game 2 = 8, game = 4 athletes) of professional players tested in the study. Salivary S-IgA levels did return to normal values when the playing schedule returned to one game per week fixtures. Morgans et al. (2014) state that monitoring S-IgA levels over intense periods of training and game schedules is crucial, implementing recovery and wellbeing interventions as required.

Incidence of URTI rate of American Football players was also found to increase during intense training periods. Fahlman and Engels (2005) reported secretion rate of S-IgA was significantly and inversely related to URTI incidence. The authors found that over the 12 month time period the secretion rate of S-IgA over a season was the variable most linked to URTI incidence rate in athletes.

Gleeson (2007) states that during chronic periods of very heavy training, several aspects of both innate and adaptive immunity are depressed, but athletes will not be clinically immune deficient. In other words, high volumes of training and exercise-induced immune dysfunction does not put athletes in danger of serious illness, but it increases the likelihood of that athlete picking up common infections such as URTI or influenza should an outbreak occur (Gleeson, 2007). These common infections may require athletes to take considerable time off their training for recovery and may result in missing games or competition. Ueno et al. (2013) found that the two week tapering or rest period was considered insufficient in restoring immune function back to resting levels following the highly intensive and extended training (Ueno et al., 2013). This study indicates, regardless of URTI incidence, significant rest periods, greater than two weeks, are required after highly intensive immune depressing training or competition periods.

Lindsay et al. (2015) found a significant change in salivary S-IgA following professional rugby union games. The significant S-IgA suppression provides sufficient evidence that it can be a measure of psychophysiological stress in rugby union. The authors suggest that salivary S-IgA may also have the ability to measure individual stress adaptation over time and may indicate over-training. Furthermore, Lindsay et al. (2015) emphasises the importance of utilising effective periodization plans, correct peaking, and sufficient recovery time to ensure athlete immune health. Coad et al. (2015) found that AFL players, exposed to weekly match play, are likely to require a recovery period of greater than 36 h post-match to restore healthy mucosal immunological function. The authors suggest that the athletes may be at higher risk of illness during the initial 36 h post-match. These results combined to those found by Morgans et al. (2014), indicate that elite footballers/athletes who have intense periods of competition (such as 5 games in 15 days) are not getting sufficient time for mucosal immunity recovery. Prolonged periods of such intense competition and a weakened mucosal immunity, would likely result in an URTI. Coad et al. (2015) supports this statement, finding a significant immune suppression was witnessed in a 3-match period where match-play exercise workloads were significantly variable between matches.

Owen et al. (2016) completed a study examining salivary S-IgA over high and low intensity training periods on 10 elite footballers. Training intensity was monitored in terms of internal and external loads with heart rate and RPE, and GPS data, respectively (Owen et al., 2016). They found significantly lower salivary S-IgA levels in the last period of high intensity training. Although non-significant, the authors found that acute prolonged high intensity training did reduce salivary S-IgA concentration, which is consistent with other literature (Mackinnon et al., 1993; MacKinnon & Jenkins, 1993). Similarly, the percentage change between variation of

salivary S-IgA concentration to baseline significantly ($P < 0.05$) differed between high and low intensity training periods. The authors encouraged coaches to monitor S-IgA in routine, particularly during high intensity training periods and to take precautions to avoid upper respiratory tract infection in highly trained football players (Owen et al., 2016). Taken together, the results of these studies show the importance of knowing the impact that intense periods of intermittent exercise can have on salivary S-IgA and the likelihood of contracting a URTI. It is suggested that monitoring salivary S-IgA compared to a 'healthy' baseline could help sport scientists to predict, and reduce workload where applicable, when an athlete is at risk of contracting such an infection.

2.5 Thermoregulatory response to exercise in hot conditions

Exercise in the heat, as compared with a neutral environment, causes a greater strain on the human body with marked alterations in the circulatory, thermoregulatory, and endocrine systems (as reviewed in Nybo, Rasmussen, and Sawka (2014)). Vigorous exercise in thermally challenging environments can overload the body's performance ability, resulting in hyperthermia, dehydration, deteriorated physical and mental performance, and a potentially serious (even fatal) exertional heat illness (Casa, 1999). Competitive football matches are commonly played in such thermally challenging environments. Major football tournaments can be located in countries with high ambient temperatures, including the upcoming 2022 FIFA World Cup, with temperatures recorded up to 45°C (Mohr et al., 2012). Mohr et al. (2010) reported high intensity running decreased markedly in the last 15 min of a football match played in moderately high environmental temperatures (30°C). In this study muscle temperatures of some players rose to excess of 41°C at the end of the match. The findings of Mohr et al. (2010) indicate that the ability to perform high intensity running in the final stages of match-play may be heavily impacted by thermally challenging environments compared to cooler conditions. Thermally challenging environments (>30°C) have been previously noted with other major football events, such as the 2014 FIFA World Cup in Brazil, Champions League and Europa League Campaigns in Spain, Greece and other hot climate countries. Drust, Cable, and Reilly (2000) state that intermittent activity, such as football, is associated with greater increases in rectal temperature compared with continuous exercise. Managing athletes in hot conditions requires a thorough understanding of the body's response to exercising in heat.

The body attempts to dissipate heat, whether heat is produced endogenously or accumulated exogenously, via conduction, convection, evaporation, and radiation (Werner, 1993). The body's use of each heat dissipation method depends on the ambient temperature, relative humidity, and exercise intensity (Casa et al., 2000). As environmental temperature rises, the use of conduction and convection are reduced, and radiation renders insignificant (Takamata, Mack, Stachenfeld, & Nadel, 1995). The primary heat dissipating method for athletes competing in hot environments is evaporation. Casa et al. (2000) suggests that in warm, humid conditions, evaporation may account for more than 80% of heat loss. Armstrong and Maresh (1993) expand

on this stating that hot, dry conditions, cause evaporative heat dissipation to increase to as much as 98% of cooling. Insufficient fluid consumption can result in offsetting the rate of water loss via sweating, with sufficient time dehydration will ensue. The sweating response is critical to body cooling during exercise in the heat (Casa et al., 2000). The level of heat stress upon an athlete is dependent on a complex array of interlinking factors, including the duration of heat exposure, the temperature of the heated environment, the athlete's abilities to complete the tasks and their heat acclimatisation status, but also level of dehydration (Cian et al., 2000; Pepler, 1958; Piwonka & Robinson, 1967). When the body is no longer able to dissipate heat faster than it is being gained, core temperature rises. González-Alonso et al. (1999) demonstrated that fatigue in trained athletes occurred at the same critical body and muscle temperature despite differences in initial internal temperature and its rate of rise. Thermal heat gain occurs via heat created from active muscles transferred to nearby skin. The resulting heat is transferred through to the body's trunk and cerebral area via the circulating blood (Crandall & Gonzalez-Alonso, 2010). A change in brain temperature, cerebral activity (Nybo, 2012; Nybo, Møller, Volianitis, Nielsen, & Secher, 2002) and altered brain neurotransmitter (Roelands & Meeusen, 2010) concentrations are believed to play a role in the onset of central fatigue. Decreased cardiac output is also a mechanism that causes a cessation of exercise. Heat stress challenges cardiac output via impaired diastolic filling of the heart. Impaired cardiac filling occurs as the large venous bed under the skin dilates during exercise in heat. As blood flow increases during exercise, the blood vessels of the skin increase in size and large amounts of blood collect under the skin, resulting in less blood available to the thorax, reducing cardiac filling (Nybo et al., 2014). Specifically, less blood in the thorax will lower end-diastolic volume and reduce stroke volume, thus requiring an increase in heart rate to sustain cardiac output (Nybo et al., 2014).

2.5.1 Effects of heat on intermittent exercise

Performance of intermittent exercise is also affected by hot environmental conditions. Mohr, Rasmussen, Drust, Nielsen, and Nybo (2006) examined intermittent exercise in hot (40°C, 17% RH) and thermoneutral (20°C, 24% RH) environments. The protocol included 40 min of intermittent 15 s loaded and unloaded intervals (corresponding to 65% of peak aerobic capacity). The 8 participants then completed a muscle biopsy followed by 5 x 15 s maximal sprints interspaced by 15 s recovery. Following exercise with heat stress, the core and muscle temperatures peaked at 39.5 ± 0.2°C and 40.2 ± 0.2°C to be 1°C higher ($P < 0.05$) than the corresponding control trials. Repeated sprint performance was impaired during the heat stress trials, and this was not mediated by traditional peripheral fatiguing agents. Rather, the cause of fatigue during high intensity intermittent exercise in the heat may relate to central factors and hyperammonemia could be a component influencing the cerebral function (Mohr et al., 2006).

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, >50% RH) compared with

temperate environments below 24°C (Nassis, Brito, Dvorak, Chalabi, & Racinais, 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 (>28°C) to temperate (<24°C) 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). Thus the variation that these parameters play in the heat (>30°C) is likely due to an altered "pacing strategy" and distribution of absolute exercise intensity across the game (Mohr et al., 2012). Three previous studies have used field based soccer specific simulations to quantify the changes in physical performance in a hot (>30°C) compared with a temperate (20°C) environment (Hughes, Doherty, Tong, Reilly, & Cable, 2006; Morris, Nevill, Lakomy, Nicholas, & Williams, 1998; Morris, Nevill, & Williams, 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. The authors stated significantly higher heart rate (hot = 186 ± 2, moderate = 179 ± 2 beats·min^{-1}, $P < 0.05$) at the end of exercise and nearly twice (hot = 1.18 ± 0.12, moderate = 0.63 ± 0.07 l·h^{-1}, $P < 0.01$) as much water was consumed in the hot trial compared to moderate temperature trial. These results occurred without any trial differences in dehydration levels, RPE, blood glucose, and blood lactate. Morris et al. (1998) found a higher rectal temperature and a faster rise in rectal temperature at 30°C, further suggesting that the final rectal temperature was the reason for the curtailment of exercise. Mohr et al (2012) reported that at 43°C ± 12% RH, male elite soccer players demonstrated a 26% reduction in high speed distance covered compared to a match within temperate conditions (21°C. 55% RH). 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.

Dehydration is also believed to play a part in the decrease in both performance and salivary flow rate in heated environments. Dehydration influences all physiological systems in the human body (Murray, 1995). It is well established in the surrounding literature that during continuous exercise, a loss as little as 1% in body mass, impairs physiological and performance responses (Murray, 1992; Sawka, 1992; Sawka, Young, Cadarette, Levine, & Pandolf, 1985). Dehydration affects a vast variety of cardiovascular and thermoregulatory responses (Sawka, 1992; Sawka & Pandolf, 1990). Sawka and Pandolf (1990) state that every 1% decrease in body mass results in

an increase in core temperature of 0.10 - 0.40 °C. Dehydration by 2% of body mass has been shown to reduce physical performance in 1,500, 5,000, and 10,000 m events by 3.1, 6.7, and 6.3%, respectively (Armstrong, Costill, & Fink, 1985). Studies show that dehydration of 3% to 4% affect muscle endurance and reduce performance (Bijlani & Sharma, 1980; Bosco, Greenleaf, Bernauer, & Card, 1974; Mnatzakanian & Vaccaro, 1982; Saltin, 1964; Serfass, Stull, Alexander, & Ewing Jr, 1984). The research concerning maximal aerobic power and the physical work capacity for extended exercise is relatively consistent (Casa et al., 2000). Dehydration levels higher than 3% have been shown to decrease maximal aerobic power (Sawka & Coyle, 1999). Further decrements in aerobic power are shown in heated environments (Sawka, Montain, & Latzka, 1996). The influence of dehydration on muscle strength has also been analysed in the academic literature. Studies generally show strength decrements in physical performance with dehydration levels of > 5% (Bosco et al., 1974; Houston, Marrin, Green, & Thomson, 1981; Sawka et al., 1996; Webster, Rutt, & Weltman, 1990). Casa et al. (2000) state the higher the level of dehydration, the more negative the impact on physiologic systems and overall athletic performance. Murray (1995) expands on this stating that the greatest decrements in exercise performance occur during prolonged exercise in hot environments at high levels of dehydration. Interestingly, dehydration not only reduces exercise performance in the heat but also induces exhaustion at a lower core temperature (Sawka et al., 1990). Dehydration has been shown to be present in footballers after a match (Guttierres, Natali, Vianna, Reis, & Marins, 2011), with football players not sufficiently replacing fluid loss after trainings (Shirreffs et al., 2005) and games(Guttierres et al., 2011). High levels of dehydration and heat stress puts further physical strain on an athlete, potentially impacting their health, and may limit the performance of that athlete.

2.5.2 Exercise in the heat: effects on salivary S-IgA

Performing exercise in thermally stressful environments produces greater disturbances on the immune system compared to heat alone (Lim & Mackinnon, 2006; Shephard et al., 1998; Walsh & Whitham, 2006). Heat exposure is a further stress during exercise, elevating body core temperature, raising heart rate (cardiovascular drift), increasing circulating hormones with coinciding alterations in immune responses (Galloway & Maughan, 1997; Sari-Sarraf et al., 2011). During vigorous exercise, particularly in warm weather, core body temperature frequently exceeds levels associated with fever and hyperthermia (over 39.5°C) (Byrne, Lee, Chew, Lim, & Tan, 2006; Pugh, Corbett, & Johnson, 1967). Core body temperatures of 40 - 42°C have been reported in conscious runners and greater than 42°C in collapsed runners (Maron, Wagner, & Horvath, 1977). Performing prolonged exercise in the heat, with the concomitant increase in fluid loss, appears to result in large reductions in saliva flow rate (Horswill et al., 2006). The study examined the salivary S-IgA flow rate of 50 (Males = 34, Females = 16) well trained endurance sport athletes consuming different fluids. The authors found that exercise with solely water consumption significantly ($P < 0.05$) decreased saliva volume and rate of stimulated saliva flow. The authors found that consuming diluted orange juice and commercially available

sports drink (Gatorade, 6% carbohydrate) did not affect saliva flow rate. Consumption of beverages was shown to maintain body fluid balance, and possibly the plasma volume. Because plasma volume is the source of fluid for saliva production, maintenance of near normal hydration status could support the capacity to generate saliva (Horswill et al., 2006). Bishop et al. (2000) suggest that the presence of carbohydrate in a beverage ingested during sustained exercise may result in significantly greater saliva flow rates than that when using a placebo devoid of carbohydrate but consumed at identical volumes.

As discussed previously in this review, Sari-Sarraf et al. (2011) completed a study examining the effect of carbohydrate ingestion on salivary S-IgA response, prior to and following intermittent soccer specific exercise. The authors found that salivary total protein increased by 51% after the soccer-specific intermittent exercise protocol during both carbohydrate and placebo trials, likely due to increased β-sympathetic activity and that effect on the salivary glands. Salivary S-IgA to protein ratio decreased significantly ($P = 0.019$) after both carbohydrate and placebo trials, a finding which has been noted in previous studies (MacKinnon & Jenkins, 1993; Moreira et al., 2009). Sari-Sarraf et al. (2011) suggest the possible cause of the changes in salivary S-IgA concentrations relative to total protein and other proteins were a result of deviation in salivary volume due to drying of oral surfaces from the intermittent exercise protocol. Housh et al. (1991) found no differences in salivary S-IgA either immediately or 1 h following a 30 min bout of exercise at 80% of VO_{2peak} at any environmental temperature from 6 to 34 °C. The results showed that moderate exercise for 30 min did not increase the susceptibility of upper respiratory tract infections by decreasing salivary S-IgA. Carrillo, Murphy, and Cheung (2008) examined whether vitamin C ingestion affected salivary immune markers in 12 healthy individuals. The participants cycled for 3 h or until exhaustion in a heated environment (34.8°C, 13% RH). The study showed significant ($P < 0.05$) increases in salivary S-IgA concentration from pre-exercise (59.6 ± 7.6 $mg \cdot L^{-1}$) to post-exercise levels (104.3 ± 13.3 $mg \cdot L^{-1}$). Post-exercise values significantly ($P < 0.05$) decreased after 72 h post-exercise (64.7 ± 7.1 $mg \cdot L^{-1}$). The authors also found that rectal temperature (37.3 - 38.9°C) and skin temperature (35.7 - 38.3°C) had significant increases from pre-exercise to post-exercise (Carrillo et al., 2008).

Further research is needed in the area to confirm the effect of exercise on salivary immune markers. Shephard (1998) suggests that the inconsistent findings in the literature may be due to some individuals exercising in hot (vs cool) conditions tend to fatigue sooner or reduce their work rate, so their exposure to exercise stress in the heat tends to be self-limiting. Utilising an exercise protocol that measures a self-paced section may provide insight to this self-limiting or reduction in work rate. It may be of interest to sport scientists to observe at what core temperature this self-limiting phenomenon occurs. A notion in thermoregulation research is that a limited effect on immune function is noted, in either hot or cold environments, in laboratory studies, when participants core temperature remains within 2 °C of normal baseline (Severs, Brenner, Shek, & Shephard, 1996; Walsh & Whitham, 2006). The core temperature of an athlete may play an important role in immune response and being able to lower an athlete's

core temperature may reduce the effect exercising in the heat has on immune markers.

2.6 Pre-cooling methods

Exercising in hot environments can cause greater strain on the human body compared to thermo-neutral conditions. It can alter the body's ability to dissipate heat, whether produced endogenously or accumulated exogenously. During the 1980's the general consensus that high ambient temperature and humidity had a detrimental effect on performance led to increased attention on how to best cool the athlete prior to competition (as reviewed in Quod, Martin, and Laursen (2006)). Literature around the topic focused on the theory that cooling an athlete, prior to exercise in the heat, will allow them to perform at their peak and reduce the detrimental effects of the heated environment. Currently, it is generally accepted that lowering or attenuating a rise in core temperature by implementing a cooling strategy prior to exercise in the heat increases the body's ability to store exogenous and endogenous heat, and can lead to an improvement in exercise performance (as reviewed in Ross, Abbiss, Laursen, Martin, and Burke (2013)). Nevertheless, the precise mechanisms associated with the improvements in performance following pre-cooling are not well understood (Ross et al., 2013). Since the 1980's, multiple pre-cooling methods have been examined, utilising cold water, cold air, and ice in various modes, and allocations. Different pre-cooling methods will have differing effects on the body; some methods primarily reduce skin temperature (cold air, mist fans, ice jackets), others reduce core temperature (ice slurry ingestion, breathing cool air, cold intravenous saline), and others reduce skin temperature as well as core temperature (cold water immersion, cold room, combinational methods) (Quod et al., 2006).

Due to the wide range of literature on this topic and relevance to the thesis, this review will focus on team sport specific studies. Price et al. (2009) examined the effects of pre and halftime cooling, solely pre-cooling, or no cooling intervention on 8 elite female football players. The cooling intervention consisted of wearing an ice vest for 20 min pre-exercise and for 15 min during the half-time interval. The participants performed two 45 min periods of intermittent running separated by 15 min seated rest on three separate occasions in the heat (30°C, 63.5% RH). The performance protocol was based off match analysis data from the league (Football Association Women's National Premier League) in which the athletes competed, consisting of three identical 15 min periods per half. The results of this study suggest that both cooling strategies were effective in reducing thermal strain during intermittent exercise in the heat. However, pre-cooling plus half-time cooling was more effective than solely pre-cooling in offsetting heat storage. Slight decreases were noted during pre-cooling for rectal temperature (0.1°C); the authors suggest this is likely due to the mixing of cooled cutaneous blood on return to the central circulation.

Whole body cooling prior to physical activity has the potential to reduce thermal strain and fatigue during subsequent endurance exercise. Thus, Drust et al. (2000) examined the effect of

pre-cooling on thermoregulatory responses during an intermittent test under “normal" environmental conditions. Six male university soccer players completed a 90 min soccer-specific intermittent protocol (developed by Drust et al. (2000)) on a non-motorised treadmill. The protocol consisted of 6 identical 15 min sections, which included walking, jogging, cruising, and sprint speed categories. The run was completed with and without pre-cooling under thermoneutral laboratory conditions (20°C) and without pre-cooling in a moderately heated laboratory (26°C). The pre-cooling strategy involved exposure to a cold shower (mean temperature 26°C) for 60 min. The cold shower started at 28°C and was reduced by 2°C every 20 min, this method is not a practical pre-cooling method and would not be advised for team sport athletes. The study concluded that there is no evidence for the beneficial effects of pre-cooling on the physiological responses to soccer-specific intermittent exercise under normal environmental conditions (Drust et al., 2000). Intermittent activity has been shown to cause higher levels of physiological strain than those associated with continuous exercise at the same mean work rate (Bangsbo, 1993). Although not accounted for in the Drust et al. (2000) study, pre-cooling prior to intermittent exercise in a heated environment (>26°C) may reduce the physiological strain upon an athlete.

Zhang et al. (2014) completed a study investigating two methods of partial skin/body cooling (forearm and hand cooling, and neck cooling) during a simulated half-time recovery on thermoregulatory responses and subsequent soccer-specific exercise performance. Following a 45 min treadmill run in the heat (30.5°C, 50% RH), participants (n = 7) undertook 15 min recovery with either passive cooling, forearm and hand cooling, or neck cooling in a simulated cooled locker room environment. After the recovery, participants performed a 6 × 15 m sprint test and Yo-Yo Intermittent Recovery Level 1 test (YYIR1) in a temperate environment (20.7°C, 45% RH). During the 15 min recovery, rectal temperature fell significantly ($p<0.05$) in all cooling conditions. The YYIR1 results were improved by forearm and hand cooling (42 ± 37%), and neck cooling (31 ± 24%) compared to passive cooling. Neck cooling (4.6 ± 0.6), however not in the forearm and hand cooling protocol, reduced thermal sensation compared to the passive cooling (air conditioned room) control (5.3 ± 0.5) ($P < 0.05$). These results suggest that body part cooling effectively improved comfort and sweat rate (pre-cooling $0.8 l \cdot h^{-1}$, passive cooling $1.2 l \cdot h^{-1}$), which delayed exercise-heat induced performance decreases during a second bout of exercise (Zhang et al., 2014). Duffield and Marino (2007) examined whether pre-cooling procedures improved both maximal sprint and sub-maximal work during intermittent-sprint exercise. Nine male rugby players performed a familiarisation session and three testing sessions of a 2 x 30 min intermittent sprint protocol, which consisted of a 15 m sprint every min separated by free-paced hard-running, jogging and walking in 32°C and 30% RH. The three sessions included a control condition, ice-vest condition and ice-bath plus ice-vest condition, with respective cooling interventions imposed for 15 min pre-exercise and 10 min at half-time. The authors found no significant improvements between conditions for the time or percentage decline in 15 m sprint efforts or the distance covered during sub-maximal work bouts. Ice-bath plus ice-vest condition showed improvements with large effect size data (ES = 0.88) for hard running distance compared to the ice vest and control trials. Similarly, lower core temperature

(ES = 1.0 – 1.8), skin temperature (ES = 1.0 – 1.2), sweat loss ($P < 0.05$), and thermal comfort ($P < 0.05$) were noted in the ice-bath plus ice-vest condition. The authors suggest that the ergogenic benefits of effective pre-cooling procedures in warm conditions for team-sports may be predominantly evident during sub-maximal bouts of exercise (Duffield & Marino, 2007). As discussed earlier in this review, submaximal running bouts, specifically high intensity running have shown to be important in football performance. It has been noted that elite players complete more high intensity running than moderate players and this speed category has shown to be reduced in the last 15 min of a game, a period which is highly related to goal scoring opportunities (Njororai, 2014). Therefore, effective pre-cooling procedures could improve performance of high intensity running and potentially reduce performance declines in the last 15 min of a game, a period of play crucial for goal scoring and match success.

It is important, though, to note that the most effective methods are not usually the most practical (Marino, 2002). For sporting competition there is a need for practical cooling methods, which do not require vast resources or equipment. As utilised in Duffield, Coutts, McCall, and Burgess (2013), ice slurry ingestion has been suggested as a practical and inexpensive method of pre-cooling (Yeo, Fan, Nio, Byrne, & Lee, 2012).

2.6.1 Effects of ice slurry ingestion as a pre-cooling method in the heat.

The ingestion of ice slurry as a pre-cooling method has been suggested due to the ability to cool the core internally. Ice slurries consist of millions of small ice particles within a liquid, providing a greater surface area. Conduction heat transfer occurs over the greater surface area and provides more heat storage capacity through the extra energy (334 kJ·kg) required for phase change (Tan & Lee, 2015). The ingestion of the ice and the resulting greater heat storage capacity is known as a 'heat sink'. The creation of a significantly larger heat sink with ice ingestion, compared to cool beverages (4°C), allows greater heat storage during exercise, thus delaying or prolonging the attainment of critically high internal temperatures (Siegel et al., 2012). Ice slurry ingestion has been shown to significantly lower core temperature prior to exercise (Ross et al., 2011; Siegel et al., 2010; Siegel et al., 2012; Stevens, Dascombe, Boyko, Sculley, & Callister, 2013; Yeo et al., 2012), allowing a greater core temperature range before reaching the critical temperatures. Hence, the ingestion of ice helps reduce the thermal load. It is also theorised that thermoreceptors in the mouth, oesophagus, and the abdominal region are cooled via ice slurry ingestion. The thermoreceptors then transmit afferent feedback signals that the body's temperature is lower than actually occurring (Hasegawa & Cheung, 2013; Villanova, Azpiroz, & Malagelada, 1997), resulting in the body 'thinking' it is cooler and hence allowing exercise to continue at high core temperatures. Such feedback may delay the anticipatory reduction in skeletal muscle activation and running velocity utilised to prevent damage to the tissue (Tucker, Rauch, Harley, & Noakes, 2004). In support of this theory, Onitsuka, Zheng, and Hasegawa (2015) examined the effect 7.5 $g \cdot kg^{-1}$ of ice slurry ingestion had on conductive cooling in facial skin and brain temperatures. Participants (n = 8) in the study ingested 2.5g·kg

every 5 min in 30°C, then remained at rest for 1 h. They found that ice slurry ingestion reduced thermal sensation via lowering core temperature and forehead skin temperature (Onitsuka et al., 2015). Hypothesising that ice slurry ingestion may reduce brain temperature by conductive cooling from the facial area, however, an indirect method was used to determine that hypothesis. If ice slurry ingestion can reduce brain temperature, this means that ice slurry could attenuate not only peripheral fatigue but also central fatigue, and that this effect might contribute to improved performance (Hasegawa & Cheung, 2013). Potential issues with ice slurry ingestion, although they are rarely reported in research studies, is the incidence of sphenopalatine ganglioneuralgia (brain freeze) (Siegel et al., 2010; Siegel et al., 2012) and gastric discomfort (Ross et al., 2011). Standardised ingestion rates can combat these adverse effects, which will be further discussed throughout this review.

Ice slurry ingestion provides an internal pre-cooling method, increasing an athlete's heat storage capacity via lowering core temperature prior to exercise. In particular, the reduction in the core temperature resulting from ice slurry ingestion may prevent decline in central neural drive that contributes to decreased performance in hot environments (Naito & Ogaki, 2015; Nybo et al., 2014).

2.6.2 Effects of ice slurry ingestion on continuous exercise performance in the heat.

Ice slurry ingestion has been shown to improve performance during continuous exercise in hot environments. Siegel et al. (2012) completed a cross over study examining ice slurry ingestion (-1°C), cold water immersion (24°C immersion) and warm fluid ingestion (37°C) on running time to exhaustion in the heat (34.0°C, 52% RH). Eight participants ran to exhaustion on three separate occasions following a pre-cooling method or control trial. The ice slurry ingestion required consuming 7.5 $g \cdot kg^{-1}$, with 1.25 $g \cdot kg^{-1}$ provided every 5 min prior to exercise. The results indicated that participants ran significantly longer in the cold water immersion (56.8 + 5.6 min; $P = 0.008$) and ice slurry ingestion (52.7 ± 8.4 min; $P = 0.005$) compared to the warm fluid trial (46.7 ± 7.2 min). Rectal temperature at exhaustion was significantly ($P < 0.05$) higher following the ice slurry ingestion protocol (39.8 + 0.4°C) compared to control (39.5 +0.4°C) and tended ($P = 0.065$) to be higher than cold water immersion (39.5 + 0.3°C). Siegel et al. (2012) concluded that ice slurry ingestion of 7.5 $g \cdot kg^{-1}$ can be used as an alternative to other pre-cooling protocols in prolonging submaximal running in the heat. Ihsan, Landers, Brearley, and Peeling (2010) found that performance time during a 40 km cycling time trial in the heat (30°C, 75% RH) was significantly quicker (6.5%) with ice slurry ingestion, in comparison to a subsequent trial when the participants solely drank tap water. Similarly, the seven participants increased their power output by 6.9% when ingesting ice slurry prior to the time trial (Ihsan et al., 2010). The performance results occurred from pre-exercise ice slurry ingestion of 6.8$g \cdot kg^{-1}$ of body mass, with servings of 150 to 200 g provided every 8-10 min, over 30 min.

Naito and Ogaki (2015) found that cycling time to exhaustion in the heat (35°C, 30% RH) was

greater with ice slurry ingestion (50.0 ± 12.2 min) when compared to cold water ingestion (42.2 ± 10.1 min). The participants were given 1.25 g·kg^{-1} of body mass of ice slurry ingestion (0.5°C) or cold water (4°C) every 5 min for 30 min, finishing 5 min before the start of exercise. Rectal temperature was significantly lower (0.32°C ± 0.09°C) in the ice slurry ingestion trial prior to exercise, although, no differences were observed between trials at the conclusion of the exercise protocol (Ice slurry 38.9 ± 0.4°C; cold water: 38.93°C ± 0.52°C; P = 0.575) (Naito & Ogaki, 2015). Stevens et al. (2013) also reported increased performance with ice slurry ingestion during triathlon performance (2.5%), as well as significantly lower intragastric temperature at the end of the cycle leg (P < 0.001), lower perceived thermal stress at 5 km (P = 0.038) and 9 km (P = 0.039), and a significantly (P = 0.039) increased VO_{2peak} during the last 500 m. The triathlon performance was simulated, with the cycle and run legs completed within a heated chamber (32 - 34°C, 20 - 30% RH). The ice slurry trial had participants ingest 10 g·kg^{-1} of body mass within the 17-45 min of the cycling leg. Concluding that lower core temperature and perceived thermal stress contributed to self-selection of a higher running intensity and improved performance time (Stevens et al., 2013).

Ross et al. (2011) used a large amount of ice slurry beverage with a time structured consumption plan, finding that ingestion of 14 g·kg^{-1} (two boluses of 7 g·kg^{-1}) of body mass, while wearing iced towels (on torso and legs), increased performance in a cycling time trial (46.4 km) and reduced core temperature in the heat (32 - 35°C, 50 - 60% RH). Ice slurry ingestion was one of 3 trials; the other two consisted of either a cold water (10°C) plunge followed by wearing a cooling jacket or a control (no intervention) trial. The authors found that ice slurry ingestion plus application of iced towels was associated with a 3.0% increase in power and a 1.3% improvement in performance time, with true likely events ranging from trivial to a large benefit in the ice slurry trial compared to the control trial. The authors found that the differences between a trial involving cool water immersions followed by a 10 min period of wearing a cooling jacket and a control trial were “unclear”. Yeo et al. (2012) examined ice slurry intervention (-1.4°C) compared to a warm drink temperature (30.9°C) on a 10km outdoor running time trial in the heat (Wet Bulb Globe Temperature 28.2°C). Mean performance time was faster with ice slurry ingestion (2715 ± 396 s) than in the control trial (2730 ± 385 s, P = 0.023). Gastrointestinal temperature reduced by 0.5 ± 0.2 °C after ice slurry ingestion compared with 0.1 ± 0.1 °C (P < 0.001) with control. During the run, the rate of rise in gastrointestinal temperature was greater (P = 0.01) with ice slurry ingestion than with the control trial for the first 15 min. At the end of time trial, gastrointestinal temperature was higher with ice slurry ingestion (40.2 ± 0.6 °C) than control (39.8 ± 0.4 °C, P = 0.005). Higher core temperature at the end of exercise was also shown in Siegel et al. (2010) and Siegel et al. (2012), indicating that ice slurry may increase core temperature range before performance decreases occur. Siegel et al. (2010) suggests that the high core temperature at exhaustion may be due to a brain cooling effect resulting from ice slurry ingestion, as previously discussed in this review. Cooler brain temperature and cooler thermoreceptors in the stomach, as a by-product of ice slurry ingestion, would also lower ratings of thermal sensation during the pre-cooling phase and during the first part of exercise, as seen in Yeo et al. (2012). The authors recommended that ice slurry ingestion was a practical and

effective pre-competition cooling strategy to improve performance in warm and humid environments.

Stanley, Leveritt, and Peake (2010) also found that ice slurry ingestion lowered core temperature by 0.4°C during a recovery period (after steady state exercise, 75 min at 58 ± 6% of peak power output), prior to a performance trial (75% of peak power output x 30 min). The authors found that, despite having a lower core temperature, ice slurry ingestion did not improve performance compared to the cool water (18.4°C) beverage trial. Stanley et al. (2010) used 1 kg of ice slurry as their pre-cooling method. In this study the ice slurry beverage was provided with an initial amount of 400 g given in the first 5 min, followed by 200 g amounts provided at 15, 25, and 35 min. This method differs to others in the surrounding literature, where ingestion is based of $g \cdot kg^{-1}$ of body mass (Naito & Ogaki, 2015; Siegel et al., 2010; Siegel et al., 2012; Stevens et al., 2013). No gastrointestinal discomfort was noted in this study, although having much higher amounts of ice slurry beverage consumed. It was noted by Stanley et al. (2010) that although ice slurry ingestion did not significantly alter exercise performance, it is an effective method of cooling athletes following exercise in hot humid environments.

Historically, the majority of ice slurry ingestion studies have examined the internal pre-cooling effects on continuous exercise. Ice slurry ingestion has been examined under various allocations and conditions in continuous exercise protocols. As discussed above, ice slurry ingestion and other pre-cooling methods have shown to benefit physical performance, physiological measures, and perceived values in continuous exercise based literature. Currently there is a need for more literature to focus on intermittent protocols, specifically simulations of team sport performance. The further literature in this area will provide sport practitioners an insight into the application of ice slurry ingestion to team sport performance.

2.6.3 Effects of ice slurry ingestion on intermittent exercise performance in the heat.

Very little literature has examined the effect of ice slurry ingestion on intermittent or sport specific protocols. To the authors knowledge only two studies (Duffield et al., 2013; Holm et al., 2015) and one part of an academic thesis (Aldous, 2016) have examined the effect ice slurry has on intermittent or football related exercise. Duffield et al. (2013) examined the effect of field-based pre-cooling strategies in a crossover design on professional football players during training and competition in the heat. Prior to one competition game and one team training, the participants underwent a 20 min pre-cooling intervention which involved wearing an ice-vest (frozen overnight), cold towels over their head and neck (soaked in 5°C water), and the internal cooling method of 350 mL ice slushy ingestion. Training sessions (9 participants) were randomised, and consisted of 2 x 10 min interval training, followed by 6 x 3 min of 5v5 small sided games. Competitions (7 participants) involved official A-League matches during the 2009/10 season. The results showed equivocal findings for the effects of pre-cooling for professional football players during competitive training and matches in the heat. Core

temperature in this study was lowered after the cooling protocol (P = 0.01 - 0.07) but only until after warm-up in training and was less evident in the games (P = 0.09-0.80). Similarly, ratings of perceived exertion (P = 0.07 - 0.60) and thermal stress (P = 0.01 - 0.89) were reduced with the cooling intervention in training and to a lesser extent in games (P = 0.13 - 0.62, P = 0.08 - 0.15, respectively). The authors suggested that the performance and thermoregulatory results in this study showed similarities to the surrounding literature, however less prominent due to the field (vs laboratory) setting.

Holm et al. (2015) examined practical pre and mid practice cooling interventions on running performance, perceived exertion, and thermal sensation during two formal pre-season practices. The authors examined 8 NCAA Division II football athletes, who played four bouts of simulated match play (11v11 or 10v10), three of which were 15 min bouts followed by a 4th 10 min bout. Following the 1st, 2nd, and 3rd bouts, players completed competitive sets of two, 27.4 meter sprints against other team members with time recorded between 2.74 and 27.4 meters. After the 4th bout, players completed an indoor shuttle running beep test. In the pre-cooling intervention ice towels were applied to the head and neck regions and draped across both legs for 10 min following a standardized warm-up and for 10 min during a 15 min break between the 2nd and 3rd scrimmage sessions. Sport beverage slurries (350 mL; -0.3 °C, 6% carbohydrate) were also served during cooling intervention; while the control received no ice towels and drank the same, uncooled sport beverage. Holm et al. (2015) found that pre-cooling had no effect on sprint performance (P = 0.51) or numbers of repetitions completed in the running beep test (P = 0.88). Ratings of perceived exertion did not change following any performance test, however, thermal sensation was lower following the 3rd sprint bout (P = 0.04) and the beep test (P = 0.005) for the pre-cooling protocol (Holm et al., 2015). Lower thermal sensation is seen in continuous exercise studies (Ihsan et al., 2010; Stanley et al., 2010; Yeo et al., 2012) and the suggested mechanism of cooling thermoreceptors, indicating to the brain that the body is cooler than actual temperatures, appears to be relevant to intermittent exercise. Although one limitation of this study was that it began in the evening (6:15 PM) with a mean ambient temperature of 25.6°C and 70% RH. Ice slurry ingestion has shown benefits to continuous exercise in a heated environment (>30°C) (Ihsan et al., 2010; Naito & Ogaki, 2015; Ross et al., 2011; Siegel et al., 2012). Utilising a hotter environment in the Holm et al. (2015) study may have provided further insights into the effects of ice slurry ingestion on football performance.

As part of the requirements of a Doctor of Philosophy degree, Aldous (2016) investigated the impact of 30 min of pre-cooling and 15 min of half-time cooling via externally placed ice packs, ice slurry ingestion, and a mixed method (ice packs and ice slurry ingestion) pre-cooling protocol on physiological and performance variables during the intermittent soccer performance test protocol in a heated environment (30°C, 50% RH). The study utilised 8 male football players, aged 18 - 33, playing for the University of Bedfordshire (UK) football team who had been free from musculoskeletal injuries for greater than 6 months. Ice slurry ingestion significantly reduced thermal sensation (ice slurry 5.2 ± 0.4, control 5.5 ± 0.2, P = 0.001) and rectal temperature by 0.5°C (P = 0.04) prior to exercise. Ice slurry ingestion did not result in any

significant improvements in any other physiological or performance variables. However, the author found that the combination of ice slurry ingestion with placed ice packs (mixed method) significantly improved ($P < 0.05$) total distance, high-speed distance and variable run distance covered by 3%, 4% and 5% during the 1st half of ISPT, respectively. No significant differences were noted during the second half of the ISPT regardless of pre-cooling method. The results of these three studies show ice slurry ingestion could provide promising results to intermittent/football protocols. These studies have shown improvements from ice slurry ingestion in performance (Aldous, 2016), improved perceived thermal sensation (Aldous, 2016; Holm et al., 2015), and ability to lower core temperature (Aldous, 2016; Duffield et al., 2013; Holm et al., 2015). Ice slurry is an effective pre-cooling method, resulting in an athlete feeling cooler and may improve performance during intermittent protocols/sport. Although, the variation in other results of these three studies (Aldous, 2016; Duffield et al., 2013; Holm et al., 2015) show further research is required to gain a clear understanding of the effect of ice slurry ingestion on exercise performance. Due to the lack of literature on ice slurry ingestion prior to intermittent exercise and the proposed mechanisms involved, comparisons have to be made from continuous based exercise protocols and their impact on performance, perceptual, and physiological variables.

2.7 Pre-cooling on mucosal immunity

To the author's knowledge no current studies have investigated the effect of pre-cooling on mucosal immunity in response to exercise in the heat. In fact, the only study that has investigated any form of pre-cooling on mucosal immunity was done under resting conditions, in a temperate environment. Janský et al. (1996) examined the effect of single cold water immersion (immersed to mid chest) on salivary S-IgA of 10 young athletic males. The results indicated minimal effect on immunity after single water immersions. The continuation of water immersions (three times per week for six weeks) resulted in small changes in immune response, with no significant changes found in salivary S-IgA concentrations. Janský et al. (1996) concluded that repeated cold water immersion caused shivering which increased metabolic rate and increased blood concentrations of catecholamines, activating the immune system to a slight extent. Both single and continued cold water immersion were completed at rest without participants exercising in any form.

As previously stated, the effect that pre-cooling prior to exercise in heat has on the immune system and more specifically salivary S-IgA is currently unknown. Theoretically, the higher core temperatures seen at exhaustion (Ross et al., 2011; Siegel et al., 2010; Siegel et al., 2012) after ice slurry ingestion could have a detrimental effect on mucosal immunity. This theory could be supported due to ice slurry ingestion cooling brain temperature, delaying central drive fatigue, which allows the body to exercise for longer. The longer duration of exercise at high core temperatures may place further strain on the mucosal immune system. In short, there is not enough literature in the area to come to a definite conclusion on the effects of pre-cooling on mucosal immunity. Further research is required to examine this proposed theory.

2.8 Conclusion

In conclusion, mucosal immunity provides the first line of defence against foreign pathogens and infections. Mucosal immunity can be negatively affected by prolonged highly intense bouts of exercise, further exacerbated by competing in hot and humid environments. Football players are exposed to long, physically draining seasons which have shown to effect mucosal immunity (Morgans et al., 2014). This effect could be further exacerbated by tournaments in hot climates, such as the 2022 FIFA World Cup in Qatar. It has been suggested that intermittent exercise provides greater thermal and physiological strain, compared to endurance exercise, although limited research has examined football specific exercise protocols. Pre-cooling provides a method to reduce the heat stress placed on an athlete, however, little research has examined the effect that pre-cooling has on mucosal immunity. Ice slurry ingestion provides an internal cooling method and may provide a method of reducing an athlete's core temperature, potentially improving performance and perceived exertion.

Chapter 3: Methods

3.1 Study design

A randomised repeated-measures, crossover design, with participants acting as their own controls, was utilised for this study. Participants were required to visit the laboratory on 4 occasions. The first visit involved measurement of height, body mass, peak speed, and peak oxygen consumption (VO_{2peak}). The peak speed assessment involved a thorough warm up and 4 x 6 s sprints on the non-motorised Woodway treadmill, acting as the first familiarisation session with this equipment. The second visit involved a 30 min familiarisation session of the Intermittent Soccer Performance Test (ISPT) protocol and two saliva samples. The next two visits were the experimental (control or intervention) trials carried out in a randomised order. The trials required participants to ingest either ice slurry or room temperature fluid (control) prior to completing the ISPT in a heated environmental chamber set to 30°C and 50% RH. Allocations of fluid, aligning with each trial, were provided during a 30 min period prior to exercise. During the 15 min half-time interval a further fluid, aligning with each trial, was ingested. Throughout the exercise protocol various thermoregulatory, cardiovascular, immunity, and performance measures were quantified. All tests were performed in the Sports Physiology laboratory at the Sports Performance Research Institute New Zealand, AUT Millennium.

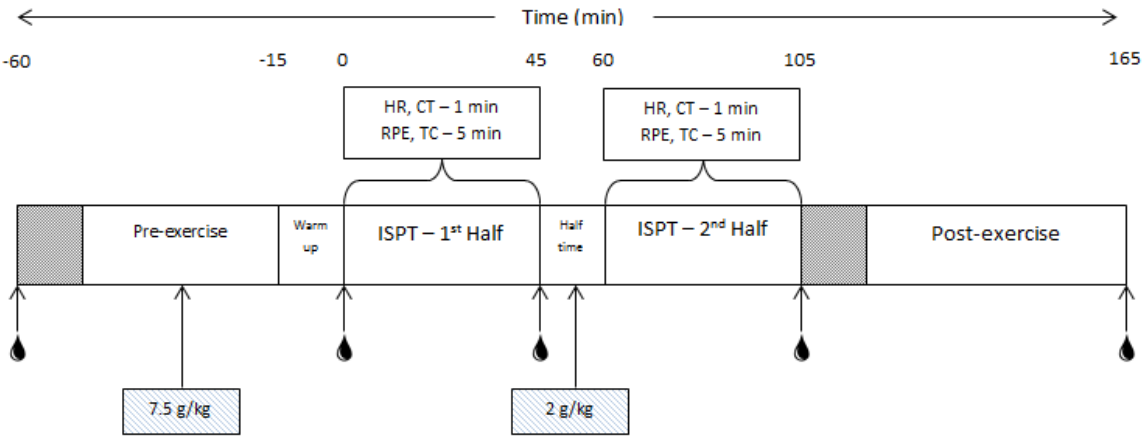

Figure 3.1 A schematic figure detailing experimental trial procedures. = Saliva sample taken. = Temperature recorders applied or removed. = Ice slurry allocations. HR = Heart rate. CT =Core temperature. RPE = Rating of perceived exertion. TC = Thermal comfort. 1 min = Reading taken every minute. 5 min = Reading taken every 5 minutes.

3.2 Participants

Participants (n = 8) were semi-professional football players aged between 18-35 years of age, playing at a regional level (Lotto Northern Regional Football League, Premier Division, Auckland

Football, New Zealand) (Table 3.1). Each participant was actively training at least twice and playing at least one full 90 min match per week (autumn season, ambient conditions: ~16°C, 81% RH). The inclusion criteria for this study included a VO_{2peak} greater than 50mL·kg^{-1}·min^{-1}. Exclusion criteria for the study included; any recent joint or musculoskeletal injuries, asthma or any other associated illnesses/diseases (i.e. upper respiratory tract infection (URTI), flu, viral infection) that would interfere with immunity markers examined in this study. All participants were informed, verbally and in written form, of the risks associated with the testing and the requirements of their participation, and were given the opportunity to have any questions answered. Prior to participation, all participants provided written, informed consent in accordance with the Research Ethics Committee at Auckland University of Technology #16/148.

Table 3.1 Anthropometric parameters and physiological values at the beginning of the study

Age (y)	21.6 ± 2.0
Body mass (kg)	76.76 ± 7.2
Height (cm)	178.2 ± 5.1
VO_{2peak} (mL·kg·min⁻¹)	53.9 ± 1.7
Peak speed (km·h⁻¹)	20.2 ± 0.9

Values are mean ± SD

3.3 Preliminary testing

Upon arrival to the laboratory the participants had their height (Stadiometer, Harpenden, HAR 98.602, Holtain Wales, UK) and body mass (Tanita, BWB0800, AlliedWeighing) measured, to the nearest 0.1 cm and 0.1 kg respectively, while wearing minimal clothing and no footwear. Prior to each use of the digital scales, they were set for zero. For height measurements, participants stood with their feet together in contact with the Stadiometer and kept their head in the Frankfurt plane. Participants were instructed to inhale deeply whilst keeping their heels 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 and take their height measurement.

3.3.1 Peak Oxygen Uptake

Participants completed a graded exercise test to volitional exhaustion on a motor driven treadmill (Saturn 4.0, h/p/ Cosmos Sports and Medical GmbH, Nussdorf-Traunstein, Germany) to determine peak oxygen consumption (VO_{2peak}), approximately 2 weeks prior to the beginning of experimental trials. The test began at 8 km·h^{-1} at a 1% incline in order to reproduce the energetic cost of outdoor running on a flat surface (Jones & Doust, 1996). The speed increased to 10 km·h^{-1} after 2.5 min, continuing to rise 1 km·h^{-1} every 2.5 min thereafter, until volitional exhaustion was achieved.

Expired air was measured continuously for concentrations of oxygen and carbon dioxide using a calibrated Parvo metabolic cart (Parvo Trueone, Sandy, Utah), connected to a HP EliteDesk computer (800 G2 SFF) utilising data acquisition/analysis software (ParoMedics OUSW 4.3.4, v.20111228).The metabolic cart was calibrated according to manufacturer's instructions prior to each test to ensure accuracy of measurement. Peak oxygen consumption was defined as the highest value achieved (averaged every 30s) during the graded test. Determination that VO_{2peak} was achieved was confirmed via reaching two of the following measures; a respiratory exchange ratio above 1.10, heart rate within 10 beats·min^{-1} of predicted maximal heart rate and a rating of perceived exertion (RPE) of 20.

Ratings of perceived exertion were measured using the Borg 6 - 20 scale (Borg, 1982). This scale is considered to be a valid and reliable marker of exercise intensity during a number of exercise tests (reviewed in Eston (2012). It has also been widely utilised during various soccer based drills (Little & Williams, 2007) and previous soccer-specific simulation studies (Oliver et al., 2007; Thatcher & Batterham, 2004).

Heart-rate measurements were recorded using a heart rate monitor (Polar RS800CX, Polar Electro Oy, Kemplele, Finland); set to record heat rate data every 5 s. These monitors have been shown to be reliable and accurate (Achten & Jeukendrup, 2003; Laukkanen & Virtanen, 1998).

3.3.2 Peak speed assessment

The peak speed assessment (PSA) was completed to assess the maximal speed the participant could achieve on the non-motorised Woodway treadmill (NMT) (see section 3.6) once familiarised with the equipment. Initially each participant was given 5 min of self-selected pacing on the NMT. In this time participants became accustomed to the machine; specifically how the belt felt while running, and how to change speeds. The peak speed assessment consisted of 4 x 6 s maximal sprints with 1 min active recovery between each bout. For each participant, the peak sprint speed was defined as the fastest speed recorded throughout the PSA. The participants' speeds were then analysed from the PSA by a bespoke spread-sheet (excel) and utilised to set the percentage of all speed thresholds in the ISPT protocol (section 3.5).

3.4 Familiarisation

As non-motorised treadmills have a unique feel and are unlike motorised treadmills found in gyms, familiarisation had to occur for accurate force readings. The familiarisation session included a standardised warm up and 30 min of the ISPT protocol (2 x 15 min blocks). This session was designed to help the participant get familiar with changing speeds on the NMT, maintain balance, and sprint effectively.

Participants were given a warm up prior to the 2 x 15 min blocks. Throughout the standardised

warm up participants were instructed to follow the change of speed categories on the pacer technology. The warm up protocol had similar speed categories as the experimental ISPT protocol, however, actual speeds completed were standardised and were based on a peak speed of 20 km·h^{-1}. The duration of time spent in each speed category was reduced during the warm up; this was designed to get participants used to changing speed quickly. The warm up consisted of two sprints near the end of the protocol, separated by 90 s. The sprints included in the warm up were reduced to 4 s, compared to the 6 s used throughout the ISPT

Thereafter, 2 x 15 min blocks (30 min total) of the ISPT protocol (section 3.5) were completed. This allowed participants to become familiar with the different movement categories within the ISPT and to quickly change between them. Participants were also acquainted with saliva collection methods (see section 3.8) prior to and after the 30 min run, to ensure accurate samples were obtained during subsequent experimental trials.

3.5 Intermittent Soccer Performance Test Protocol

The ISPT has previously been shown to be a valid and reliable soccer specific performance test (Aldous et al. (2014). The protocol consists of 2 x 45 min halves, separated by a 15 min half-time interval. Each 45 min half is made up of 3 x 15 min blocks, the activity profile of which is based on several soccer match play studies (Bangsbo et al., 1991; Mayhew & Wenger, 1985; Reilly & Thomas, 1976). Activity profile of the ISPT is shown in Appendix A. During the protocol, participants interact with a computer program (Pacer Performance System Software, Innervation) by following two lines on the screen, which display their target speed and current speed. Participants were instructed to match their current speed with the target speed as closely as possible throughout the full protocol. Visual and audio cues were given prior to every change in movement category. The participants were able to see and hear what speed they were changing to in the next category, for example ‘three, two, one, sprint’. As per Aldous et al. (2014), speed categories were determined on percentages from each participant’s PSA. Speed categories were further adjusted if the participant achieved higher speed in the second familiarisation session to ensure an accurate representation of the athletes peak speed. Adjusting the categories for each participant allowed the free running ability of the NMT to be quantified and specific to the individual, as used previously (Abt et al., 2003; Sirotic & Coutts, 2008). The percentages used (Table 3.2) determined the speed targets for each of the movement categories. For example, if a participant had a peak speed of 20 km·h^{-1}, the following speeds would be set for each movement category; stand (0 km·h^{-1}), walk (4 km·h^{-1}), jog (7 km·h^{-1}), run (10 km·h^{-1}), fast run (12 km·h^{-1}), sprint (20km·h^{-1}). A bespoke spreadsheet (Microsoft Excel 2013, Microsoft) was used to alter the speeds of the original ISPT protocol to suit each participant.

Table 3.2 Movement categories with percentage of PSA, example speed, and frequency of occurrence

Movement category	% of PSA	Example (25km·h^{-1})	Frequency per ISPT
Stand	0	0	120
Walk	20	4	228
Jog	35	7	150
Run	50	10	96
Fast run	60	12	36
Variable run	Unset	Unset	24
Sprint	100	20	36

Each 15 min activity profile consisted of 6 sprints. Verbal encouragement was given for each sprint to ensure maximal effort. Participants were instructed to ignore the target speed/screen for the sprints and told to sprint as fast as they could. A verbal countdown to the end of the sprint was given (three, two, one) to let the participant know when to stop sprinting. The sprints utilised in this protocol were 6s, as this duration was found to be highly reliable on the NMT (Hughes et al., 2006).

The variable run is a speed category designed to be a self-selected speed above the second ventilatory threshold. The speed category is a 6 s run occurring four times in the 13th - 14th min of each 15 min block. Participants were instructed to cover as much distance as possible in the 6 s without sprinting.

3.6 Woodway treadmill

The ISPT protocol and familiarisation sessions were completed on a non-motorised Woodway treadmill (Woodway 3.0, Eugene, OR, USA). While on the treadmill the participants' wore a harness around their waists, which was tethered to an anchor point behind, allowing them to overcome the inertia of the treadmill belt to perform the locomotor movements required during the ISPT protocol. During each locomotor movement measures of speed, distance, horizontal and vertical force were collected at a sampling rate of 200 Hz by the XPV7 PCB interface (Fitness Technology, Adelaide, Australia) and analysed with Force 3.0 software.

Vertical force was measured by 4 individual vertical load cells that were mounted under the running surface. The vertical load cells were calibrated before and after each testing session by placing a range of known weights on the treadmill deck according to the manufacturer's instructions. The first weight was at the low end of the range (i.e., 60 kg), and the second weight

was at the top end of the expected forces measured (i.e., 300 kg). The horizontal load cell was attached to a metal vertical strut with a sliding gauge, which locked into place to avoid any movement during testing. The sliding gauge allowed the horizontal load cell to be adjusted vertically in accordance with the participant's height, so that the tether was horizontal to the load cell during the running bouts. The load cell was calibrated before and after each testing session using a range of known weights hanging from the load cell. The first weight was at the lower end of the range (i.e., 10 kg), and the second weight was toward the top end (i.e., 30 kg) of the expected forces to be measured. Two different force inputs were required so that a line could be fitted, the slope of which was the calibration factor and the y-intercept was the zero offset. Force was calibrated into Newtons (N) by multiplying the mass of the calibration object by 9.81 $m \cdot s^{-1}$ (i.e., the acceleration due to gravity) (Brughelli, Cronin, & Chaouachi, 2011).

3.7 Experimental trial procedures

Prior to arrival, participants were instructed to attend trials in a performance ready state, being well rested and hydrated. Participants were asked to refrain from exercise 24 h prior to experimental trials to ensure they were free of fatigue and muscle soreness. Participants abstained from consumption of alcohol and caffeine in the 24 h prior to trials. Participants were also required to fill out a diet diary (Appendix B) in the 24 h prior to an experimental trial and were asked to replicate this for 24 h prior to the subsequent trial. Participants were allowed to eat prior to trials, with consumption no later than 06:00 h. The pre-trial meal was recorded on the food diary and was repeated on the following trial. Participants were required to consume 500 mL of fluid prior to both trials.

Participants completed two experimental trials which consisted of completing 90 min of the ISPT protocol with either ice slurry (<1°C) or a control fluid (~20 °C) prior to exercise. Upon arrival to the lab (08:00 h), participants had their body mass measured to determine the amount of ice slurry or control fluid they received. To combat any potential placebo effect the participants were informed that different temperature beverages would be used in the trials and that both fluids had the same amount of carbohydrate. Body mass was also measured after the exercise protocol to calculate body mass loss. An initial saliva sample (see section 3.8) was taken for 3 min to obtain a baseline measure.

The participants were then led to a private bathroom where they inserted the rectal thermometer. A piece of gauze, tied at 12cm, was used as an indicator of depth for the rectal thermometer. A rectal thermometer (Monatherm Thermistor; 400 Series, Mallinckrodt Medical, St Louis, MO) was used to measure rectal temperature (T_{re}) during all experimental trials. 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. T_{re} measurements were taken every min throughout the trials which was recorded onto a data logger (1 Hz; Grant Instruments, Shepreth, UK)

Following placement of the rectal thermistor, skin thermometers were taped onto specific points in order to record skin temperatures. Thermistors were located on the mid belly of the left bicep, chest, quadriceps, and gastrocnemius. Skin temperature was measured with four reusable skin thermistors (DS-1922L; Maxim Integrated, San Jose, CA) which were attached via adhesive tape. Measurements of skin temperature were taken every min throughout testing and retrieved from a data logger program (0.5 Hz; DS-1922L, Maxim Integrated, San Jose, CA). Various formulas, as shown below, were utilised for mean skin, mean body, and muscle temperatures;

Mean skin temperature: $Tsk = 0.3 \cdot (Tchest + Tarm) + 0.2 \cdot (Tthigh + Tcalf)$.

Mean body temperature: *Tbody = 0.79 (Tcore) + 0.21 (Tskin).*

Muscle temperature: *Tmu= 1.02*Tsk + 0.89*

At this point an initial core temperature measure was recorded. The participants then consumed the ice slurry or control beverage (8:10 a.m.). Both solutions included the same flavouring and consisted of 5% carbohydrate (92% sucrose, 8% glucose). To standardise consumption rates, the amount of ice slurry or control consumed prior to exercise was divided into 3 portions of 2·5 $g \cdot kg^{-1}$ or 2·5 $ml \cdot kg^{-1}$ every 10 min until reaching 7·5 $g \cdot kg^{-1}$ or 7·5 $ml \cdot kg^{-1}$ at 30 min (ice slurry and control beverage, respectively). Standardisation of consumption rates were utilised to limit the amount of gastric discomfort of consuming the cold beverage too quickly. The ice slurries were made via blending (Sunbeam, Multiblender platinum, Sunbeam Corporation Limited, Auckland,) 275 g of ice from an ice flaker machine (Scotsman af103, Hubbard Systems, Ipswich, Suffolk), 200 ml of water, and 35 g of Gatorade powder (Sports drink, lemon and lime flavour). Amounts of ice slurry consumed were standardised according to the participant's body mass. Prior to exercise, participants consumed 7.5 $g \cdot kg^{-1}$ of pre-cooling beverage, with a further 2 $g \cdot kg^{-1}$ at the half-time interval. Half-time consumption was completed in the final 10 min of the interval after collection of the saliva sample. Participants were given the same amount of fluid (7.5 $ml \cdot kg^{-1}$) as the ice slurry protocol but at room temperature. The fluid contained the same amount of carbohydrates as the ice slurry trial to avoid any changes in immune or performance responses. Serving allocations and times stayed consistent between trials.

Immediately following ingestion (8:40 a.m.) of ice slurry or control fluid, a belt was fitted for attachment to the Woodway Treadmill. The standardised warm up protocol (section 3.4) from the familiarisation sessions was used prior to both trials and were completed inside the heat chamber. To generate reliable heated conditions a heat chamber (Design Environmental Ltd, Gwent, UK) was utilised. The chamber was programmed to 30°C and 50 % RH.

Once the warm up was completed (8:50 a.m.) the participants exited the heat chamber to undertake any dynamic and static stretches they felt were necessary prior to the ISPT protocol. Exiting the chamber at this point replicated soccer athletes utilising the field of play in a stadium to warm up. A procedure commonly used in FIFA organised tournaments. Athletes then returned to an air conditioned changing room for final instructions from the coach and a final preparation period before gameplay. Upon exiting the chamber the participants had 5 min of

stretching and general preparation, and then a pre-exercise saliva sample was taken. (Lab conditions: 18.6 ± 0.8ºC and 60 ± 7% RH).

On entering the heat chamber (9:00 a.m.), participants stated their RPE and thermal comfort. Thermal Comfort was measured using a modified 10 point scale based on Gagge, Stolwijk, and Hardy (1967). Participants were asked 'how comfortable do you feel with the temperature of your body?'. The scale was set from 1.0 (comfortable) to 10 (beyond extremely uncomfortable) and gave an indication of their satisfaction with the thermal environment during the ISPT protocol. Heat-rate and core temperature were also recorded at this time. Participants were then clipped onto the NMT using the belt worn, provided a drink bottle and towel for wiping sweat. The drink bottle provided was filled with room temperature water, to which the athlete could consume *ab libitum* during the first trial. The ISPT protocol began once the participant was comfortable and ready to partake. Heart rate and core temperature were taken every min during the exercise protocol. Every 5 min the participant was asked their RPE and thermal comfort. Participants were given verbal encouragement for every sprint to ensure maximal effort throughout the protocol. On 45 min, the participants were asked for their RPE before exiting the heat chamber (9:45 a.m.).

Similar to the warm up procedure, the participants left the chamber to replicate air-conditioned changing rooms. This was chosen to replicate a professional team environment where players would enter an air-conditioned changing room found in stadiums. At this time-point a mid-exercise saliva sample was obtained. Immediately following saliva collection, participants consumed 2 $g \cdot kg^{-1}$ or 2 $ml \cdot kg^{-1}$ of the beverage aligning with the trial (ice slurry and control beverage, respectively). Throughout the half-time period core temperature, heart rate, RPE, and thermal comfort were taken. The provided water bottle was weighed at the half-time interval to measure how much water was consumed. A new bottle, filled with room temperature water, was provided for the second half. On 15 min post the first half, participants re-entered the heat chamber and prepared for the next half (10:00 a.m.). The second half runs were in the same manner as the first with variables recorded at the same times. On completion of the second half, participants were asked their final RPE and exited the heat chamber (10:45 a.m.). A post-exercise saliva sample was taken before all of the temperature recorders were removed. Participants were then instructed to remove their footwear and have their BM measured. The provided water bottle was again weighed to calculate total water consumption throughout the trial. The amount of water consumed in both halves of the first trial were calculated and replicated in the following trial. One hour following exercise cessation, a final saliva sample was collected (1 h post-exercise). During the hour between post-exercise and 1 h post-exercise saliva samples, participants were permitted to consume water.

3.8 Saliva Collection

Prior to each saliva collection, participants were instructed about the collection protocol and encouraged to minimise orofacial movement. Participants were asked to swallow anything in

their mouth prior to the collection of saliva to ensure an unstimulated sample. During collection participants were seated, leaning forward, with their heads tilted down. The participant then allowed saliva to collect in a provided tube (7 ml-capacity with screw top, Labserve, Auckland, NZ) which was weighed pre and post collection to measure saliva flow rate.

Completed saliva sample volume was estimated by weighing to the nearest milligram assuming salvia density to be 1.0 $g \cdot ml^{-1}$ (Cole, McGivan, Eastoe, Hayes, & Smillie, 1988). Saliva flow rate ($ml \cdot min^{-1}$) was analysed by dividing the total volume of saliva by the amount of time needed for collection. Samples were frozen at -80°C until analysis was completed.

3.9 Saliva Analysis

The saliva samples were subsequently analysed for S-IgA, cortisol and osmolality. Samples were initially thawed and spun at 13,400 rpm for 2 min prior to analysis.

The concentration of S-IgA ($mg \cdot L^{-1}$) was determined by an enzyme linked immunosorbent assay (ELISA) method using a commercially available kit (DRG SLV-4636, DRG Instruments, Marburg, Germany). Additionally osmolality was determined using a freeze point depression osmometer (Model 3320 Micro-Osmometer, Advanced Instruments, Massachusetts, USA) with 20 µl of saliva. Saliva cortisol concentration was determined using the Elecsys Cortisol immunoassay analyser.

The secretion rate of S-IgA ($\mu g \cdot min^{-1}$) was calculated by multiplying saliva flow rate ($mL \cdot min^{-1}$) by its concentration ($mg \cdot L^{-1}$). All samples from one participant were analysed on the same microplate and run in duplicate. The intra assay coefficients of variation for the analytical methods were 3.0%, 1.3% and 1.5% for saliva S-IgA, cortisol and osmolality assays, respectively.

3.10 Statistical analysis

All data are presented as mean values and the standard deviation. The performance, physiological, and perceived data were analysed three different ways; examining the difference between trial means, means of trial halves, and the means of the 15 min exercise blocks within trials.

Mean comparisons between trials and halves for all performance, physiological, perceived, and saliva variables were made using Student's paired t-tests. Differences between trials and halves were also examined using Student's paired t-tests at pre-exercise and post-exercise for physiological measures. Statistical significance was accepted at $P < 0.05$. Analysis of the 15 min blocks used a 2 (trial) x 6 (time of measurement) ANOVA with a repeated measures design. Prior to analysis, data was initially checked for normality of distribution. If a data set was not normally distributed, a logarithmic transformation was performed before further analysis. Assumptions of homogeneity and sphericity in the data were also checked. If required and

appropriate, adjustments in the degrees of freedom for the ANOVA were made using the Huynh-Feldt method of correction.

Any statistically significant findings were further analysed using post-hoc testing with paired samples t-tests, with Holm-Bonferroni adjustments for multiple comparisons applied to the unadjusted P value. Saliva results utilised a similar analysis method, except examining 5 time-points; baseline, pre-exercise, half-time, post-exercise, and 1 h post-exercise. The observed powers of the reported main and interaction effects were all >0.8.

Further differences in performance, physiological, subjective and saliva measurements were analysed using a magnitude-based inference approach (Hopkins, Marshall, Batterham, & Hanin, 2009) and were determined using published spreadsheets (xParrallelGroupsTrial.xls) from sportsci.org (Hopkins et al., 2009). Magnitude based inferences were used due to the sport specific nature of the statistical approach.

Uncertainties in performance, physiological, subjective and saliva variables were expressed as probabilities of harm or benefit in relation to the coefficient of variation (±1.0%). This system was utilised to specify the possible benefit or harm of ice slurry ingestion compared to the control trial. To make inferences for performance measures an estimate of the smallest worthwhile change (SWC) was required. The SWC was set at 0.2, based on published literature examining the validity of a repeated-sprint test for football players (Impellizzeri et al., 2008).

Magnitude thresholds were utilised to determine the SWC for differences between trials and halves in which the possible range of change was transformed into a full scale of deflection (Hopkins, 2010). The range used to analyse such differences was made from 0- 100% and magnitude thresholds were defined as 10%, 30%, 50%, 70% and 90% for small, moderate, large, very large and extremely large changes, respectively. Quantitative changes throughout performance, physiological, subjective and saliva measures were assessed qualitatively as follows: <1%, *most unlikely*; 1-5%, *very unlikely*; 25-75%, *possible*; 75-95%, *likely*; 95-99%, *very likely*; 99%, *most likely* (Hopkins et al., 2009). An effect higher than 5% in both beneficial and harmful categories was described as *unclear*.

Section 4: Results

4.1 Physiological characteristics

The physiological characteristics of the participants are presented in *Section 3.2.* All participants met the entry criteria by possessing a $VO_{2peak} \geq 50ml \cdot kg^{-1} \cdot min^{-1}$.

4.2 Performance outputs

The mean physical running outputs from the ISPT performed in both ice slurry and control conditions are presented in Table 4.1. There were few differences between trials as highlighted in the following sections.

Magnitude based inferences that have been shown to be *most likely trivial* or *very likely trivial* have been presented in table form (refer to Table 4.3) and have not been discussed further in this section.

4.2.1 Total Distance

There was no significant interaction or trial effects found for total distance. A significant effect for time ($P < 0.001$) was found, with greater distance being covered within the first 15 min block (0 - 15 min) compared to the third (30 - 45 min) and the final 15 min (75 - 90 min) block ($P < 0.001$). Mean total distance covered was similar between the ice slurry and control trial (8726.2 ± 252.9 m and 8698.4 ± 215.4 m, respectively) (Table 4.2).

Magnitude based inferences showed that ice slurry provided *possibly beneficial* results to total distance in the last 15 min block (75 - 90 min) of exercise. Other differences between trials and halves were shown to be *very likely trivial* (Table 4.3).

4.2.2 Sprint performance

No significant interaction or trial effects were found for peak or mean sprint speed. However, there was a significant main effect for time ($P < 0.001$). Peak and mean sprint speed in the first 15 min block (0 - 15 min) were significantly faster when compared to the third (30 - 45 min) and final (75 - 90 min) 15 min blocks ($P < 0.001$). The same time effect ($P < 0.001$) was also apparent for total sprint distance, with the greatest sprint distance being covered in the first 15 min block (0 - 15 min) when compared to the third (30 - 45) and final (75 - 90) 15 min blocks ($P < 0.001$) (Table 4.2). No significant differences were found between mean sprint speed between the ice slurry (16.69 ± 1.31 $km \cdot h^{-1}$) and control (16.7 ± 1.38 $km \cdot h^{-1}$) trial. Total sprint distance covered and mean sprint (per sprint) distance covered were similar in the ice slurry (1001.63 ± 78.28; 27.82 ± 2.17, respectively) and control (1002.50 ± 82.92; 27.85 ± 2.30, respectively) trials (Table 4.2).

Magnitude based inferences showed ice slurry ingestion to have a possibly beneficial effect on mean sprint speed compared to control. All other sprint results showed *likely trivial* or *very likely trivial* differences between trials and halves (Table 4.3).

4.2.3 High speed Distance

There was no significant interaction or trial effects found for high speed distance. However, a significant time effect (P = 0.035) was found between the first (0 - 15) and the third (30 - 45) block with greater speeds found in the first block (Table 4.2).

Magnitude based inferences showed ice slurry to have a *possibly beneficial* effect on high speed distance covered compared to control. Similarly, ice slurry showed *possibly beneficial* effects on high speed distance covered in the second half (45 - 90 min) compared to control (Table 4.3).

4.2.4 Variable run

There were no significant interaction or trial effects, however there was a main effect for time (P < 0.001). Irrespective of trial, more variable run distance was covered in the first 15 min block (0 - 15 min) compared to the third (30 - 45 min) and the final 15 min (75 - 90 min) block (P < 0.001) (Table 4.2). No significant differences in peak and mean variable run speed were found between trials. Mean variable run speed were similar in the ice slurry (11.09 ± 1.18 km·h^{-1}) and control trial (11.12 ± 1.45 km·h^{-1}) (Table 4.2).

Magnitude based inferences showed that differences between trials were *very likely trivial*. Peak variable run speed differences in the first half and second half were very *likely trivial* (Table 4.3).

Table 4.1 Mean performance variables between trials.

	Ice slurry	Control
Peak sprint (km·h ⁻¹)	20.91 (1.38)	20.88 (1.19)
Mean sprint (km·h ⁻¹)	16.69 (1.31)	16.7 (1.38)
Variable run distance (m)	11.09 (1.18)	11.12 (1.45)
High speed distance (m)	16.85 (0.62)	16.63 (0.51)
Mean sprint distance (m)	27.82 (2.17)	27.85 (2.30)
Sprint distance (m)	1001.63 (78.28)	1002.50 (82.92)
Sprint difference (%)	3.48 (1.34)	3.47 (1.53)
Distance covered (m)	8726.2 (252.9)	8698.4 (215.4)

Values are means ± (SD)

Table 4.2 Performance variables between 15 minute blocks and halves.

	0-15 min	15-30 min	30-45 min	45-60 min	60-75 min	75-90 min	1st half (0-45 min)	2nd Half (45-90 min)
Peak sprint speed (km·h ⁻¹) [a]								
Ice slurry	20.5 (1.4)	19.9 (1.5)	19.5 ± (1.6)	19.5 (1.8)	19.3 (1.7)	20.2 (1.8)	20.5 (1.4)	20.5 (1.5)
Control	20.7 (1.3	20.1 (1.3	19.4 (1.4	19.5 (1.5	19.1 (1.7	19.3 (1.9	20.7 (1.3	20 (1.7
Mean sprint speed (km·h ⁻¹) [a]								
Ice slurry	17.7 (1.2)	16.9 (1.4)	16.4 (1.4)	16.6 (1.4)	16.3 (1.4)	16.3 (1.5)	17 (1.3)	16.4 (1.4)
Control	17.9 (1.2	17.1 (1.5	16.4 (1.4	16.8 (1.4	16.4 (1.6	15.7 (1.7	17.1 (1.2	16.3 (1.7
Variable run distance (m) [a]								
Ice slurry	20.7 (1.7)	18.3 (1.8)	17.9 (2.5)	18.1 (2.3)	17.8 (2.5)	18.3 (2.6)	18.9 (2.0)	18.1 (2.5)
Control	20.8 (2.3)	19 (3.1)	17.5 (2.4)	18.8 (2.2)	17.4 (3.2)	17.7 (3.2)	19.1 (2.6)	18 (2.9)
High speed distance (m)								
Ice slurry	17.0 (0.7)	17.0 (0.6)	16.8 (0.6)	16.7 (0.6)	16.5 (1.2)	17.1 (0.8)	16.9 (0.6)	16.8 (0.7)
Control	17.1 (0.8)	16.9 (0.6)	16.6 (0.7)	16.8 (0.6)	16.2 (1.2)	16.2 (0.7)	16.8 (0.6)	16.4 (0.7)
Sprint distance covered (m) [a]								
Ice slurry	29.4 (2.0)	28.5 (2.3)	27.4 (2.2)	27.6 (2.3)	27.1 (2.3)	27.1 (2.2)	28.4 (2.1)	27.3 (2.3)
Control	29.8 (1.9)	28.5 (2.4)	27.3 (2.3)	28 (2.3)	27.3 (2.7)	26.2 (2.3)	28.5 (2.1)	27.2 (2.8)
Sprint difference (%) [b]								
Ice slurry	2.5 (1.2)	3.3 (1.5)	3.7 (1.3)	3.6 (1.5)	3.9 (1.4)	3.9 (1.5)	3.2 (1.3)	3.8 (1.4)
Control	2.3 (1.0)	3.1 (1.3)	3.8 (1.4)	3.4 (1.8)	3.8 (2.0)	4.5 (2.3)	3.0 (1.2)	3.9 (2.0)
Distance covered (m) [a]								
Ice slurry	1494.9 (45.9)	1462.5 (35.8)	1442.2 (39.4)	1439.2 (57.2)	1433.7 (55.2)	1453.7 (51.8)	4399.6 (116.0)	4326.6 (144.7)
Control	1499.3 (47.7)	1466.6 (43.2)	1435.1 (45.1)	1441.5 (43.3)	1424.6 (51.2)	1419.2 (70.5)	4400.9 (119.5)	4297.5 (141.0)

Values are means ± (SD). [a] Main effect of time; significantly lower at all 15 min blocks when compared to the first 15 min block ($P < 0.001$).

[b] Main effect of time; significantly higher at all 15 min blocks when compared to the first 15 min block ($P < 0.001$).

Table 4.3 Magnitude based inferences of performance differences between ice slurry and control in halves, and trials.

			Confidence limits			
Performance	Qualitative outcome	MDiff (90% CI)	Lower	Upper	"±"	positive/trivial/negative
Variable run (first half)	very likely trivial	-0.00 (0.05)	-0.2	0.15	0.17	1/97/2
Variable run (second half)	very likely trivial	0.01 (0.06)	-0.17	0.19	0.18	2/97/1
Variable run	very likely trivial	0.00 (0.05)	-0.17	0.15	0.16	1/98/1
Variable run (peak speed)	Likely trivial	-0.02 (0.03)	-0.17	0.15	0.15	1/98/1
Total distance	very likely trivial	0.00 (0.02)	-0.11	0.2	0.15	3/97/0
Total distance (first half)	very likely trivial	-0.00 (0.01)	-0.15	0.15	0.15	1/99/1
Total distance (second half)	likely trivial	0.01 (0.01)	-0.11	0.25	0.18	8/92/0
Total distance (last 15 min)	possibly beneficial	0.03 (0.03)	-0.03	0.31	0.17	28/72/0
Mean sprint speed	possibly beneficial	0.00 (0.02)	-0.04	0.34	0.19	32/68/0
Mean sprint speed (first half)	most likely trivial	-0.01 (0.02)	-0.14	0.08	0.11	0/100/0
Mean sprint speed (second half)	very likely trivial	0.01 (0.04)	-0.15	0.19	0.17	2/98/1
Peak sprint speed	very likely trivial	0.00 (0.3)	-0.19	0.21	0.2	2/95/3
Peak sprint speed (first half)	very likely trivial	-0.01 (0.02)	-0.18	0.04	0.11	0/99/1
Peak sprint speed (second half)	likely trivial	0.03 (0.06)	-0.15	0.36	0.26	22/77/1
Sprint distance	most likely trivial	0.00 (0.02)	-0.13	0.12	0.13	0/100/0
Sprint distance (first half)	most likely trivial	-0.01 (0.02)	-0.14	0.08	0.11	0/100/0
Sprint distance (second half)	very likely trivial	0.01 (0.04)	-0.15	0.18	0.17	2/98/1
Sprint difference	most likely trivial	0.01 (0.03)	-0.11	0.12	0.12	0/100/0
High speed distance	possibly beneficial	0.01 (0.02)	-0.04	0.34	0.19	32/68/0
High speed distance (first half)	most likely trivial	0.01 (0.01)	-0.05	0.16	0.1	0/100/0
High speed distance (second half)	possibly beneficial	0.02 (0.03)	-0.05	0.4	0.22	41/58/1

Qualitative outcomes for performance data indicate positive and negative values for beneficial or harmful chances, respectively. MDiff = mean difference. CI= confidence interval.

4.3 Physiological and perceptual measures

4.3.1 Core temperature

Pre-exercise core temperature was significantly lower in the ice slurry trial than in the control trial (main effect of trial; p=0.03) (Figure 4.1).

There were no significant interaction or trials effects, however there was a main effect for time ($P < 0.001$) when 15 min blocks throughout exercise were compared. Core temperature values significantly increased ($P < 0.001$), regardless of trial, across all time-points when compared to pre-exercise. Irrespective of trial, significant changes were also noted when comparing the 0 - 15 block to the 30 - 45 ($P < 0.001$) and 75 - 90 ($P < 0.001$) blocks (Table 4.5).

Differences between trials in mean core temperature were found to be *possibly trivial* with similar temperatures recorded in the ice slurry trial (38.5°C ± 0.4) and control trial (38.5°C ± 0.4). Differences in the first half between the ice slurry and control trial were *likely trivial* (Figure 4.1). Second half values indicated that ice slurry had a *possibly beneficial* effect on core temperature compared to the control trial (Figure 4.2). End of exercise core temperature in the first half were *likely trivial* differences between trials. Similarly, end of exercise core temperature showed *possibly beneficial* effects from ice slurry ingestion, with mean core temperatures of 39.1 ± 0.3°C and 39.3 ± 0.5°C, in the ice slurry and control trial, respectively. Core temperature differences at the start of the second half were *unclear* between trials, although finding the ice slurry trial was 0.2°C lower than in the control trial (Table 4.6).

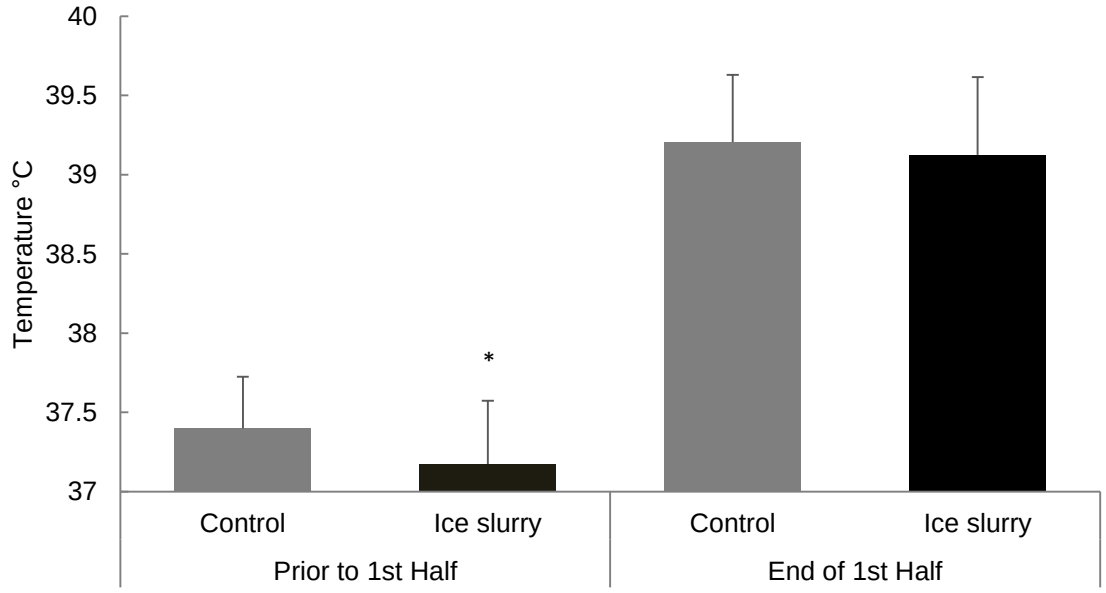

Figure 4.1 First half core temperatures in the control and ice slurry trials. Values are means + SD. * main effect of trial; significantly lower in ice slurry than the control trial.

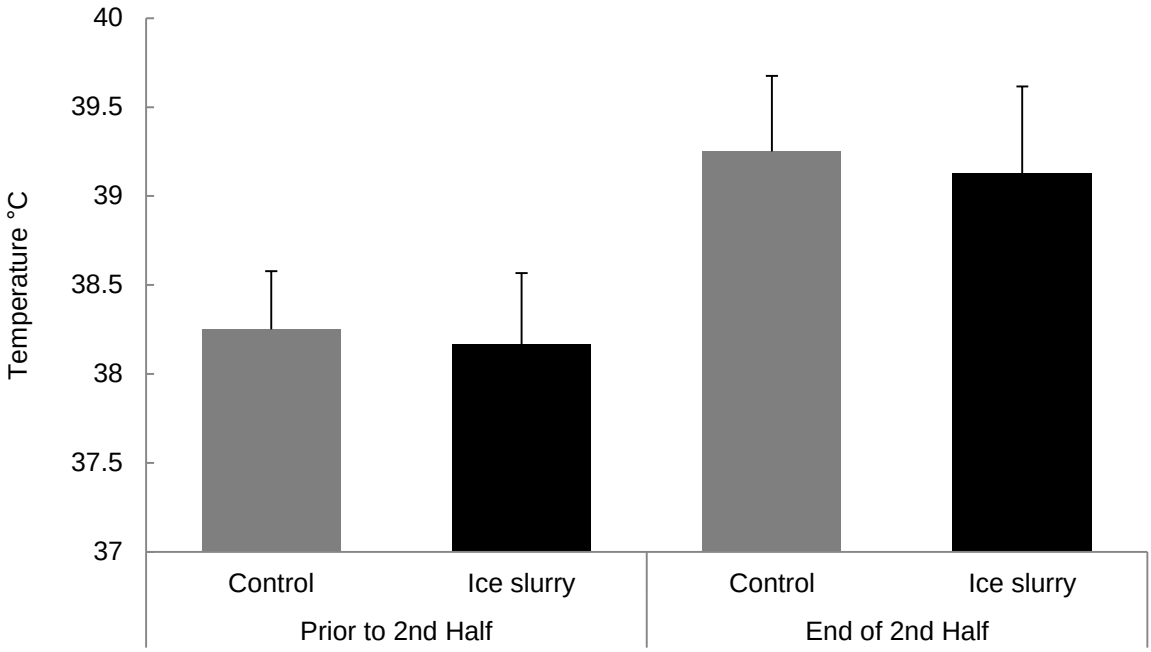

Figure 4.2 Second half core temperatures in the control and ice slurry trials. Values are means ± SD

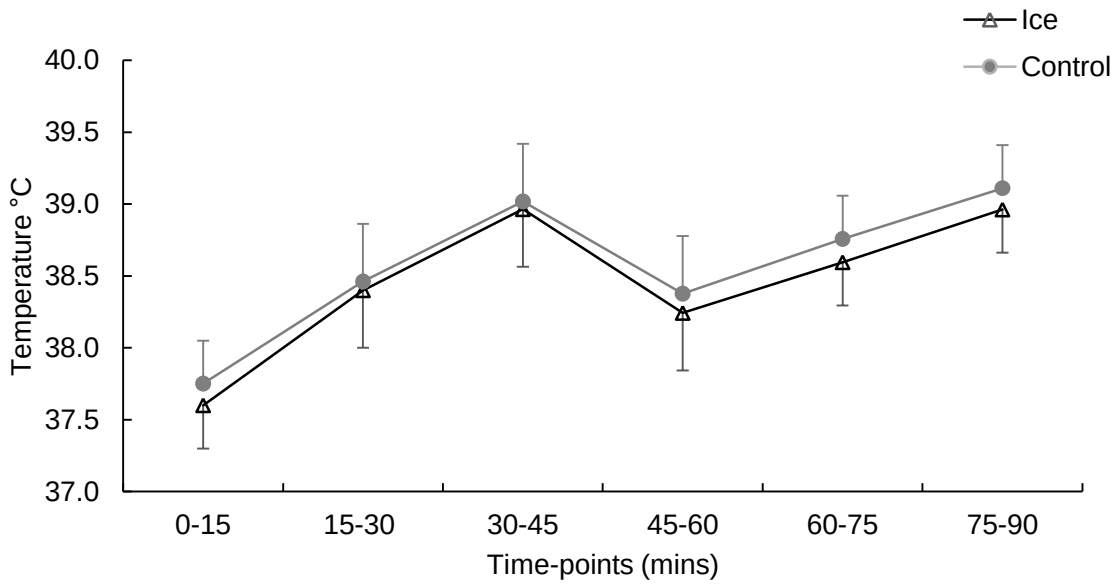

Figure 4.3 Core temperatures across each 15 min ISPT exercise block. Values are means ± SD

4.3.2 Skin temperature

No significant interaction or time effects were found for skin temperature, however there was a main effect between trials ($P < 0.001$). Significantly lower skin temperature was noted in the

control trial (33.6°C ± 0.6) compared to the ice slurry trial (34.2°C ± 0.7). No significant differences were noted between halves of each trial (Table 4.5).

Magnitude based inferences showed ice slurry had *likely harmful* effects on mean skin temperature compared to the control trial. First and second half skin temperature values indicated *possibly harmful* effects from the ice slurry trial compared to the control trial (Table 4.6).

4.3.3 Body temperature

No significant interaction or trial effects were found for body temperature, however there was a main time effect ($P < 0.001$). The main time effect showed body temperature to be significantly higher at all 15 min blocks when compared to the first 15 min block ($P < 0.001$).Mean body temperature showed no significant differences between the ice slurry trial (37.5°C) and control trial (37.6°C). Similarly, no significant differences were noted between halves of each trial (Table 4.5).

Magnitude based inferences showed *unclear* results when examining trials and halves (Table 4.6).

4.3.4 Muscle temperature

No significant interaction or time effects were found for muscle temperature, however there was a main effect between trials ($P < 0.001$). A significantly lower muscle temperature was noted in the control trial (35.2°C ± 0.6) compared to the ice slurry trial (35.7°C ± 0.7) (Table 4.5).

Magnitude based inferences showed ice slurry had *likely harmful* effects on mean muscle temperature compared to the control trial. First and second half skin temperature values indicated *possibly harmful* effects from the ice slurry trial compared to the control trial (Table 4.6).

4.3.5 Heart rate

There were no significant interaction or trial effects, however there was a main effect for time ($P < 0.001$). Mean heart rate during the 15 min blocks significantly ($P < 0.001$) increased as the exercise progressed, with the exception being between 15 - 30 and 30 - 45. First (0 - 15) block mean heart rates were significantly ($p<0.001$) lower compared to the last block, irrespective of trial. Heart rate showed a further significant ($P < 0.001$) increase from the first (0 - 15) block to the third (30 - 45) block (Table 4.5).

Mean trial heart rate was shown to be *possibly trivial*, with values being lower in the control trial compared to the ice slurry beverage trial with values of 156 ± 9 and 159 ± 9 beats·min^{-1}),

respectively. Mean heart rate values over the first half were *likely trivial*. Heart rate values in the second half were higher in the ice slurry trial (161 ± 11 beats·min^{-1}), stating a *possibly harmful* effect, compared to the control trial (158 ± 7 beats·min^{-1}). Differences between ice slurry and control were *unclear* for pre-exercise heart rate. Although not statistically significant, this data shows ice slurry may increase heart rate in the second half of exercise (Table 4.6).

4.3.6 Thermal comfort

No significant interaction or trial effects were found for thermal comfort, however there was a main effect for time ($P < 0.001$). As exercise progressed in the heat chamber, thermal comfort values significantly increased ($P < 0.001$) from block to block, regardless of trial. Thermal comfort values were significantly increased from the first (0 - 15 min) block to the last (75 - 90 min) in both trials ($P < 0.001$). The first (0 - 15) block had a significant ($P < 0.001$) increase compared to the third (30 - 45 min) (Table 4.5).

Magnitude based inferences showed a *possibly benefic*ial effect on overall mean thermal comfort, resulting in a score 15% higher in the control trial (6.0 ± 1.4) than in the ice slurry trial (5.2 ± 2.0). There was also a *possibly beneficial* effect of ice slurry on 1st half comfort measures, being lower (5.5 ± 1.2) compared to the control trial (4.9 ± 1.7). Similarly, *possibly beneficial* effects were seen in the second half, with participants in the ice slurry trial reporting they felt cooler in the second half with thermal comfort scores of 6.4 ± 1.8, as opposed to 5.5 ± 2.3 in the control trial (Table 4.6).

Ice slurry ingestion also showed a *possibly beneficial* effect on end of exercise thermal comfort scores. Similarly, ice slurry showed a *possibly beneficial* effect on pre-exercise thermal comfort (Table 4.6).

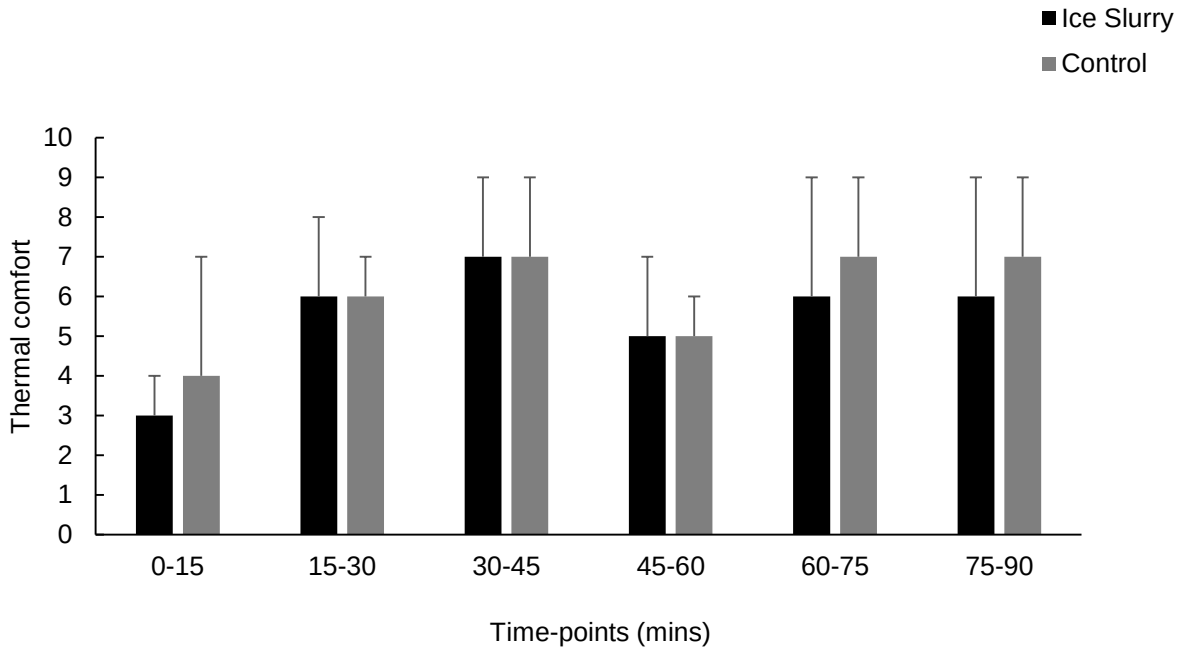

Figure 4.4 Perceived thermal comfort across each 15 min ISPT exercise block. Values are means + SD.

4.3.7 RPE

No significant interaction or trial effects were found for RPE, however there was a main effect for time ($P < 0.001$). RPE values changed significantly ($P < 0.001$) across all time-points in both trials. Irrelevant of trial, significant ($P < 0.001$) differences were found between the 0 - 15 and the 30 - 45 blocks. As expected a significant ($P < 0.001$) difference was found between the first (0 - 15) and last (75 - 90) block of exercise. Significant changes ($P = 0.006$) were also noted between the 30 - 45 and 75 - 90 blocks (Table 4.5).

Ice slurry had a *possibly beneficial* effect on mean RPE between trials, being lower in the ice slurry ingestion (14 ± 2) than in the control trial (15 ± 1). While differences in RPE between trials in the first half were *likely trivial*, a *possibly beneficial* effect of ice slurry on RPE in the second half (16 ± 1) was observed compared to control (15 ± 2). Likewise, the ice slurry trial had a *likely beneficial* effect on end of exercise RPE when compared to the control trial (Table 4.6).

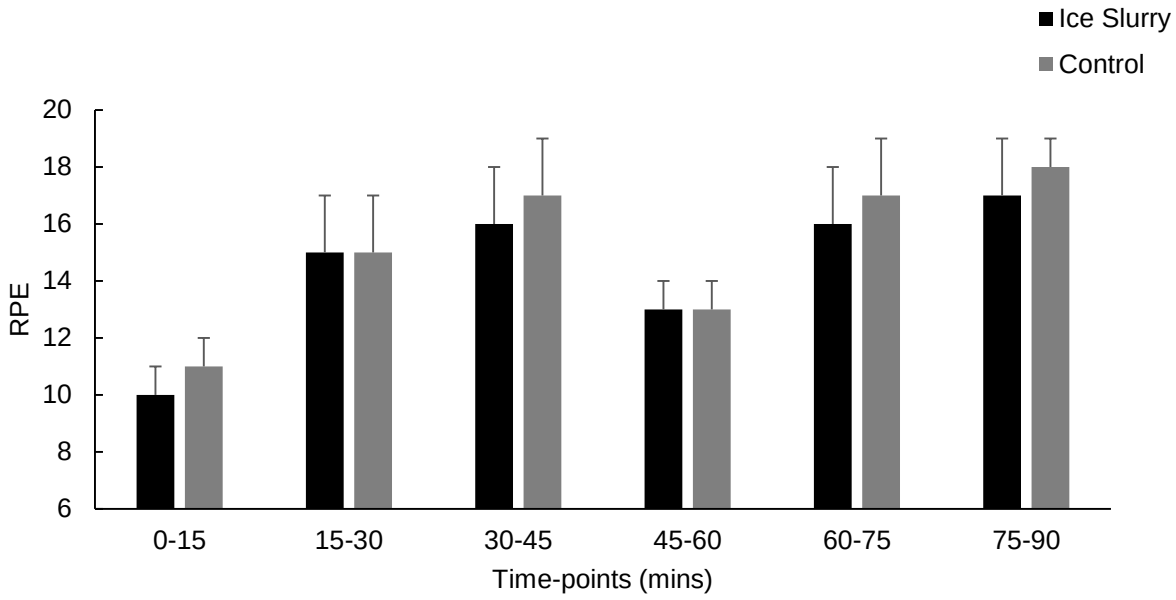

Figure 4.5 Rating of perceived exertion across each 15 min ISPT exercise block. Values are means + SD

Table 4.4 Mean physiological and perceptual variables between trials.

	Ice slurry	Control
Core Temp (°C)	38.5 (0.4)	38.5 (0.4)
Skin Temp (°C)	34.2 (0.7)	33.6 (0.6)
Body Temp (°C)	37.5	37.6
Muscle temp (°C)	35.7 (0.7)	35.2 (0.6)
Heart rate (beats·min $^{-1}$)	159 (9)	156 (9)
Thermal comfort	5 (2)	6 (1)
RPE	14 (2)	15 (1)
Body mass loss (L)	1.7	1.6

Values are means ± (SD)

Table 4.5 Physiological variables between 15 minute blocks and halves.

	Pre-exercise	0-15	15-30	30-45	45-60	60-75	75-90	1st half (0-45)	2nd Half(45-90)
Core temp (°C) [b]									
Ice slurry	37.2 (0.4)	37.6 (0.2)	38.4 (0.4)	39.0 (0.4)	38.2 (0.5)	38.6 (0.5)	39.0 (0.5)	38.4 (0.3)	38.6 (0.5)
Control	37.4 (0.3)	37.8 (0.3)	38.5 (0.4)	39.0 (0.4)	38.4 (0.4)	38.8 (0.3)	39.1 (0.3)	38.4 (0.4)	38.7 (0.3)
Heart rate (beats·min $^{-1}$) [b]									
Ice slurry	89 (12)	147 (9)	163 (12)	167 (12)	155 (11)	162 (12)	166 (14)	159 (11)	161 (12)
Control	88 (12)	146 (15)	163 (12)	163 (14)	156 (10)	161 (10)	165 (10)	157 (14)	161 (10)
Thermal Comfort [b]									
Ice slurry	1 (1)	3 (1)	6 (2)	7 (2)	5 (2)	6 (3)	6 (3)	5 (2)	5 (2)
Control	2 (1)	4 (3)	6 (1)	7 (2)	5 (1)	7 (2)	7 (2)	6 (1)	6 (2)
RPE [bc]									
Ice slurry	6 (0)	10 (1)	15 (2)	16 (2)	13 (1)	16 (2)	17 (2)	13 (2)	15 (2)
Control	6 (0)	11 (1)	15 (2)	17 (2)	13 (1)	17 (2)	18 (1)	14 (1)	16 (1)
Skin temp (°C)									
Ice slurry [a]	33.4 (0.8)	34.3 (0.3)	34.5 (0.8)	34.4 (1.0)	34.0 (0.8)	33.7 (0.6)	34.1 (0.9)	34.4 (0.7)	34.0 (0.8)
Control	32.5 (2.0)	33.3 (1.3)	33.9 (1.3)	33.8 (1.0)	33.6 (1.1)	33.2 (0.9)	33.8 (1.1)	33.7 (1.2)	33.5 (1.0)
Body temp (°C) [b]									
Ice slurry	36.8 (0.4)	36.9 (0.2)	37.6 (0.3)	37.9 (0.3)	37.3 (0.4)	37.5 (0.4)	37.9 (0.4)	37.5 (0.3)	37.6 (0.4)
Control	36.6 (0.4)	36.8 (0.5)	37.5 (0.6)	37.9 (0.5)	37.4 (0.3)	37.6 (0.3)	38.0 (0.3)	37.4 (0.5)	37.7 (0.3)
Muscle temp (°C)									
Ice slurry [a]	34.8 (0.9)	35.9 (0.3)	36.0 (0.8)	36.0 (1.0)	35.6 (0.8)	35.2 (0.6)	35.7 (0.9)	36.0 (0.7)	35.5 (0.8)
Control	33.9 (2.3)	34.9 (1.3)	35.5 (1.4)	35.4 (1.1)	35.1 (1.1)	34.8 (0.9)	35.3 (1.1)	35.2 (1.2)	35.1 (1.1)

Values are means ± (SD). [a] Main effect of trial; significantly higher in the ice slurry than control trial ($P < 0.05$).

[b] Main effect of time; significantly higher at all 15 min blocks when compared to the first 15 min block ($P < 0.001$).

[c] Main effect of time; significantly higher at the third 15 min block compared to the last 15 min block ($P < 0.05$).

Table 4.6 Magnitude based inferences of physiological differences between ice slurry and control in pre-exercise, post-exercise, halves, and trials.

			Confidence limits			
Physiological	Qualitative outcome	MDiff ± 90% CI	Lower	Upper	"±"	positive/trivial/negative
Thermal comfort	possibly beneficial	-0.14 (0.12)	-0.4	0.01	0.21	0/50/49
Thermal comfort (pre-exercise)	possibly beneficial	0.02 (0.37)	-0.37	0.14	0.26	0/68/30
Thermal comfort (post-exercise)	possibly beneficial	-0.18 (0.15)	-0.45	0	0.22	0/43/57
RPE	possibly beneficial	-0.04 (0.04)	-0.42	0.05	0.24	0/55/44
RPE (first half)	possibly beneficial	-0.03 (0.04)	-0.29	0.04	0.16	23/77/0
RPE (second half)	possibly beneficial	-0.05 (0.05)	-0.45	0.02	0.23	0/45/54
RPE (post-exercise)	Likely beneficial	-0.07(0.05)	-0.77	-0.02	0.37	1/19/81
Core temp	possibly trivial	0.00 (0.00)	-0.38	0.01	0.2	0/55/44
Core temp (first half)	unclear	-0.00 (0.01)	-0.26	0.23	0.24	7/83/10
Core temp (second half)	possibly beneficial	-0.00 (0.01)	-0.41	0.13	0.27	2/63/35
Core temp (pre-exercise)	unclear	-0.01 (0.01)	-0.44	-0.05	0.19	0/35/65
Core temp (post-exercise)	possibly beneficial	-0.00 (0.01)	-0.43	0.16	0.3	3/62/34
Heat rate	possibly trivial	0.02 (0.03)	-0.38	0.01	0.2	0/55/44
Heat rate (first half)	likely trivial	0.02 (0.04)	-0.09	0.29	0.19	19/80/1
Heat rate (second half)	possibly harmful	0.02 (0.04)	-0.17	0.42	0.29	33/63/4
Heart rate (pre-exercise)	unclear	0.02 (0.07)	-0.34	0.21	0.27	5/75/20
Skin temp	likely harmful	0.02 (0.01)	0.06	0.54	0.24	81/19/0
Skin temp (first half)	possibly harmful	0.02 (0.02)	-0.05	0.41	0.23	42/58/0
Skin temp (second half)	possibly harmful	0.01 (0.02)	-0.09	0.35	0.22	27/73/0
Body temp	unclear	-0.00 (0.01)	-0.38	0.32	0.35	9/75/16
Body temp (first half)	unclear	-0.00 (0.01)	-0.28	0.31	0.3	10/82/7
Body temp (second half)	unclear	-0.00 (0.01)	-0.48	0.27	0.37	5/65/29
Muscle temp	likely harmful	0.02 (0.01)	0.06	0.54	0.24	81/19/0
Muscle temp (first half)	possibly harmful	0.02 (0.02)	-0.05	0.41	0.23	42/58/0
Muscle temp (second half)	possibly harmful	0.01 (0.02)	-0.09	0.35	0.22	27/73/0

Qualitative outcomes for physiological data indicate negative and positive values for beneficial or harmful chances, respectively. MDiff = mean difference. CI= confidence interval.

4.4 Saliva analysis

4.4.1 Saliva flow rate

No significant interaction or trial effects were found for saliva flow rate, however there was a main effect for time ($P < 0.001$). Irrespective of trial, a significant rise in the rate was found from post-exercise to 1h post-exercise ($P = 0.004$). Significant decreases ($P < 0.001$) were also noted between pre-exercise and post-exercise, and pre-exercise and half-time (Figure 4.6).

Magnitude based inferences showed *unclear* effects of ice slurry ingestion on saliva flow rate at pre-exercise, and halftime. The effect ice slurry ingestion had *possibly positive* effects on post-exercise and 1h post-exercise saliva flow rate.

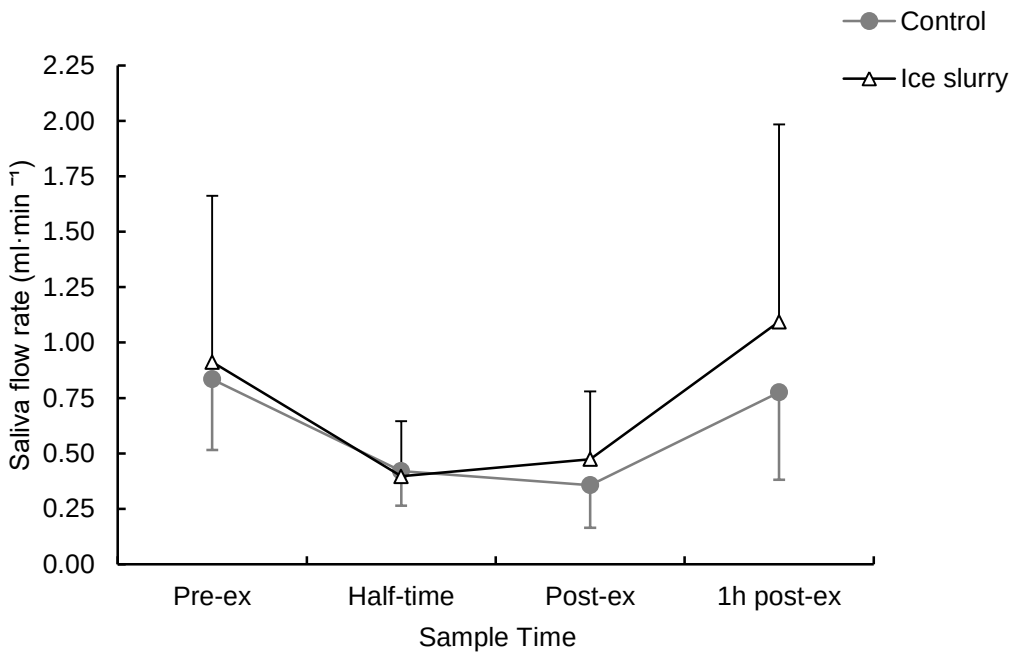

Figure 4.6 Saliva flow rate at pre-exercise, half-time, post-exercise, and 1h post-exercise. Values are means ± SD

4.4.2 Saliva S-IgA Concentration

No significant interaction, trial, or time effects were found for salivary S-IgA (Figure 4.7).

Magnitude based inferences showed *likely trivial* effects of ice slurry ingestion on salivary S-IgA at post-exercise, and 1h post-exercise. Pre-exercise showed *possibly negative* differences between ice slurry ingestion and control, indicating ice slurry ingestion possibly reduced salivary S-IgA prior to exercise. Half-time differences were *unclear*.

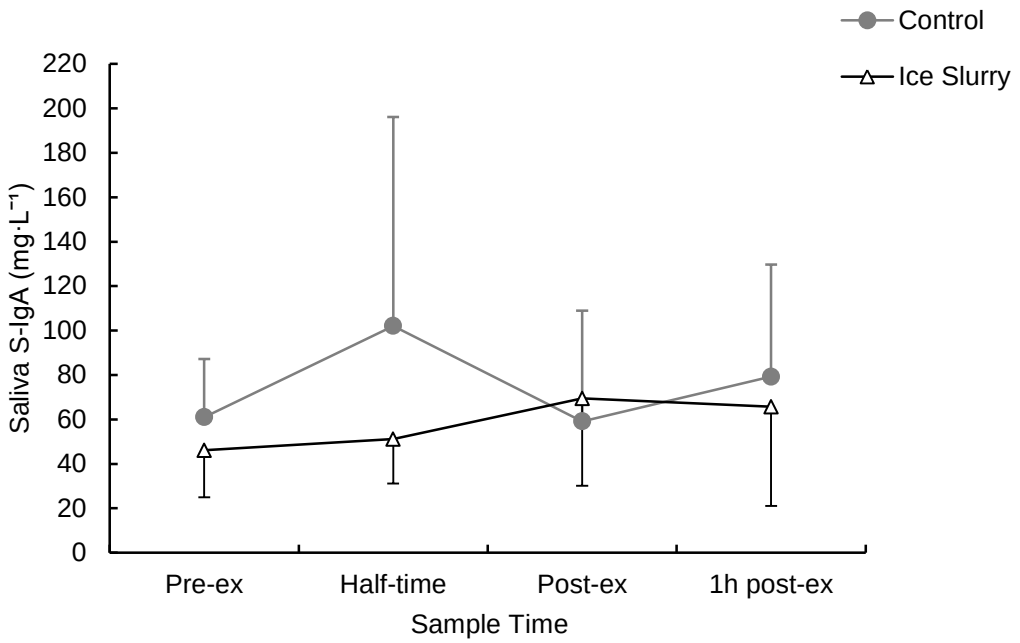

Figure 4.7 Salivary S-IgA at pre-exercise, half-time, post-exercise, and 1h post-exercise. Values are means ± SD

4.4.3 Saliva S-IgA secretion rate

No significant interaction, trial, or time effects were found for saliva S-IgA secretion rate (Figure 4.8).

Magnitude based inferences showed ice slurry ingestion to have *possibly negative* results for saliva S-IgA secretion rates at pre-exercise and half-time. Post- exercise values showed *possibly positive* results from ice slurry ingestion. Saliva S-IgA secretion rate showed *likely trivial* differences at 1h post-exercise.

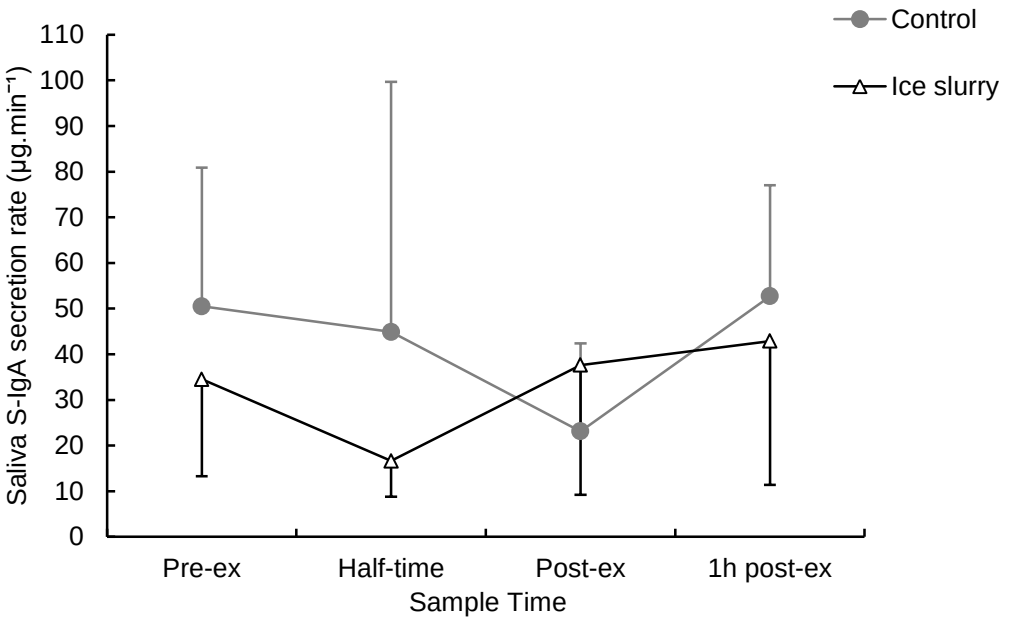

Figure 4.8 Saliva S-IgA secretion rate at pre-exercise, half-time, post-exercise, and 1h post-exercise. Values are means ± SD

4.4.4 Saliva Cortisol Concentration

No significant interaction or trial effects were found for saliva cortisol concentration; however there was a main effect for time (P < 0.001). Significant decreases were noted between post-exercise and 1h post-exercise (P = 0.005). Significant increases were found between half-time and post-exercise (P = 0.028), and pre-exercise and post-exercise (P = 0.022) (Figure 4.9).

Magnitude based inferences showed *unclear* effects of ice slurry ingestion on saliva cortisol concentration at 1h post-exercise. The effect ice slurry ingestion had likely trivial effects on pre-exercise and half-time saliva flow rate. Post-exercise values showed *possibly positive* effects on salivary flow rate from ingestion of ice slurry.

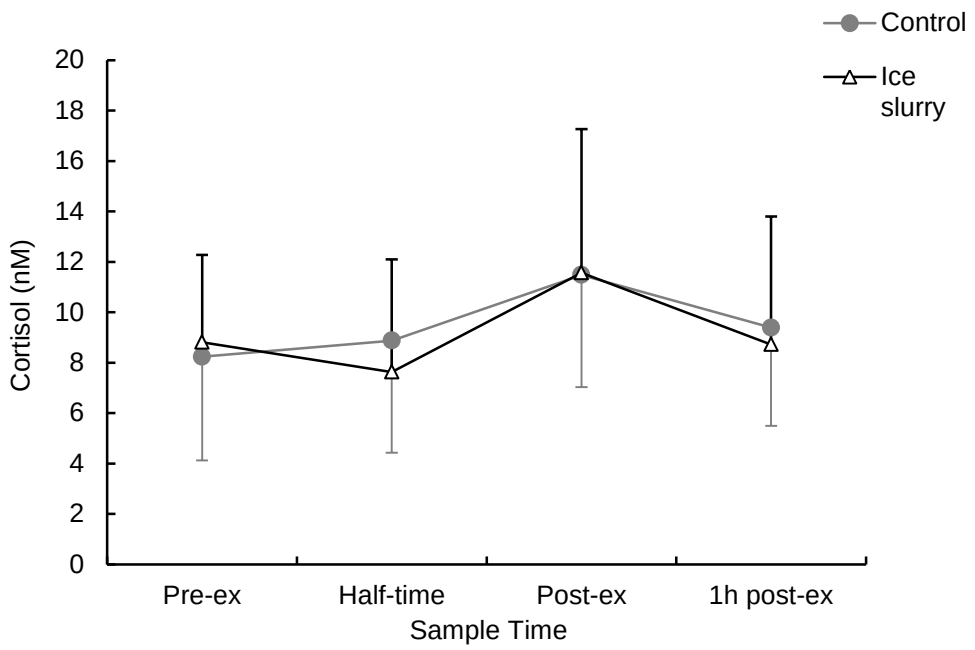

Figure 4.9 Saliva cortisol at pre-exercise, half-time, post-exercise, and 1h post-exercise. Values are means ± SD

4.4.5 Saliva osmolality

No significant interaction or trial effects were found for saliva osmolality, however there was a main effect for time (P = 0.005). Irrelevant of trial, significant increases were found between pre-exercise and half-time (P = 0.039), and pre-exercise and post-exercise (P < 0.01). A significant decrease was noted from post-exercise to 1h post-exercise (P = 0.004) (Figure 4.10).

Magnitude based inferences showed *very likely trivial* results for post-exercise saliva osmolality levels. Ice slurry ingestion had *unclear* results on pre-exercise osmolality. *Possibly positive* effects to saliva osmolality occurred due to ice slurry ingestion at half-time and 1h post-exercise.

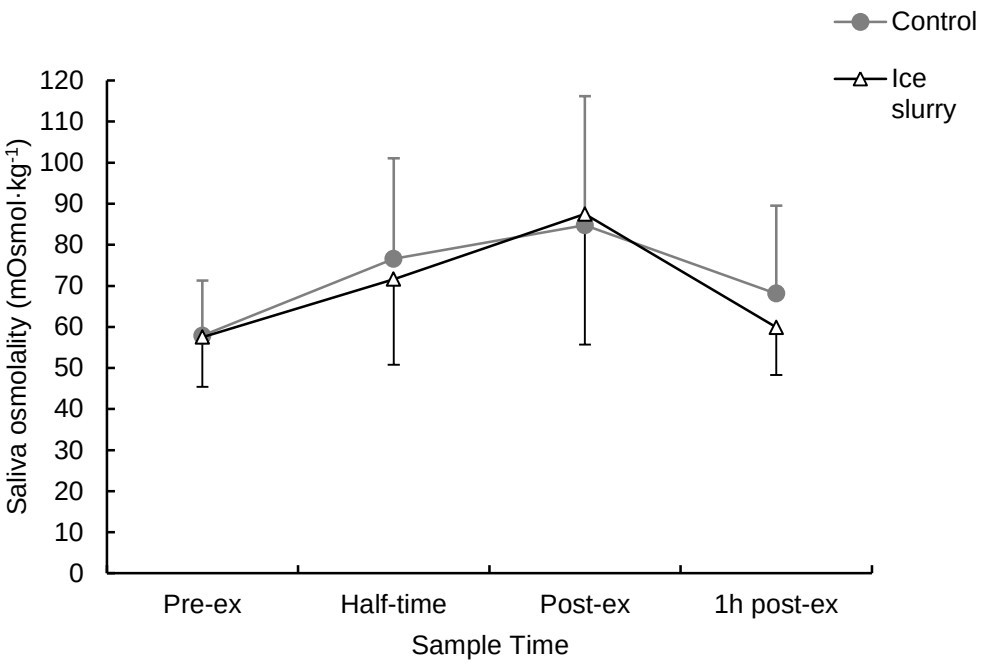

Figure 4.10 Saliva osmolality at pre-exercise, half-time, post-exercise, and 1h post-exercise. Values are means ± SD

Table 4.7 Salivary immune markers at baseline, pre-exercise, half-time, post-exercise, and 1h post-exercise.

	Baseline	Pre-ex	Half-time	Post-ex	1h post-ex
Saliva flow rate (ml·min $^{-1}$) [ab]					
Ice slurry	0.76 (0.59)	0.91 (0.75)	0.40 (0.25)	0.47 (0.31)	1.09 (0.89)
Control	0.67 (0.32)	0.84 (0.32	0.42 (0.16)	0.36 (0.19)	0.78 (0.39)
S-IgA concentration (mg·L^{-1})					
Ice slurry	73.4 (53)	46.1 (21)	51.1 (20)	89.6 (68)	56.7 (39)
Control	95.9 (78)	61 (26)	102.1 (94)	94.3 (110)	79.2 (50)
S-IgA secretion rate (µg.min^{-1})					
Ice slurry	42 (23)	35 (8)	17 (8)	38 (31)	55 (45)
Control	58.8 (37)	50.5 (30)	25.9 (12)	23.1 (19)	51.1 (26)
Cortisol (nM) [cde]					
Ice slurry	11.1 (4)	8.2 (4)	8.9 (4)	11.5 (4)	9.4 (4)
Control	13.2 (4)	8.8 (3)	7.6 (4)	11.6 (6)	8.7 (5)
Osmolality (mOsmol·kg-1) [cde]					
Ice slurry	58.4 (26)	57.5 (12)	71.6 (21)	87.5 (32)	59.9 (12)
Control	65.9 (21)	57.9 (13)	76.6 (24)	84.8 (31)	68.1 (21)

Values are means ± (SD). [a] Main effect of time; significant decrease from pre-exercise to post-exercise ($P < 0.001$). [b] Main effect of time; significant decrease from pre-exercise to half-time ($P < 0.03$). [c] Main effect of time; significant decrease from post-exercise to 1h post-exercise ($P = 0.004$). [d] Main effect of time; significant increase from pre-exercise to post-exercise ($P < 0.01$). [e] Main effect of time; significant increase from pre-exercise to half-time ($P < 0.04$).

Section 5: Discussion

5.1 Main findings

The primary aim of this research was to examine the effect of ice slurry ingestion on mucosal immunity when performing intermittent football specific exercise in the heat. The secondary aim was to examine the effect of ice slurry ingestion on performance during intermittent football specific exercise in a hot environment. Given the surrounding literature around the topic, it was hypothesised that 1) ice slurry ingestion could help reduce depression in salivary immune response caused by the exercise protocol and heated environment, and 2) ice slurry ingestion would aid performance during intermittent football performance, due to lowering core temperature prior to exercise. The main finding of this study was that ice slurry ingestion within a heated environment had no effect on post-exercise salivary immune response. Another main finding of this study was that ice slurry ingestion lowered core temperature prior to exercise and showed improvements in football specific performance variables. The main findings will be discussed further with special reference made to salivary immune response, pre-cooling on performance, physiological and perceived variables, responders and non-responders to ice slurry ingestion, and limitations and implications of this thesis.

5.2 Salivary immune response

This study showed that salivary S-IgA was not significantly affected (p=0.36, pre-exercise S-IgA = 85 ± 66 mg·L^{-1}, post-exercise S-IgA = 92 ± 89 mg·L^{-1}) following 90 min of intermittent simulated football performed in the heat (30°C, 50RH). Although the participants had physiological and performance measures that indicated high levels of physical exertion were applied, this acute bout of exercise did not appear to stress salivary S-IgA immune response. High physiological and subjective responses during the protocol, such as final core temperature (Ice slurry = 39.1 ± 0.3°C, Control = 39.2 ± 0.5°C), and final RPE (Ice slurry = 18 ± 2, Control = 19 ± 1), show that the athletes were at high workloads throughout the ISPT. Aligned with the decline in performance during the last 15 min of exercise, high final ratings of perceived exertion (Ice slurry = 18 ± 2, Control = 19 ± 1) and heated environment (30°C, 50RH), it could be hypothesised that the exertion applied would have been enough to strain salivary S-IgA. This could have reasonable theoretical grounds due to the reactions that occur when exercising in heat; with potential for greater sympathetic nervous system activity, greater fluid losses, and greater plasma cortisol concentrations (Nybo et al., 2014). However, other studies have found that heated environments do not appear to affect salivary S-IgA immune response. Laing et al. (2005) found that 2 h of cycling at 62% of VO_{2peak} in the heat (30.3°C, 76RH) with *ad-libitum* water intake did not influence salivary S-IgA responses in trained cyclists. There were no trial·time effects for salivary flow rate, S-IgA concentration, S-IgA secretion rate, saliva osmolality, or S-IgA to osmolality ratio. The current study found that ice slurry ingestion was *possibly beneficial* for increasing post-exercise saliva cortisol levels, although this was not

correlated with a subsequent decrease in salivary S-IgA levels. Ice slurry ingestion was found to have a *possibly negative* effect on saliva S-IgA secretion rate at pre-exercise and half-time. These effects seemed to be transient as all succeeding time points showed *trivial* or *unclear* results. It appears that ice slurry ingestion may cause a momentary drop in salivary S-IgA concentration and secretion rate after consumption. However, once an athlete starts exercising, this exercise stress overrides any transient drops in salivary S-IgA and these effects seem to be short lived as post-exercise and 1h post-exercise were beginning to return to pre-exercise values. This is an important finding due to the previously stated open window effect that can occur after intense exercise. During this period, when athletes are at high risk of infectious episodes, salivary S-IgA response is unaffected by ice slurry ingestion and/or a heated environment. Hence, these results suggest that athletes who ingest ice slurry beverages prior to exercise are unlikely to be at an increased risk of contracting a URTI. As sport scientists it is important to be aware of this transient drop noted pre-exercise (after ice slurry ingestion), however, given the small change in mucosal values and transient nature of the response it is unlikely that these effects would impact incidence of URTI.

To the author's knowledge, this is the first study to show *possibly negative* effects on salivary S-IgA after ingestion of ice slurry. Similarly, ice slurry ingestion had *possibly positive* effects on saliva flow rate at post-exercise and 1h post-exercise. Laing et al. (2015) found that although there was a significant increase (91%) in plasma cortisol concentration from baseline readings, there was no significant corresponding drop in salivary S-IgA compared to the same exercise in thermo-neutral conditions. Similarly, a study done by Housh, Johnson, Housh, Evans, and Tharp (1991) found that submaximal running at various temperatures (6°C, 19°C, 34°C) had no effect on salivary S-IgA and did not increase the susceptibility of contracting a URTI. Gill et al. (2013) examined salivary S-IgA response and incidence of URTI during a 230-km multistage ultramarathon in hot conditions (32-40°C). The results showed a depression in salivary S-IgA secretion rates which were offset by favourable increases in salivary α-amylase and unchanged lysozyme responses in the majority of runners during the competition. The study reported that only 1 ultra-endurance runner reported upper respiratory symptoms during and 1 month after competition. Gill et al. (2013) suggest that ensuring euhydration throughout a multistage ultramarathon competition in the heat may play a role in preventing URTI from occurring. It is worth noting that individuals exercising in hot conditions tend to fatigue sooner or reduce their work rate so their exposure to exercise stress in the heat tends to be self-limiting (González-Alonso et al., 1999). Based on the current literature and the results of this study, it could be suggested that perhaps salivary S-IgA is unaffected when exercising in the heat.

The elevation of core temperature is seen during a fever, which is to be a sign of the acute inflammatory response triggered by the body as a defence mechanism (Duff, 1986; Hanson, 1997; Roberts Jr, 1979). It is believed to be part of the body's own disease-fighting arsenal: rising body temperatures are capable of controlling many disease-producing organisms by decreasing their growth rate and reducing their ability to invade the host's circulation (Zhang, Mehta, Cohen, & Guha, 2008). Therefore, the rise in core temperature due to exercising in a hot environment is probably a response that the immune system is accustomed to and in fact tends

to utilise for protection. Hence, it could therefore be assumed that exercising in the heat does not negatively affect salivary S-IgA immune response, a main finding in the results of this study.

Ingestion of sufficient carbohydrates both before, during and after exercise has been extensively found to minimise the potential immunosuppressive effects of exercise on both measures of innate and cell mediated immunity (as reviewed in Walsh et al. (2011)). However, carbohydrate has been shown to play a minimal role in a football specific exercise protocol, finding relatively small changes in plasma glucose, cortisol, saliva S-IgA concentration and secretion rate (Bishop, Blannin, Robson, Walsh, & Gleeson, 1999). This finding that carbohydrate has limited influence on salivary S-IgA response following exercise has also been supported by other literature examining carbohydrate ingestion on a competitive marathon race (Nieman et al., 2002) and continuous cycling performance (Bishop et al., 2000; Li & Gleeson, 2005).

The current study utilised an ice slurry ingestion and control trial, both consuming 7.5 $g \cdot kg^{-1}$ of body mass prior to exercise and $2g \cdot kg^{-1}$ of body mass during a half-time period. Both trials in this study had the same amount of carbohydrate provided (5%), this was utilised to solely examine the response of salivary S-IgA and limit other factors that may influence this response. An extra control trial without carbohydrates in this study would have provided further insight into the potential mechanism behind the influence of carbohydrate on cortisol. However, the performance benefit of ingesting carbohydrate during exercise is well known and is common practice in professional football (Hawley, Dennis, & Noakes, 1994; Kirkendall, 1998; Maughan & Leiper, 1994). The sporting application of the additional trial (no carbohydrate) is limited, hence, was not utilised in this study.

Salivary S-IgA and cortisol have been shown to have an inverse relationship in the literature (Hucklebridge et al., 1998). Cortisol is the major circulatory glucocorticoid and is highly responsive to various stress states, including physical stress through exercise (Cunniffe, 2012). As exercise stress increases, so does circulating salivary cortisol. As cortisol is also a powerful immunosuppressant, this increase of cortisol has been suggested to negatively affect salivary S-IgA. However, this inverse relationship was not supported by the results of this study. Allgrove et al. (2009) suggest that increased cortisol may inhibit transepithelial transport of S-IgA and may inhibit S-IgA synthesis by B cells in the submucosa. The ISPT protocol may not have caused sufficient stress on the athlete to raise saliva cortisol to levels to have affected salivary S-IgA. It would be beneficial for future research in the area to utilise protocols that require exercising to exhaustion, unlike the ISPT. The exhaustive protocols could increase saliva cortisol levels which may provide further information on the inverse relationship to S-IgA.

5.3 Pre-cooling on performance and core temperature

The performance effects of ice slurry ingestion were a main finding of this study, showing that ice slurry ingestion had *beneficial* performance effects on high intensity running (between trials, during the second half, and in the final 15 min), total distance in the last 15 min, and mean sprint speed. An important factor to note is that ice slurry ingestion did not have any harmful effects on

exercise performance compared to a control temperature fluid. In fact, several studies have looked at cycling time trial (Ihsan et al., 2010; Ross et al., 2011), repeated sprint (Brade, Dawson, & Wallman, 2014), and exercise to exhaustion performance (Siegel et al., 2012) and have found performance improvements with the aid of ice slurry ingestion.

As hypothesised the positive effect ice slurry ingestion had on core temperature was another main finding of the study, with a significantly lower core temperature at the start of exercise (37.2 ± 0.3°C) than in the control trial (37.4 ± 0.4°C). This study also showed core temperature at the start of the second half being lower, showing *possibly beneficial* effects, in the ice slurry trial (38.1 ± 0.7 °C) compared to the control trial (38.3 ± 0.5°C). The ability of ice slurry ingestion to lower core temperature prior to exercise may improve performance and perceptual measures, when exercising in hot ambient temperatures. Naito and Ogaki (2015) and Stanley et al. (2010) showed that ice slurry ingestion significantly lowered core temperature below that seen with cold water ingestion. However, despite the reduced starting core temperature seen by Stanley et al. (2010), no increase in performance was found when compared to cold water ingestion. Contrasting these findings, Siegel et al. (2010) found that running time to exhaustion increased with the aid of ice slurry ingestion. In that study, ice slurry ingestion decreased core temperature by 0.66°C ± 0.14°C compared with 0.25°C ± 0.09°C in the cool water beverage study. Both Naito and Ogaki (2015) and Siegel et al. (2010) studies discovered that core temperature in the ice slurry ingestion trial remained lower than in the cold water ingestion trial for the first 30 min of exercise, indicating that ice slurry ingestion is an effective pre-cooling tool.

Focusing on the last 15 min period in this study showed promise for ice slurry ingestion, finding *possibly beneficial* improvements in total distance. The last 15 min of a game has been shown to be vital during football performance, Njororai (2014) found that more goals were scored in the last 15 min of regulation time compared to any other period in a game. Distance covered was 3% higher in the ice slurry trial over the control trial, supporting the results of Aldous (2016). High speed distance in the second half also showed *possibly beneficial* effects from the use of ice slurry. High speed or high-intensity running is a crucial element of football performance (Mohr et al., 2003). High-intensity efforts are critical to the outcome of matches as they relate to activities that are key to the final match result, such as movements to win the ball and actions with agility to go past defending players (Stølen et al., 2005). Fatigue also appears to occur towards the end of a game (Mohr et al., 2003), this is supported by the current study with participants performing less high-intensity work during the last 15 min of a game. Mohr et al. (2003) found that not only the total amount of high-intensity running but also the frequency of high-intensity running bouts decreased may indicate that fatigue manifests over the course of the game and therefore affects the style of play. Players' increased need for recovery and less frequent bouts of high-intensity actions appear to impact upon the tactical possibilities of a team and may indicate that players cannot maintain high-intensity running in support of their team-mates (Bradley et al., 2009). The increase in high-speed distance covered in the second half may have been due to the ice slurry ingestion and how it made the athlete feel towards the difficult part of the protocol. Ice slurry ingestion showed *possibly beneficial* effects on trial mean (ice slurry = 14 ± 2, control = 15 ± 1), first half (ice slurry = 13 ± 2, control = 14 ± 1), and second

half (ice slurry = 15 ± 2, control = 16 ± 1) RPE values. During the ice slurry trial, participants felt that ISPT protocol was easier compared to the control trial, having mean RPE values of 14 and 15, respectively. Siegel et al. (2010) also found lower RPE values for submaximal running with ice slurry ingestion, at 10 (P = 0.04), 20 (P = 0.04) and 30 min (P = 0.012). Naito and Ogaki (2015) found a trend for ice slurry ingestion to lower RPE values during the first 5 min of exercise (P = 0.07). RPE at the end of exercise is another aspect of interest to sports professionals as showing how difficult exercise is perceived can be important when analysing interventions. Final RPE in this study showed that the ice slurry trial (18 ± 1) was lower compared to the control (19 ± 2) trial, indicating a *likely beneficial* effect. This finding in combination with improved performance has real benefit for using ice slurry ingestion as a pre-cooling method prior to and during football games, as it could allow players to perceive the game as 'easier' and as such exert more effort for longer, prior to reaching fatigue.

To the author's knowledge, there is only one other study that has examined the effect of ice slurry ingestion on a sports simulated lab protocol. Aldous (2016) examined the effects of pre-cooling and halftime cooling on football specific physical performance. The performance results of Aldous (2016) were similar to the current study, however, they found that a mixed pre-cooling method (ice slurry ingestion and ice packs) had further improvements on total distance (3%), high-speed distance (4%) and variable run distance (5%) covered during the first half compared to a control trial without pre-cooling. Aldous (2016) found that ice slurry ingestion, without ice packs, did not have the same improvements on performance. However, similar to the current study, ice slurry ingestion resulted in a significant improvement (P < 0.05) between the first 15 min period and the final 15 min period. The current study showed decrements in the last 15 min of exercise compared to the first 15 min, specifically in distance covered, sprint distance, variable run distance, mean and peak sprint speed, and high-intensity running. Bradley et al. (2009) showed similar findings throughout a football game; the amount of high-intensity running decreased gradually but found the greatest reduction of high-intensity running was most pronounced (reduced by 18 - 21%) in the last 15 min period of the game. Likewise Krustrup et al. (2005) observed that the amount of high-intensity running decreased markedly within each half and that all but one player did the least high-intensity running in the last 15 min period of the first or second half. The amount of high intensity running has shown to increase with the amount completed by the opposing team and the competitive level of that team (Rampinini et al., 2007). Aligned with these findings, the ability to maintain high-intensity running, with the aid of ice slurry ingestion, may be of importance in games against higher quality opponents and in the final stages of a match.

The improvements seen in the last 15 min of exercise may have stemmed from the lower core temperature noted in the second half. In support of this, Stevens et al. (2013) found that a lower core temperature in the last section of exercise (last 500m) showed a significant (P = 0.039) performance increase and lower perceived thermal stress. In contrast, a few studies (Siegel et al., 2012; Yeo et al., 2012) have shown that ingestion of ice slurry before exercise resulted in an increased core temperature at the end of exercise. Increases in performance could still be noted with an increased core temperature due to a proposed theory from Siegel et al. (2010). The

authors suggest that as ice slurry is ingested through the mouth, it may have a meaningful impact on brain temperature. Ice slurry ingestion may reduce brain temperature, delaying the attainment of critically high brain temperature, allowing the participants to run longer and store more metabolic heat in their core (Siegel et al., 2010). Hasegawa and Cheung (2013) supported this theory suggesting that if ice slurry ingestion can reduce brain temperature, this means that ice slurry could attenuate not only peripheral fatigue but also central fatigue, and that this effect might contribute to improved performance. Although much more research is required in the area, Hasegawa and Cheung (2013) further suggested that ice slurry may change afferent feedback signals. It is known that in the stomach and small intestine thermoreceptors are present (Villanova et al., 1997) and that the glossopharyngeal (ninth cranial) nerve carries impulses for temperature sensation from the posterior third of the tongue and upper pharynx to the brain (Pallett & O'Brien, 1985). It is theorised that ice slurry ingestion cools thermoreceptors in the mouth, oesophagus, and the abdominal region, then transmit afferent feedback signals that the body's temperature is lower than actually occurring (Hasegawa & Cheung, 2013), resulting in the body 'thinking' it is cooler and hence allowing exercise to continue at high core temperatures.

In the heat stress literature it is generally accepted that there is a critical core temperature which once achieved prevents physical performance to continue optimally (González-Alonso et al., 1999; Nielsen, Strange, Christensen, Warberg, & Saltin, 1997; Thomas, Cheung, Elder, & Sleivert, 2006). The critical core temperature is thought to be around 40°C for lab based protocols (González-Alonso et al., 1999). Real world events have shown physical performance maintained with core temperatures above this level. Maron et al. (1977) found that elite marathon runners had core temperatures rise up to 41.9°C without exhibiting signs of heat stress or decreasing running pace. Generally reaching core temperatures above 40°C can result in exertional heat exhaustion, exertional heat cramps, or in extreme cases exertional heatstroke (Binkley, Beckett, Casa, Kleiner, & Plummer, 2002). In the present study, the ice slurry ingestion at halftime simulated the ability of professional footballers to ingest fluid during the halftime period in games. Interestingly, on commencement of the second half some participants in the ice slurry trial were observed to still be decreasing their core temperature during the first 5 min of exercise. The further drop in core temperature seen during this period can probably be attributed to the heat sink created by the ice slurry, where even after ingestion and the continuation of exercise, heat is drawn to the core to regulate body temperature (Mündel, King, Collacott, & Jones, 2006). The current study found that the ice slurry trial had a 0.2°C (39.1 ± 0.5°C) lower core temperature at the completion of exercise compared to the control trial (39.3 ± 0.3 °C), showing *possibly beneficial* effects. The results of this study demonstrate that perhaps ice slurry ingestion can be an effective way to lower core temperature prior to exercise and help maintain a lower core temperature throughout exercise. Lowering core temperature may prevent a football player reaching a critical core temperature that limits/reduces performance. Another potential mechanism for the increase in performance from a lower core temperature may be a result of a glycogen sparing effect. The glycogen sparing effect is due to net muscle glycogen utilization being reduced when rises in core temperature occur during exercise (Febbraio, Snow,

Stathis, Hargreaves, & Carey, 1996) and that high temperatures have been shown to increase glycogen breakdown as core temperature increases and increased circulating catecholamines (Jeukendrup, 2003). However, there are studies that have found performance increases align with increases in core temperature at exhaustion. Siegel et al. (2010) found running time to exhaustion increased by 19% (±6%) with core temperature being 0.1°C higher in the ice slurry beverage trial compared to the control trial. Ihsan et al. (2010) also found that ice slurry ingestion increased performance (6.5%) and had a higher core temperature (gastric) at the end of the cycling time trial. The potential rationale between the differences in core temperature noted in these studies may be that participants exercise to exhaustion compared to end of exercise. Ice slurry ingestion may allow participants to exercise longer than ingestion of thermoneutral fluid, resulting in participants being able to continue exercise for longer, resulting in core temperatures increasing to higher levels. The preceding studies utilised running time to exhaustion or cycling time trials, protocols vastly different from the ISPT, hence direct comparisons are difficult to make. The mechanism behind the performance increase with a high core temperature may be due to the previously mentioned brain cooling effect of ice slurry ingestion.

The brain cooling effect via ice slurry may be responsible for this study's finding that thermal comfort was lower at the end of exercise in the ice slurry trial (6 ± 3) compared to the control (8 ± 2), showing a *possibly beneficial* effect. Lower thermal sensation values have also been noted in other ice slurry literature, with Stanley et al. (2010) reporting significant improvements ($P < 0.001$, ice slurry = -0.5, cold water beverage = 1) at the end of exercise. Conflicting results can be seen in other studies. For example, Naito and Ogaki (2015) showed no significant difference between ice slurry ingestion and control trial at exhaustion. Likewise, Aldous (2016) mirrored these findings with similar thermal sensation values found at exhaustion between ice slurry and control, and expanded on this to find no differences between trials in the second half. In the current study, ice slurry ingestion showed *possibly beneficial* effects on mean thermal comfort, finding lower values during the second half in the ice slurry trial (5.5 ± 2.3) compared to the control (6.4± 1.8). A consistent finding in the literature is that thermal sensation is lower after consumption of ice slurry compared to the control trial (Aldous, 2016; Ihsan et al., 2010; Naito & Ogaki, 2015; Siegel et al., 2010). Similarly, ice slurry ingestion is successful at improving thermal sensation at the start of exercise (Ihsan et al., 2010; Naito & Ogaki, 2015; Siegel et al., 2010). Lower thermal comfort at the start of exercise was seen in the current study. *Possibly beneficial* effects were seen in the first half of exercise from the aid of ice slurry consumption compared to the control trial. Ice slurry ingestion has also been shown to be effective as a way of significantly lowering thermal sensation during a recovery period (Stanley et al., 2010). These results show that ice slurry ingestion may be beneficial not only to pre-exercise but at the end of exercise, indicating that the athlete may be feeling less heat fatigued. Ice slurry allows sport professional to effectively pre-cool an athlete, making an athlete feel cooler when competing in hot environments. Reducing the feeling of heat strain on athletes may be of importance to competitions located in hot climates. Thermal comfort has shown to be high in team sports during game play in hot conditions (29.5°C, 64.9 RH) (Duffield, Coutts, & Quinn, 2009). Multiple

factors play a part in the thermal comfort of an athlete, including environmental temperature, humidity, amount and breathability of clothing, metabolic heat production, sweat rate, and more. Metabolic heat increase with activity metabolism, through increased oxygen demands of the muscles and their contractions, which is variable according to a person's age, gender, and fitness level (Vanos, Warland, Gillespie, & Kenny, 2010). Yao, Lian, Liu, and Shen (2007) found that the conscious mind makes conclusions about actual thermal sensation via thermoreceptors located on the skin. Although, the findings in the current study conflict that of Yao et al. (2007) showing that *possibly beneficial* thermal comfort results were noted with concurrent skin temperature increases (*possibly harmful*). A possible explanation for these inconsistent findings is that other thermoreceptors may be influencing thermal comfort. The aforementioned thermoreceptors in the mouth and stomach may be responsible for this mechanism. Therefore, it is a possibility that ice consumed during ingestion has a significant cooling effect on these thermoreceptors and through inhibitory feedback makes an athlete feel cooler.

Variable run is a speed category during the ISPT, introduced by Aldous (2016), which examines a self-selected run speed. This unique speed category shows the participants 'willingness' to perform high speed running, providing a further insight into how the athlete is feeling during this category. Although not significant, there was a tendency for participants to achieve slower speeds in the first variable run section in the ice slurry trial compared to the control trial, noticeably in the first 15 min period in the first and second half. This may be due to the large volume of ice slurry ingested and the amount of time required for phase change (Tan & Lee, 2015). Alternatively, the slower speeds witnessed in this period may be a pacing strategy utilised by the participants, due to fatigue from the first period. As expected variable run speed slowed as the exercise protocol progressed, with fatigue the 'willingness' to complete faster variable run speeds was lower. This 'willingness' to perform high speed exercise has been shown to be reduced during football match-play in hot (Mohr et al., 2003) and hypoxic (Garvican et al., 2014) environments but such decrements appear not to be alleviated by ice slurry ingestion, at least in a physical soccer simulation trial.

Therefore, ice slurry ingestion is an effective method of pre-cooling an athlete prior to exercise and in this study, maintaining a lower core temperature compared to a control beverage. Ice slurry ingestion could be *beneficial* to performance, specifically in the second half and final 15 min of football related intermittent exercise. The mechanism behind this improved performance is believed to be due to lower core temperature, a glycogen sparing effect, and/or cooled thermoreceptors from ingestion of ice. The last 15 min of football has been shown to be vital to match success (Mohr et al., 2003) and goal scoring (Njororai, 2014). The ability of ice slurry ingestion to reduce core temperature during this period of play, along with the reduced perceived exertion and perceived thermal comfort, allows players to exert more high intensity running and may result in a greater chance of winning a football match.

5.4 Responders and non-responders to ice slurry ingestion.

Participants in this study can be distinguished into responders and non-responders with regard to pre-cooling via ice slurry ingestion. There were individuals (n = 5) that showed marked improvements in core temperatures during the second half of the ice slurry trial. The remaining 3 participants showed either no change or an increase of core temperature in the second half of the ice slurry trial. The participants, who sustained an increased core temperature in the ice slurry trial, still stated higher perceived difficulty and greater thermal discomfort in the control trial.

Thermal comfort is another area in which some participants responded better to ice slurry ingestion than others. Certain participants had marked improvements, especially noticeable in the second half. For example, one participant's final thermal comfort rating was 5 points lower on the scale than in their control trial. Another two participants had a score 3 points lower on the thermal comfort scale in the final 5 min of the ice slurry trial compared to the control. On the other hand, some participants (n=2) had higher thermal discomfort prior to starting the exercise protocol in the ice slurry trial than in the control trial. The variation in thermal comfort scores shows how varied the response can be between individuals.

Acknowledging that in this study some participants appeared to respond to ice slurry ingestion better than others, it is suggested that ice slurry ingestion is piloted for individuals prior to utilisation in training and major competition. Piloting ice slurry ingestion for athletes should provide insight into the potential for that athlete to get sphenopalatine ganglioneuralgia (brain freeze) and gastric discomfort, although it is rare, some surrounding literature have stated these adverse effects (as reviewed in Siegel and Laursen (2012)). One participant in the current study experienced mild gastric discomfort during the ice slurry trial. It was noted, however, that this participant had experienced gastric discomfort previously from ingestion of sports drinks prior to exercise. Ingesting carbohydrates during exercise can often cause gastrointestinal distress, commonly noted in endurance based events (Burke, Wood, Pyne, Telford, & Saunders, 2005; Pfeiffer, Cotterill, Grathwohl, Stellingwerff, & Jeukendrup, 2009; Pfeiffer et al., 2012). In previous literature an emphasis has been placed on limiting the amount of ice slurry ingestion via standardised ingestion rates to reduce or prevent any gastric discomfort caused during exercise (as reviewed in Siegel and Laursen (2012)). A vast array of different standardised ingestion rates are seen in the literature (Onitsuka et al., 2015; Ross et al., 2011; Siegel et al., 2010; Stanley et al., 2010), hence it is hard to make concise conclusions about the recommended amount of ice slurry for optimal benefits. The current study utilised a standardised ingestion amount of 7.5 $g \cdot kg^{-1}$ of body mass. This meant that participants, depending on their body mass, consumed 485 – 656 g of ice slurry prior to exercise. Ingestion was further standardised with specific amounts (2.5 $g \cdot kg^{-1}$) provided every 10 min for a half hour period. This protocol was adapted from Siegel et al. (2010) who used 7.5$g \cdot kg^{-1}$ of ice slurry with amounts of 1.25$g \cdot kg^{-1}$ provided every 5 min.

The variation of ice slurry amounts provided to athletes in the surrounding literature and potential for athletes to not respond well to ice slurry ingestion suggests that athletes should pilot the pre-cooling method prior to training and competing in competition. For athletes who do not respond to ice slurry ingestion or for athletes to whom ingestion results in gastric discomfort, it is recommended that the athletes consume the same beverage at a cool temperature (4°C), or perhaps experiment with other pre-cooling methods.

5.5 Limitations

A limitation in this study was the lack of a non-carbohydrate control trial. The extra control trial would have provided further insight into the effect of carbohydrate on salivary S-IgA immune response. Carbohydrate is believed to influence cortisol levels, which may suppress transepithelial transport of salivary S-IgA. The results would have furthered the research of Allgrove et al. (2009), examining the extent of the inverse relationship between salivary S-IgA and cortisol in intermittent football-specific exercise.

The Woodway treadmill was utilised in this study due to its non-motorised design and accuracy of performance measures. Non-motorised treadmills have their advantages, especially with the ability to vary speeds with ease. Although their use can also be seen as a limitation due to the increased trunk flexion noted in studies utilising the treadmill (Aldous et al., 2014; Aldous, 2016; Gonzalez et al., 2013) As discussed in section 3.6, each participant wore a harness attached to a fixed position allowing the treadmill belt to be controlled by the user. Using the harness belt requires the athletes to overcome an initial resistance prior to completing sprint demands, resulting in a slightly unorthodox and different running style compared to running during football match-play (Lakomy, 1987). The protocol of the ISPT helps with limiting this initial resistance via having a jog protocol prior to and after each sprint bout. As the participant is already jogging prior to the sprint the inertia required to overcome is lower than sprinting from a standing start. Through the current study it was observed that the change in running style was similar to towing a weight sled. Visually, this was seen as a slightly increased trunk angle when accelerating into a sprint. The increased trunk angle was mentioned by two participants, stating that the sensation felt like they would fall forward. After familiarisation and warm up protocols this sensation passed. It has been previously suggested that the flat design of the Woodway treadmills is a limitation, as it impedes natural running style dynamics due to the use of a harness and its instrumentation (Gonzalez et al., 2013; Lakomy, 1987). Curved Woodway treadmills have recently been used in studies to mimic field based sprint performance without the need for harnesses and other instrumentation. The curve treadmill was found to be reliable with sprint performance (Gonzalez et al., 2013) and self-paced intermittent team protocol (Tofari, McLean, Kemp, & Cormack, 2015). Use of the curve treadmill correlated well with actual sprint performance, that only one familiarisation session was adequate for good levels of test-retest reliability (Tofari et al., 2015). Use of the ISPT on the curved Woodway treadmill may provide further insights to changes any sprint variables seen in this study, without the altered sprint mechanics.

A further limitation to this study was the inability to replicate multidirectional and sport specific movements on the non-motorised treadmill. Although performance was the secondary outcome measure in this study, perhaps the way of measuring 'performance' in this study on the Woodway limited/masked the potential 'true' improvement of ice slurry on performance. Therefore, future studies should utilise more specific performance tests, perhaps with multiple changes of directions and football specific movements to gain a further understanding of the impact of ice slurry on football. Bloomfield et al. (2007) found that FA Premier league football players completed on average 727 changes of direction or swerving motions per game. They also found that 'other' type movement frequency was dependant on playing position, with midfielders and strikers completing significantly more of the these movements (jumping, landing, diving, sliding, slowing down, falling and getting up). Additional movements with the ball are performed during a football match (tackling, passing, shooting, heading, and receiving the ball), which are unable to be replicated with the non-motorised protocol design. The ISPT protocol was designed to counteract the physiological demands of the multidirectional and sport specific movements by increasing the percentage of peak sprint speed by 5% to account for the different movement (Aldous, 2016). Regardless of the counter measures utilised by Aldous (2016), the inability of the ISPT to address change of direction seen in football is a limitation of this study.

One issue with the use of peak speed assessment for determining speed categories of participants is the distance covered between fast and slower sprinters. Regardless of VO_{2peak}, participants who are faster sprinters get faster speed categories during the ISPT. The faster speed categories result in greater work and distance being completed compared to the slower participants. In this study the fastest individual sprinted a further 195 m compared to the slowest individual. Although more time consuming, GPS data from participants' match footage would allow for greater individualisation. The GPS data would provide accurate speed categories and top speeds of each individual meaning a high level of specificity to the athlete and sport. Such individualisation would be of use, especially on return from injury. The ability of the ISPT to replicate game performance without the need for change of direction and excessive joint rotation, while matching speeds seen in games, would be of use for injury rehabilitation and as a monitoring tool.

5.6 Implication of this thesis

This thesis examined the effect of pre-cooling via ice slurry ingestion on salivary S-IgA immune response and performance throughout and following the ISPT in a heated environment. Providing further insights into how pre-cooling may influence team-sport athletes during and after intermittent performance. Events in the heat commonly arise in the professional sport world, some in conditions that athletes are not used to competing in. The 2022 Football World Cup in Qatar will provide a further strain on athletes via hot environmental temperatures.

Ice slurry ingestion provides a unique method of cooling athletes prior to competition and in the present study showed *beneficial* improvements in subsequent performance. Specifically ice

slurry related improvements were witnessed in the last 15 min of exercise, a period of play crucial for match success and goal scoring opportunities (Njororai, 2014). Ingesting ice slurry may allow players to perform at their peak longer during a football match in a hot environment via reduced thermal strain, meaning they are less fatigued at vital moments during games. Ice slurry ingestion also reduced RPE and thermal comfort compared to a control temperature beverage. Ingestion of ice reduced core temperature but also resulted in an athlete feeling cooler and perceiving the same protocol to be easier. During vital moments in game play, if an athlete perceives the difficulty or thermal strain to be less it may increase the amount of high intensity running they are able to perform. As previously stated, high performance running is a speed category that is performed more by elite players (than moderate players), and is believed to be the best measure of physical performance during a game (Mohr et al., 2003).

Salivary S-IgA was not significantly affected by exercising in the heat, with saliva collected upon arrival to the laboratory similar with that collected after exercise. Ice slurries appeared to temporarily decrease salivary S-IgA concentration and secretion rate after ingestion, at rest; however, in response to exercise ice slurry did not appear to continue to reduce mucosal immunity. Collectively, these results show that ice slurry ingestion is an effective way of pre-cooling athletes, which improves performance and does not negatively impact salivary immune markers following exercise. Future research in the area should examine salivary S-IgA response during football specific exercise protocols that include brief intense actions, such as changing direction, shuffling, jumping, skill sections, etc. Previously in this thesis, these movements have been shown to be an important part of football, with up to 250 brief intense actions performed in a match. The inclusion of intense actions may provide further insight into the application of ice slurry ingestion to specific football performance and salivary immune response. Another area for future research is the use of a non-carbohydrate control trial. This extra control would allow the researchers to examine if the inverse cortisol and salivary S-IgA relationship is present in football or team sport related intermittent exercise.

A further recommendation from this study; is to be aware of individual differences in response to ice slurry ingestion. Piloting the use of ice slurry ingestion is recommended prior to competition to ensure athlete comfort. For players who respond well to ice slurry ingestion, it is an effective pre-cooling method prior to exercising in hot environments. Ingestion of $7.5 g \cdot kg^{-1}$ of body mass of ice slurry ingestion over a 30 min pre-cooling period showed improvements to performance and did not affect post-exercise salivary immune markers.

5.7 Conclusion

In conclusion, the results of the present study show that ice slurry ingestion provides *possibly beneficial* improvements in intermittent football performance in a heated environment, by pre-cooling an athlete prior to exercise. It appears that ice slurry ingestion creates an internal heat sink, due to heat in the core being drawn to the ice consumed, that results in a lower core temperature prior to exercise and during a half-time interval compared to a control, neutral

temperature beverage. This study shows that utilising ice slurry ingestion as a pre-cooling method when exercising in a hot environment does not influence salivary immune response post-exercise. These findings suggesting that athletes are unlikely to be at any increased risk or susceptibility for illness, particularly URTI, when the open window period for infection is most apparent

Football has been shown to be a highly taxing, intermittent sport, where players are required to perform high intensity movements (sprints, jumps, change of directions) and activities (passing, tackling, shooting). Football tournaments have physically demanding game scheduling and can be hosted in thermally challenging environments, which can further fatigue a player. Pre-cooling and half-time cooling via ice slurry ingestion provides potentially *beneficial* results to football performance, especially during the last 15 min of exercise. This period of play has been shown to be crucial in determining the result of a match and provides a real opportunity for goal scoring. Therefore, ice slurry ingestion is suggested, with appropriate pilot testing, to be an effective tool for Sport Scientist to utilise prior to football events in hot environments, such as the 2022 FIFA World Cup in Qatar.

References

Abt, G., Reaburn, P., Holmes, M., & Gear, T. (2003). Changes in peak sprint speed during prolonged high-intensity intermittent exercise that simulates team sport play. *Journal of Sports Sciences, 21*(4), 256-257.

Achten, J., & Jeukendrup, A. E. (2003). Heart rate monitoring. *Sports Medicine, 33*(7), 517-538.

Aldous, J. W., Akubat, I., Chrismas, B. C., Watkins, S. L., Mauger, A. R., Midgley, A. W., Taylor, L. (2014). The reliability and validity of a soccer-specific nonmotorised treadmill simulation (intermittent soccer performance test). *The Journal of Strength & Conditioning Research, 28*(7), 1971-1980.

Aldous, J. W. F. (2016). Environmental stressors and cooling interventions on simulated soccer performance. *Retrieved from http://hdl.handle.net/10547/601084*.

Ali, A., & Farrally, M. (1991). Recording soccer players' heart rates during matches. *Journal of Sports Sciences, 9*(2), 183-189.

Ali, A., Foskett, A., & Gant, N. (2014). Measuring intermittent exercise performance using shuttle running. *Journal of Sports Sciences, 32*(7), 601-609.

Ali, A., & Williams, C. (2009). Carbohydrate ingestion and soccer skill performance during prolonged intermittent exercise. *Journal of Sports Sciences, 27*(14), 1499-1508.

Allgrove, J. E., Geneen, L., Latif, S., & Gleeson, M. (2009). Influence of a fed or fasted state on the s-IgA response to prolonged cycling in active men and women. *International Journal of Sport Nutrition and Exercise Metabolism, 19*(3), 209-221.

Armstrong, L., & Maresh, C. (1993). The exertional heat illnesses: A risk of athletic participation. *Medicine Exercise Nutrition and Health, 2*(3), 125-147.

Armstrong, L. E., Costill, D. L., & Fink, W. J. (1985). Influence of diuretic-induced dehydration on competitive running performance. *Medicine and Science in Sports and Exercise, 17*(4), 456-461.

Bangsbo, J. (1993). Energy demands in competitive soccer. *Journal of Sports Sciences, 12*, S5-12.

Bangsbo, J., Mohr, M., & Krustrup, P. (2006). Physical and metabolic demands of training and match-play in the elite football player. *Journal of Sports Sciences, 24*(07), 665-674.

Bangsbo, J., Nørregaard, L., & Thorsoe, F. (1991). Activity profile of competition soccer. *Canadian Journal of Sport Sciences, 16*(2), 110-116.

Baum, B. (1987). Neurotransmitter control of secretion. *Journal of Dental Research, 66*(2_suppl), 628-632.

Bijlani, R., & Sharma, K. (1980). Effect of dehydration and a few regimes of rehydration on human performance. *Indian Journal of Physiology and Pharmacology, 24*(4), 255-266.

Binkley, H. M., Beckett, J., Casa, D. J., Kleiner, D. M., & Plummer, P. E. (2002). National Athletic Trainers' Association position statement: exertional heat illnesses. *Journal of Athletic Training, 37*(3), 329.

Bishop, N., Blannin, A., Robson, P., Walsh, N., & Gleeson, M. (1999). The effects of carbohydrate supplementation on immune responses to a soccer-specific exercise protocol. *Journal of Sports Sciences, 17*(10), 787-796.

Bishop, N., & Gleeson, M. (2009). Acute and chronic effects of exercise on markers of mucosal immunity. *Frontiers in Bioscience, 14*, 4444-4456.

Bishop, N. C., Blannin, A. K., Armstrong, E., Rickman, M., & Gleeson, M. (2000). Carbohydrate and fluid intake affect the saliva flow rate and IgA response to cycling. *Medicine and Science in Sports and Exercise, 32*(12), 2046-2051.

Blannin, A., Robson, P., Walsh, N., Clark, A., Glennon, L., & Gleeson, M. (1998). The effect of exercising to exhaustion at different intensities on saliva immunoglobulin A, protein and electrolyte secretion. *International Journal of Sports Medicine, 19*(8), 547-552.

Bloomfield, J., Polman, R., & O'Donoghue, P. (2007). Physical demands of different positions in FA Premier League soccer. *Journal of Sports Science and Medicine, 6*(1), 63-70.

Bosco, J., Greenleaf, J., Bernauer, E., & Card, D. (1974). Effects of acute dehydration and starvation on muscular strength and endurance. *Acta Physiologica Polonica, 25*(5), 411.

Brade, C., Dawson, B., & Wallman, K. (2014). Effects of different precooling techniques on repeat sprint ability in team sport athletes. *European Journal of Sport Science, 14* (1), 84-91.

Bradley, P. S., Sheldon, W., Wooster, B., Olsen, P., Boanas, P., & Krustrup, P. (2009). High-intensity running in English FA Premier League soccer matches. *Journal of Sports Sciences, 27*(2), 159-168.

Brandenberger, G., & Follenius, M. (1975). Influence of timing and intensity of musclar exercise on temporal patterns of plasma cortisol levels. *The Journal of Clinical Endocrinology and Metabolism, 40*(5), 845.

Brandenberger, G., Follenius, M., & Hietter, B. (1982). Feedback from meal-related peaks determines diurnal changes in cortisol response to exercise. *The Journal of Clinical Endocrinology and Metabolism, 54*(3), 592.

Brandtzaeg, P. (2003). Role of secretory antibodies in the defence against infections. *International Journal of Medical Microbiology, 293*(1), 3-15.

Brandtzaeg, P. (2009). Mucosal immunity: induction, dissemination, and effector functions. *Scandinavian Journal of Immunology, 70*(6), 505-515.

Brandtzaeg, P., Baekkevold, E. S., Farstad, I. N., Jahnsen, F. L., Johansen, F.-E., Nilsen, E. M., & Yamanaka, T. (1999). Regional specialization in the mucosal immune system: what happens in the microcompartments? *Immunology today, 20*(3), 141-151.

Brughelli, M., Cronin, J., & Chaouachi, A. (2011). Effects of running velocity on running kinetics and kinematics. *The Journal of Strength & Conditioning Research, 25*(4), 933-939.

Burke, L. M., Wood, C., Pyne, D. B., Telford, R. D., & Saunders, P. U. (2005). Effect of carbohydrate intake on half-marathon performance of well-trained runners. *International Journal of Sport Nutrition and Exercise Metabolism, 15*(6), 573-589.

Byrne, C., Lee, J., Chew, S. A. N., Lim, C. L., & Tan, E. (2006). Continuous thermoregulatory responses to mass-participation distance running in heat. *Medicine and Science in Sports and Exercise, 38*(5), 803.

Carli, G., Bonifazi, M., Lodi, L., Lupo, C., Martelli, G., & Viti, A. (1986). Hormonal and metabolic effects following a football match. *International Journal of Sports Medicine, 7*(1), 36-38.

Carrillo, A. E., Murphy, R. J., & Cheung, S. S. (2008). Vitamin C supplementation and salivary immune function following exercise-heat stress. *International Journal of Sports Physiology and Performance, 3*(4), 516-530.

Casa, D. J. (1999). Exercise in the heat. I. Fundamentals of thermal physiology, performance implications, and dehydration. *Journal of Athletic Training, 34*(3), 246.

Casa, D. J., Armstrong, L. E., Hillman, S. K., Montain, S. J., Reiff, R. V., Rich, B. S., Stone, J. A. (2000). National Athletic Trainers' Association position statement: fluid replacement for athletes. *Journal of Athletic Training, 35*(2), 212.

Chicharro, J. L., Lucía, A., Pérez, M., Vaquero, A. F., & Ureña, R. (1998). Saliva composition and exercise. *Sports medicine, 26*(1), 17-27.

Cian, C., Koulmann, N., Barraud, P., Raphel, C., Jimenez, C., & Melin, B. (2000). Influences of variations in body hydration on cognitive function: Effect of hyperhydration, heat stress, and exercise-induced dehydration. *Journal of Psychophysiology, 14*(1), 29.

Coad, S., Gray, B., & McLellan, C. (2015). Seasonal Analysis of Mucosal Immunological Function and Physical Demands in Professional Australian Rules Footballers. *International Journal of Sports Physiology and Performance*.

Cole, A. S., McGivan, J., Eastoe, J. E., Hayes, M. L., & Smillie, A. C. (1988). *Biochemistry and Oral Biology* (Second edition ed.) [Electronic document]: London ; Boston : Wright, 1988. Retrieved from http://ezproxy.aut.ac.nz/login?url=http://ebookcentral.proquest.com/lib/aut/detail.action?docID=1675167

Crandall, C., & Gonzalez-Alonso, J. (2010). Cardiovascular function in the heat-stressed human. *Acta Physiologica, 199*(4), 407-423.

Cunniffe, B. (2012). *Effect of acute and chronic exercise on immunoendocrine responses in professional rugby union*. University of Glamorgan. Retrieved from https://pure.southwales.ac.uk/files/987824/PhD_Brian_Cunniffe.pdf

Cunniffe, B., Griffiths, H., Proctor, W., Davies, B., Baker, J. S., & Jones, K. P. (2011). Mucosal immunity and illness incidence in elite rugby union players across a season. *Medicine and Science in Sports and Exercise, 43*(3), 388-397.

Czyzewska-Buczyńska, A., Lewandowicz-Uszyńska, A., & Jankowski, A. (2006). IgA, an essential part of the immune system: selected issues. *Postepy higieny i medycyny doswiadczalnej (Online), 61*, 38-47.

Daly, W., Seegers, C., Rubin, D., Dobridge, J., & Hackney, A. C. (2005). Relationship between stress hormones and testosterone with prolonged endurance exercise. *European Journal of Applied Physiology, 93*(4), 375-380.

Davison, G. (2011). Innate immune responses to a single session of sprint interval training. *Applied Physiology, Nutrition, and Metabolism, 36*(6), 395-404.

Dawes, C. (1974). Rhythms in salivary flow rate and composition. *International Journal of Chronobiology, 2*(3), 253-279.

Di Mascio, M., & Bradley, P. S. (2013). Evaluation of the most intense high-intensity running period in English FA premier league soccer matches. *The Journal of Strength and Conditioning Research, 27*(4), 909-915.

Drust, B., Cable, N., & Reilly, T. (2000). Investigation of the effects of the pre-cooling on the physiological responses to soccer-specific intermittent exercise. *European Journal of Applied Physiology, 81*(1), 11-17.

Duff, G. W. (1986). Is fever beneficial to the host: a clinical perspective. *The Yale Journal of Biology and Medicine, 59*(2), 125.

Duffield, R., Coutts, A., McCall, A., & Burgess, D. (2013). Pre-cooling for football training and competition in hot and humid conditions. *European Journal of Sport Science, 13*(1), 58-67.

Duffield, R., Coutts, A. J., & Quinn, J. (2009). Core temperature responses and match running performance during intermittent-sprint exercise competition in warm conditions. *The Journal of Strength and Conditioning Research, 23*(4), 1238-1244.

Duffield, R., & Marino, F. E. (2007). Effects of pre-cooling procedures on intermittent-sprint exercise performance in warm conditions. *European Journal of Applied Physiology, 100*(6), 727-735.

Ekblom, B. (1986). Applied physiology of soccer. *Sports medicine, 3*(1), 50-60.

Engels, H.-J., Fahlman, M. M., Morgan, A. L., & Formolo, L. R. (2004). Mucosal iga response to intense intermittent exercise in healthy male and female adults. *Journal of Exercise Physiology Online, 7*(5).

Engels, H.-J., Fahlman, M. M., & Wirth, J. C. (2003). Effects of ginseng on secretory IgA, performance, and recovery from interval exercise. *Medicine and Science in Sports and Exercise, 35*(4), 690-696.

Eston, R. (2012). Use of ratings of perceived exertion in sports. *International Journal of Sports Physiology and Performance, 7*(2), 175-182.

Fahlman, M., Engels, H., Morgan, A., & Kolokouri, I. (2001). Mucosal IgA response to repeated wingate tests in females. *International Journal of Sports Medicine, 22*(02), 127-131.

Fahlman, M. M., & Engels, H.-J. (2005). Mucosal IgA and URTI in American college football players: a year longitudinal study. *Medicine and Science in Sports and Exercise, 37*(3), 374-380.

Fan, Y., Tang, Y., Lu, Q., Feng, S., Yu, Q., Sui, D., Li, S. (2009). Dynamic changes in salivary cortisol and secretory immunoglobulin A response to acute stress. *Stress and Health, 25*(2), 189-194.

Febbraio, M. A., Snow, R. J., Stathis, C. G., Hargreaves, M., & Carey, M. F. (1996). Blunting the rise in body temperature reduces muscle glycogenolysis during exercise in humans. *Experimental Physiology, 81*(4), 685-693.

Foster, C., Daines, E., Hector, L., Snyder, A. C., & Welsh, R. (1996). Athletic performance in relation to training load. *Wisconsin Medical Journal, 95*(6), 370-374.

Fricker, P., Gleeson, M., Flanagan, A., Pyne, D., McDonald, W., & Clancy, R. (2000). A Clinical Snapshot: Do Elite Swimmers Experience More Upper Respiratory Illness Than Nonathletes? *Clinical Exercise Physiology, 2*, 155-158.

Gagge, A. P., Stolwijk, J., & Hardy, J. (1967). Comfort and thermal sensations and associated physiological responses at various ambient temperatures. *Environmental Research, 1*(1), 1-20.

Galloway, S., & Maughan, R. J. (1997). Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Medicine and Science in Sports and Exercise, 29*(9), 1240-1249.

Garvican, L. A., Hammond, K., Varley, M. C., Gore, C. J., Billaut, F., & Aughey, R. J. (2014). Lower running performance and exacerbated fatigue in soccer played at 1600 m. *International Journal of Sports Physiology and Performance, 9*(3), 397-404.

Gill, S. K., Teixeira, A. M., Rama, L., Rosado, F., Hankey, J., Scheer, V., Costa, R. J. S. (2013). Salivary antimicrobial protein responses during multistage ultramarathon competition conducted in hot environmental conditions. *Applied Physiology, Nutrition, and Metabolism, 38*(9), 977-987.

Gleeson, M. (1999). Mucosal immune responses and risk of respiratory illness in elite athletes. *Exercise immunology review, 6*, 5-42.

Gleeson, M. (2000). Mucosal immunity and respiratory illness in elite athletes. *International Journal of Sports Medicine, 21*, 33-43.

Gleeson, M. (2007). Immune function in sport and exercise. *Journal of Applied Physiology, 103*(2), 693-699.

Gleeson, M., Bishop, N., & Walsh, N. (2013). *Exercise Immunology*: Routledge.

Gleeson, M., Cripps, A. W., & Clancy, R. L. (1995). Modifiers ofthe human mucosal immune system. *Immunology and Cell Biology, 73*, 397-404.

Gleeson, M., Ginn, E., & Francis, J. L. (2000). Salivary immunoglobulin monitoring in an elite kayaker. *Clinical Journal of Sports Medicine, 10*(3), 206-208.

Gleeson, M., McDonald, W., Cripps, A., Pyne, D., Clancy, R., & Fricker, P. (1995). The effect on immunity of long-term intensive training in elite swimmers. *Clinical & Experimental Immunology, 102*(1), 210-216.

Gleeson, M., McDonald, W., Pyne, D., Clancy, R., Cripps, A., Francis, J., & Fricker, P. (2000). Immune status and respiratory illness for elite swimmers during a 12-week training cycle. *International Journal of Sports Medicine, 21*(4), 302-307.

Gleeson, M., McDonald, W. A., Pyne, D. B., Cripps, A. W., Francis, J. L., Fricker, P. A., & Clancy, R. L. (1999). Salivary IgA levels and infection risk in elite swimmers. *Medicine and Science in Sports and Exercise, 31*(1), 67-73.

Gleeson, M., McFarlin, B., & Flynn, M. (2006). Exercise and Toll-like receptors. *Exercise Immunological Review, 12*(1), 34-53.

Gleeson, M., & Pyne, D. B. (2000). Exercise effects on mucosal immunity. *Immunology and Cell Biology, 78*(5), 536-544.

Gleeson, M., Pyne, D. B., & Callister, R. (2004). The missing links in exercise effects on mucosal immunity. *Exercise Immunological Review, 10*(10).

González-Alonso, J., Teller, C., Andersen, S. L., Jensen, F. B., Hyldig, T., & Nielsen, B. (1999). Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *Journal of Applied Physiology, 86*(3), 1032-1039.

Gonzalez, A. M., Wells, A. J., Hoffman, J. R., Stout, J. R., Fragala, M. S., Mangine, G. T., Emerson, N. S. (2013). Reliability of the Woodway CurveTM non-motorized treadmill for assessing anaerobic performance. *Journal of Sports Science & Medicine, 12*(1), 104.

Gregson, W., Drust, B., Atkinson, G., & Salvo, V. (2010). Match-to-match variability of high-speed activities in premier league soccer. *International Journal of Sports Medicine, 31*(4), 237-242.

Guttierres, A., Natali, A., Vianna, J., Reis, V., & Marins, J. (2011). Dehydration in soccer players after a match in the heat. *Biology of Sport, 28*(4).

Hanson, D. F. (1997). Fever, temperature, and the immune response. *Annals of the New York Academy of Sciences, 813*(1), 453-464.

Hasegawa, H., & Cheung, S. S. (2013). Hyperthermia effects on brain function and exercise capacity. *The Journal of Physical Fitness and Sports Medicine, 2*(4), 429-438.

Hawley, J., Dennis, S., & Noakes, T. (1994). Carbohydrate, fluid, and electrolyte requirements of the soccer player: a review. *International Journal of Sport Nutrition, 4*(3), 221.

Holm, R. L., Pribyslavska, V., Johnson, S. L., Lowe, J. B., Stevenson-Wilcoxson, M. C., Scudamore, E. M., O'Neal, E. K. (2015). Performance and perceptual responses of collegiate female soccer players to a practical external and internal cooling protocol. *International Journal of Exercise Science, 8*(4), 3.

Hopkins, W., Marshall, S., Batterham, A., & Hanin, J. (2009). Progressive statistics for studies in sports medicine and exercise science. *Medicine and Science in Sports and Exercise, 41*(1), 3.

Hopkins, W. G. (2010). Linear models and effect magnitudes for research, clinical and practical applications. *Sports Science, 14*(1), 49-57.

Horswill, C., Stofan, J., Horn, M., Eddy, D., & Murray, R. (2006). Effect of exercise and fluid consumption on salivary flow and pH. *International Journal of Sports Medicine, 27*(6), 500-504.

Housh, T., Johnson, G., Housh, D., Evans, S., & Tharp, G. (1991). The effect of exercise at various temperatures on salivary levels of immunoglobulin A. *International Journal of Sports Medicine, 12*(05), 498-500.

Houston, M. E., Marrin, D. A., Green, H. J., & Thomson, J. A. (1981). The effect of rapid weight loss on physiological functions in wrestlers. *The Physician and Sportsmedicine, 9*(11), 73-78.

Hucklebridge, F., Clow, A., & Evans, P. (1998). The relationship between salivary secretory immunoglobulin A and cortisol: neuroendocrine response to awakening and the diurnal cycle. *International Journal of Psychophysiology, 31*(1), 69-76.

Hughes, M. G., Doherty, M., Tong, R., Reilly, T., & Cable, N. (2006). Reliability of repeated sprint exercise in non-motorised treadmill ergometry. *International Journal of Sports Medicine, 27*(11), 900-904.

Ihsan, M., Landers, G., Brearley, M., & Peeling, P. (2010). Beneficial effects of ice ingestion as a precooling strategy on 40-km cycling time-trial performance. *International Journal of Sports Physiology and Performance, 5*(2), 40-51.

Impellizzeri, F., Rampinini, E., Castagna, C., Bishop, D., Bravo, D. F., Tibaudi, A., & Wisloff, U. (2008). Validity of a repeated-sprint test for football. *International Journal of Sports Medicine, 29*(11), 899-905.

Ispirlidis, I., Fatouros, I. G., Jamurtas, A. Z., Nikolaidis, M. G., Michailidis, I., Douroudos, I., Katrabasas, I. (2008). Time-course of changes in inflammatory and performance responses following a soccer game. *Clinical Journal of Sport Medicine, 18*(5), 423-431.

Janský, L., Pospíšilová, D., Honzova, S., Uličný, B., Šrámek, P., Zeman, V., & Kaminkova, J. (1996). Immune system of cold-exposed and cold-adapted humans. *European Journal of Applied physiology and Occupational Physiology, 72*(5-6), 445-450.

Jeukendrup, A. (2003). Modulation of carbohydrate and fat utilization by diet, exercise and environment: Portland Press Limited.

Jones, A. M., & Doust, J. H. (1996). A 1% treadmill grade most accurately reflects the energetic cost of outdoor running. *Journal of Sports Sciences, 14*(4), 321-327.

Kirkendall, D. T. (1998). Fluid and electrolyte replacement in soccer. *Clinics in Sports Medicine, 17*(4), 729-738.

Klentrou, P., Cieslak, T., MacNeil, M., Vintinner, A., & Plyley, M. (2002). Effect of moderate exercise on salivary immunoglobulin A and infection risk in humans. *European Journal of Applied Physiology, 87*(2), 153-158.

Kreusser, W., Heidland, A., Hennemann, H., Wigand, M., & Knauf, H. (1972). Mono-and Divalent Electrolyte Patterns, pCO2 and pH in Relation to Flow Rate in Normal Human Parotid Saliva. *European Journal of Clinical Investigation, 2*(6), 398-406.

Krustrup, P., Mohr, M., Ellingsgaard, H., & Bangsbo, J. (2005). Physical demands during an elite female soccer game: importance of training status. *Medicine & Science in Sports & Exercise, 37*(7), 1242-1248.

Kunz, H., Bishop, N. C., Spielmann, G., Pistillo, M., Reed, J., Ograjsek, T., Simpson, R. J. (2015). Fitness level impacts salivary antimicrobial protein responses to a single bout of cycling exercise. *European Journal of Applied Physiology, 115*(5), 1015-1027.

Laing, S., Gwynne, D., Blackwell, J., Williams, M., Walters, R., & Walsh, N. (2005). Salivary IgA response to prolonged exercise in a hot environment in trained cyclists. *European Journal of Applied Physiology, 93*(5-6), 665-671.

Lakomy, H. (1987). The use of a non-motorized treadmill for analysing sprint performance. *Ergonomics, 30*(4), 627-637.

Lamm, M. E. (1997). Interaction of antigens and antibodies at mucosal surfaces. *Annual Reviews in Microbiology, 51*(1), 311-340.

Laukkanen, R. M., & Virtanen, P. K. (1998). Heart rate monitors: state of the art. *Journal of Sports Sciences, 16*(1), 3-7.

Li, T.-L., & Gleeson, M. (2005). The effects of carbohydrate supplementation during repeated bouts of prolonged exercise on saliva flow rate and immunoglobulin A. *Journal of Sports Sciences, 23*(7), 713-722.

Lim, C. L., & Mackinnon, L. T. (2006). The roles of exercise-induced immune system disturbances in the pathology of heat stroke. *Sports Medicine, 36*(1), 39-64.

Lindsay, A., Lewis, J., Scarrott, C., Gill, N., Gieseg, S., & Draper, N. (2015). Assessing the effectiveness of selected biomarkers in the acute and cumulative physiological stress response in professional rugby union through non-invasive assessment. *International Journal of Sports Medicine, 36*(06), 446-454.

Little, T., & Williams, A. G. (2007). Measures of exercise intensity during soccer training drills with professional soccer players. *The Journal of Strength & Conditioning Research, 21*(2), 367-371.

Mackinnon, L. (1996). Immunoglobulin, antibody, and exercise. *Exercise Immunology Review, 2*, 1-34.

Mackinnon, L. T., Chick, T. W., Van As, A., & Tomasi, T. B. (1989). Decreased secretory immunoglobulins following intense endurance exercise. *Research in Sports Medicine: An International Journal, 1*(3), 209-218.

Mackinnon, L. T., Ginn, E., & Seymour, G. J. (1993). Decreased salivary immunoglobulin A secretion rate after intense interval exercise in elite kayakers. *European Journal of Applied Physiology and Occupational Physiology, 67*(2), 180-184.

MacKinnon, L. T., & Jenkins, D. G. (1993). Decreased salivary immunoglobulins after intense interval exercise before and after training. *Medicine and Science in Sports and Exercise, 25*(6), 678-683.

Macpherson, A., McCoy, K., Johansen, F., & Brandtzaeg, P. (2008). The immune geography of IgA induction and function. *Mucosal Immunology, 1*(1), 11-22.

Majkowska-Skrobek, G., & Augustyniak, D. (2004). The structure and function of human IgA subclases. *Kosmos, 53*, 155-165.

Malm, C. (2006). Susceptibility to infections in elite athletes: the S-curve. *Scandinavian Journal of Medicine and Science in Sports, 16*(1), 4-6.

Marino, F. (2002). Methods, advantages, and limitations of body cooling for exercise performance. *British Journal of Sports Medicine, 36*(2), 89-94.

Maron, M. B., Wagner, J. A., & Horvath, S. (1977). Thermoregulatory responses during competitive marathon running. *Journal of Applied Physiology, 42*(6), 909-914.

Mastorakos, G., Pavlatou, M., Diamanti-Kandarakis, E., & Chrousos, G. P. (2005). Exercise and the stress system. *Hormones (Athens), 4*(2), 73-89.

Maughan, R., & Leiper, J. (1994). Fluid replacement requirements in soccer. *Journal of Sports Sciences, 12*, 29-34.

Mayhew, S., & Wenger, H. (1985). Time-motion analysis of professional soccer. *Journal of Human Movement Studies, 11*(1), 49-52.

McGhee, J. R., Mestecky, J., Elson, C. O., & Kiyono, H. (1989). Regulation of IgA synthesis and immune response by T cells and interleukins. *Journal of Clinical Immunology, 9*(3), 175-199.

McGregor, S., Nicholas, C., Lakomy, H., & Williams, C. (1999). The influence of intermittent high-intensity shuttle running and fluid ingestion on the performance of a soccer skill. *Journal of Sports Sciences, 17*(11), 895-903.

Mnatzakanian, P., & Vaccaro, P. (1982). Effects Of 4% Dehydration And Rehydration On Hehatological Profiles, Urinary Profiles And Muscular Endurance Of College Wrestlers. *Medicine & Science in Sports & Exercise, 14*(2), 117.

Mohr, M., Krustrup, P., & Bangsbo, J. (2003). Match performance of high-standard soccer players with special reference to development of fatigue. *Journal of Sports Sciences, 21*(7), 519-528.

Mohr, M., Mujika, I., Santisteban, J., Randers, M. B., Bischoff, R., Solano, R., Krustrup, P. (2010). Examination of fatigue development in elite soccer in a hot environment: a multi-experimental approach. *Scandinavian Journal of Medicine and Science in Sports, 20*(3), 125-132.

Mohr, M., Nybo, L., Grantham, J., & Racinais, S. (2012). Physiological responses and physical performance during football in the heat. *PloS one, 7*(6).

Mohr, M., Rasmussen, P., Drust, B., Nielsen, B., & Nybo, L. (2006). Environmental heat stress, hyperammonemia and nucleotide metabolism during intermittent exercise. *European Journal of Applied Physiology, 97*(1), 89.

Moreira, A., Arsati, F., Cury, P. R., Franciscon, C., de Oliveira, P. R., & de Araújo, V. C. (2009). Salivary immunoglobulin a response to a match in top-level brazilian soccer players. *The Journal of Strength and Conditioning Research, 23*(7), 1968-1973.

Morgans, R., Orme, P., Anderson, L., Drust, B., & Morton, J. P. (2014). An intensive winter fixture schedule induces a transient fall in salivary IgA in english premier league soccer players. *Research in Sports Medicine, 22*(4), 346-354.

Morris, J., Nevill, M., Lakomy, H., Nicholas, C., & Williams, C. (1998). Effect of a hot environment on performance of prolonged, intermittent, high-intensity shuttle running. *Journal of Sports Sciences, 16*(7), 677-686.

Morris, J. G., Nevill, M. E., & Williams, C. (2000). Physiological and metabolic responses of female games and endurance athletes to prolonged, intermittent, high-intensity running at 30 and 16 C ambient temperatures. *European Journal of Applied Physiology, 81*(1), 84-92.

Mündel, T., King, J., Collacott, E., & Jones, D. A. (2006). Drink temperature influences fluid intake and endurance capacity in men during exercise in a hot, dry environment. *Experimental Physiology, 91*(5), 925-933.

Murray, R. (1992). Nutrition for the marathon and other endurance sports: environmental stress and dehydration. *Medicine and Science in Sports and Exercise, 24*(9), 19-23.

Murray, R. (1995). Fluid needs in hot and cold environments. *International Journal of Sport Nutrition, 5*(1), 62-73.

Naito, T., & Ogaki, T. (2015). Comparison of the effects of cold water and ice ingestion on endurance cycling capacity in the heat. *Journal of Sport and Health Science*.

Nassis, G. P., Brito, J., Dvorak, J., Chalabi, H., & Racinais, S. (2015). The association of environmental heat stress with performance: analysis of the 2014 FIFA World Cup Brazil. *British Journal of Sports Medicine, 49*(9), 609-613.

Neville, V., Gleeson, M., & Folland, J. P. (2008). Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes. *Medicine and Science in Sports and Exercise, 40*(7), 1228-1236.

Nicholas, C. W., Nuttall, F. E., & Williams, C. (2000). The Loughborough Intermittent Shuttle Test: a field test that simulates the activity pattern of soccer. *Journal of Sports Sciences, 18*(2), 97-104.

Nielsen, B., Strange, S., Christensen, N. J., Warberg, J., & Saltin, B. (1997). Acute and adaptive responses in humans to exercise in a warm, humid environment. *Pflügers Archiv, 434*(1), 49-56. doi:10.1007/s004240050361

Nieman, D. C. (1994). Exercise, infection, and immunity. *International Journal of Sports Medicine, 15*(3), 131-141.

Nieman, D. C., Henson, D. A., Fagoaga, O. R., Utter, A. C., Vinci, D. M., Davis, J. M., & Nehlsen-Cannarella, S. (2002). Change in salivary IgA following a competitive marathon race. *International Journal of Sports Medicine, 23*(01), 69-75.

Nieman, D. C., Johanssen, L. M., Lee, J., & Arabatzis, K. (1990). Infectious episodes in runners before and after the Los Angeles Marathon. *Journal of Sports Medicine and Physical Fitness, 30*(3), 316-328.

Njororai, W. (2014). Timing of goals scored in selected European and South American soccer leagues, FIFA and UEFA Tournaments and the critical phases of a match. *International Journal of Sports Science, 4*(6A), 56-64.

Nybo, L. (2012). Brain temperature and exercise performance. *Experimental Physiology, 97*(3), 333-339.

Nybo, L., Møller, K., Volianitis, S., Nielsen, B., & Secher, N. H. (2002). Effects of hyperthermia on cerebral blood flow and metabolism during prolonged exercise in humans. *Journal of Applied Physiology, 93*(1), 58-64.

Nybo, L., Rasmussen, P., & Sawka, M. N. (2014). Performance in the Heat—Physiological Factors of Importance for Hyperthermia-Induced Fatigue. *Comprehensive Physiology, 4*(2).

Oliver, J. L., Armstrong, N., & Williams, C. A. (2007). Reliability and validity of a soccer-specific test of prolonged repeated-sprint ability. *International Journal of Sports Physiology and Performance, 2*(2), 137-149.

Onitsuka, S., Zheng, X., & Hasegawa, H. (2015). Ice slurry ingestion reduces both core and facial skin temperatures in a warm environment. *Journal of Thermal Biology, 51*, 105-109.

Orysiak, J., Malczewska-Lenczowska, J., Szyguła, Z., & Pokrywka, A. (2012). The role of salivary immunoglobulin A in the prevention of the upper respiratory tract infections in athletes–an overview. *Biology of Sport, 29*(4), 311.

Owen, A. L., Wong, D. P., Dunlop, G., Groussard, C., Kebsi, W., Dellal, A., Zouhal, H. (2016). High-Intensity Training and Salivary Immunoglobulin A Responses in Professional Top-Level Soccer Players: Effect of Training Intensity. *The Journal of Strength & Conditioning Research, 30*(9), 2460-2469.

Pallett, P. J., & O'Brien, M. T. (1985). *Textbook of neurological nursing*: Little, Brown.

Parry-Billings, M., & Newsholme, E. A. (1992). The overtraining syndrome: some biochemical aspects. In *Integration of Medical and Sports Sciences* (pp. 281-287): Karger Publishers.

Peñailillo, L., Maya, L., Niño, G., Torres, H., & Zbinden-Foncea, H. (2015). Salivary hormones and IgA in relation to physical performance in football. *Journal of Sports Sciences, 33*(20), 2080-2087.

Pepler, R. (1958). Warmth and performance: An investigation in the tropics. *Ergonomics, 2*(1), 63-88.

Peters, E. (1997). Exercise, immunology and upper respiratory tract infections. *International Journal of Sports Medicine, 18*, 69-77.

Peters, E. M., Goetzsche, J. M., Grobbelaar, B., & Noakes, T. D. (1993). Vitamin C supplementation reduces the incidence of postrace symptoms of upper-respiratory-tract infection in ultramarathon runners. *The American journal of clinical nutrition, 57*(2), 170-174.

Petrovsky, N., McNair, P., & Harrison, L. C. (1998). Diurnal rhythms of pro-inflammatory cytokines: regulation by plasma cortisol and therapeutic implications. *Cytokine, 10*(4), 307-312.

Pfeiffer, B., Cotterill, A., Grathwohl, D., Stellingwerff, T., & Jeukendrup, A. E. (2009). The effect of carbohydrate gels on gastrointestinal tolerance during a 16-km run. *International Journal of Sport Nutrition and Exercise Metabolism, 19*(5), 485-503.

Pfeiffer, B., Stellingwerff, T., Hodgson, A. B., Randell, R., PÖttgen, K., Res, P., & Jeukendrup, A. E. (2012). Nutritional intake and gastrointestinal problems during competitive endurance events. *Medicine & Science in Sports & Exercise, 44*(2), 344-351.

Pietrowsky, R., Meyrer, R., Kern, W., Born, J., & Fehm, H. L. (1994). Effects of diurnal sleep on secretion of cortisol, luteinizing hormone, and growth hormone in man. *The Journal of Clinical Endocrinology & Metabolism, 78*(3), 683-687.

Piwonka, R., & Robinson, S. (1967). Acclimatization of highly trained men to work in severe heat. *Journal of Applied Physiology, 22*(1), 9-12.

Price, M. J., Boyd, C., & Goosey-Tolfrey, V. L. (2009). The physiological effects of pre-event and midevent cooling during intermittent running in the heat in elite female soccer players. *Applied Physiology, Nutrition, and Metabolism, 34*(5), 942-949.

Proctor, G. B., & Carpenter, G. H. (2007). Regulation of salivary gland function by autonomic nerves. *Autonomic Neuroscience, 133*(1), 3-18.

Pugh, L., Corbett, J., & Johnson, R. (1967). Rectal temperatures, weight losses, and sweat rates in marathon running. *Journal of Applied Physiology, 23*(3), 347-352.

Pyne, D., & Gleeson, M. (1998). Effects of intensive exercise training on immunity in athletes. *International Journal of Sports Medicine, 19*, 183-194.

Quod, M. J., Martin, D. T., & Laursen, P. B. (2006). Cooling athletes before competition in the heat. *Sports Medicine, 36*(8), 671-682.

Rampinini, E., Coutts, A. J., Castagna, C., Sassi, R., & Impellizzeri, F. (2007). Variation in top level soccer match performance. *International Journal of Sports Medicine, 28*(12), 1018-1024.

Reilly, T., & Thomas, V. (1976). A motion analysis of work-rate in different positional roles in professional football match-play. *Journal of Human Movement Studies, 2*(2), 87-97.

Rienzi, E., Drust, B., Reilly, T., Carter, J. E. L., & Martin, A. (2000). Investigation of anthropometric and work-rate profiles of elite South American international soccer players. *Journal of Sports Medicine and Physical Fitness, 40*(2), 162.

Roberts Jr, N. J. (1979). Temperature and host defense. *Microbiological Reviews, 43*(2), 241.

Robson-Ansley, P. J., Blannin, A., & Gleeson, M. (2007). Elevated plasma interleukin-6 levels in trained male triathletes following an acute period of intense interval training. *European Journal of Applied Physiology, 99*(4), 353-360.

Roelands, B., & Meeusen, R. (2010). Alterations in central fatigue by pharmacological manipulations of neurotransmitters in normal and high ambient temperature. *Sports Medicine, 40*(3), 229-246.

Ross, M., Abbiss, C., Laursen, P., Martin, D., & Burke, L. (2013). Precooling methods and their effects on athletic performance. *Sports Medicine, 43*(3), 207-225.

Ross, M. L. R., Garvican, L. A., Jeacocke, N. A., Laursen, P. B., Abbiss, C. R., Martin, D. T., & Burke, L. M. (2011). Novel precooling strategy enhances time trial cycling in the heat. *Medicine and Science in Sports and Exercise, 43*(1), 123-133. doi:10.1249/MSS.0b013e3181e93210

Russell, M., Benton, D., & Kingsley, M. (2011). The effects of fatigue on soccer skills performed during a soccer match simulation. *International Journal of Sports Physiology and Performance, 6*(2), 221-233.

Sabbadini, E., & Berczi, I. (1995). The submandibular gland: a key organ in the neuro-immuno-regulatory network? *Neuroimmunomodulation, 2*(4), 184-202.

Saltin, B. (1964). Circulatory response to submaximal and maximal exercise after thermal dehydration. *Journal of Applied Physiology, 19*(6), 1125-1132.

Sari-Sarraf, V., Doran, D., Clarke, N., Atkinson, G., & Reilly, T. (2011). Effects of carbohydrate beverage ingestion on the salivary IgA response to intermittent exercise in the heat. *International Journal of Sports Medicine, 32*(9), 659-665.

Sawka, M., Montain, S., & Latzka, W. (1996). Body fluid balance during exercise-heat exposure: Body Fluid Balance: Exercise and Sport. New York, NY: CRC Press.

Sawka, M., Young, A., Latzka, W., Neufer, P., Wenger, C., & Pandolf, K. (1990). Influence of hydration and aerobic fitness on tolerance to exercise-heat strain. *Medicine and Science in Sports and Exercise, 22*(2), S118.

Sawka, M. N. (1992). Physiological consequences of hypohydration: exercise performance and thermoregulation. *Medicine and Science in Sport and Exercise, 24*(6), 657-670.

Sawka, M. N., & Coyle, E. F. (1999). Influence of body water and blood volume on thermoregulation and exercise performance in the heat. *Exercise and Sport Sciences Reviews, 27*(1), 167-218.

Sawka, M. N., & Pandolf, K. B. (1990). Effects of body water loss on physiological function and exercise performance. *Perspectives in Exercise Science and Sports Medicine, 3*, 1-38.

Sawka, M. N., Young, A. J., Cadarette, B. S., Levine, L., & Pandolf, K. B. (1985). Influence of heat stress and acclimation on maximal aerobic power. *European Journal of Applied Physiology and Occupational Physiology, 53*(4), 294-298.

Schneyer, L. H., Young, J. A., & Schneyer, C. A. (1972). Salivary secretion of electrolytes. *Physiological Reviews, 52*(3), 720-777.

Sear, J. A., Hoare, T. K., Scanlan, A. T., Abt, G. A., & Dascombe, B. J. (2010). The effects of whole-body compression garments on prolonged high-intensity intermittent exercise. *The Journal of Strength and Conditioning Research, 24*(7), 1901-1910.

Serfass, R. C., Stull, G. A., Alexander, J. F., & Ewing Jr, J. L. (1984). The effects of rapid weight loss and attempted rehydration on strength and endurance of the handgripping muscles in college wrestlers. *Research Quarterly for Exercise and Sport, 55*(1), 46-52.

Severs, Y., Brenner, I., Shek, P., & Shephard, R. (1996). Effects of heat and intermittent exercise on leukocyte and sub-population cell counts. *European Journal of Applied Physiology and Occupational Physiology, 74*(3), 234-245.

Shephard, R., & Shek, P. (1998). Acute and chronic over-exertion: do depressed immune responses provide useful markers? *International Journal of Sports Medicine, 19*(3), 159-171.

Shephard, R. J. (1998). Immune changes induced by exercise in an adverse environment. *Canadian Journal of Physiology and Pharmacology, 76*(5), 539-546.

Shephard, R. J., Castellani, J. W., & Shek, P. N. (1998). Immune deficits induced by strenuous exertion under adverse environmental conditions: manifestations and countermeasures. *Critical Reviews™ in Immunology, 18*(6).

Shirreffs, S., Aragon-Vargas, L., Chamorro, M., Maughan, R., Serratosa, L., & Zachwieja, J. (2005). The sweating response of elite professional soccer players to training in the heat. *International Journal of Sports Medicine, 26*(2), 90-95.

Siegel, R., & Laursen, P. B. (2012). Keeping Your Cool. *Sports Medicine, 42*(2), 89-98.

Siegel, R., Maté, J., Brearley, M. B., Watson, G., Nosaka, K., & Laursen, P. B. (2010). Ice slurry ingestion increases core temperature capacity and running time in the heat. *Medicine and Science in Sports and Exercise, 42*(4), 717-725.

Siegel, R., Maté, J., Watson, G., Nosaka, K., & Laursen, P. B. (2012). Pre-cooling with ice slurry ingestion leads to similar run times to exhaustion in the heat as cold water immersion. *Journal of Sports Sciences, 30*(2), 155-165. doi:10.1080/02640414.2011.625968

Sirotic, A. C., & Coutts, A. J. (2008). The reliability of physiological and performance measures during simulated team-sport running on a non-motorised treadmill. *Journal of Science and Medicine in Sport, 11*(5), 500-509.

Slattery, K. M., Wallace, L. K., Bentley, D. J., & Coutts, A. J. (2012). Effect of training load on simulated team sport match performance. *Applied Physiology, Nutrition, and Metabolism, 37*(2), 315-322.

Small, K., McNaughton, L., Greig, M., & Lovell, R. (2010). The effects of multidirectional soccer-specific fatigue on markers of hamstring injury risk. *Journal of Science and Medicine in Sport, 13*(1), 120-125.

Stanley, J., Leveritt, M., & Peake, J. M. (2010). Thermoregulatory responses to ice-slush beverage ingestion and exercise in the heat. *European Journal of Applied Physiology, 110*(6), 1163-1173. doi:10.1007/s00421-010-1607-3

Stevens, C. J., Dascombe, B., Boyko, A., Sculley, D., & Callister, R. (2013). Ice slurry ingestion during cycling improves Olympic distance triathlon performance in the heat. *Journal of Sports Sciences, 31*(12), 1271-1279.

Stewart, A. M., & Hopkins, W. G. (2000). Seasonal training and performance of competitive swimmers. *Journal of Sports Sciences, 18*(11), 873-884.

Stølen, T., Chamari, K., Castagna, C., & Wisløff, U. (2005). Physiology of soccer. *Sports Medicine, 35*(6), 501-536.

Takamata, A., Mack, G., Stachenfeld, N. S., & Nadel, E. R. (1995). Body temperature modification of osmotically induced vasopressin secretion and thirst in humans. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 269*(4), 874-880.

Tan, P., & Lee, J. (2015). The role of fluid temperature and form on endurance performance in the heat. *Scandinavian Journal of Medicine and Science in Sports, 25*(S1), 39-51.

Teeuw, W., Bosch, J. A., Veerman, E. C., & Nieuw Amerongen, A. V. (2004). Neuroendocrine regulation of salivary IgA synthesis and secretion: implications for oral health. *Biological Chemistry, 385*(12), 1137-1146.

Thatcher, R., & Batterham, A. (2004). Development and validation of a sport-specific exercise protocol for elite youth soccer players. *Journal of Sports Medicine and Physical Fitness, 44*(1), 15.

Thomas, M. M., Cheung, S. S., Elder, G. C., & Sleivert, G. G. (2006). Voluntary muscle activation is impaired by core temperature rather than local muscle temperature. *Journal of Applied Physiology, 100*(4), 1361-1369.

Tofari, P. J., McLean, B. D., Kemp, J., & Cormack, S. (2015). A self-paced intermittent protocol on a non-motorised treadmill: A reliable alternative to assessing team-sport running performance. *Journal of Sports Science and Medicine, 14*(1), 62.

Tomasi, T. B., Trudeau, F. B., Czerwinski, D., & Erredge, S. (1982). Immune parameters in athletes before and after strenuous exercise. *Journal of Clinical Immunology, 2*(3), 173-178.

Trochimiak, T., & Hübner-Woźniak, E. (2012). Effect of exercise on the level of immunoglobulin a in saliva. *Biology of Sport, 29*(4), 255.

Tucker, R., Rauch, L., Harley, Y. X., & Noakes, T. D. (2004). Impaired exercise performance in the heat is associated with an anticipatory reduction in skeletal muscle recruitment. *Pflügers Archiv, 448*(4), 422-430.

Ueno, Y., Umeda, T., Takahashi, I., Iwane, K., Okubo, N., Kuroiwa, J., Nakaji, S. (2013). Changes in immune functions during a peaking period in male university soccer players. *Luminescence, 28*(4), 574-581.

Vanos, J. K., Warland, J. S., Gillespie, T. J., & Kenny, N. A. (2010). Review of the physiology of human thermal comfort while exercising in urban landscapes and implications for bioclimatic design. *International Journal of Biometeorology, 54*(4), 319-334.

Villanova, N., Azpiroz, F., & Malagelada, J. R. (1997). Perception and gut reflexes induced by stimulation of gastrointestinal thermoreceptors in humans. *The Journal of Physiology, 502*(1), 215-222.

Walsh, N. P., Gleeson, M., Pyne, D. B., Nieman, D. C., Dhabhar, F. S., Shephard, R. J., Kajeniene, A. (2011). Position statement part two: maintaining immune health. *Exercise Immunological Review, 17*, 64-103.

Walsh, N. P., & Whitham, M. (2006). Exercising in environmental extremes. *Sports Medicine, 36*(11), 941-976.

Webster, S., Rutt, R., & Weltman, A. (1990). Physiological effects of a weight loss regimen practiced by college wrestlers. *Medicine and Science in Sports and Exercise, 22*(2), 229-234.

Werner, J. (1993). Temperature regulation during exercise: an overview. *Exercise, Heat, and Thermoregulation. Dubuque, IA: Brown and Benchmark*, 49-77.

Williams, A. E. (2011). *Immunology: mucosal and body surface defences*: John Wiley & Sons.

Yao, Y., Lian, Z., Liu, W., & Shen, Q. (2007). Experimental study on skin temperature and thermal comfort of the human body in a recumbent posture under uniform thermal environments. *Indoor and Built Environment, 16*(6), 505-518.

Yeo, Z., Fan, P., Nio, A., Byrne, C., & Lee, J. (2012). Ice slurry on outdoor running performance in heat. *International journal of sports medicine, 33*(11), 859-866.

Zhang, H.-G., Mehta, K., Cohen, P., & Guha, C. (2008). Hyperthermia on immune regulation: a temperature's story. *Cancer letters, 271*(2), 191-204.

Zhang, Y., Nepocatych, S., Katica, C. P., Collins, A. B., Casaru, C., Balilionis, G., Bishop, P. A. (2014). Effect of half time cooling on thermoregulatory responses and soccer-specific performance tests. *Montenegrin Journal of Sports Science and Medicine, 3*(1), 17-22.

Appendix A.

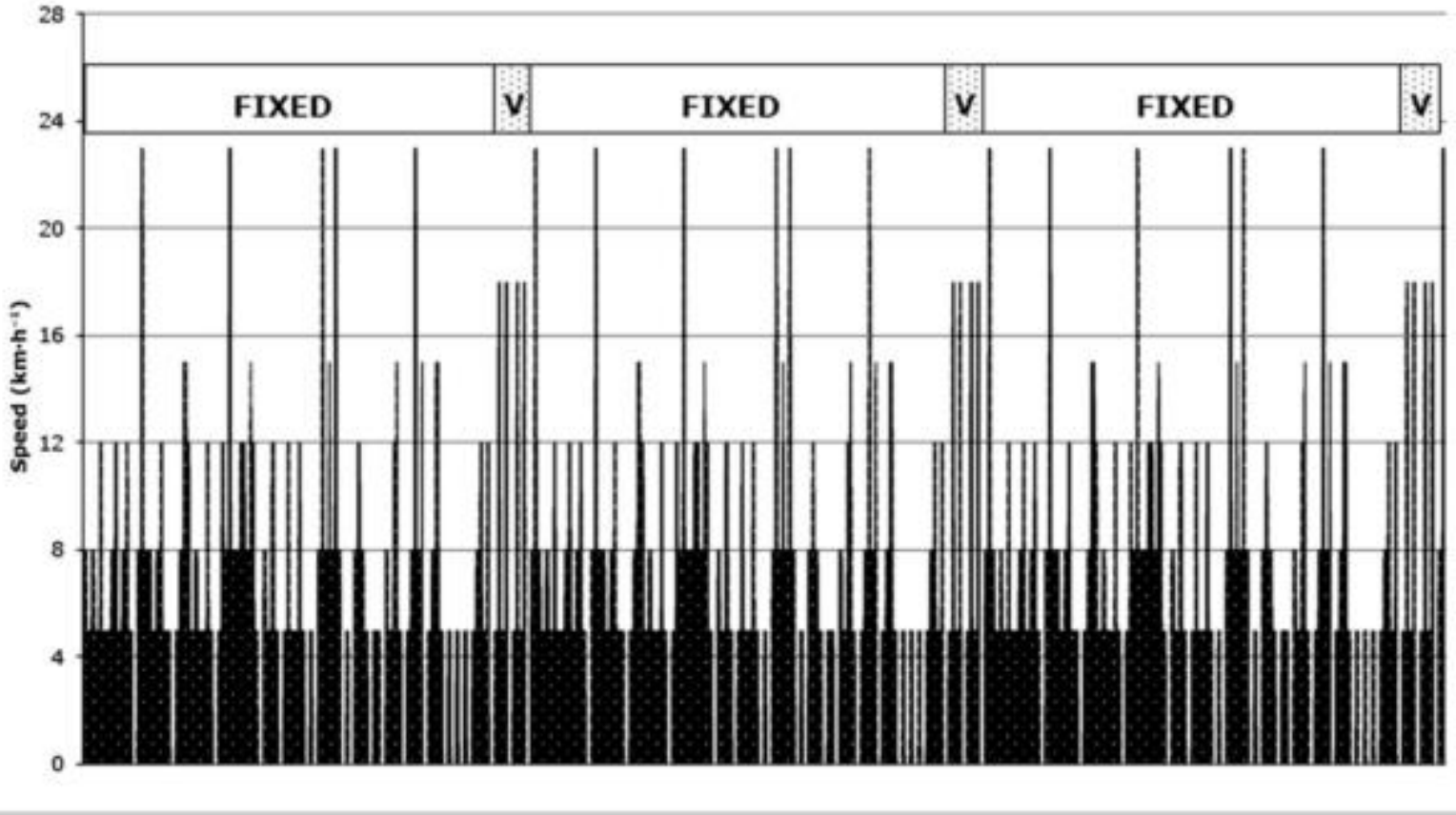

Figure 1. The 45-minute activity profile of iSPT for a participant with a peak sprint speed of 23 km·h^{-1}.

Appendix B

SPRINZ

Diet Diary

It is important to ensure that conditions are kept similar for all trials, therefore we ask you to keep a record of all food and fluid you consume during the 1 day before the first experimental trial, and ask you to reproduce this in the days prior to the second trial. An example is given on the following page.

The following guidelines may help when completing the diary:

1. All foods and beverages including snacks should be recorded (NOTE: No caffeine should be consumed before each trial. No alcohol should be consumed 24 hours before each trial).
2. Quantities of foods drinks consumed can be estimated using approximate portion sizes, with amount left over after eating.

e.g.	Breads:	Brown, wholemeal, white
	Milk:	Whole, skimmed, semi-skimmed
	Biscuits:	Shortcake, digestive
	Cheeses:	Kraft processed slices, Scottish cheddar
	Fish	Mackerel, tuna, haddock
	Fruits:	Large apple, tinned fruit in syrup
	Drinks:	Fruit juice, sports drink, decaf coffee

3. Try to describe each item fully, giving type and brand of food.
4. When eating ready-made food please include brand name and description of the food.
5. Please include use of sauces and condiments (tomato ketchup, salad cream etc).
6. Include method of cooking - boiled, fried, grilled etc
7. Indicate whether skins are eaten.
8. Include all food, vitamin and mineral supplements used.
9. Use as many pages as required for each day.
10. Please attempt to record all items immediately after consumption. Do not wait until the end of the day as you may forget some items.

It is important that you are rested prior to the trials, so no exercise should be undertaken in the day before each trial.

If you have any problems completing this diary, or with any aspect of the study, please contact me on 021 296 9416.

DAY:	EXAMPLE	
TIME	DESCRIPTION OF FOOD OR DRINK CONSUMED	PORTION SIZE
8:30am	Kellogg's cornflakes	Small bowl
	Semi-skimmed milk	250 ml
	Toast, Tip Top white bread	2 slices
	Countdown olive margarine	Thin spread
	Tropicana fresh orange juice	1 small glass
11:00am	Coffee, Nescafe decaffeinated	1 mug
	Whole milk	splash
1:15pm	Sandwich:	1 sandwich
	Tip Top White bread	2 slices
	Margarine	Thin spread
	Grated cheddar cheese	20 g
	Tomato	3 slices
4:30pm	Lucozade Sport – orange	500ml bottle
6:00pm	Grilled lean pork chops	2 medium
	Boiled new potatoes with skins	7 small
	Cross & Blackwell peas	2 tablespoons
	Banana	1 medium
7:30pm	Green tea (decaffeinated)	1 mug
	McVities Digestives	4 biscuits

DAY:	Trial one	
TIME	DESCRIPTION OF FOOD OR DRINK CONSUMED	PORTION SIZE

Appendix C

Participant Information Sheet

Date Information Sheet Produced:

24 May 2016

Project Title

Does ice slurry ingestion influence mucosal immunity and performance during intermittent exercise in the heat?

An Invitation

Hello, my name is Ryan Morrow and I am a Masters Student at AUT University, based at AUT-Millennium. I would like to invite you to assist me in some research on team sport performance and immune response that I am conducting with Dr Deborah Fletcher and Dr Andrew Kilding. I am conducting this study to determine the effect of precooling ice slurry ingestion on immune system response during exercise in hot environments. This research will help inform the practices elite-level athletes and coaches, as well as serious sub-elite athletes.

It is entirely your choice as to whether you participate in the project or not. If at any time you decide you no longer want to participate, you are free to withdraw from the study without consequences. Your consent to participate in this research will be indicated by your signing and dating the consent form. Signing the consent form indicates that you have read and understood this information sheet, freely given your consent to participate, and that there has been no coercion or inducement to participate by the researchers from AUT.

What is the purpose of this research?

We want to determine whether or not it matters what temperature of fluid athletes drink while competing in team sport in hot environments. We wish to publish these results in a scientific journal so as to help coaches and athletes improve their performance.

How was I identified and why am I being invited to participate in this research?

You have been invited to participate because you are a competitive football player aged between aged between 18 and 35, and are currently competing in a competitive football league.

What will happen in this research?

Eligibility

To take part in this study you will need to meet the following criteria

- You have to be male and aged between 18 and 35.
- You will have recently competed in an Auckland football competition
- You will be actively training at least twice a week and playing at least one game per week.
- You will not smoke or be on any current medications
- You are not currently suffering from any injuries or infections

You will be required to visit the SPRINZ lab on four occasions:

Visit 1 - During the first session, you will be asked to complete two assessments on a treadmill to determine your peak speed, and aerobic fitness, or maximum oxygen uptake ($\dot{V}O_{2max}$). The peak speed assessment involves completing 4 sprints lasting 6 seconds on a non-motorised treadmill. The aerobic fitness test will require running at a relatively easy intensity, and this will progressively increase until you can no longer keep up. The idea is that you are exhausted by the end of the test! Throughout the test you will be wearing a facemask. This collects the air you breathe out in order to determine how much oxygen you are taking in. You will also wear a heart rate monitor round your chest.

Visit 2 – This will be a familiarisation trial in which you will complete a mock intermittent soccer performance test in the heat chamber to get used to the testing procedures. You will be allowed to drink water throughout this trial which will be available in a bottle near the treadmill.

You will then need to visit the laboratory on two further occasions, all lasting approximately 3 hours.

Visits 3 - 4 – These visits will feature the same intermittent soccer performance test, only this time you will be drinking either an ice slurry, or room temperature beverage. Again, you will wear a heart rate monitor and your skin and core temperature will be measured. Measurement of skin temperature involves places small 'buttons' on your skin which transmit temperature to a device. Core temperature will be determined using a rectal probe. This is a thin, pliable, sterile probe that you insert yourself in a private room. Instruction of how to do this will be given. It is a pain-free procedure and will not inhibit or impede your comfort or performance in anyway and is a commonly used method of gaining accurate temperature readings in sport and exercise research. Samples of saliva will be taken at several stages through the research for analysis of immune system response. You (and your drink bottle, which will be provided) will also be weighed before and after the time-trial, to determine how much you sweated. Also, your diet in the day before the last two experimental trials will have to be similar to make sure that the comparison is fair. This basically means drinking at least a set amount of fluid and having at least a set amount of carbohydrates. You will be given written guidance on how to do this after your second visit (the mock trial). You will also be asked to refrain from consuming any alcohol or caffeine 24 hours prior to the trials.

What are the discomforts and risks?

There may be some discomfort ($\dot{V}O_{2max}$) and risk associated with measures in this research, but the running tasks will be similar to your own experiences. You will be asked to complete the tasks in normal exercise attire. If you are experiencing discomfort at any stage you are

encouraged to inform the researcher with you at the time in order that they can best address the problem. If you have any questions regarding and risk or comfort that you anticipate, please feel free to address these concerns to the researcher so that you feel comfortable at all times throughout the process. You will be able to withdraw from this research at any point prior to the completion of data collection.

What are the benefits?

You will be given the results of your $\dot{V}O_{2max}$ test for your interest and perusal (this test would normally cost $150), and you will experience exercising in a heat chamber.

What compensation is available for injury or negligence?

In the unlikely event of a physical injury as a result of your participation in this study, rehabilitation and compensation for injury by accident may be available from the Accident Compensation Corporation, providing the incident details satisfy the requirements of the law and the Corporation's regulations.

How will my privacy be protected?

All your personal details will remain confidential and will only be available to Deborah, Andrew, and myself during the period of the study. These personal details will be kept in a locked cabinet for ten years before being destroyed. All trial data will be stored on password protected computers or in locked files. Following completion of data analysis, your data will be anonymised and stored by the SPRINZ research officer in the secure SPRINZ ethics storage room for ten years. We store the data as it is not uncommon for analysis techniques to change and improve. Therefore, in the future researchers not currently involved with this project may have access to your performance data. However, it will be anonymised. This research may be published in an academic journal at a later date, you will not be identifiable in any way during this process.

What are the costs of participating in this research?

You will be required to attend the SPRINZ laboratories at AUT-Millennium four times, 5-7 days apart. The first two visits are familiarisation trials lasting approximately 1 hour each, the following two visits being experimental trials, approximately 3.5 hours (total of 90 minutes exercising time) each. All participants will only perform one maximum oxygen consumption test (on the first lab visit).

What opportunity do I have to consider this invitation?

We would appreciate it if you could let us know within one week whether you would be available to take part in the study or not. After consideration you may withdraw your participation at any time.

How do I agree to participate in this research?

If you agree to participate please fill in the attached consent form and return to Ryan Morrow.

Will I receive feedback on the results of this research?

If you would like feedback on the results of the research please indicate on the consent form.

What do I do if I have concerns about this research?

Any concerns regarding the nature of this project should be notified in the first instance to myself via *ryan.morrow@outlook.com*

Concerns regarding the conduct of the research should be notified to the Executive Secretary of AUTEC, Kate O'Connor, *ethics@aut.ac.nz* 921 9999 ext. 6038.

Whom do I contact for further information about this research?

Researcher contact details

Ryan Morrow, Auckland University of Technology, Millennium Campus, Phone: 0212969416, Email; ryan.morrow@outlook.com

Deborah Dulson Sport Performance Research Institute New Zealand, AUT-Millennium Campus, AUT University, Private Bag 92006, Auckland 1020, Phone: 09 921 9999 Ext 7417, Email: deborah.dulson@aut.ac.nz

Andrew Kilding, Sport Performance Research Institute New Zealand, AUT-Millennium Campus, AUT University, Private Bag 92006, Auckland 1020, Phone: 09 921 9999 x7056, Email: andrew.kilding@aut.ac.nz.

Approved by the Auckland University of Technology Ethics Committee on *30/5/16*, AUTEC Reference number *16/148*.

Appendix D

Consent Form

Project title:

Does ice slurry ingestion influence mucosal immunity and performance during intermittent exercise in the heat?

Primary Researcher: ***Ryan Morrow***

(Please Tick)

- O I have read and understood the information provided about this research project in the Information Sheet.
- O I have had an opportunity to ask questions and to have them answered.
- O I am not suffering from heart disease, high blood pressure, any respiratory condition (mild asthma excluded), any illness or injury that impairs my physical performance.
- O I understand that I may withdraw myself or any information that I have provided for this project at any time prior to completion of data collection, without being disadvantaged in any way.
- O I understand there may be some discomfort associated with measures in this research, but this will not be beyond your normal high intensity level of training.
- O I am not suffering from any illness or injury that may prevent me from being able to complete the tasks detailed in the information sheet.
- O I have not experienced an upper respiratory tract infection in the past 4 weeks, am currently smoke-free, and competing in an Auckland football competition.
- O I agree to my de-identified performance data being kept indefinitely in storage for future analysis/research purposes, and my personal details being stored in a locked cabinet for a period of ten years before being destroyed.
- O I agree to take part in this research.
- O I wish to receive a copy of the report from the research (please tick one): YesO NoO
- O I wish to have my saliva samples returned to me at the end of the research: YesO NoO

Participant's signature:.....

Participant's name: .....

Participant's contact details (if appropriate):

.....

.....

Date:

Approved by the Auckland University of Technology Ethics Committee on 1/6/16 AUTEC Reference number 16/148

Note: The Participant should retain a copy of this form.

Appendix E.

SPORTS PERFORMANCE
RESEARCH IN NEW ZEALAND AT AUT MILLENNIUM INSTITUTE

AUT UNIVERSITY

WANTED

Football players for immunity response research

Are you:

Male

18-35 years old

Recently competed in an Auckland football competition

My name is Ryan Morrow and I am a Masters student at the Sports Performance Research Institute, at AUT University. I am conducting research comparing the effects of ingested fluid temperature on immune system response in the heat.

What the research will involve:

Five visits to the laboratory (each 5-7 days apart) at AUT Millennium. Days will include:

1. Running test to exhaustion to determine aerobic fitness ($\dot{V}O_{2max}$) and peak speed Test (lasting ~1hour)
2. Getting familiar with the test and equipment (lasting ~1hour)
3-5. Three football specific tests in a heat chamber with different fluids (Protocol lasting 90minutes each)

Would you like to participate?

If you would like to find out more information and/or want to register your interest to take part in this study, please contact myself at ryan.morrow@outlook.com for a detailed participant information sheet.

Thank you for your consideration,

Ryan Morrow, AUT University

Appendix F

AUTEC Secretariat

Auckland University of Technology
D-88, WU406 Level 4 WU Building City Campus
T: +64 9 921 9999 ext. 8316
E: ethics@aut.ac.nz
www.aut.ac.nz/researchethics

1 June 2016
Deborah Dulson
Faculty of Health and Environmental Sciences

Dear Deborah

Re Ethics Application: **16/148 Does ice slurry ingestion influence mucosal immunity and performance during intermittent exercise in the heat.**

Thank you for providing evidence as requested, which satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 24 May 2019.

As part of the ethics approval process, you are required to submit the following to AUTEC:

- A brief annual progress report using form EA2, which is available online through http://www.aut.ac.nz/researchethics. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 24 May 2019;
- A brief report on the status of the project using form EA3, which is available online through http://www.aut.ac.nz/researchethics. This report is to be submitted either when the approval expires on 24 May 2019 or on completion of the project.

It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are responsible for ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application.

AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to obtain this.

To enable us to provide you with efficient service, please use the application number and study title in all correspondence with us. If you have any enquiries about this application, or anything else, please do contact us at ethics@aut.ac.nz.

All the very best with your research,

Kate O'Connor
Executive Secretary
Auckland University of Technology Ethics Committee

Cc: Ryan Morrow ryanmorrow92@gmail.com; Andrew Kilding