Does caffeine ingestion influence mucosal immunity and performance in males during intermittent exercise in the heat?

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Contents

Executive Summary

Mucosal immunity provides the first line of defence against Upper Respiratory Tract Infections (URTI). Recent literature suggests that secretory immunoglobulin A (SIgA) constitutes the predominant immunoglobulin isotype in secretions, including saliva. SIgA could be used to help indicate an athlete's risk of URTI. Athletic training and competitions often occur in hot environments and therefore performing exercise in these conditions may result in reductions in SIgA concentrations and provides a further stress on immune response. The use of caffeine in athletic population has increased in recent years. Caffeine is a central nervous system stimulant found in ordinary foods and beverages and its metabolic and performance effects have been widely reported. However, the effects of caffeine on mucosal immunity in team sport athletes in hot temperatures is currently unknown. Therefore, the purpose of this study was to investigate the effects of caffeine on mucosal immunity in team sport athletes when exercising at high intensities in heat.

In a double-blinded randomised cross-over design, 12 semi- professional team sport athletes completed two trials which consisted of six 3-minute intervals at 108% of their maximal oxygen uptake ($VO_{2\ max}$) interspersed with 2 minutes of brisk walking in a heat chamber, set at 30 ^{o}C and 50% relative humidity (RH). $VO_{2\ max}$ is the measurement of the maximum amount of oxygen a person can utilise during exercise. It is a common measurement used to establish the aerobic endurance of an athlete prior to or during the course of training. Participants consumed, at a standardised rate, 6mg/kg/BM of caffeine or a placebo pill. Unstimulated saliva samples were collected at baseline, pre-exercise, immediately post exercise and 1-hour post exercise. Caffeine had no significant effects on salivary concentration rate, salivary flow rate and salivary secretion rates in all participants. The decreases in salivary concentration rate and secretion rate were short lived as post exercise values returned to baseline levels. High intensity exercise in heat may cause a transient drop in immune function after caffeine consumption, however, acute ingestion of caffeine in heat in team sport athletes does not appear to influence SIgA following exercise when the open window period is most apparent.

In conclusion, taking an acute dose of caffeine before completing high intensity exercise in heat did not provoke any changes in mucosal immunity or physiological markers in highly trained male athletes. Results showed that athletes can take a moderate dose of caffeine in heat. However, these results should be treated with caution by coaches as well as athletes and should be tested well in advance before trialling it pre-competition or game.

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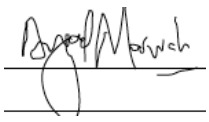
List of Abbreviations

ANOVA	analysis of variance
ANS	autonomic nervous system
ATP	adenosine triphosphate
BM	body mass
BP	blood pressure
CAF	caffeine
CM	centimetre
CNS	central nervous system
CV	coefficient of variation
FFA	free fatty acids
g	grams
h	hour
HIE	high intensity exercise
HIIT	high intensity interval training
HR	heart rate
Kg	kilogram
Km	kilometre
LIST	Loughborough intermittent shuttle test
m	meters
MALT	mucosal associated lymphoid tissues
mg	milligram
min	minutes
ml	millilitre
Pcr	phosphocreatine
PLA	placebo
RER	respiratory exchange ration
RH	relative humidity
RPE	ratings of perceived exertion
SD	standard deviation
SIgA	salivary secretory immunoglobulin A
URTI	upper respiratory tract infection
$VO_{2\ max}$	maximal oxygen uptake
W_{max}	maximal watts
y	years
^{o}C	degrees Celsius
µg	microgram
µl	microlitre

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 acknowledgements), 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: 

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Intellectual Property Rights

Ethical Approval

Ethical approval for the study undertaken in this thesis was granted from Auckland University of Technology's Ethics Committee (AUTEC) on 31 July 2017; ethics application number: 17/153.

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 1 introduces the thesis topic. Chapter 2 (Literature Review), introduces the reader to mucosal immunity and how different exercise protocols affect these measures. The effect of exercising in heat on mucosal immunity in team sport athletes is also discussed in this section. The effect of caffeine during high intensity exercise is discussed and finally the effects of caffeine on mucosal immunity are examined. Chapter 3 includes the study design and methodology. Chapter 4 consists of the main findings and statistical analysis of the results, with relevant tables and figures provided. Chapter 5 provides a discussion around the study's findings, alludes to the limitations, potential future research in the area, and applications to the sport physiology field.

Chapter 1 Introduction

Mucosal immunity provides the first line of defence against URTI (Brandtzaeg et al., 1999) the most commonly reported illness among athletes (Fricker et al., 2000; Peters, 1997; Pyne & Gleeson, 1998). Factors such as training, exposure to environmental conditions and nutrition have the potential to have a negative effect on mucosal immunity. URTI represents the most common acute illness in the general population (Page & Diehl, 2007). Athletes training at high loads are often considered to be at an elevated risk for URTI as a result of a suppressed immune system. It has been widely hypothesized that a J-Shaped (Figure 1.1) curve model may represent the immunologic capacity of an individual (Nieman et.al., 1994). This model suggests that the lowest risk of URTI is found among individuals who are moderately active and that risk of URTI is increased for both physically inactive and highly active individuals. This means that athletes training at high intensity and loads are at a greater risk for contracting URTI than healthy inactive individuals.

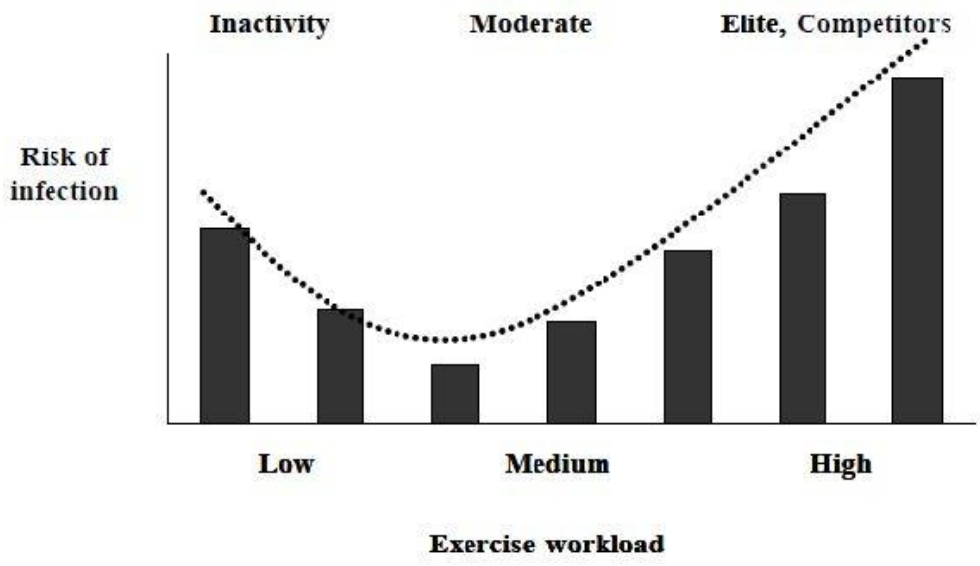

Figure 1.1: 'J' shaped curve of infection risk. Adapted from Nieman (2008)

The intensity and duration of exercise are known to be the main determinants of sport related changes in mucosal immunity. The 'open window' hypothesis by Pederson and Ullum (1994) suggests that immune function is stimulated during high-intensity exercise, quickly followed by a period of immune depression that may last between 3 and 72 hours depending on the intensity, duration and type of activity performed. The above authors proposed that it is during this time that athletes are most susceptible to infection. Gleeson (2013) also reported that

intensive endurance training was also associated with increased episodes of clinically confirmed URTI. This relationship has also been reported in athletes involved in physically demanding intermittent sports including football and rugby (Foskett, Ali, & Gant, 2009; Stuart et al., 2005).

While the 'J' shaped model has been supported by several studies (Foster,1998; Matthews et al., 2002) , Malm (2006) indicated that elite athletes most likely possess an immune system that is capable of enduring infections during periods of high psychological and physiological stress to sustain their performances required at a high level. As a result, Malm (2006), therefore recommended an 'S' shaped curve (Figure 1.2) which depicts elite athletes have a diminished risk of contracting infection compared to non-elite athletes who train or perform exercise at higher intensities.

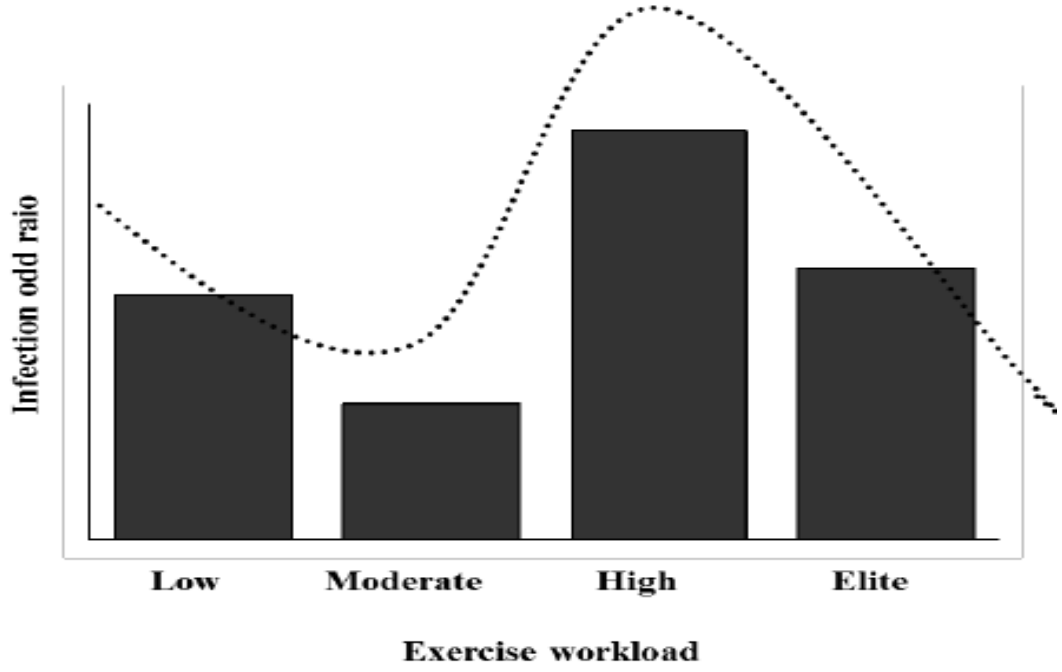

Figure 1.2: S-shaped relationship between training load and infection rate. Adapted from Malm (2006)

Strenuous exercise in both moderately and highly trained athletes is commonly followed by reports of depressed mucosal immune response and upper respiratory symptoms (Gleeson, 2000). Mucosal secretions play an important role in innate immunity and provide a mechanical washing effect against foreign pathogens (Bishop et al., 2000). These defence systems are mediated by secretory immunoglobulins (Ig), particularly IgA and IgM. Salivary IgA (SIgA) is considered to provide the first line of defence against pathogens and antigens presented at mucosal surfaces. SIgA protects the body's mucosal surfaces by constraining pathogen adherence, virus neutralisation and excretion of immune complexes (Lamm, 1997a). SIgA

prevents viral and bacterial material from replicating and attaching itself to mucosal epithelium of the mouth, throat and upper respiratory tract (Cunniffe et al., 2011). SIgA has been proposed as the most promising immune marker for identifying those athletes at risk of URTI (Neville, Gleeson, Folland, 2008; Pyne & Gleeson, 1998; Shephard & Shek 1998). One group applied a different method of research to investigating SIgA in team sport athletes by employing an experimental protocol similar to the exercise requirements of a soccer match. (Sari-Sarraf, Reilly, & Doran, 2006). They used a 90 min soccer-simulated exercise protocol. Pre to post exercise SIgA concentration and secretion rate were found to be similar by Sari-Sarraf et al., (2006), on the other hand increases in both SIgA concentration and secretion rate were observed by Sari-Sarraf et. al., (2007). It could be postulated that these differences in findings may be due to variability in between or within subject responses. Psychological motivation could also be a factor in the difference in the findings as completing an exercise protocol is different to actual game situations. Similarly, Moreira et al., (2009) reported SIgA concentration and secretion rate to remain unchanged following a friendly 70- minute football game. However, two highly competitive futsal games resulted in decreases to both SIgA concentration and secretion rates (Moreira, Arsati, de Oliveira Lima-Arsati, de Freitas, & de Araujo, 2011). Although lengthy exercise is generally thought to have greater effects on immune function as it is more likely to provoke a stress response capable of affecting SIgA (Nieman et al., 2002,2006; Pacque et al., 2007), it is also suggested that non- competitive prolonged exercise may not negatively affect SIgA at intensities of 50 - 80% $VO_{2\ max}$ (Blannin et al., 1998). As such, it maybe be that prolonged exercise must be performed at a high intensity (such as playing in a competitive game) in order to reduce secretion of SIgA. Overall, the findings by Moreira et al., (2009) and Moreira et al., (2011) may suggest that SIgA responses may be more influenced by exercise intensity rather than duration.

Sports performance is said to decrease in hot environments because exercising in extreme conditions increases demands on the central nervous system, cardiovascular system and on metabolism (Walsh et al., 2011). Exercising in hot conditions where core temperature rises by more than 1 ^{o} C compared with thermoneutral conditions has been shown to result in an increase in circulating stress hormones, including catecholamines and cytokines (Gleeson, 2007). Shephard (1998) hypothesised that exercise in adverse environments with increased stress hormone (e.g. cortisol and adrenaline) responses as compared to exercise in favourable conditions, may cause greater disruption to the immune system. Performing exercise in thermally stressful environments appears to produce greater disturbances on the immune system compared to heat alone (Lim & Mackinnon, 2006; Shephard et al., 1998; Walsh & Whitham, 2006). Heat exposure provides a further stress during exercise, elevating core temperature with coinciding alterations in immune responses (Sari-Sarraf et al., 2011). Performing prolonged exercise in the heat, with the increase in fluid loss, appears to result in

large reductions in saliva flow rate (Horswill et al., 2006) and it also appears to suggest that salivary IgA concentration reduces when exercising at the same intensity in thermo-neutral conditions (Sari-Sarraf et al., 2011).

On the other hand, high intensity intermittent exercise in the heat (35 ^{o} C) has formerly been proven to provide greater thermal strain than continuous exercise and may therefore be a more powerful stimulus for acclimation.

A rapid increase in body temperature (>1.8^{o} C) in women has been reported within 30 minutes of high intensity intermittent running in heat (Morris et al., 2000; Sunderland & Neville., 2003). A high core body temperature is alluded to be a key determinant in adaptations to heat acclimation and thus the achieving a high absolute temperature in a short time period may be advantageous for heat acclimation.

The major tournaments for team sports such as FIFA World Cup, Summer Olympic Games, often take place in summer months and often in countries with hot environmental temperatures (Burke, 2007). Therefore, the need for team sport performers to acclimatise is evident, however, time is often limited due to the need to complete technical and tactical sessions. Therefore, the use of prolonged low-intensity exercise for acclimation for team sport activity is time consuming and not specific to the requirements or the nature of the game. Also, the players would be expected to taper during the final few weeks leading up to a competition rather than looking to increase their training volume. Therefore, a protocol that consisted of six high intensity running intervals interspersed with active recovery in hot temperature was used for this research.

Caffeine is a member of the methylxanthine family of drugs and is probably the most extensively consumed psychoactive ingredient due to its presence in various foods and fluids (Fredholm et al.,1999). It is estimated that the mean daily intake of caffeine in New Zealand is 3.5 mg/kg/ body mass (BM), which is similar to approximately 2-4 cups of coffee (Thomson & Schiess, 2011). Doses of 2-6 mg/kg/BM of caffeine have been previously reported to increase performance from short sprints to endurance activities (Goldstein et al., 2010). Schneiker et al., (2006) explored the effects of caffeine supplementation on 10 recreationally competitive team sport athletes. The results of the study found that a caffeine dose of 6 mg/kg/BM was successful in inducing more total sprint work as compared to a placebo. Stuart et al., (2005) also examined the effects of a 6 mg/kg/BM dose of caffeine in well trained amateur rugby players. Results from this study showed a 10% improvement in ball passing accuracy and also indicated that participants who took caffeine were able to maintain sprint times at the end of the circuit, relative to the beginning of the protocol.

While caffeine in thermoneutral conditions has been shown to have ergogenic effects on performance, there is conflicting evidence of the benefits of caffeine ingestion while exercising in heat. Del Coso et al., (2008) showed that trials conducted with caffeine ingestion in 36 °C

and 29 % relative humidity increased cycling power by 3% above trials without caffeine in endurance cyclists. Similarly, Pitchford et al., (2014) reported that time trial performance performed by 9 well trained male cyclists (35 °C and 25% relative humidity) was faster with caffeine compared with placebo. However, a study by Roelands et al (2011) showed no changes in performance. This study however, reported an increase in core temperature and as a result have concluded that this may have neutralized the ergogenic effects of caffeine. Factors such as inter-individual differences in response to caffeine and changes in neurotransmitter concentrations might also be accountable for the lack of performance improvement of caffeine in high ambient temperature. Bishop et al., (2005) conducted a study investigating SIgA responses to prolonged intensive exercise following caffeine ingestion. Eleven endurance-trained male participants cycled for 90 min at 70% $VO_{2\ max}$ on two occasions, having ingested 6 mg/kg/BM of caffeine or placebo 1h before exercise. The results showed that SIgA was higher on caffeine than placebo at mid and post exercise and SIgA secretion rate was higher with caffeine at mid exercise only. They also found that caffeine ingestion did not affect saliva flow rate. Saliva α- amylase activity and secretion were higher with caffeine compared to placebo. These findings suggest that caffeine ingestion before intensive exercise is associated with elevated SIgA responses during exercise. To the best of our understanding, the effect of caffeine ingestion on SIgA responses in humans either at rest or following exercise in the heat has not been extensively researched.

Therefore, the primary purpose of this research is to determine the effect of caffeine ingestion on mucosal immune response following intermittent exercise in the heat in male team sport athletes with a secondary focus on performance.

Chapter 2 Literature Review

2.1 Mucosal Immunity

The mucosal immune system is the largest immune network in the human body and defends several organs as well as the respiratory system from infection (Gleeson et al. 2013) . Mucosal immunity is often regarded as an initial line of defence, reducing the need for systemic immunity intervention (Gleeson, 2006a). Systemic immunity is predominantly inflammatory and could cause damage to the tissues, therefore, whilst systemic immunity offers protection, it can also be harmful to the body.

The mucosal immune system is made up of several mucosal associated lymphoid tissues (MALT), which includes tissues in the salivary gland, nasal passages and respiratory tract (Williams, 2011). This network of tissues found at mucosal surfaces can provide protection not only at the surfaces but at sites distal to the original site of antigen presentation as well (Brandtzaeg et al., 1999). The humoral arm of the immune system begins this process where circulating ß-Cells separate to become antibody-producing ß plasma cells (Gleeson, 2006a). These cells can produce 5 antibody isotopes; immunoglobulin (Ig) A,G,M,D and E, depending on which antigen is presented at the cell surface. Mucosal immune status is most frequently assessed by measurement of salivary IgA concentration, due to the ease of collection of this secretion.

In addition, α-amylase, produced by acinar cells in salivary glands contributes to mucosal immunity by diminishing bacterial adherence and growth to epithelial surfaces (Scannapieco, Solomon, & Wadenya, 1994).

These antibodies and enzymes work together to form the first line of defence to shield the body against the pathogens, antigens and allergens presented at the mucosal surfaces (Gleeson et al 2000). This protection is accomplished through the activation of an immune response that aims to avert pathogen adherence, neutralize viruses within epithelial cells and emit immune complexes i.e. antigen bound to an antibody across mucosal epithelial cells to the cell surface (Lamm, 1997b).

2.2 Saliva

The composition of saliva consists of numerous antibodies, enzymes, peptides, hormones, mucus and antibacterial compounds (Chicharro, Lucía, Pérez, Vaquero, & Ureña, 1998) which makes it an important component of the mucosal immune system. About 750-1500 ml of saliva is secreted each day (Bishop & Gleeson, 2009). Research has also indicated that some compounds such as cortisol, testosterone and aldosterone provide a reliable reference for their respective blood concentrations (Cadore et al., 2008; Dawes, 1972)

Secretion of saliva is largely controlled by the autonomic nervous system (ANS). Sub-maxillary, parotid, sub-lingual and several minor mucous glands found in the oral cavity are responsible for saliva secretion in humans (Dawes, 1972). Sub maxillary glads are responsible for up to 65% of total unstimulated saliva secretion with parotid glands contributing up to 23%, sublingual glands 4% and minor mucous glands up to approximately 8% (Dawes, 1972). Schneyer, Young, and Schneyer (1972) suggested that while normal saliva secretion is achieved by a combination of both sympathetic and parasympathetic innervation, parasympathetic activity provides the primary stimulus for increased saliva secretion.

Sympathetic activity has been suggested to trigger a decrease in blood flow to salivary glands and hence reduce the flow of saliva (Ferreira & Hoffman 2013). Whilst the saliva flow rate is low due to sympathetic activation, the saliva is high in organic and inorganic compounds containing α-amylase which has been indicated to be a potential indicator of increased sympathetic activity and is often seen to be elevated with sympathetic activation (Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007). On the other hand, parasympathetic activity results in the opposite response with increased blood flow and high saliva flow rate that is low in organic and inorganic compounds (Baum, 1987).

Saliva flow rate and composition can also be modified via other mechanisms that include chewing polythene tubes, sucking on mints and holding citric acid or salt on tongue. It is suggested that the stimulation of saliva flow rate and the alterations in composition is likely due to changes in the gland secretions, with parotid secretions contributing to over 50% of stimulated saliva secretion (Humphrey & Williamson, 2001; Sreebny, 2000). Therefore, to allow for accurate comparison between studies, it is important for researchers to state whether saliva collected was from different glands or a specific gland, and if the saliva collected was stimulated or not.

Other factors such as fasting, dehydration and circadian rhythm may also affect saliva flow rate. For example, Ship and Fischer (1997) reported a decrease in resting parotid flow rate and saliva osmolality after 24h of fasting with no food or water. Circadian rhythm is also thought to possibly affect saliva flow rate (Dawes, 1972). However, there is conflicting evidence with some studies suggesting a decrease in saliva flow rate in the morning (Palmai & Blackwell, 1965) and other reporting increases in saliva flow rate over the course of a morning in resting participants (Nehlsen-Cannarella et al., 2000). It is unclear as to why there are discrepancies between the studies and as such it appears that further research is required in this area.

2.3 Immune Markers in Saliva

2.3.1 IgA

Secretory IgA is considered to be the first line of defence against pathogens and antigens at mucosal surfaces (Gleeson et al. 2013). SIgA is the most abundant immunoglobulin found in mucosal secretions, including saliva (Proctor & Carpenter, 2001). It consists of two monomeric IgA units and two additional polypeptide chains, J chains & secretory component (Teeuw, Bosch, Veerman, & Amerongen, 2004). The secretion and formation of IgA is a multistep process. The two IgA monomers are secreted by plasma cells (B Cells) located in the basolateral surface. Dimeric IgA is formed when two IgA monomers are joined by the J- Chain. This then binds to the polymeric immunoglobulin receptor (pIgR) on the basolateral membrane of the epithelial cell and is transported to the apical surface where the pIgR is separated leaving on the secretory component bound to the secreted IgA.

However, the remaining secretory component makes the secretory IgA more resistant to protease degradation. The IgA bound to pIgR across the mucosal epithelium essentially protects SIgA through prevention of pathogen adherence and penetration of the mucosal epithelium. It also helps neutralise the viruses within the epithelial cells during transcytosis (Lamm 1997a).

Pathogen adherence is commonly considered to be the most important function of SIgA as it prevents bacteria from penetrating mucosal epithelium at the apical surface (Teeuw, Bosch, Veerman, & Amerongen, 2004). This is accomplished when IgA binds to antigen making it too large to penetrate cells or by blocking the ability of antigen to bind to cells (Williams, 2011).

2.3.2 α-amylase

Antimicrobial action that contribute to mucosal immunity are as a result of the various enzymes and proteins present in the saliva (Papacosta & Nassis, 2011). α-amylase is an antimicrobial enzyme and is most influential in both pancreatic and salivary fluids (Papacosta & Nassis, 2011). It contributes to innate mucosal immunity as it inhibits the ability of bacteria to grow and attach to mucosal surfaces (Walsh et al., 2011) and is also seen as part of the body's first line of defence against infection (Scannapieco et al., 1994). α-amylase is secreted from acinar cells in salivary glands innervated by both sympathetic and parasympathetic nerves and is primarily secreted by parotid glands (Speirs et al., 1974).

α-amylase has been shown to be affected by physiological as well as psychological mechanisms. For example, Mackie & Pangborn (1990) reported that chewing stimulated α-amylase activity, in order to help breakdown food. α-amylase activity has also been shown to

be affected by food with salt and lemon juice resulting in increased levels of α-amylase (Noble, 2000).

Chatterton Jr et al., (1996) suggest that psychological stress can also result in an increase in α-amylase activity through interactions with the ANS. They reported levels of α-amylase and norepinephrine to increase following a written examination when compared to α-amylase activity prior to the exam. As a result of these findings, they suggested that α-amylase activity maybe predictive of catecholamine levels. These findings were also supported by Takai et al. (2004) who examined the effects of watching a stressful video on α-amylase activity and saliva cortisol concentration. They reported an increase in the α-amylase activity as well as increase in saliva cortisol following the video. Nater et al (2007) also concluded that α-amylase was appropriate for use as a non-invasive marker of biological stress and autonomic nervous system activity.

Although studies have consistently shown increases in α-amylase activity to be correlated with an increase in psychological stress, reasons as to how or why increases in α-amylase levels may be useful to the body are yet to be investigated. Nater et al., (2007) suggest that α-amylase could increase metabolism and hence provide more energy to the body, however no recommendations regarding potential antibacterial implications are yet to be investigated.

2.4 Are athletes more susceptible to infection?

It is well known that SIgA levels are an important measure of mucosal immunity and resistance to URTI. Studies have proposed that heavy loads experienced by athletes bring about temporary suppression of functioning of the immune system that is likely to make them more susceptible to infections (so called “open-window” theory) (Campbell & Turner, 2018). This could be a result of depression in different immune measures, predominantly IgA (Mckinnon & Jenkins 1993; Nieman et al., 2003; Pacque et al; 2007). Research has indicated that sIgA levels may be related to an associated risk of URTI and vice versa (Fahlman & Engels, 2005; Neville et al., 2008).

Previous studies have shown a correlation between exercises, SIgA and incidences of URTI. For example, Nieman et al. (2003) reported that a 160km endurance running race was associated with a 53% decrease in SIgA secretion rate from pre to post race in 25 runners who reported URTI compared to the 81 runners who did not report any signs of URTI. In a follow up study using the same athletes, they found that decreases in SIgA secretion rate were related to incidences of URTI in the two weeks following the event, with those athletes showing greatest decreases in SIgA secretion rate (Nieman et al., 2006). Participants in these studies included males and females from a broad age group (between 19 and 68 y). Despite the

variability in the participants sex and age group, the research found no significant differences related between the participants and pattern of change of SIgA secretion rate or incidence of infection.

Several longitudinal studies have investigated the effects of training on SIgA concentration and secretion and the occurrence of URTI. The effects of a 7- month training period on SIgA levels and URTI incidence in elite swimmers was investigated by Gleeson et al., (2000). Resting SIgA concentrations were measured and found to decline by 4% after each subsequent month of training. Additionally, athletes displaying lower pre-season SIgA levels were thought to have a higher incidence of infection as resting SIgA levels were identified to be a possible indicator of infection risk. The occurrence of respiratory infections rose over the season and were linked with a decrease in SIgA secretion rate (Fahlman & Engels, 2005). Secretion rate is said to be the strongest predictor of URTI as it identifies the rate at which IgA is being secreted into saliva. (Nieman et al., 2003; Nieman et al., 2002).

2.5 Mucosal Responses to Acute Exercise

Exercise is often demonstrated to affect both saliva flow rate and composition, which is generally ascribed to sympathetic stimulation and parasympathetic withdrawal in salivary glands (Bishop & Gleeson, 2009). A reduction in saliva flow rate is generally observed in the wake of strenuous or prolonged exercise (Hall, Fahlman, & Engels, 2007; Nieman et al., 2003). While α-amylase levels generally increase with exercise, the increases are said to be reliant on exercise intensity (Bishop et al., 2000). However, the response of SIgA to acute exercise is equivocal. Researchers have reported an increase (Gleeson, 2007), no significant change (Moreira et al., 2009), or a decrease (Palmer et al., 2003) following exercise. However, the discrepancies in the results are perhaps associated with differences in the duration and intensity of the exercise, methods used for collection of saliva samples, and the way the findings are reported.

2.5.1 Saliva Flow Rate

Decreases in saliva flow rate are often reported after intermittent, short term high-intensity exercise (Engels, Fahlman, & Wirth, 2003; Leicht, Goosey-Tolfrey, & Bishop, 2018) and continuous exercise such as endurance races (Henson et al., 2008; Nieman et al; 2006; Nieman et al., 2002). Despite saliva flow rate generally showing a decrease following exercise (Dimitriou et al., 2002), some authors have found no effect of exercise on saliva flow rate (Davison 2011; Li & Gleeson, 2004). Additionally, several authors have chosen to exclude saliva flow rate data from their studies which has made the understanding of saliva flow rate

responses to exercise very challenging. It is therefore suggested that researchers should be more consistent in reporting variables such as changes in body mass or measures of osmolality (Gleeson et al., 2011) that may help justify a change, or lack of change in saliva flow rate.

2.5.2 Saliva IgA

The SIgA responses to acute exercise are variable and have been suggested to be influenced by several factors. As reported earlier, many authors found increases (Sari-Saraf et al., 2007; Leicht et al., 2011) and some found no change in both SIgA concentration and secretion rate (Bishop et al., 2000; Reid et al., 2001; Sari-Sarraf et al., 2006; Davison, 2011). The different methodologies used may be suggested for such disparity in the findings, particularly, exercise protocols, duration of exercise, exercise intensity, mode of exercise and saliva collection methods (Bishop & Gleeson, 2009; Gleeson et al., 2004). The fitness levels of participants (elite vs non- elite) is also thought to possibly affect the results (Gleeson & Pyne, 2000).
Studies that have been used to understand SIgA responses to acute exercises usually involve either continuous or intermittent exercises (Pacqué, Booth, & Dwyer, 2002). Researchers have used continuous exercise protocols to investigate the consequences of different durations and intensities on SIgA whereas intermittent protocols examine SIgA responses to repeated sprints, acute effects of a single training session or a game. For the purpose of this review, the use of intermittent protocols and its effects on SIgA are discussed below.

2.5.2.1 Intermittent Exercise

Several studies have reported on SIgA measures before and after intermittent exercise. Studies conducted by Engles et al., (2003, 2004), Fahlman et al., (2001) and Hall et al., (2007) required participants to complete 3 x 30 s Wingate tests with 3 min recovery between each test. While the studies found SIgA secretion rate to decrease by 40-55% from pre- exercise to immediately post- exercise, the effect on SIgA concentration was not consistent. SIgA concentration was reported to be unaffected by Engels et al., (2003) and Fahlman et al., (2001), however it was shown to decrease by up to 69% in the study conducted by Hall et al., (2007). Similarly, Mackinnon and Jenkins (1993) reported SIgA secretion rate to decrease by 52% after participants completed 5 x 60 s of maximal cycling with 5 min rest between each effort. On the other hand, the study reported an increase in SIgA concentration by up to 22% from pre to post- exercise. Since saliva flow rate was not reported, it is expected that the participants elicited a similar response as seen in the study by Engels et al., (2003, 2004) by experiencing a significant decrease in saliva flow rate. Davison (2011) also used a similar protocol as the above studies in which participants completed 4 x 30 s Wingate tests

interspersed with 4 minutes of rest. In contrast to the above findings, Davison (2011) reported a rise in SIgA concentration from pre-to-post exercise, but no effect on SIgA secretion rate. Overall, the findings from the above research suggest that maximal effort exercise is most likely to reduce SIgA secretion rates despite the inconsistency in the responses with SIgA concentration.

2.5.2.2 Team Sport and Field Testing

The SIgA response to participation in team sport is wide-ranging. Moreira et al., (2009; 2011) reported that SIgA concentration levels and secretion rate were unchanged following a friendly football game (70 min) and a kickboxing fight (3 x 4 min rounds with 1 min rest) respectively. In contrast, a study by Moreira et al (2011) reported decreases to both SIgA concentration and secretion rates following a highly competitive futsal game (4 x 10 min quarters with 5 min recovery intervals). Since the exercise protocols are clearly different, it is difficult to compare the above findings. Therefore, more research is required in this area to further elucidate the effects of these types of exercises on SIgA.

Sari- Sarraf et al (2006) employed a 90 min soccer specific exercise protocol to investigate SIgA responses to soccer simulated exercise. The study found no changes in SIgA concentration or secretion rate from pre-to-post exercise. However, Sari-Sarraf, Reilly, Doran and Atkinson (2007) did show increases in both SIgA concentration and secretion rate after using the exact same soccer simulated exercise protocol as Moreira et al (2011). It could be postulated that the discrepancies in findings may be due to the variability in between or within subject responses. It is also possible that experimental protocols involving team sports may not be applicable to game situations due to differences in psychological stimulation.

2.5.2.3 Intensity and Duration

Although there are several differences in SIgA literature, it is however commonly reported that a reduction in SIgA levels can be seen post prolonged and high intensity exercise compared to exercises that are lack intensity and are shorter in duration (Bishop et al., 2006). Exercise intensities in research range from 50-85% $VO_{2\ max}$ with many authors using an intensity between 65-70% $VO_{2\ max}$. Similarly protocols ranging from, 5 mins-2.5 hours (Davison et al., 2009; Mackinnon & Jenkins, 1993) and races ranging from 2-27 hours (Henson et al., 2008; Libicz et al., 2006) have also been used in research . Exercise protocols requiring repeated sprints generally seem to result in a lower SIgA secretion rate. Repeated sprint cycling has also shown to reduce SIgA secretion rate from pre-to-post exercise by up to 55% (Nieman et al., 2003). Therefore, it could be suggested that for SIgA secretion levels to decrease the exercise intensity needs to be near maximal or severely taxing.

2.5.3 α amylase

α-amylase activity is predominantly affected by sympathetic stimulation and is often said to increase after completing exercises with varied intensities and durations (Allgrove et al., 2008; Bishop et al., 2006; Walsh, 1999).

Marathon running has been associated with increased α-amylase secretion rate by 60% pre-to-post exercise with Ljungberg et al (1997) reporting the α-amylase activity to remain 20% above baseline levels after 1 hour of the race. Similarly, Walsh (1999) reported α-amylase activity to rise almost 6-fold following 60 min of intermittent sprint cycling. In a more recent study conducted by Bishop et al (2006), α-amylase responses were investigated following a dose of caffeine (6mg/kg/ BM). α-amylase activity increased from pre-to-post exercise following 90 min cycling at 70% $VO_{2\,max}$. However, α-amylase levels were increased three-fold after caffeine ingestion compared to placebo trials. Exercise of shorter durations such as investigated by Allgrove et al (2008), compared the effects of approximately 22 mins of exercise at 50% and 75% $VO_{2\,max}$ with an incremental exercise test to exhaustion. α-amylase secretion rate was reported to increase by up to 70% during exhaustive exercise and exercise completed at 75% $VO_{2\,max}$. However, it was also found that α-amylase was unaffected by exercise completed at 50% $VO_{2\,max}$. Similarly, Chatterton Jr et al (1996) reported α-amylase activity to increase with jogging (48%) and running (158%) but not with walking. Overall, the findings from the above studies suggest that exercise and associated sympathetic activity has a marked effect on α-amylase activity.

2.6 Heat

2.6.1 What is Heat Training and Why is it Important

Heat acclimation is said to induce numerous physiological adaptations that have shown to aid in athletic performance (Ruell, Hoffman, Chow, & Thompson, 2004). These physiological adaptations from heat acclimation include: reduced oxygen uptake at a given power output, muscle glycogen sparing, reduced blood lactate at a given power output, plasma volume expansion, improved myocardial efficiency and increased ventricular compliance (Corbett, Neal, Lunt, & Tipton, 2014). It is suggested that exercise capability can be improved by enhancing cardiovascular, metabolic, perceptual and thermoregulatory functions through repeated heat stress exposure, heat acclimation (response to a controlled environment setting) or acclimatization (response to naturally occurring environment) processes (Chalmers et al., 2014).

Traditionally, heat acclimation protocols have consisted of at least ten exposures, which is often considered the point at which full heat acclimation is usually obtained (Armstrong & Maresh, 1991). However, Sunderland, Morris & Nevill (2008) suggest that as little as four heat training sessions have been reported to confer partial heat acclimation. In this study, all participants completed an intermittent exercise protocol (The Loughborough Intermittent Shuttle Test- LIST) in hot conditions (30.5 ^{o}C, 27% RH) on two occasions. The LIST requires participants to complete repeated 15 min sets of variable speed shuttle running over a 20m distance. The key finding of the study was that the distance run during intermittent exercise simulating a game was greater after heat acclimation but did not change in the training or control group. The study also reported that high-intensity intermittent running distance was increased by 33% following four short sessions of acclimation, over a 10-day period. Gisolfi and Cohen (1979) suggest that interval training casued a swift increase in deep body temperature and thus was a powerful stimulator of thermoregulatory responses. The protocol used in this study resulted in lower rectal temperature following acclimation. Thus, a reduction of deep body temperature and an increase in thermal comfort may be partly responsible for the upturn in exercise capacity. The magnitude of positive change in performance in the acclimation group suggests that a short-term high intensity intermittent protocol could enhance performance for team sport athletes competing in tournaments in hot climates.

In order to achieve partial or full heat acclimation most athletes use repeated bouts of exercise in hot conditions. Recently, athletes have sought heat acclimation training as an ergogenic aid with the objective of enhancing physical performance in both hot and temperate conditions (Buccheit, Voss, Nybo, 2011; Sunderland, Morris, Nevill, 2008; Lorenzo, Halliwell, Sawka, 2010). Heat acclimation studies of longer duration (8 weeks) have demonstrated improved physical maximal oxygen consumption, time- trial performance, power at lactate threshold and 400 m swimming time, have been observed in temperate conditions (Lorenzo, Halliwill, Sawka, & Minson, 2010). Thus, it seems apparent from the above research that physical performance can be enhanced in hot and temperate conditions following long term heat acclimation training.

2.6.2 Heat Training and Team Sport Athletes

Heat Acclimation training has traditionally been undertaken by athletes from individual sports, however, team-sport coaches are now showing an increased interest in heat acclimation training for their athletes (Chalmers, Esterman, Eston, Bowering, & Norton, 2014). Training and competing in team sport events requires an all-encompassing multifactorial approach which includes endurance, skill, tactical and resistance training (Gabbett, King, & Jenkins, 2008). Many team sport competitions are played in hot environments, often requiring teams

to complete inter-continental travel. A brief short-term heat acclimation training protocol may induce partial heat acclimation for players preparing for hot competition and theoretically improve physical performance (Minson & Cotter, 2016). This short-term heat acclimation may also provide the opportunity for team sport players in regions with temperate climate to achieve physiological adaptations that might not be developed to the same magnitude in cooler climates (Buchheit et al., 2011).

2.6.3 Effects of heat training

The ergogenic physiological adaptations observed from acute or short-term exposure to a heat stress include; plasma volume expansion, improved cardiac and skeletal muscle ventricular compliance, lower resting core temperature, increased sweating, and heat shock protein induction in multiple tissues (Ruell et al., 2004; Corbett et al., 2014). Potential ergogenic outcomes also include; increased maximal cardiac output or reduced sub-maximal heart rate, improved fluid balance, increased aerobic power and efficiency, enhanced cellular protection and increased metabolic capacity (Corbett et al., 2014; Periard et al., 2015). Lorenzo et al (2010) showed 5-8 % improvements in sub elite male and female cyclists in a lab based 1h time trial following 10 daily 90-minute bouts of cycling at matched absolute intensity in hot or temperate conditions. They also reported improvements in anaerobic threshold, $VO_{2\ max}$ and cardiac output at 13 ^{o}C. The above studies utilised a lab-based performance test which may underestimate the cooling effects of airflow compared to high-speed cycling outdoors (Saunders et al., 2005). Regardless, the studies have still shown evidence of an ergogenic benefit with heat acclimation. Intermittent running test (5-minute run at 9km/h) following uncontrolled heat acclimation in team sports players during a 7-day heat training camp showed significant increases in plasma volume expansion after examining adaptive responses to exercising heart rate, post exercise heart rate recovery and heart rate variability (Buchheit et al, 2011). Collectively the above studies demonstrate that heat acclimation provides an ergogenic benefit in sub-elite athletes, potentially due to their sub-optimised haematological, myocardial and vascular adaptations.

On the other hand, acute heat acclimation may be less beneficial for exercise. No improvements in peak aerobic power or outdoor time-trial performance was reported in cool conditions in a group of sub-elite cyclists that travelled to a hot environment for a training camp (Minson & Cotter, 2016). Convertino et al, 1983 reported that exercise in heat often causes dehydration since rates of sweat loss are rarely matched by rates of rehydration, which further elevates the situation. Sawka et al (1985) reported a 7% decrease in maximal aerobic power in the heat as compared with euhydrated subjects in cool temperatures. They also suggest that heat stress and dehydration can act independently to compromise physiologic function

when the extreme demands for skin blood flow caused decreased cardiac output, which then poses restrictions on the supply of oxygenated blood to the entire body. In addition to decrements in performance, the potential for exertional heat illness increases as the environmental conditions worsen.

2.6.4 Exercise in Heat: Effects on Salivary SIgA

Evidence supports an interaction between neuroendocrine and immune responses to exercise when exercising in heat (Laing et al., 2005). A decrease in saliva concentration of IgA has been implicated as a possible causal factor in the increased susceptibility of athletes to URTI (Gleeson et al., 1999). Exercising in hot environments may also indirectly influence mucosal immunity via the influence it may have on central nervous system and specifically the branches which control the release of adrenal hormones (Jonsdottir, 2000). Since the sympathetic nervous system activation appears to be closely related to the exercise associated changes in SIgA, any additional stimulation of these pathways through exercising in hot conditions may cause greater disturbance to SIgA (Bishop & Gleeson, 2009). Exercising in hot conditions in which core temperature rises by more than 1^{o}C compared with thermoneutral conditions augments increases in circulating stress hormones, including catecholamine and cytokines, with associated elevations in circulating leukocyte counts (Starkie et al., 2005; Suzuki et al., 1999). In addition to a rise in core temperature and stress hormone responses, exercising in a hot environment provokes a significant sweat response that causes pronounced dehydration. The loss of body fluids exemplifies an additional homeostatic disturbance that may interact with heat and exercise to further alter immune system function (Ohira, Girandola, Simpson, & Ikawa, 1981). Exercising while dehydrated causes an elevation in heart rate that is due to the reduced stroke volume associated with blood volume reductions and increases in subcutaneous blood flow (Mitchell, Dugas, Mcfarlin, & Nelson, 2002). As the redistribution of immune cells during exercise is influenced by both cardiac output and catecholamine responses, the reductions in cardiovascular function as a result of dehydration may produce a more pronounced disturbance in immune cell function compared with exercise in a euhydrated state (Francesconi et al., 1985). Euhydration did not affect cell number or function when ten trained men completed four cycle ergometer rides at 55% $VO_{2\ max}$ compared with a dehydrated state (Mitchell et al., 2002). However, the findings of the study also showed more severe disturbances with leukocyte, lymphocyte, neutrophil and NK cell count, elevating post exercise in hot environment compared with neutral environment.

Laing et al. 2005 conducted a study to determine the effects of prolonged exercise in hot conditions on SIgA responses in trained cyclists. Participants cycled for 2 hours on a stationery ergometer at 63% VO_2 max in 30^{o}C heat 76% RH and 20^{o}C 60% RH on two separate

occasions. They reported that saliva flow rate in participants decreased by 43% returning to pre-exercise levels by 2h post exercise with no difference between trials. Saliva IgA concentration increased post exercise with no difference between trials. Saliva IgA secretion rate decreased by 34% returning to pre exercise levels by 2h post exercise (Laing et al., 2005). The data from this study shows that a prolonged bout of exercise results in a reduction in SIgA secretion rate. Additionally, the study reported that performing prolonged exercise in the heat with ad libitum water intake does not influence SIgA responses to prolonged exercise.

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. Utilizing 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^{o}C of normal baseline (Severs et al., 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.

Therefore, performing exercise in hot conditions with associated elevated circulating stress hormones and catecholamine would be expected to cause greater immune disturbance compared with exercise in thermoneutral conditions (Galbo et al., 1979).

2.7 Caffeine

Caffeine (1,3,7- trimethylxanthine) is the world's most consumed pharmacologic and psychoactive substance (Fredholm et al., 1999). It is commonly found in over the counter medications, tea, coffee, chocolate and in various other products. It is metabolised in the liver and is proposed to have an effect on peripheral and central tissues in the body (Fredholm, 1995). Caffeine is found as an odourless crystalline powder in various plants (Debry, 1994). In New Zealand, the mean daily intake of caffeine is approximately estimated to be 3.5 mg/kg/BM: approximately 2-4 cups of coffee (Thomson & Schiess 2011). However, caffeine intake varies widely between individuals globally. Caffeine intake amongst New Zealanders was found to be like that in the United Kingdom (3.6 mg/kg/BM) (Thomson & Schiess 2011). Caffeine can be commonly found in the form of tea, coffee and other energy drinks, however the levels of caffeine in foods can vary greatly, particularly in coffee or tea due to different

preparation methods. The caffeine content in commonly found caffeine-containing products is presented in the Table 2.1 as below.

Table 2.1: Caffeine content in most common beverages (Burke, 2008)

Food/Drink	Serving	Caffeine, mg
Instant Coffee	250ml	60
Brewed Coffee	250 ml	80
Short Black or Espresso	1 Standard Serving	107
Tea	250 ml	27
Hot Chocolate	250ml	5
Dark Chocolate	60g	10
Coca-Cola	375ml can	49
Red Bull Energy Drink	250 ml can	80
No-Doz	1 Tablet	100

Caffeine is swiftly absorbed through the gastrointestinal tract and moves quickly through cellular membranes to tissue. Caffeine has been suggested to influence the body's central nervous system, cardiovascular, pulmonary and renal functions during rest and exercise (Magkos & Kavouras, 2005).Caffeine stimulates bronchodilation of alveoli, vasodilation of blood vessels, neural activation of muscle contraction, blood filtration in the kidneys and lipolysis (Graham & Spriet, 1995). During endurance exercise, these metabolic and physiologic effects of caffeine are suggested to have a diminishing effect on the rating of perceived exertion (RPE), the respiratory exchange ratio and also suggested to increase circulating levels of epinephrine, increase metabolic rate, cardiac output and ventilation in trained and untrained individuals (Engels et al., 1999).

2.7.1 Caffeine and Sport

Caffeine research in exercise and sport has advanced more recently. In addition to examining the possible ergogenic effects of low caffeine doses in a variety of settings, researchers have also examined: Time trial performance tests to simulate real world situations vs exercise to exhaustion; administering lower doses of caffeine prior to and during exercise; caffeine administration in team sport environments with sport specific simulations of performance and the ergogenic effects of caffeine in semi-professional and professional athletes (Spriet, 2014). Most sporting activities rely on bursts of activities where energy production is provided by oxidative or anaerobic energy sources. Phosphocreatine and adenosine triphosphate

production in the glycolytic pathway are the two main pathways to produce anaerobic energy. These pathways can produce energy rapidly and in large quantities over short durations to allow athletes to complete very powerful movements. In intermittent sports, the ability to repeatedly perform quick burst of exercises or sprint is essential to success. In power-based sports, these bursts often occur on the back of high energy production from the aerobic system.

Caffeine studies involving endurance exercise have shown to increase work output and time to exhaustion (Bell & McLellan, 2002; Pasman et al., 1995) . Ingestion of caffeine has also shown an increase in performance during intense, short term cycling and running events of approximately 5 minutes. Studies have also reported an increase in peak power output, speed and isokinetic strength in sprint and power events following caffeine ingestion (Wiles et al., 1992). Early research generally found no benefit of caffeine use for activities involving short bursts as the majority of the studies conducted reported no effects. However, recent research in this area suggested that there were benefits in repeated high-intensity intermittent exercise with caffeine ingestion.

Many researchers have shown caffeine to be ergogenic with doses between 3-6 mg/kg/BM improving performance in events lasting as little as 30s or as long as 2h (Graham, 2001; Goldstein et al., 2010). For example, Paton, Lower, and Irvine (2010) investigated the effects of a 240 mg dose of caffeine on repeated sprint cycling. Participants completed 4 sets of 5 x 30 s sprints and maintained a higher mean power output with caffeine ingestion (5.4%) compared to placebo.

Studies that have used 3-6mg/kg/BM dosage of caffeine have reported improvements in performance, suggesting an ergogenic effect. Del Coso Munoz- Fernandez et al., (2012) and Lara et al., (2014) have reported that ingestion of 3mg/kg/BM of caffeine in the form of energy drinks increased the running distance covered at high intensity, sprint intensity in male and female soccer players as well as rugby players during simulated or real games. Abian- Vicen et al., (2014) also reported an improved jump performance in basketball players with the ingestion of 3mg/kg/BM doses of caffeine via energy drinks. A study conducted by Strecker et al (2007) reported that male collegiate tennis players demonstrated some improvements in forehand shot performance when 3mg/kg/BM was consumed 90 min before a simulated tennis match. Furthermore, two recent studies by Perez-Lopez et al (2014) and Del-Coso et al (2014) examined the effects of ingesting 3mg/kg/BM on volleyball performance in females and males. Caffeine was administered in the form of an energy drink, with caffeine free energy drink serving as the control. The players were required to complete a series of volleyball-based performance tests and played in a simulated match on both occasions. The results revealed that ball velocity in a spike test, several jump tests, the duration it took to complete an agility test all showed improvements in the caffeine trials for both females and males.

A 6mg/kg/BM dose of caffeine has been said to increase the distance cycled in a 60m time trial (McNaughton et al., 2008). In this study, the caffeine dose was coupled with an increase in cycle distance of ~1km. Stuart et al (2005) also examined the effects of a 6mg/kg/BM dose of caffeine in well trained rugby union players. Participants in this study completed a circuit emulating the actions of a rugby player which included sprinting and ball passing. The results showed a 10% improvement in ball passing accuracy when compared to placebo. Moreover, the results also showed that the participants who were under the caffeine condition were able to maintain their sprint times at the end of the circuit, relative to the beginning of the protocol. Conversely, Collomp et al (1991) reported caffeine (5mg/kg/BM) did not result in any significant increase in performance for peak power or total work performed in subjects who participated in only 2-3 hours of non-specific sporting activity per week. These findings are in agreement with Greer and colleagues (1998) where subjects classified as non-trained reported a lack of performance enhancement with a dose of 6mg/kg/BM caffeine. They also reported a decline in power, as compared to placebo during the last two of four Wingate bouts. Crowe et al (2006) reported significantly slower times to reach peak power in untrained subjects in the second of two bouts of 60 s maximal cycling when 6mg/kg/BM caffeine was consumed. Finally, Lorino et al., (2006) investigated the effects of 6mg/kg/BM caffeine on athletic agility and the Wingate test. They reported that the performance in non- trained males did not significantly improve in either the pro-agility run or the 30 s Wingate test. However, a study by Goldstein et al., (2014) showed that well- conditioned participants achieved greater peak power during the Wingate test after consuming caffeine at a dose of 5mg/kg/BM. Therefore, it can be drawn from the above literature that caffeine is not effective for non-trained individuals participating in high intensity exercise. Based on the above research, it is apparent that caffeine supplementation in the range of 4-6mg/kg/BM can be advantageous to either short term intermittent / prolonged high intensity performance, but only in trained athletes.

In addition to the above effects, caffeine has also been suggested to augment cognition, alertness and mood (Davis et al., 2003; Evans & Griffiths, 1992; Yeomans, Ripley, Davies, Rusted, & Rogers, 2002). Additionally, academics have also recommended the use of caffeine to help athletes tolerate higher intensities of during endurance training, as well as for its capability to decrease perceived exertion (Sokmen et al., 2008). This might offer an insight as to why caffeine consumption is widespread amongst a broad range of athletes from different sporting backgrounds and it might also offer an explanation as to why consumption has amplified since being accepted by the World Anti-Doping Association (WADA) in 2004 (Chester & Wojek, 2008).

2.7.2 Caffeine and Saliva IgA during rest and exercise

As reported earlier, a reduction in Salivary IgA concentration and secretion rate has been suggested as a risk factor for subsequent episodes of URTI in athletes. Caffeine ingestion is associated with enhanced sympathetic nervous system activity and increases in plasma epinephrine and is widely used among athletes for its ergogenic properties. Therefore, it can be argued that caffeine ingestion prior to any physical activity may affect salivary responses to exercise. Bishop et al. (2006) have investigated the effects of caffeine on SIgA and α-amylase at rest. Participants in this study ingested either 6mg/kg/BM or placebo and rested in a lab for 3.5 hours, while providing saliva samples at specific time points. They found that saliva flow rate, SIgA concentration and secretion rate were unaffected after caffeine ingestion, however, α-amylase secretion levels were reported to be over four times higher 1h post caffeine ingestion compared to placebo. Therefore, it can be suggested that while α-amylase secretion rate increased after caffeine ingestion, its effects alone did not appear to cause a strong enough stimulus so as to alter SIgA responses at rest.

However, Bishop et al (2006) also investigated the effects of a 6mg/kg/BM dose of caffeine on SIgA responses following 90 min cycle at 70% $VO_{2\ max}$. They reported a 50% increase in the SIgA concentration at the mid exercise point with caffeine intake and reported the SIgA concentration levels to remain 40% higher post exercise when compared with placebo.

Similarly, SIgA secretion rates were almost twice as high compared to placebo, although no differences were recorded post exercise. α-amylase secretion was reported to increase after caffeine intake from baseline to post exercise and was almost twice as high than the levels reported during the resting trial. Similar results were reported in the placebo trial; however, the increase of α-amylase secretion was reported to be 30% lower than that with caffeine intake, suggesting that exercise also stimulated sympathetic activity. Saliva flow rate was reported to be unaffected by caffeine ingestion, however reduced by 30% from baseline to post exercise.

2.7.3 Caffeine, Heat and Exercise Performance

There appears to be a paucity of research investigating how caffeine affects exercise performance in heat. What's more, the findings of these studies are inconsistent. Beaumont & Lewis (2016) investigated the influence of 6mg/kg/BM caffeine on endurance cycle performance and thermoregulation during prolonged exercise in heat (30 ^{o}C & 50% RH). Exercise included 60 minutes cycling at 55% W_{max}, followed by a 30- min performance task. Performance was enhanced in the caffeine trial compared with placebo. Performance improved without differentially influencing thermoregulation during the exercise period at a fixed work-rate vs placebo. The study therefore concluded that moderate caffeine doses which typically enhance performance in temperate environmental conditions, also appear to benefit

endurance performance in the heat. Similarly, Ganio et al., 2009 reported enhanced work production during a 15- min cycle performance task with no difference in core temperature between trials when 3mg/kg/BM caffeine was ingested 60 min before and 45 min during exercise in 33ºC. However, in the study by Roelands et al., a 6mg/kg/BM caffeine dose administered 60 mins before exercise failed to enhance time–trial performance but increased core temperature during exercise in 30ºC heat. Additionally, Roti et al. (2006) reported that five days of controlled caffeine intake (3 and 6 mg/kg/BM) showed no influence on the core temperature response whilst exercising in 37 ºC heat. Another study reported no influence on 21km race time after caffeine intake of 5 or 9mg/kg/BM in hot and humid conditions (Cohen et al., 1996). However, dehydration levels in the race participants were reported to be approximately 4%. Therefore, it is unknown if intake of caffeine would have attributed to performance enhancement had the fluid balance been maintained. Ganio et al., (2011) reported increase in performance following caffeine ingestion in 33ºC heat when hydration status of the participants was controlled across cool and hot environments.

2.7.4 Effect of Caffeine on RPE During Exercise

Doherty and Smith (2005) conducted a meta-analysis in which they tested the results of 21 studies comparing the differences in RPE responses between caffeine and placebo conditions. The exercise protocols were narrowed down to either exercise completed with constant load or protocols that included exhaustive exercise. For constant load exercises to be included in this investigation, the intensity of exercise was set between 50 and 80% $VO_{2\ max}$. It was reported that RPE, on an average was 5.6% lower in the caffeine trials when compared to placebo. There was a concomitant increase of 11.2 % in exercise test performance. Mean exercise RPE justified 29 % of the discrepancy in performance variation between caffeine and placebo conditions following regression analysis. Therefore, it can be said that RPE response during constant load exercise post caffeine ingestion reduces which might allow individuals to exercise for longer periods of time before subjective fatigue becomes intolerable (Doherty & Smith, 2005).

The above findings agree with the investigations that have studied the effects of caffeine during exercise performed using constant RPE as supposed to constant workload. Research conducted by Ivy et al. (1979) and (Cole & Eastoe, 2014) reported that subjects in their studies chose to perform exercise at higher intensities post caffeine ingestion compared to a placebo, even though the subjects were asked to self- regulate the intensity of exercise similar to that of the RPE for both conditions. The meta- analysis by Doherty and Smith (2005) also found that RPE, following exhaustive exercise, did not fluctuate between caffeine and placebo conditions. This response is instinctive, given that the individuals were instructed to perform

the exercise until they were no longer able to do so. Such exercise protocols should therefore result in RPE levels that are maximal or at least near maximal.

Reduced perceptions in fatigue have been linked with caffeine ingestion in endurance as well as resistance exercise protocols (Backhouse et al. 2011; Demura et al. 2007; Hadjicharalambous et al. 2006; Spriet, 2014). Demura et al., (2007) examined the effects of 6 mg/kg/BM caffeine intake on physiological variables and RPE during 60 min of submaximal endurance cycling at 60 % $VO_{2\,max}$. RPE was reported to be significantly lower and as the only major difference after caffeine ingestion during a submaximal cycle for 60 mins (Demura et al, 2007).

Hadjicharalambous et al., (2006) investigated how 7–7.5 mg/kg/BM of caffeine intake affected different RPE consisting of legs and chest and performance during constant load (73% VO_{2max}) and incremental exercise in endurance trained men after consuming a high fat meal. This was done to remove the possible ergogenic effect of an increase in free fatty acids (FFA) as elevated FFA concentration can enhance performance by producing a glycogen sparing effect. However, it is critical to acknowledge that such a reaction may only be seen in certain individuals (Battram et al. 2007; Graham et al. 2008; Martin et al. 2006). Hadjicharalambous et al. (2006) reported that the results from both exercise tests showed a significantly lower RPE for the legs whereas RPE for the chest was significantly lowered during caffeine condition only. However, it is important to note that performance was not improved because of caffeine intake.

Laurence et al., (2012) studied the effects on maximal 30- min cycling performance, RPE and RER post 6 mg/kg/BM caffeine ingestion in sedentary men. Performance (total work) was considerably better post caffeine ingestion when compared with placebo. RPE and RER were similar between trials across different time-points. The improved performance and similar RPE data suggest that after caffeine intake, an increase in work rate was not complemented by a corresponding increase in RPE. The participants were able to achieve a higher intensity while reporting the same level of perceptual fatigue. Additionally, by measuring RER, the researchers were able to assess prospective differences in energy substrate utilisation following caffeine ingestion. The similar RER between the two intensities might therefore allude to the fact that regardless of the exercise intensity the relative contribution of carbohydrate and fat as fuel for the muscles was similar between experimental conditions (Laurence et al. 2012).

Dose response effects of caffeine have also been investigated in two studies looking at caffeine and ratings of leg muscle pain during cycle exercise (60% $VO_{2\,max}$) and leg muscle pain in subjects who associated themselves as being low habitual caffeine users. O'Connor et al. (2004) used males as participants in their study, and Motl et al., (2006) used female participants. Both studies reported a significant reduction in leg muscle pain ratings for the

duration of exercise after caffeine ingestion (5 and 10 mg/kg/BM) compared to placebo, without reporting any alterations in blood pressure (BP), HR, and VO_2. In addition, there was no statistically significant variation in leg muscle pain between the two caffeine conditions. Participants rating of perceived pain was reported to be lower during the 10mg/kg/BM condition than 5mg/kg/BM condition, yet the total group mean difference was not statistically significant. These results suggest that there may be a dose–response relationship between caffeine ingestion and the reduction muscle pain in males caused by exercise. Moreover, a research by Gliottoni & Motl (2008) compared 5mg/kg/BM of caffeine ingestion to placebo during high intensity cycling (80 % $VO_{2\ max}$) in women. Leg muscle pain was reported to be significantly lower during the caffeine trial than placebo conditions (Gliottoni & Motl, 2008).

2.8 Potential Mechanisms

2.8.1 Glycogen Sparing

The ergogenic effect of caffeine intake on endurance exercise performance entails glycogen sparing. Caffeine enhances plasma epinephrine concentration (Graham & Spriet 1995) which can result in an increased release of FFA from adipose tissue triglycerides. As a result, an increase in FFA accessibility for skeletal muscle metabolism should enhance the use of fat as an energy source and spare muscle glycogen (Essig et al. 1980; Ivy et al. 1979).

However, some contradiction provided in the literature suggests that the increased levels of plasma FFA is not always as a result of caffeine ingestion and increased plasma epinephrine. (Arogyasami et al. 1989a; Graham 2001; Graham & Spriet 1995). Additionally, while muscle glycogen sparing post caffeine intake has been shown in some studies (Erickson et al. 1987; Essig et al. 1980; Spriet et al. 1992), the findings of most studies contradict this notion (Arogyasami et al., 1989a, 1989b; Graham et al., 2000; Graham 2001; Greer et al., 2000; Laurent et al., 2000; Roy et al. 2001). Graham et al., (2008) assembled data from multiple studies (Chesley et al., 1998; Erickson et al., 1987; Graham et al., 2000; Greer et al., 2000; Laurent et al., 2000; Spriet et al., 1992) to attain a total sample size of 37 from whom muscle glycogen content throughout exercise was measured. They observed no significant glycogen sparing as a result of caffeine intake. Other studies have been unsuccessful to link the overall effect of escalated plasma epinephrine levels on endurance exercise performance (Graham & Spriet 1995; Kovacs et al., 1998).

Possible reasons for the differing results are that an increase in plasma epinephrine has been shown to increase muscle glycogen breakdown. This action offsets the impact of increased FFA availability, and enhances the production of lactate, a precursor of muscular fatigue (Arogyasami et al. 1989a; Kovacs et al. 1998; Laurent et al. 2000). In addition, although

majority of evidence indicates no significant effect of caffeine on carbohydrate or fat metabolism within skeletal muscle, noticeable inter-individual differences have been reported. The results show that some individuals appear to respond well to caffeine and/or epinephrine causing glycogen sparing while most people are non-responders (Battram et al. 2007; Graham et al. 2008; Martin et al. 2006).

2.8.2 Adenosine Antagonism

There is a notion that the primary mechanism of caffeine action that causes an ergogenic effect is the blocking of receptors for the neurotransmitter adenosine (Davis et al. 2003; Fredholm et al. 1999). Structurally, adenosine is classified as a purine along with a larger molecule of which it is a component, adenosine triphosphate (ATP) (Hoehn & Marieb, 2007). Adenosine on its own and can have extensive effects throughout both the central and peripheral nervous systems as it considered to be a powerful inhibitor of neurotransmission in the brain (Hoehn & Marieb, 2007). This can diminish the release of neurotransmitters such as dopamine and thus decrease overall brain arousal (Davis et al. 2003; Fredholm et al. 1999). While adenosine increases in skeletal muscle and the blood with muscular contraction (Davis et al. 2003), the intake of caffeine can neutralize its effects, thus allowing constant release of dopamine and increased stimulation.

Dopamine is well known to increase attention, memory, motivation and reward (Meeusen et al., 2006a, 2006b) and plays a key role in exercise performance. Three studies have shown that clinically inhibiting the re uptake of dopamine causes an increase in dopamine concentration and improves endurance exercise performance (Bridge et al., 2003; Roelands et al., 2012; Watson et al., 2005). Therefore, the effect of caffeine on dopamine might increase attention and memory, leading to a more accurate pacing strategy. This along with an increased motivation for reward can contribute towards enhanced exercise performance capacity (Roelands et al., 2013).

Caffeine's ergogenic effects have been credited to its capacity to blunt RPE and naturally occurring muscle fatigue. The diminished effect of caffeine on both RPE and muscular pain may be because of its antagonism of adenosine receptors. First, adenosine antagonism may allow an increased secretion of dopamine (Davis et al. 2003; Fredholm et al. 1999) which has been found to be inversely related to central fatigue during exercise. This might be because of its ability to offset the tiredness and fatigue caused by high levels of serotonin (Davis and Bailey 1997). Studies have shown that blocking dopamine re uptake improved the exercise intensity, as well as performance without related increases in the RPE response (Watson et al. 2005; Roelands et al. 2012).

Secondly, adenosine is one of many chemicals that can initiate pain receptors in both the central and peripheral nervous systems (Sawynok & Liu, 2003). Therefore, it is believed that adenosine antagonism may cause a decrease in the activation of pain receptors. Increase in adenosine levels during exercise naturally produces muscle pain, just like the pain caused by the production of hydrogen ions. However, caffeine does not have a known effect on these chemicals. Therefore, caffeine may delay muscle pain and diminish the pain response to exercise as the intensity and/or duration of exercise increases. Improvements in performances during testing protocols and the related reduction in the RPE, both due to caffeine ingestion, have been shown during endurance (time-to-exhaustion and time-trials) (Backhouse et al. 2011; Demura et al. 2007; Doherty and Smith 2005; Gliottoni and Motl 2008; Hadjicharalambous et al. 2006; Laurence et al. 2012; O'Connor et al. 2004) as well as resistance exercise (Bellar et al., 2011; Hudson et al., 2008).

2.9 High Intensity Interval Training Protocols

High Intensity Interval Training (HIIT) involves repeated short to long bouts of high-intensity exercise interspersed with recovery periods. For team sport athletes, the inclusion of sprints and maximal efforts into HIIT programmes has proven to be very effective. It has been recommended that protocols which produce maximal oxygen uptake ($VO_{2\ max}$) or at least a very high percentage of $VO_{2\ max}$ and provide a great stress to the oxygen transport and utilization systems should therefore provide the most effective stimulus for increasing $VO_{2\ max}$ (Laursen & Jenkins, 2002; A. Midgley & Mc Naughton, 2006). While there is a lack of evidence clear enough to specify exercising at such high intensities, it can be argued that only exercise intensities near $VO_{2\ max}$ allow for both large motor unit recruitment and attainment of near-to-maximal cardiac output, which, in turn, jointly signals for oxidative muscle fibre adaptation and myocardium enlargement and hence, $VO_{2\ max}$. For optimal stimulus, it is believed that athletes should spend more time per HIIT session in their "Red Zone", which generally means attaining an intensity greater than 90% of $VO_{2\ Max}$ (Billat 2001; Laursen & Jenkins 2002; Midgely et al., 2007).

Acute physiological response to a HIIT session can be determined by several factors. The sport that the athlete is involved in and the athlete's profile or their specialty should first be taken into consideration in relation to the desired long-term training adaptations. Secondly, on a short- term basis, training periodisation needs to be considered as majority of the desired training adaptations are likely to be dependent of the training cycle that the athlete is in. Additionally, for athlete training twice a day or team sport athletes training a combination of metabolic and neuromuscular systems simultaneously, the physiological strain associated with a given HIIT sessions needs to be considered in relation to the demands of other physical and technical/tactical sessions to avoid training overload and allow for appropriate adaptation.

Some of the variables that can be manipulated to prescribe different HIIT intervals include total number of intervals, work-to-rest ratios, exercise modality i.e. running based vs cycling or rowing. The manipulation of each variable in isolation can have a direct impact on metabolic and cardiopulmonary and/or neuromuscular response.

Prescribing the intensity of HIIT bouts using a RPE based method is highly efficient because of its simplicity and versatility. Using RPE as a variable, coaches generally prescribe the total session duration or distance as well as total amount of work and rest intervals. In return, the athlete can self- regulate their exercise intensity. RPE responses may reflect a conscious feeling of how strenuous the exercise is, relative to the combined physiological, biomechanical and psychological stress that is imposed on the body during exercise. In a practical setting, the first benefit of RPE based HIIT sessions is that they do not require any knowledge of the athletes' fitness level. Finally, RPE is considered to be a universal exercise regulator, irrespective of locomotor mode and variations in terrain and different environmental conditions. Clearly, more research is required in trained athletes to confirm the efficacy of RPE guided training sessions. However, pilot testing demonstrated that RPE was a more valid method of controlling exercise intensity compared to target HR and fixed workload to achieve a target metabolic stimulus during a 5 x 3-minute high intensity interval training protocol (HIIT) (unpublished findings by Matthew Wood, 2018). Given the effect of caffeine on RPE, this method is somewhat limited as it may not allow for the precise manipulation of the physiological response to a given HIIT session. There are also potential limitations to the use of HR and workload-based methods for controlling exercise intensity during HIIT with caffeine.

High intensity interval training is a widely used and effective training method in various sports, including both endurance and sprint/power events (Milanovic et al., 2015). This type of training requires an integration of several physiological systems. The contributions of the ATP-PCr and glycolytic metabolic pathway is crucial for maintaining high exercise intensity for as long as possible (Buchheit & Laursen, 2013; Tschakert & Hoffman, 2013). Furthermore, protocols with high intensity exercise have reported to produce cardiovascular, metabolic and skeletal muscle adaptations that are similar or superior to traditional endurance training despite the total volume of exercise being low. Therefore, for the above reasons, high intensity exercise protocols are preferred to improve cardiorespiratory fitness in healthy as well as diseased populations with protocols differing in the work-to-rest ratios.

The 4x4 "Norwegian" HIE protocol which consists of four 4-minute intervals performed at 90-95% of peak heart rate interspersed with 3 minutes of active recovery is one of the examples of such a protocol. In this study, it was shown that the 4x4 HIE protocol when compared to 16 x 1 HIE protocol, elicited a higher (~ 3 %) overall mean VO_2 (Moholdt et al., 2014). The authors have also reported that mean VO_2 and HR during the high intensity intervals were also significantly higher ($p < 0.001$) with the 4x4 HIE compared with the 16 x 1 HIE. Conversely,

the study found the mean VO_2 and HR during the recovery periods to be significantly higher ($p < 0.001$) in the 16 x 1 HIE than 4x4 HIE. The highest VO2 attained during the 4x4 protocol ranged between 90 and 99% of $VO_{2\,max}$ overall compared to the ranges of VO_2 found between 76 and 85% in the 16x 1 HIE protocol. Buchheit and Laursen (2013), suggest that intervals with long work durations induce a higher anaerobic glycolytic energy contribution and higher neuromuscular load than intervals with short work duration. Cumulated high intensity (>90% $VO_{2\,max}$) exercise time during typical sessions in well trained athletes ranges from 12 minutes (6 x 2-minute intervals), 15 minutes (5 x 3 minute intervals), 16 minutes (4 x 4 minute intervals) and 30 minutes (6 x 5 minute intervals) which enabled athletes to accumulate, depending on the HIIT format, from 10 mins >90% to 4-10 mins >95% at $VO_{2\,Max}$. In a study by Seiler et al., (2005) it is suggested that larger volumes of HIIT performed at a lower intensity i.e. 4 x 8 minutes at 90% HR max may be more effective than using a 4 x 4 “traditional” HIIT model. However, it is also suggested that further research is required to examine the effects of these sessions in highly trained athletes to confirm the above findings.

2.10 Overall Conclusions

Saliva IgA is the most profuse antibody in mucosal secretions and its levels are often reported to be affected by exercise. Research suggests that exercise is a formidable stimulator of sympathetic activity and enhances adrenaline which has been shown to cause constraints on several immune functions that are often suppressed for several hours post exercise. Exercising in hot environments may also influence mucosal immunity because of the effect it may have on the central nervous system which controls the release of adrenal hormones. Since the sympathetic nervous system activation appears to be closely related to the exercise associated changes in SIgA, any additional stimulation for example exercising in hot temperatures may cause greater disturbances to SIgA. It is also reported that since caffeine consumption is known to increase adrenaline, ingesting it before exercise in the heat may exacerbate any exercise-induced immunosuppression.

Considering that the research on caffeine ingestion and its effects on mucosal immunity whilst exercising in the heat is scarce, it would be beneficial to investigate the effect of a 6mg/kg/BM dose of caffeine in team sport athletes that are required to complete multiple high intensity running bouts in hot temperatures.

Chapter 3. Methods

3.1 Participants

Twelve semi- professional team sport athletes (mean SD: Age: 20 ± 1.65; Body Mass: 97.59 ± 18.01 kg; $VO_{2\ max}$ 45.73 ± 3.83 ml/kg/min^{-1}) volunteered to participate in this study with rationale and experimental protocols of the study given prior to acceptance. Participants were also advised of the inclusion and exclusion criteria prior to participating in the study. Male team sport athletes (amateur or semi-professional) between the age of 18 to 35, regular consumers of caffeine, playing at top club/ regional level and training twice a week for at least 70 minutes each week were included in this study. People with existing injuries, smokers and anyone on anti-inflammatory medication, asthma or any other illness were excluded from the study. Approval was granted by Auckland University of Technology Ethics Committee with participants providing written and informed consent before taking part in the study. Anyone on medication or had reported symptoms of infection in the 4 weeks prior to the commencement of the study were excluded.

3.2 Preliminary Testing

One week prior to beginning experimental trials, each participant was required to perform a continuous incremental exercise test on a treadmill to determine their $VO_{2\ max}$ and to prescribe interval training intensity levels. Participants started running at a speed of 10-12 km/hr and the treadmill gradient was set at 1.0%. Speed of the treadmill was increased by 1 km/hr every 3 minutes while gradient remained constant throughout. This was continued until participants reached volitional exhaustion. Three-minute stages were used to match the length of intervals used in the experimental protocols as opposed to the more commonly used <1-minute stages. Starting speed was adjusted to cater for the participants fitness levels and to ensure each participant completed at least 6 but no more than 9 stages. Encouragement was given verbally to each participant to help maximise their efforts. HR was measured throughout the test using short-range radio telemetry (Polar RS 800, Polar Electro, Finland) and minute ventilation, O_2 consumption and CO_2 production were determined from expired samples using a metabolic system (Parvo Medics True One 2400, Sandy UT). The metabolic system flow-volume and gas analysers were calibrated before each test. Peak oxygen consumption was determined as the highest 30-second average period of oxygen consumption.

After completing the $VO_{2\ max}$ test, participants were allowed to rest for 15 minutes before they were asked to enter the heat chamber, pre-heated at 30^{o}C and 50% RH and were asked to perform one 3-minute interval. This was done so participants would become familiar with running inside the heat chamber. During this session, participants were advised of all the

details i.e. saliva collection methods and rest periods after completing the exercise protocol in the heat.

3.3 Familiarisation

As the participants were going to be running in the heat chamber preheated at 30^{o}C and 50% RH, it was important for them to familiarise themselves with the protocol as this is something they would not have done before. The familiarisation session included a standard warm up which was to be replicated in the experimental trial. Participants were given a progressive 5-minute warm-up which included 3 minutes of Brisk walking, 1 minute of light jogging followed by 1 minute of light running. Following the warmup, participants completed 3 intervals for 3 minutes at an intensity equivalent to a rating of perceived exertion (RPE) of 15 or “hard” using the 6-20 Borg Scale (Borg, 1982), interspersed with 2 minutes of brisk walking. It was decided to not complete the entire protocol as participants may either get potential training benefits or immune system suppression by running at high intensity in the heat chamber. Treadmill speeds were pre-determined and maintained for 45 seconds at the start of each interval, followed by either increasing/ decreasing the speed by 0.5km/hr every 15 seconds or keeping it the same, as advised by the participants. This was done to ensure that the participants maintained their RPE levels throughout the duration of their 3-minute intervals. During the 2-minute brisk walk, tympanic (ear) temperature (Genius Model 3000A, Intelligent Medical System Inc, Carlsbad, CA) was recorded along with Thermal Stress and Thermal comfort.

3.4 Experimental Trial Procedures

Pre- trial information was provided to the participants in an information sheet. A list including caffeine containing foods and beverages was provided and participants were asked to refrain from consuming any food items on the list 24 h prior to each experimental trial. Participants were also asked to avoid any alcohol and any strenuous exercise 24 h before the trial day. In order to standardize nutritional practice, participants were asked to record everything they ate a day before testing and to mirror it for the next trial.

In a double-blinded repeated-measure cross over design participants completed 2 experimental trials, separated by approximately 1 week. Double blinding was achieved by having a laboratory technician prepare the capsules for each participant. Participants were randomly assigned to Caffeine or Placebo groups (CAF or PLA) and acted as their own controls.

They rested for 15 minutes and during that time a pre-supplement saliva sample was collected. Immediately after this, the participants ingested either 6mg/kg/BM of caffeine powder or cornflour taken in the form of cellulose capsules, with 5ml/kg/BM of plain water. Participants

then rested quietly for 30 minutes in the lab and a further saliva sample was collected, after which pre-exercise mass (in shorts only) was recorded. Immediately after this, participants entered the heat chamber, pre-heated to 30^{o}C and 50% RH. Participants were given a progressive 5-minute warm-up which included 3 minutes of brisk walk, 1 min of light jogging followed by 1 min of light running. Once the warm-up was completed, participants then began their exercise protocol. This included completing six 3-minute high intensity running intervals on the treadmill interspersed with 2 minutes of brisk walking (in shorts only).

The prescribed running intensities pre-calculated from $VO_{2\,max}$ scores. Participants were asked to maintain the predetermined RPE for the 3-minute intervals. The predetermined RPE for each participant corresponded to 108% of secondary gas exchange threshold. Treadmill speeds were pre-determined and maintained for 45 seconds at the start of each interval, followed by either increasing/ decreasing the speed by 0.5km/hr every 15 seconds or keeping it the same, as advised by the participants. This is done to ensure that the participants maintained their RPE levels throughout the duration of their 3-minute intervals. Participants heart rate (HR) was recorded during the last 10 seconds of each minute of their intervals.

During the 2-minute brisk walk, tympanic (ear) temperature was recorded along with Thermal Stress and Thermal comfort. Participants were advised that the exercise will be ceased immediately if their core temperature increased >39^{o}C.

Once the exercise protocol had been completed, another saliva sample was collected before post exercise BM (in shorts only) was recorded. Participants were given a 300ml bottle of water and their water consumption was recorded so that it could be replicated for their next trial. Participants then rested quietly in the lab for a further hour before a final (1h post exercise) saliva sample was collected, no additional fluid or food was permitted. Participants were allowed to go have a shower during this time however, they were not allowed to go do the toilet until their last saliva sample had been taken.

3.5 Saliva Collection

All saliva collections required participants to be seated, leaning forward and with their heads tilted down. Participants were then advised to swallow in order to empty their mouth of saliva prior to the collection of an un-stimulated whole saliva sample which was done over a pre-determined time into a pre-weighed sterile bijou tube (7ml capacity with screw top, Labserve, Auckland, NZ). Care was taken to allow saliva to dribble into the collection vial with minimal orofacial movement. All saliva collections were obtained at least 5 min after any scheduled drink ingestion. Samples were frozen and stored at -80 ^{o}C until analysis (Cobas Modular P800 Analyser, Roche Diagnostics, New Zealand).

3.6 Saliva Analysis

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

The concentration of SIgA ($mg \cdot l^{-1}$) 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 SIgA ($\mu g \cdot min^{-1}$) 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 SIgA, cortisol and osmolality assays, respectively.

3.7 Statistical Analysis

A double-blind crossover design was used as the experimental approach. A one-way ANOVA was used to compare baseline measurements. Linear mixed models were used to evaluate differences between groups. Mean differences between groups, order of intervention and interaction of intervention and intervals have been reported. A robust standard error was applied to account for Type 1 error. Statistical significance was accepted at $p < 0.05$ for all analyses. All analyses were performed using STATA version 15 (College Park, TX).

Chapter 4. Results

Baseline age, $VO_{2\ max}$ and anthropometric measures are presented in Table 4.1 . Participants in this study all played semi-professional rugby and included 7 forwards and 5 backs. All participants completed a $VO_{2\ max}$ test prior to completing their experimental protocol. The average $VO_{2\ max}$ was measured at 45.73 l/min ± 3.83.

Table 4.1: Anthropometric parameters and physiological values at the beginning of the study

Age (y)	20 ± 1.65
Body mass (kg)	97.59 ± 18.01
Height (cm)	179.94 ± 6.23
$VO_{2\ max}$ (ml/kg/min^{-1})	45.73 ± 3.83

Values are mean ± SD

Table 4.2: The effect of Placebo (PLA) and Caffeine (CAF) ingestion on Saliva measures

Saliva Measure	Pre-Supplement	Pre-Exercise	Post Exercise	1h Post Exercise
Concentration mg.l $^{-1}$				
PLA	77±8	76±22	88±29	82±23
CAF	96±76	70±28	93±29	87±31
Secretion µg.min^{-1}				
PLA	58±22	73±38	58±31	62±21
CAF	62±13	63±27	56±26	72±15
Flow Rate µl.min^{-1}				
PLA	0.73±0.33	0.91±0.46	0.62±0.21	0.76±0.21
CAF	0.66±0.22	0.86±0.48	0.58±0.21	0.90±0.30

The results from both trials (Table 4.2) show that acute caffeine intake whilst completing high intensity exercise in heat did not influence mucosal immunity.

The results showed that the change in Salivary Flow rate, Secretion rate and the Concentration rate between Caffeine and Placebo groups was non-significant (p= 0.898; p= 0.862; p= 0.237).

The results also showed no differences in Saliva Flow Rate (p= 0.612), Secretion Rate (p= 0.897) and Concentration Rate (p= 0.705) between caffeine and placebo groups across all four time points.

A weak interaction appears to be trending for Salivary Secretion rate and Salivary Concentration rate between the order in which the participants received the treatment when measured across four time points (p= 0.374; p= 0.216).

Similarly, there were no significant inter group differences found when assessing VO_2 (p= 0.797). As with the saliva samples, only 10 samples were analyzed due to missing data and outliers. There were also no differences found in the order in which the groups received caffeine or placebo (p= 0.910). There was also no significant interaction between the order in which the participants received caffeine or placebo and the exercise intervals (p= 0.614).

Table 4.3: Between group VO2, order of caffeine and placebo ingestion differences, interaction between order of ingestion and exercise intervals

VO_2 (ml/kg/min^{-1})	Coef.	Std. Err.	z	P>|z|
Group	-.086	.336	-0.26	0.797
Order	-.038	.341	-0.11	0.910
Order and interval				
	.011	.023	0.50	0.614

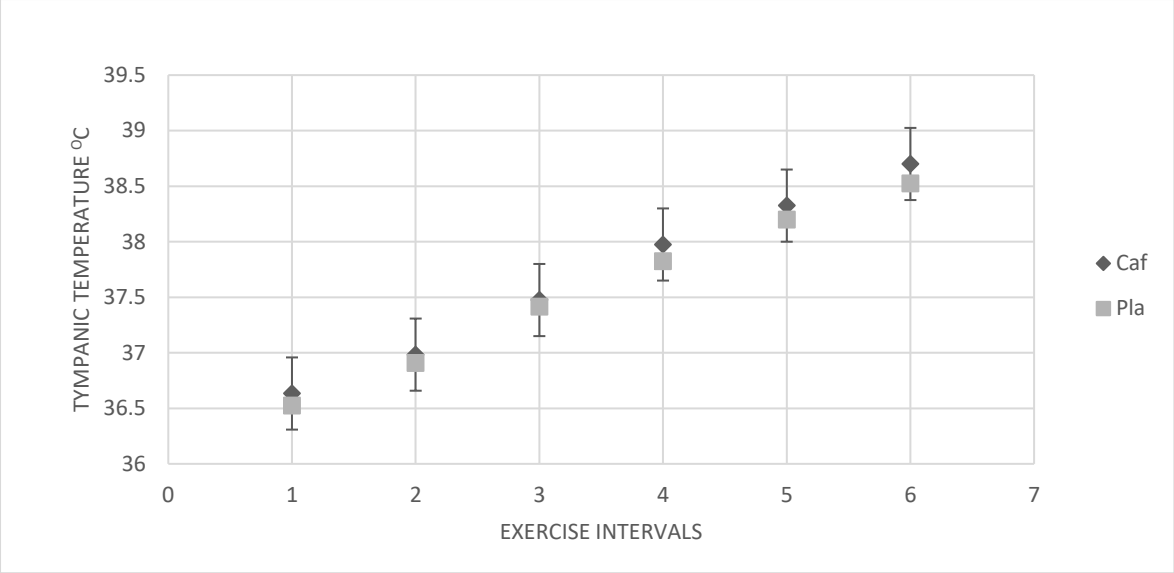

Figure 4.1: Caffeine and Placebo Tympanic Temperature

Tympanic temperature increased steadily across the intervals in both trials. However, this increase is predictable due to the participants going from a resting state to exercising at high intensities in a short period of time. Tympanic temperature in the caffeine trial was reported to be marginally higher than the placebo trials (38.7 ^{o}C ±.50 vs 38.5 ^{o}C ±0.51), however this was found to be statistically non-significant (p= 0.087).

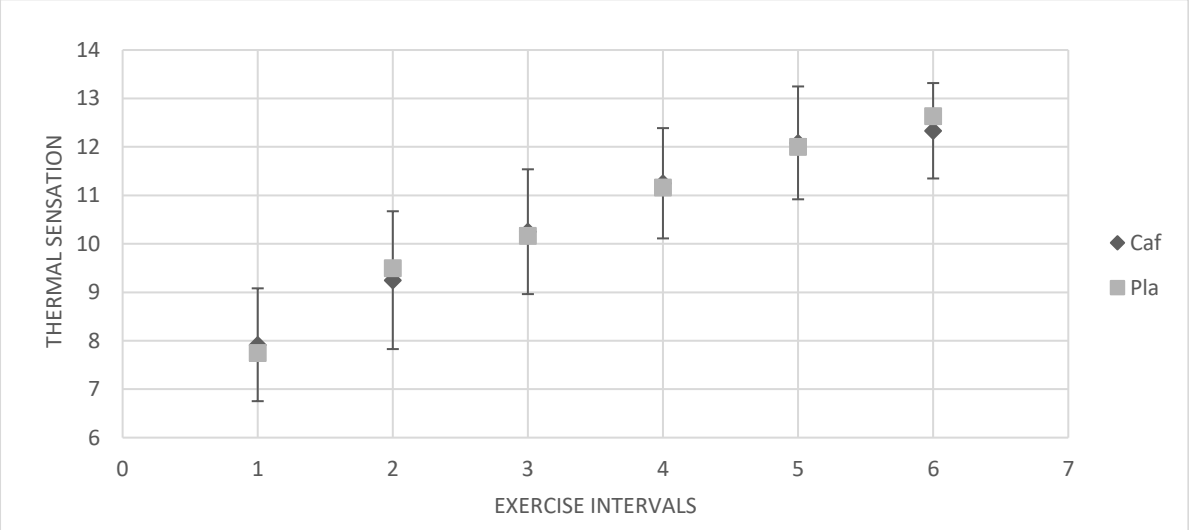

Figure 3.2: Caffeine and Placebo Thermal Sensation

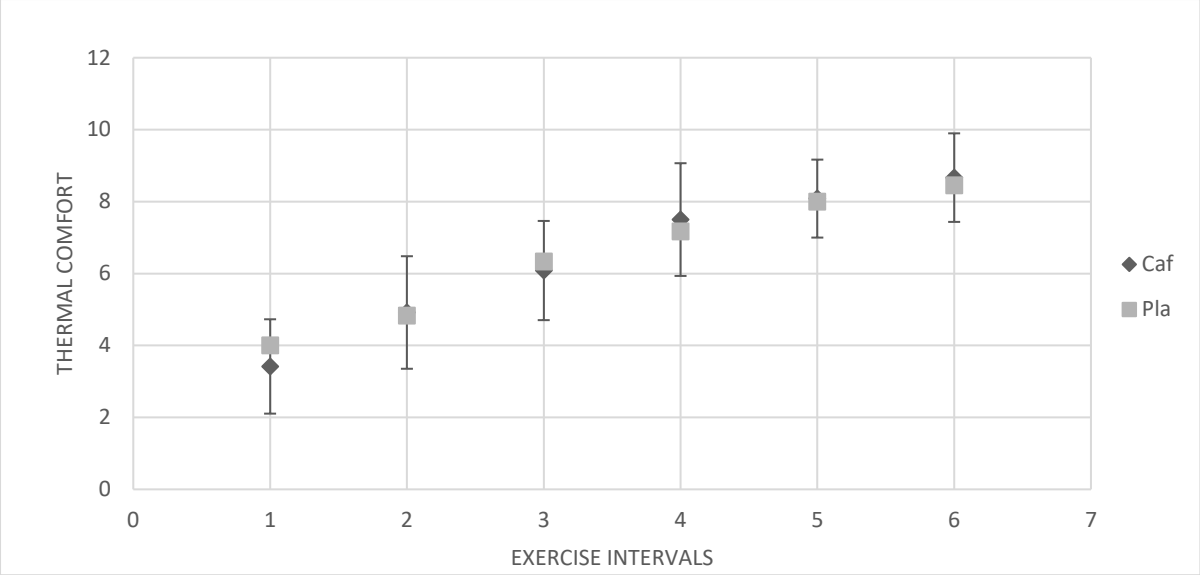

Figure 4.3: Caffeine and Placebo Thermal Comfort

Thermal sensation and Thermal comfort increased in a similar pattern, almost overlapping, in both caffeine and placebo trials throughout the duration of the exercise protocol. Thermal sensation was reported to be lower in placebo trial when compared to caffeine. Except, it was reported to be higher at the end of the final exercise interval (12.63±1.02 vs 12.33±0.98) (p=0.30). Slight increase in thermal sensation in the placebo trial was recorded in the second exercise interval (9.5±1.5) vs (9.25±1.42). However, these were marginal. Similarly, thermal comfort was reported lower in placebo trial compared to caffeine (p=0.25).

Heart Rate before, during and after exercise was not altered by caffeine administration (mean HR Caf = 177bpm ; mean HR Pla = 178bpm).

Chapter 5. Discussion

The primary aim of this research was to examine the effect of caffeine ingestion on mucosal immunity in team sport athletes when training in heat. The secondary aim was to examine the effect of caffeine ingestion on performance during a high intensity intermittent protocol in heat. Given the surrounding literature around the topic, it was hypothesised that 1). Caffeine ingestion could help reduce depression in salivary immune response as a result of high intensity intermittent running in heat and 2). Caffeine ingestion would aid running performance during the intermittent running protocol prior to exercise. The main finding of this study was that caffeine ingestion had no significant effect on post-exercise mucosal immunity in male team sport athletes performing high intensity interval training in hot environmental conditions.

5.1 Salivary Immune Response

This study found that acute caffeine intake whilst completing high intensity exercise in heat did not influence mucosal immunity. The results showed that changes in salivary flow rate, secretion rate and concentration rate between caffeine and placebo groups were insignificant. The results also showed no differences when the above measures were analysed across four time points (pre-supplement, pre-exercise, immediate post exercise, 1h post exercise). Decreased secretion rate of salivary markers, in particular SIgA, have been identified as risk factors for following episodes of infection in athletes (Bishop 2009).

The acute reduction in immunity is short-lived and is dependent on the intensity of exercise and its duration. Repeated activity during heavy periods of training with insufficient recovery can lead to a continuing deterioration of several aspects of immune function. Although most studies have concentrated on activities such as distance running, cycling and swimming, indication to support the concept of an exercise-induced immune depression has also been found after completing high intensity intermittent activities such as football, tennis as well as resistance exercises completed at high intensities (Bishop & Gleeson, 2009).

The exercise protocol in this study required participants to exercise at high intensity for approximately 30 minutes. The participants were required to complete 6 intervals in which they ran for 3 minutes at an RPE corresponding to 108% of their $VO_{2\ max}$ interspersed with 2-minutes of brisk walking. In response to acute bouts of high intensity exercise, many studies report a decrease in SIgA concentration following exercise that returns to resting levels within 1h of completion (Gleeson and Pyne., 2000; Tharp and Barnes., 1990; Tomasi et al., 1982), although some studies have reported either no change or even increases in SIgA concentrations (Sarri-Sarraf et al., 2007). In the current study elevated saliva concentration levels were reported immediately post exercise, however, these levels fell 1-hour post exercise. These changes, as reported earlier were insignificant. The different methods that

have been used to express IgA data makes it difficult to make direct comparisons between some studies and hence this could be a reason for the inconsistency in the findings that have been previously reported in these studies.

Overall exercise intensity appears to be a major factor that effects SIgA concentration and secretion rate. SIgA concentration was reported to increase by 30-45% whilst saliva secretion rate showed no changes after 30 minutes of cycling at 30% and 60% of maximal heart rate in male and females of different fitness levels (Bishop & Gleeson, 2009). Long and sustained exercise has been reported to decrease (Walsh et al., 2002) and increase (Blannin et al., 1998) SIgA secretion rate. The inconsistencies in the findings may be explained by disparities in the methods that may include exercise protocols, saliva collection storage methods and the hydration status of subjects before and during the trials. It is plausible that the protocol used for the current study affected the results as participants were not allowed to drink water while exercising. However, participants were given 5ml/kg/bw of plain water to either take with their pill at the very beginning or immediately post exercise after their post exercise saliva sample was collected.

The observed increase in SIgA secretion and concentration rate immediate post-exercise in the present study is supported by reports that the exercise induced fall in SIgA concentration occurs between 2 and 24 hours after prolonged strenuous exercise (Mackinnon et al., 1987; Gleeson et al., 2001). The reason for this increase may be partly because of the higher sympathetic activity which plays a role in the enhancement of the immune function in the short term. Further research of long-term responses to experimental exercise interventions such as above are warranted.

Decreases in saliva volume is said to be associated with exercise (Blannin et al., 1998; Walsh et al., 1999; Bishop et al., 2000) and if alterations in flow rate have not been considered, an increase in saliva levels may reflect a concentrating effect on absolute SIgA concentration. Similarly, chewing can result in a diluting effect on secreted SIgA which can give the impression of a decrease in SIgA concentration. Saliva flow rate has been suggested as the key factor in protection against oral pathogens (Walsh et al. 1999). Therefore Walsh et al., (1999) suggest that to represent the total availability of SIgA at the oral surface as well as to correct for any drying effect of breathing, it should be reported as a secretion rate (flow x concentration).

Saliva glands are innervated by both parasympathetic and sympathetic nerves. A decrease in saliva flow rate is said to be caused by vasoconstriction of the blood vessels to the salivary glands which has been proposed to be caused by an increase in sympathetic nervous system activity (Chicharro et al., 1998; Dawes, 1981). Exercise in the heat for long durations when compared to thermoneutral conditions is linked with a greater plasma norepinephrine response, indicating increased sympathetic activity (Galbo et al., 1979). Studies that have

used exercise and fluid restriction methods in thermoneutral (Bishop et al., 2000) and hot environments (Laing et al., 1990; Walsh et al 2004b) show greater reductions in saliva flow rate when compared to the saliva flow rate recorded when subjects received adequate water to compensate for the fluid loss whilst exercising. These studies suggest that the decrease in saliva flow rate with prolonged exercise is more likely due to dehydration rather than as a result of neuroendocrine responses. Nevertheless, athletes who perform exercise for long periods in hot environments, might exhibit larger reductions in saliva flow with associated increased sympathetic activity and increased fluid losses when compared to exercising in thermoneutral conditions.

A study by Bishop et al (2005) investigated the effects of prior caffeine ingestion on SIgA flow rate responses to prolonged, intensive exercise. The study found that ingesting caffeine 1 hour prior to prolonged exercise was associated with elevations in SIgA concentration and secretion rate but did not affect saliva flow rate. Intake of caffeine prior to exercise did not affect saliva cortisol responses however it was associated with advancements in serum caffeine and plasma epinephrine levels. Additional sympathetic stimulation through caffeine stimulation may therefore be expected to cause further reductions in saliva flow rate. However, in this study the saliva flow rate decreased during exercise but was unaffected by caffeine ingestion. The lack of statistical significance is likely attributable to the large intra-individual and inter-individual variation in salivary IgA.

5.2 Effect of caffeine on exercise performance in heat

Research conducted in high (30^{o}C) temperature has shown to have beneficial (Hulston and Jeukendrup 2008; Walker et al., 2008) effects on endurance performance in ambient temperature. On the other hand a study by Cheuvront et al., (2009) that applied high ambient temperature (40^{o}C) in their protocol did not detect any performance differences in a short time trial (Cheuvront et al., 2009). The results of the study conducted by Roelands et al (2010) are similar to those of Cheuvront et al., (2009), showing that caffeine intake did not change performance during prolonged exercise, however the intake of caffeine significantly increased core temperature when compared to placebo.

The mechanism of action of caffeine have been linked to result in an increased availability of free fatty acids (Spriet et al. 1992), resulting in the sparing of muscle glycogen. Graham et al., (2000) have disputed the above theory and have shown that these mechanisms are not due to glycogen sparing effect. Caffeine may allow subjects to perform at higher workloads for a longer period of time by reducing the perception of effort and pain whilst exercising (Motl et al 2003). The findings by Roelands et al. (2010) do not support this hypothesis, as they found no differences in RPE and thermal sensation between caffeine and placebo. Furthermore, a study by Hanson et al., (2019) examined the possible ergogenic benefits of both low

(3mg/kg/BM) and moderate (6mg/kg/BM) dosage of caffeine on endurance running performance in the heat. Perceived exertion and thermal sensation were measured throughout the duration of the 10km runs. The participants in this study could adjust the speed and run a self-paced 10km simulated race. This allowed the athletes to adjust their perceived exertion if the level became too high by lowering their speeds to maintain or decrease RPE. The study also reported no differences in thermal sensation, showing that participants experienced temperature and humidity in a similar manner across conditions.

Current research supports a central nervous system effect due to the antagonism of adenosine receptors as a likely cause (Glaister et al., 2008; Davis et al 2003). Caffeine is known to antagonise adenosine receptors in the brain, while adenosine inhibits the release of dopamine. Logically, caffeine will induce higher dopamine concentrations in the brain (Davis et al. 2003).

The findings from a study conducted by Spriet (2014) also confirmed that there were no significant differences among caffeine treatments of 0, 5 or 9mg/kg/BM on race performance. They reported that physiological responses along with the athletes' perception of the difficulty as well as the race times were unchanged. Therefore, it can be concluded that caffeine ingestion of 5 or 9 mg/kg/BM body mass by itself did not enhance the performance of the 21km race performed during conditions of high heat stress. However, it should be noted when considering the above findings that many runners do not practice well advised physiological habits during a race in a hot and humid environment. They do not take advantage of available opportunities to maintain fluid balance out of fear of compromising their performance. The athletes in this study were all reported to have experienced dehydration and therefore it can be concluded that dehydration was of enough magnitude to influence their performance and this effect may have masked any possible benefits from glycogen sparing.

In the present study, the lack of performance outcomes after caffeine administration without co-ingestion of carbohydrates might be explained by several factors. A recent study by Cheuvront et al., (2009) stated that environmental conditions may impact the effectiveness of well-established nutritional ergogenic supplements like caffeine. This may explain the lack of positive effect on exercise performance after administration of two different adenosine receptor antagonists (caffeine and quercetin). Undeniably, elevated internal body temperature and increased heat storage have been attributed to reduce CNS drive for exercise performance and cause feeling of fatigue (Nielsen et al 1990) during prolonged exercise in the heat (Gonzalez-Alonso et al. 1999). It seems that the rate at which the core temperature escalates post caffeine intake could cause a progressive inhibition of different brain regions accountable for motor activation (Belza et al., 2009), which could result in the disappearance of the caffeine's ergogenic effects (Nybo et al., 2010).

5.3 Caffeine and Other Physiological Markers

In addition to the primary outcomes, as discussed above, a number of secondary outcomes of this study showed that caffeine intake did not have a major effect on the physiological markers when training in hot temperature. Several studies have researched the effects of caffeine on thermal responses to temperate and warm environments and found that caffeine doses of 5-7.5 mg/kg/BM did not produce significant changes in core body temperature during exercise when the temperature was less than 30^{o}C (Anderson & Hickey, 1994; Daniels et al., 1998; Dunagan,et al., 1998). Caffeine doses as high as 10mg/kg BW, although not altering core body temperature during exercise in temperate conditions (21^{o}C), have reported lower skin temperatures, possibly due to peripheral vasoconstriction driven by increased epinephrine release (Dunagan et al., 1998). Peripheral vasoconstriction in hot temperatures could possibly interfere with heat loss, increase core temperature, and can therefore increase the predisposition of individuals to heat illness. However, Stebbins, Daniels and Lewis (2001) reported that active vasodilation may override caffeine's vasoconstrictor activity at moderate doses (6mg/kg/BM) during exercise in hotter environments (35^{o}C). This may be another contributing factor why the findings of the current study did not show any improvements of caffeine ingestion (6mg/kg/BM) in any physiological markers that were assessed. Tympanic temperature, Thermal sensation and Thermal comfort levels all showed a steady increase, however, the changes in these measures, as reported earlier were insignificant. The increases in the above measures were predictable as participants went from a resting state in thermoneutral conditions, to exercising at high intensities in hot environments. The study conducted by Ely et al., (2009), supports the above findings and concluded that a large caffeine intake of 9mg/kg, given as an acute dose to non-heat acclimated, low caffeine users, increased body heat production at rest and during exercise in a hot, dry environment. However, the magnitude of the increase in heat production was small and heat losses appeared unaffected. Therefore, increases in mean body temperature were minimal, physiologically trivial and unlikely to predispose athletes to a heightened risk of heat illness. Laboratory studies where core temperature remains within 2 ^{o}C of normal baseline indicate a rather limited effect of either hot or cold environments on immune function, except for T-Cell immunity that has been reported to decrease. As such, most of the available evidence does not support the argument that exercising in 30 ^{o}C heat poses a greater threat to immune function when compared with thermoneutral conditions.

Caffeine also did not appear to have any significant effect on perceived intensity during RPE regulated HIIT as evidenced by the VO_2 trends. A study by Berry et al., (1991) reported no significant differences in VO_2 when participants ingested 7 mg/kg/BM caffeine when compared to placebo. The participants in this study were caffeine naïve. Therefore, it can be speculated

that the use of caffeine naïve participants could have resulted in higher catecholamine levels during the caffeine trial as compared with placebo trial.

5.4 Limitations

As with all studies, there are limitations to the findings. A motorized treadmill was used for all testing. The treadmill that was initially being used broke down and therefore had to be replaced with a different model of a motorized treadmill. To increase or decrease speed required pressing a button on the treadmill display. This is not a natural process as outdoor running where individuals can simply speed up or slow down. Additionally, the nature of testing was a limitation. As with all laboratory-based studies, eliciting enough motivation was challenging, especially when participants are expected to arrive at early hours of the morning, in a fasted state and are not allowed to eat or drink anything until after their final saliva collection. This challenge becomes even greater when exercising at very high intensity with a gas mask in hot, humid conditions in a closed laboratory. No fluid intake was allowed while the participants completed the exercise protocol. Normally in a game setting, athletes would have access to water and other fluids that they can take to reduce fatigue and perform optimally. Additionally, some authors have reported that 6mg/kg/BM dose of caffeine might be considered high and is therefore unlikely to be used by athletes in a typical training situation (Chester and Wojek, 2004).

Caffeine concentration at rest without exercise was not measured in this study. However, as reported by Dulson et al., (2019), caffeine concentration did not appear to influence SIgA concentration rate or secretion rate at rest or in response to prolonged submaximal exercise. It would have also been beneficial to test the intra individual responses to caffeine to see if there are any differences between responders vs non responders. Perhaps future studies should consider testing this.

It would also be ideal to test the participants over a longer period to see if there are any changes in IgA responses, as our study measured acute responses over a 2-week period.

Additionally, using a high intensity intermittent protocol with participants that are semi-professional/ professional team sport athletes may not reflect the physical and psychological demands of rugby. The protocol used is not a direct reflection of their running habits during a game. Semi-professional/ professional rugby players would almost never run at high speeds for a continuous 3-minute period during a match. The participants also play at different positions, therefore would have different aerobic/anaerobic demands and their level of conditioning will be varied. Moreover, not only are they physically fit, but they are also more mentally resilient than amateur athletes and as such push themselves through repeated bouts of high intensity exercise.

A control arm for thermoneutral conditions was not included in this study to see whether caffeine alone would have had any effects. Future studies should consider investigating and comparing the effects of caffeine on mucosal immunity in different environmental conditions.

5.5 Implication of this Thesis

Results showed that athletes can take a moderate dose of caffeine (6mg/kg/BM) and can use it in hot temperature while exercising without seeing any ill effects on immune function. However, due to previously observed significant individual responses to caffeine, the results should be treated with caution by coaches and athletes looking to use caffeine during competition. Caffeine should be trialled in advance before using it during pre-competition or game conditions.

5.6 Conclusion

In conclusion, taking an acute dose of caffeine (6mg/kg/BM) before completing high intensity exercise in heat did not provoke any changes in participants mucosal immunity or their physiological markers in highly trained male athletes. A gradual increase in RPE and tympanic temperature was noted along with thermal sensation and thermal comfort in both conditions (caffeine and placebo), however this might have been as a result of fatigue as well as completing a high intensity exercise protocol in heat.

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Appendix A

Participant Information Sheet

Date Information Sheet Produced:
3 May 2017

Project Title
Does caffeine ingestion influence mucosal immunity and performance males during intermittent exercise in the heat?

An Invitation
Hello, my name is Angad Marwah 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 Matt Wood. I am conducting this study to determine the effects caffeine ingestion on immune system response during exercise in hot environments. This research will help inform the practices for 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 intake of caffeine helps with performance while competing in team sports in hot environments. We wish to publish these results in a scientific journal and academic conferences 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? Inclusion Criteria:
You have responded to the advertisement to participate in the above study and that you are currently playing team sports, aged between 18 and 35, and are current competing at a top club and/or regional level. You also have no current injuries, and have not been injured within the past two months. Additionally, to participate in this research, you should be actively training at least 2 times and competing in your sport for at least 70 minutes each week.

Exclusion criteria consists of the following:
If you are currently injured or ill, smoke or on anti-inflammatory medication, suffer from asthma (mild asthma is ok), or have any other illnesses that may interfere with you taking part in this research, then you will be excluded from this research study.

What will happen in this research?
You will be required to visit the SPRINZ lab on three occasions:

Visit 1 - During the first session, you will be asked to complete two assessments on a treadmill to assess

1). Aerobic fitness or maximum oxygen uptake ($\dot{V}O_{2max}$). 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 around your chest.

2. The second assessment will require you to complete six 3 min high intensity interval runs on a treadmill spread out by 2 min of brisk walking in between, in the heat chamber (preheated at 30 Degrees, 50% Humidity). This is for you to get familiar with what you will be required to do on Visits 2 and 3.

After completing the first visit, you will be required to attend the lab on 2 more occasions and these visits will last for about 2.5 hours each.

Visits 2 and 3 – These visits will feature the same High intensity intermittent running test, only this time you will be given a capsule to ingest with water. One hour prior to the starting the exercise, you will ingest opaque gelatin capsules containing either caffeine (6mg/kg/BM) or corn flour (Edmonds, Auckland, NZ), along with 5ml. kg^{-1} BM of plain water. The exercise protocol will be 30 min of high intensity interval running. The test will consist of 6 bouts of 3 min high intensity running (HIIT) which will be interspersed with 2 minutes of brisk walking (6km/hr) on the treadmill within an environmental chamber, creating an environment of 30°C and 50% relative humidity. The relative high intensity target for HIIT will be based on the rating of perceived exertion (RPE) achieved at 110% of secondary gas exchange threshold during your $\dot{V}O_{2max}$ test. Treadmill speed will be adjusted to ensure that you reach your set RPE target. Average running speed will be recorded as a measure of your performance. Whole unstimulated saliva will be collected to measure the response of the mucosal immune system.

Tympanic (ear) temperature will be recorded at the beginning of each 2-min active recovery between running intervals. You will enter the heat chamber (preheated to 30°C and 50% RH) to complete a standardised warm-up, which will include slow pace walk for 2 mins, then a jog for 2 mins followed by a light run for a minute. After the warmup is completed you will undertake the high intensity interval running test lasting approximately 30 minutes.

Whole unstimulated saliva will be collected upon arrival, prior to exercise, post-exercise, and 1 h post-exercise. Oxygen consumption, carbon dioxide production, heart rate, rating of perceived exertion (20 point Borg scale) and rating of thermal sensation (1-7) scale will also be recorded after every 3 minutes of running, along with tympanic temperature. Body weight will be measured post exercise to calculate the participants sweat loss.

Water will be provided to you in a 300ml bottle and the amount of water consumed will be measured so that this can be replicated at your 3rd visit. Also, your diet in the day before each experimental trial 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 food prior to testing days. It is important that you refrain from consuming any alcohol and or caffeine 24h prior to testing days. A diet diary has been provided for you to keep a record of your food intake.

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 running 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 any risk or discomfort that you may anticipate, please feel free to address these concerns to the researcher so that you feel comfortable at all times throughout the process.

What are the benefits?

You will be given the results of your $\dot{V}O_{2max}$ test for your interest and you will experience exercising in a heat chamber. Your participation in this study will help the researcher attain a Masters degree in Sport and Exercise.

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, Matt, 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?

There are no financial costs for participating in this research however you will be required to attend the trials at the SPRINZ lab for 45 minutes during the first visit and 2.5 hours each for the following 2 visits.

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 Angad Marwah.

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 *amarw002@gmail.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:

Angad Marwah, Auckland University of Technology, AUT-Millennium Campus, Phone: 0212592421, Email: amarw002@gmail.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

Matt Wood, Lecturer, Sport and Exercise Science, Human Potential Clinic Manager, AUT-Millennium Campus, Private Bag 92006, Auckland, 1020. Phone: 09 921 9999 ext 7848, Email: mwood@aut.ac.nz

Approved by the Auckland University of Technology Ethics Committee on *31 July 2017*, AUTEC Reference number *17/153*.

Appendix B

Consent Form

Project title:

Does caffeine ingestion influence mucosal immunity and performance in males during intermittent exercise in the heat?

Primary Researcher: ***Angad Marwah***

(Please Tick)

- ○ I have read and understood the information provided about this research project in the Information Sheet.
- ○ I have had an opportunity to ask questions and to have them answered.
- ○ 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.
- ○ 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.
- ○ 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.
- ○ 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.
- ○ I agree to my 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.
- ○ I agree to take part in this research.
- ○ I wish to receive a copy of the report from the research (please tick one): Yes○ No○
- ○ I wish to have my saliva samples returned to me at the end of the research: Yes○ No○

Participant's signature:

Participant's name:

Participant's contact details (if appropriate):

Date:

Approved by the Auckland University of Technology Ethics Committee on 31 July 2017 AUTEC Reference number 17/153

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

Appendix C

SPORTS PERFORMANCE
RESEARCH IN NEW ZEALAND AT AUT MILLENNIUM INSTITUTE

AUT UNIVERSITY

WANTED

Team Sport athletes for immunity response research

Are you:

Male ?

18-35 years old ?

Take Caffeine?

Currently competing in a competitive league in Auckland ?

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

What the research will involve:

Four 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&4. Two intermittent running tests in a heat chamber (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 amarw002@gmail.com for a detailed participant information sheet.

Thank you for your consideration,

Angad Marwah, AUT University

Appendix D

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

31 July 2017

Matthew Wood
Faculty of Health and Environmental Sciences

Dear Matthew

Re Ethics Application: **17/153 Does caffeine ingestion influence mucosal immunity and performance in males 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 31 July 2020.

Standard Conditions of Approval

1. A progress report is due annually on the anniversary of the approval date, using form EA2, which is available online through http://www.aut.ac.nz/researchethics.
2. A final report is due at the expiration of the approval period, or, upon completion of project, using form EA3, which is available online through http://www.aut.ac.nz/researchethics.
3. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form: http://www.aut.ac.nz/researchethics.
4. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
5. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.

Please quote the application number and title on all future correspondence related to this project.

AUTEC grants ethical approval only. If you require management approval for access for your research from another institution or organisation then you are responsible for obtaining it. You are reminded that it is your responsibility to ensure that the spelling and grammar of documents being provided to participants or external organisations is of a high standard.

For any enquiries, please contact ethics@aut.ac.nz

Yours sincerely,

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

Cc: amarw002@gmail.com; Deborah Dalson

Appendix E

Deborah Fletcher

AUT UNIVERSITY
TE WĀNANGA ARONUI O TAMAKI MAKAU RAU

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 day 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 or 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:	Silver (whole), blue (approx. 2% fat), green etc.
	Biscuits:	Toffee pops, digestive
	Cheeses:	Anchor processed slices, Mainland cheddar
	Fish	Hoki, tuna, gurnard
	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 021 259 2421.

DAY:	EXAMPLE	
TIME	**DESCRIPTION OF FOOD OR DRINK CONSUMED**	**PORTION SIZE**
8:30am	Kellogs cornflakes	Small bowl
	2% fat milk	250 ml
	Toast, Tip Top white bread	2 slices
	Countdown olive margarine	Thin spread
	Charlie's 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	Powerade Ion	500ml bottle
6:00pm	Grilled lean pork chops	2 medium
	Boiled new potatoes with skins	7 small
	Watties frozen peas	2 tablespoons
	Banana	1 medium
7:30pm	Healthries green tea (decaffeinated)	1 mug
	McVities Digestives	4 biscuits