The Effects of Soccer- Specific Intermittent Exercise on Salivary IgA Responses

by

Vahid Sari-Sarraf

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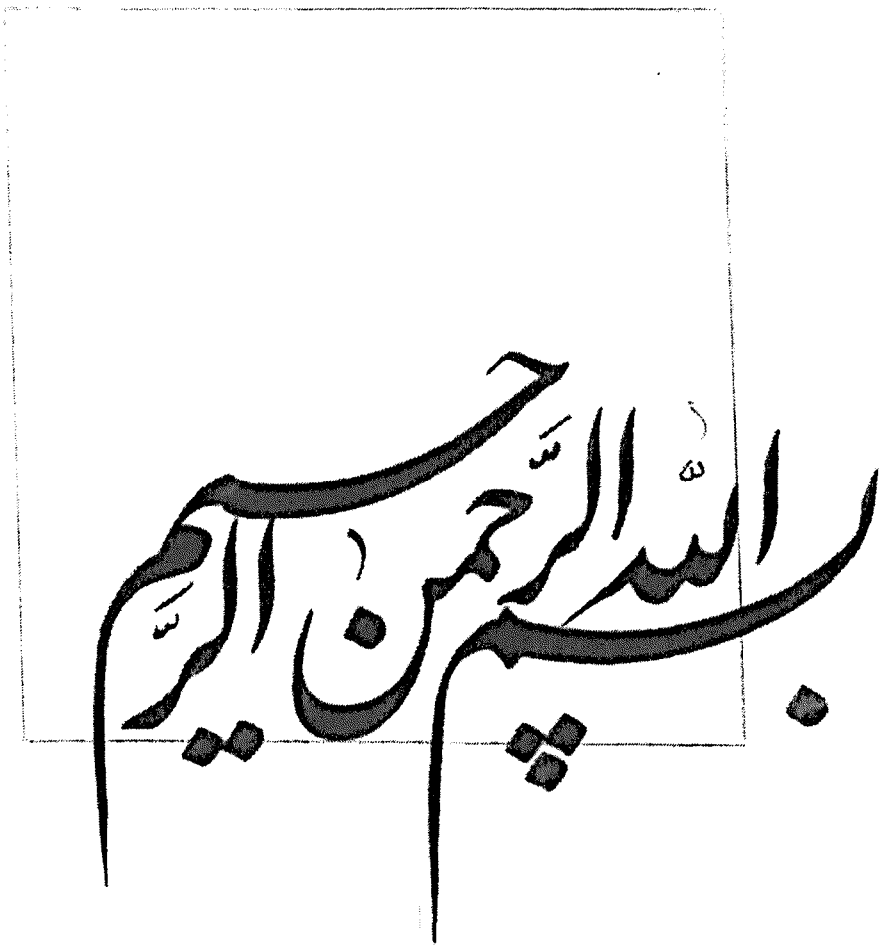
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Chapter 4

Chapter 5

Appendix E

Figure 2.2 page 12

Contact information in Appendix D

Abstract

Exercise has been demonstrated to influence susceptibility to upper respiratory tract infections (URTI) because various aspects of immune function including mucosal immunity are temporarily changed after exercise. Lower concentrations of salivary IgA (s-IgA) as a predominant immunoglobulin and component of saliva or a chronic deficiency in s-IgA have been associated with an increased frequency of URTI episodes. Previous studies of aerobic exercise have incorporated the performance of running or cycling. Responses of s-IgA to intermittent exercise patterns as they occur in soccer remain to be resolved. A laboratory based soccer-specific intermittent exercise protocol was used within this thesis to mimic the physiological stress associated with soccer, characterised by high-intensity activity as noted during soccer play. The aims of this thesis were to determine the s-IgA responses to a bout or repeated bouts of soccer-specific intermittent exercise and investigate the effects of carbohydrate ingestion on s-IgA when such exercise is performed in increased ambient temperature.

Laboratory based soccer-specific intermittent exercise and continuous exercise at the same moderate exercise intensity evoked insufficient stimulation of the hypothalamic-pituitary-adrenal axis to modify s-IgA responses. Changes in s-IgA and cortisol did not differ between exercise types during or immediately following exercise, or 6 h, 24 h and 48 h afterwards. Physiological responses to intermittent exercise also conforming to the activity pattern of soccer match-play were similar to those for continuous exercise at the same average work-rate, despite the higher perceived exertion during intermittent exercise.

Two bouts of soccer-specific intermittent exercise 48 h apart that were designed to provide a repeatable physiological stress comparable to strenuous soccer training induced s-IgA responses that were similar following both bouts of exercise. Performing the second bout of exercise did not significantly suppress s-IgA concentration after 48 h recovery although a small progressive reduction in s-IgA was observed.

Performance of a second soccer-specific exercise bout in one day with a 2.25 h rest in between bouts elicited an increase in heart rate and perceived exertion, compared with a single session at the same time of day, but did not appear to suppress s-IgA outcomes. There was also no difference between responses of s-IgA concentration and secretion rate or salivary cortisol at the different times of day.

Soccer can be played under hot environmental conditions and it is thought that addition of carbohydrate to fluids may prevent adverse changes in mucosal immune responses. Carbohydrate supplementation before and at regular intervals whilst performing soccer-specific intermittent exercise at 30 °C, did not influence s-IgA responses or salivary cortisol when compared to placebo.

In view of the failure of these experimental interventions to discern effects on s-IgA responses, a meta-analysis of the literature was conducted. The meta-analysis revealed an overall elevation in s-IgA concentration post acute exercise and following chronic exercise. In contrast, in term of s-IgA secretion rate, an overall significant decline after both acute and chronic exercise was a consistent finding.

In conclusion, one exercise bout or repeated soccer-specific intermittent exercise in the present experiments did not induce compromises in s-IgA responses. There was no adverse effect upon s-IgA responses to intermittent exercise performance under conditions of heat stress with or without carbohydrate treatment. Although, 32 percent of studies included in the meta-analysis have indicated similar results, the disparity with overall findings may arise from differences in the exercise protocols used and/or bias for s-IgA changes in published studies.

Key words: salivary immunoglobulin A, salivary cortisol, soccer-specific exercise, repeated exercise, carbohydrate, meta-analysis

Declaration

I declare that the work presented in this thesis is entirely my own, assistance is acknowledged where appropriate.

Table of Contents

List of figures

Number *Page*

List of Tables

Number *Page*

Acknowledgments

IN THE NAME OF ALLAH THE COMPASSIONATE THE MERCIFUL

Laudation to the God of majesty and glory! Obedience to him is a cause of approach and gratitude in increase of benefits. Every inhalation of the breath prolongs life and every expiration of it gladdens our nature; wherefore every breath confers two benefits and for every benefit gratitude is due.

Whose hand and tongue is capable
To fulfil the obligations of thanks to him?

"Sheikh Muslih-uddin Sa'di Shirazi
World famous Persian (Iranian) 13th century poet"

Soccer is a team game and any investigation into the sport will require an individual contribution, collaboration and advice. There are some institutions and many individuals who merit special thanks for helping to realise completing of this thesis.

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This project would not have taken shape without the motivation of subjects. They deserve thanks for the time they gave me and their remarkable patience, and careful attention to detail.

I am deeply appreciative of my wife; my daughter also deserves hugs and kisses. I would like to thank our relatives, families and friends for their spiritual support and long patience.

This thesis is dedicated to the memory of my brother, Hamid. Your memory will live on with me forever.

Publications

Journal papers

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Sari-Sarraf, V., Reilly, T. Doran, D.A., and Atkinson, G. (2007) Effects of repeated bouts of soccer-specific intermittent exercise on salivary IgA. *International Journal of Sports Medicine*. In press

Sari-Sarraf, V., Reilly, T., Doran, D.A. and Atkinson, G. (2007) The effects of single and repeated bouts of soccer-specific exercise on salivary IgA. *Archives of Oral Biology*. In press

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GLOSSARY

Term	Explanation
ADH	Anti-diuretic hormone
Antibody	A synonym for immunoglobulin. It is a glycoprotein produced by B cells and binds to antigen. It neutralizes antigens, promotes phagocytosis and activates the complement system.
Antigen	A molecule recognized by the immune system.
B Cell	A lymphocyte that play a large role in the humoral immune response as opposed to the cell-mediated immune response that is governed by T cells. The principal function of B cells is to make antibodies against soluble antigens.
Cytokine	A protein produced by many different cell types. It mediates inflammatory response and communication between cells of the immune system and other cell types.
ELISA	Enzyme-linked-immunosorbent-assay
HPA	Hypothalamic-pituitary-adrenal
Ig	Immunoglobulin, synonym for antibody
NK cell	Kills microbe-infected cells and cancer cells by lysis and IFN-γ secretion.
s-IgA	Salivary IgA
T cell	T_H: activation of B and $T_{S/C}$ cells. T_{H1} Cells mediate cellular immunity. T_{H2} cells mediate humoral immunity. $T_{S/C}$ cells lyse virus infected and cancer cells.

Chapter 1

Introduction

Chapter 1

Introduction

1.1 Background

Soccer is probably the most popular sport worldwide in terms of its both participation and spectator levels (Ekblom, 1986). The game is characterised by intermittent activity. Hence, soccer differs from other sports that demonstrate a more continuous exercise profile (e.g. running), which results in the physiological demands being more complex. The methods that can be applied in real competition to determine the physiological stresses associated with match-play have been examined by making observations during such play and obtaining physiological measures during real and simulated games (Bangsbo, 1994). The data are not necessarily collected under the controlled conditions as found in experimental investigations (Reilly, 1990). Several researchers have devised soccer-specific laboratory-based protocols that not only replicate the exercise patterns observed during match-play but also induce broadly similar physiological responses to intermittent exercise as noted during game play (Holmyard et al., 1988; Balsom et al., 1992). The patterns of the intermittent activity used in these investigations have consisted of repeated bouts of high-intensity actions of short duration separated by low-intensity or static recoveries that are observed during soccer match-play (e.g. walking, jogging, cruising and sprinting).

Exercise has been shown to induce disruption to the immune system. Immunological alterations in response to a number of different exercise protocols are well documented (Pedersen and Nielsen, 1997; Mackinnon, 2000). Various research groups have investigated changes in the quantity and function of circulating cell populations in response to one bout of acute exercise (Gabriel and Kindermann, 1997; Bishop et al., 1999) and repeated exercise (McCarthy et al., 1992; Rohde et al., 1998; Ronsen et al., 2001a; Ronsen et al., 2001b; Ronsen et al., 2002b; Ronsen et

al., 2002a; McFarlin et al., 2003; Li and Gleeson, 2004). There is scientific support for a dual effect of exercise i.e. intense exercise or training accentuates susceptibility whereas moderate exercise attenuates susceptibility to the upper respiratory tract infections (URTI). The cause of the increased incidence of infection in athletes is likely to be multifactorial: a variety of stressors, physical, psychological, environmental, or nutritional, can suppress immune function. These effects could make the athlete more susceptible to infection, but degree of exposure to pathogens is also an important factor in determining actual infection incidence (Gleeson, 2006b).

A paradoxical relationship between exercise and upper respiratory tract infection has been proposed as a "j-Curve" model (Nieman, 1994). This model describes the relationship between exercise volume (intensity and duration combined) and susceptibility to URTI, in which moderate activity reduces the risk of symptom development and expression but intense exercise progressively elevates it. However, there is evidence that does not support the theory that high volume/high intensity training results in an increase in URTI (Shephard and Shek, 1999). The infections during periods of hard training or competition stress may be part of the overtraining syndrome (except a primary medical case), perhaps indicating that the stress has initiated immune dysfunction, and increased vulnerability to infection (Sharp and Koutedakis, 1992). An epidemiological study on infection incidence and habitual physical activity disclosed that the regular performance of about 2 h of moderate exercise per day was associated with a 29% reduction in risk of picking up URTI compared with the risk of infection associated with a sedentary lifestyle (Matthews et al., 2002). More recently an S-shaped model has been proposed by Malm (2006), suggesting that at the highest level of performance elite athletes need to have an immune system capable of withstanding infections. The model was derived from a re-evaluation of previously published data and longitudinal observations in a single athlete, it therefore needs to be verified by compiling data from a large sample of athletes.

The first line of defence against respiratory infection comprises the saliva/mucosal barrier. In fact, saliva contains a number of bacterial components such as immunoglobulin A (IgA). Hence, a change in secretory IgA that is induced by exercise, may be a possible mechanism for the higher incidence of infection observed in athletes (Tomasi et al., 1982; Mackinnon and Hooper, 1994; Mackinnon, 1996a). However, it is likely that the effect of exercise on the immune system not only depends on the fitness of the subjects, intensity of exercise, and the duration of the exercise (Keast et al., 1988; Hickson, 1991; Nieman and Nehlsen-Cannarella, 1991; Hoffman-Goetz and Pedersen, 1994; Pedersen et al., 1994; Mackinnon, 1996a; Pedersen and Nielsen, 1997) but also is responsive to a combination of multiple stressors.

Various authors have examined how salivary IgA (s-IgA) concentration or secretion rate is modulated by exercise stress. There are reports demonstrating s-IgA concentrations are suppressed in response to high-intensity exercise (Tomasi et al., 1982; Mackinnon et al., 1987; Tharp and Barnes, 1990; Gleeson and Pyne, 2000), remain either unaltered (McDowell et al., 1991; Walsh et al., 1999) or are elevated in response to moderate-low intensity exercise (Tharp, 1991; Ljungberg et al., 1997; Blannin et al., 1998; Li and Gleeson, 2004). In addition, s-IgA concentrations vary widely between individuals and although some studies indicated that s-IgA concentrations are lower in endurance athletes compared with sedentary individuals, the majority of studies indicate that s-IgA levels are generally not lower in athletes compared with non-athletes, except when athletes are engaged in very heavy training (Gleeson, 2004a; Gleeson, 2006a). It seems that specific detailed information about questions related to s-IgA responses to exercise is still to be resolved.

Soccer players during the course of a competitive season, are exposed to a number of physical and psychological stressors from practice, conditioning, and competition (Ekstrand et al., 2004). Professional players are committed to more than 30 games per season (in England) with concomitant stresses such as position in the league table, and nowadays training occasionally twice per day. However, only in a handful of studies has soccer been used as a model for experimental research and

investigations of immunological changes in soccer players have been researched in few previous publication (Grasso et al., 1997; Rebelo et al., 1998; Malm et al., 2004; Klapcinska et al., 2005).

Epidemiological and experimental reports indicate that strenuous training/competition increases the perturbations in cellular and secretory immune function and also susceptibility to illness, predominantly URTI whereas moderate exercise reduces susceptibility. Given these related factors to modality, intensity and duration, study 1 (Chapter 4) sought to examine s-IgA responses to intermittent and steady-rate exercise performed at the same average work-rate.

Daily intense exercise may have a cumulative suppressant effect on s-IgA output. The incidence of upper respiratory tract infections (URTI) is higher than normal in athletes and may be linked to repetitive intensive exercises sessions without sufficient recovery between them. Failure to recover fully between exercise sessions has been suggested to evoke immunodepression. Soccer players have a little opportunity to avoid repeated stress when competitive matches are scheduled close together. Therefore, in study 2 (Chapter 5) a cumulative effect of soccer-specific intermittent exercise undertaken on different days 48 h apart on s-IgA was investigated to assess whether immune function is suppressed and then compare with the literature.

Soccer players often train twice in a day which itself may be physiologically stressful. Li and Gleeson (2004) recommended that a 3-hours rest was enough for s-IgA to recover from previous strenuous exercise. In order to investigate the effects of repeated bouts of exercise on mucosal immunity, an experimental protocol was employed to examine the effect of both single and repeated bouts of soccer-specific intermittent exercise on s-IgA (Chapter 6).

The purpose of exercise immunological studies is not only to define the impact of exercise on immune system but also to look for resources in preventing immunodepression in athletes. Nutritional strategies particularly carbohydrate

supplementation have been demonstrated to attenuate adverse responses of the immune system to exercise (Coyle, 1991; Gleeson et al., 2004b) and decrease the potential for developing symptoms of respiratory tract infections (Nieman and Pedersen, 1999). Exercise in a thermally stressful environment appears to have an additive effect on the hormonal and immune system disturbances compared to heat alone (Shephard, 1998; Shephard et al., 1998; Shephard and Shek, 1999). Therefore the effects of carbohydrate supplementation on s-IgA to soccer-specific intermittent exercise in the heat were investigated (Chapter 7).

The association between exercise and s-IgA has been reviewed previously in the form of a qualitative summary of studies findings (Gleeson, 2000 b, a; Gleeson et al., 2004a). Despite acceptance of this traditional narrative, it is usually non-quantitative and often unsystematic, and therefore conclusions are often open to subjectivity (Hunt, 1997). Consequently, there is still missing links in our understanding of the interactions between exercise and s-IgA responses, which might emerge with a more robust and quantitative research synthesis in the form of a meta-analysis that has been proposed. This technique minimises subjectivity by standardising selections, data subjectivity pooling, and presented a non-biased method of data analysis to draw conclusions with the new version of comprehensive meta-analysis software (Biostat, USA). A meta-analysis to determine whether s-IgA responses are altered by modality of acute exercise or chronic exercise at the time of writing is presented in Chapter 8. The results could be composed with those arising from the experimental studies within this thesis.

This thesis is concluded with a general discussion summarising of findings of these experimental studies. Issues arising from discrepancies with the outcomes of the meta-analysis are considered and recommendations are outlined for further work (Chapter 9).

1.2 Aims and objectives

This thesis has been designed to investigate the salivary immunoglobulin A (s-IgA) response to soccer-specific intermittent exercise. Therefore, the immunological response to laboratory based soccer-specific intermittent protocol will be delineated in separate studies through the fulfilment of the following aims:

1. To evaluate the s-IgA response associated with laboratory-based simulations of soccer match-play work rates (Study 1). This aim will be accomplished by evaluating the s-IgA responses to soccer-specific intermittent and continuous exercise protocols.

2. To study the s-IgA responses to two consecutive laboratory-based soccer-specific intermittent exercises (Study 2). This aim will be accomplished by evaluating the s-IgA responses to two successive soccer-specific intermittent exercises separated by 48 h.

3. To study the s-IgA response to two laboratory-based soccer-specific intermittent exercise sessions in day one (Study 3). This aim will be accomplished by evaluating the s-IgA responses to soccer-specific intermittent exercise sessions conducted once and twice a-day.

4. To investigate the s-IgA response to an experimental sports drink on the performance of soccer-specific intermittent exercise in the heat (Study 4). This will be accomplished by evaluating the s-IgA responses to soccer-specific intermittent exercises with and without sports drinks separated by one week.

5. To quantify the results of a meta-analysis on the effects of exercise on s-IgA responses, the main aim of this meta-analysis will be accomplished by evaluating whether the modality of acute exercise or chronic exercise interventions cause significant change in s-IgA responses concomitant to pre-exercise.

1.3 Hypotheses

The hypotheses explored in the four experimental studies are as follows:

1. The s-IgA responses associated with soccer-specific intermittent exercise is different from responses to continuous exercise that is performed at the same average work-rate.

2. The s-IgA responses associated with a second bout of soccer-specific exercise are greater than for a first bout of exercise 48 h earlier.

3. The s-IgA responses associated with one bout of soccer-specific exercise in one day is lower than for a second bout of exercise performed after 2.25 h recovery.

4. The s-IgA responses to soccer-specific intermittent exercise in the heat are attenuated with carbohydrate (CHO) supplementation compared with a placebo (PLA).

Chapter 2

Review of Literature

Chapter 2

Review of literature

2.1 The immune system

2.1.1 Structure and function

Humans are continually exposed to infectious microbes or pathogens. Such microorganisms include bacteria, fungi, protozoa, helminthes, viruses, and parasites that are capable of causing serious diseases. The fact that individuals in daily living are not overcome by these foreign invaders is a testimony to the importance and efficiency of the body's defence mechanisms, comprised primarily of the immune system (Figure 2.1).

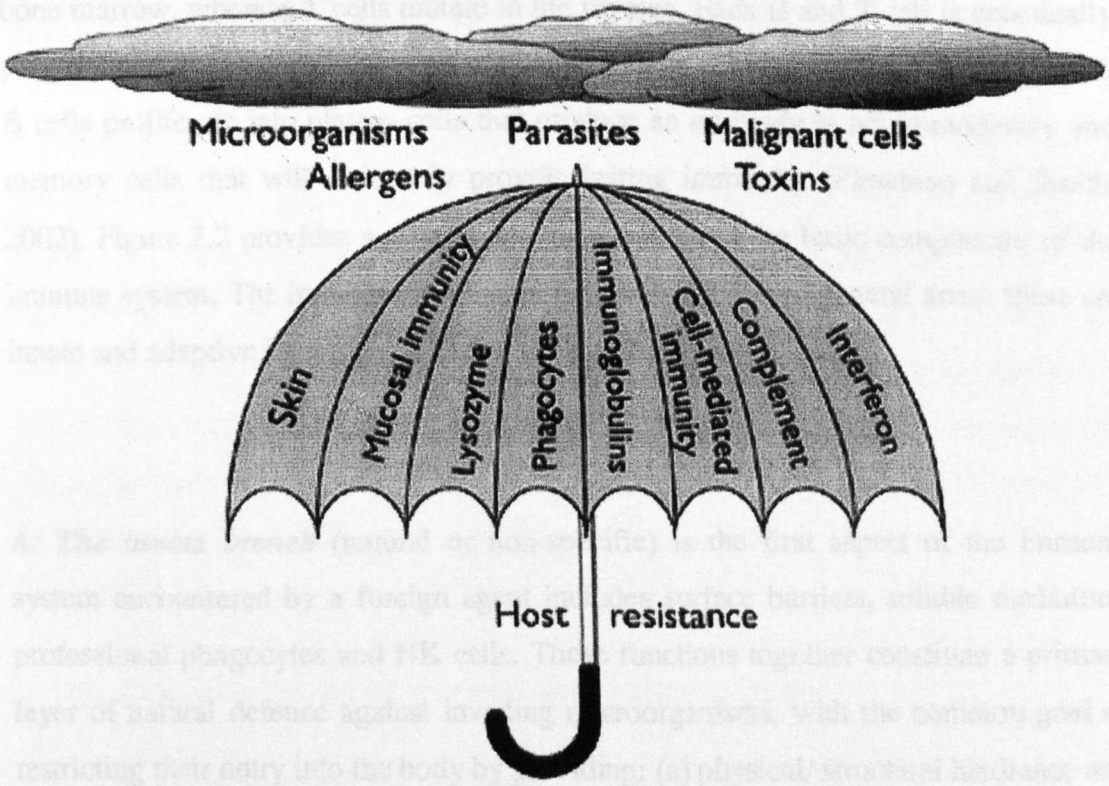

Figure 2. 1 Host resistance against infectious microbes or pathogens.

The **immune system** is a complex and precisely ordered system of cells, hormones, and chemicals that regulate susceptibility to, severity of, and recovery from infection and illness (Nash, 1994; Marieb, 2001). *Immunology* is the study of the physiological responses of the body against destroys or neutralizes foreign substances (Smith, 1995). Thus, due to these organization and operation, the immune system can be considered parallel to the nervous and endocrine systems. The immune system, in turn consists predominantly of free mobile cells that move within and outside the bloodstream, although some may be anatomically specific (Roitt et al., 1998).

In fact, B *lymphocytes* and T *lymphocytes* (B *cells* and T *cells*) specifically recognize individual pathogens. Any molecular pathogen that can be specifically recognised by B cells and /or T cells is called an *antigen*. Both B cells and T cells are derived from bone marrow, whereas T cells mature in the thymus. Each B and T cell is genetically programmed to recognize only one particular antigen. Once an antigen is recognized, B cells proliferate into plasma cells that produce an *antibody* to act immediately and memory cells that will ultimately provide lasting immunity (Plowman and Smith, 2002). Figure 2.2 provides a general structural outline of the basic components of the immune system. The immune system can be divided into two general arms: these are innate and adaptive immunity, which work together synergistically.

A: ***The innate branch*** (natural or non-specific) is the first aspect of the immune system encountered by a foreign agent includes surface barriers, soluble mediators, professional phagocytes and NK cells. These functions together constitute a primary layer of natural defence against invading microorganisms, with the common goal of restricting their entry into the body by providing: (a) physical/ structural hindrance and clearance mechanisms via epithelial linings of skin and mucosal barriers, mucus, ciliary function and peristalsis; (b) chemical factors such as the low PH of stomach fluids, numerous antimicrobial peptides and proteins; (c) phagocytic cells including

Figure 2. 2 Components of the immune system (Source: Based on Roitt et al., 1998).

neutrophils, eosinophils, blood monocytes, tissue macrophages and dendritic cells (DCs) capable of ingesting and killing microorganisms, and (d) NK cells which are non-specific killer cells that can destroy host cells that become infected with viruses, thus preventing further viral replication (Plowman and Smith, 2002; Mackinnon, 1999). Cells involved in innate immunity can protect against foreign substances or cells without having a prior exposure. Central to the functioning of the innate branch of the immune system is the *inflammatory response*. Mobilisation of the immune cells is necessary when an injury or infection occurs. Four processes combine to accomplish this convergence:

(1) Vasodilatation and an increased blood supply to the area;

(2) Increased capillary permeability;

(3) Chemotaxis; and

(4) Leukocytosis (an increased number of leukocytes).

*B: **The adaptive (specific) branch*** (lymphocytes: B and T cells) is characterised by specificity to the infectious agent and a short lag period (a few days) to become fully activated. The purpose of acquired or adaptive immunity is primarily to combat infections by preventing colonization of pathogens and keep them out of the body (immune exclusion), and to seek out specifically and destroy invading microorganisms (immune elimination). Adaptive immunity requires that the immune cells recognize a foreign material and react specifically and selectively to destroy it. The adaptive response generates memory of prior exposure that provides a faster and more effective level of protection with subsequent exposure to the same agent; this is the basis for immunisation to prevent disease. This branch includes humoral and cell-mediated immunity.

The body's ability to mount a specialized immune response is based on specific proteins called major histocompatibility component (MHC) that exist on the membrane of the body's own cells and pathogens. Major compatibility proteins are different in each individual, except between identical twins (Plowman and Smith, 2002).

The phagocytosis (ingestion) of the invading microorganism by an antigen-presenting cell (APCs) is the first step in a chain of events leading to the eventual elimination of the pathogen. Stimulation of mature B lymphocytes results in their proliferation and differentiation into immunoglobulin-secreting plasma cells. Immunoglobulins, or antibodies, are important to antigen recognition and memory of earlier exposure to specific antigens (Figure, 2.3). They also help to eliminate pathogens in the extracellular fluids but they cannot enter cells and so are not effective against pathogens that have infected host cells. The effector cells of the B-cell system are the terminally differentiated antibody producing plasma cells. These constitute the basis for so-called humoral (fluid borne) immunity, which is mediated by circulating antibody proteins or immunoglobulins (Ig) comprising five subclasses: IgA, IgD, IgE, IgG and IgM.

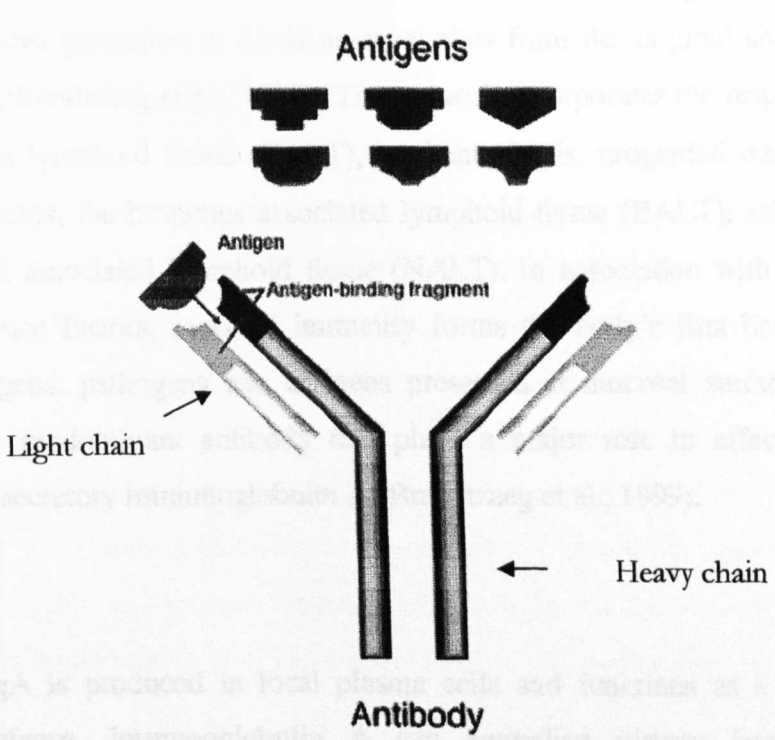

Figure 2. 3 Schematic of antibody binding to an antigen. An antibody made up of two heavy chains and two light chains. The unique variable region allows an antibody to recognize its matching antigen

Immunoglobulin A is the predominant immunoglobulin secreted at mucosal surfaces. Immunoglobulin A is a dimmer of 350 kD. The two monomers are joined by a J chain and protected from proteolysis by another peptide, the secretory component, made by epithelial cells. It is acquired by IgA molecules as they pass through the epithelium on their journey from the plasma cell to the mucosal surface. Immunoglobulin A (IgA) can immobilize micro-organisms or prevent their attachment to mucosal surfaces. It is believed that most IgA in the blood is later available for transport to mucosal surfaces. Immunoglobulin A is also secreted in saliva in the mouth. This IgA comes from B cells in the surrounding mucosal tissue. Salivary IgA is thought to be important in defence against infections of upper respiratory tract (Gleeson, 2006a).

2.1.2 The mucosal immune system

The mucosal immune system is a complex network of immune structures at cellular level in the mucosal surfaces throughout the body. The function of this network is to

provide effective protection at distal mucosal sites from the original site of antigen presentation (Brandtzaeg et al., 1999). The network incorporates the respiratory tract, gut associated lymphoid tissue (GALT), lacrimal glands, urogenital tracts, lactating mammary glands, the bronchus associated lymphoid tissue (BALT), salivary glands and the nasal associated lymphoid tissue (NALT). In association with innate, non-specific defence factors, mucosal immunity forms the body's first line of defence against allergens, pathogens and antigens presented at mucosal surfaces (Gleeson, 2000b). The predominant antibody that plays a major role in effective, specific immunity is secretory immunoglobulin A (Brandtzaeg et al., 1999).

Secretory IgA is produced in local plasma cells and functions as a multi-layered mucosal defence. Immunoglobulin A can neutralize viruses intracellulary by replication (Mazanec et al., 1992). Immunoglobulin A prevents antigens from adhering to and penetrating the epithelium, interrupts replication of interacellular pathogens during transcytosis through epithelial cells and binds antigens in the lamina propria facilitating their excretion through the epithelium back into the lumen (Lamm, 1998). Given IgA's ability to inhibit bacterial attachment and cause bacterial aggregation, reductions in salivary concentration of IgA have been implicated as causal in increasing the susceptibility to URTI (Nieman and Nehlsen-Cannarella, 1991; Mackinnon, 1996a). The concentration of secretory IgA present in mucosal fluids correlates more closely than serum antibodies with resistance to certain infections caused by viruses (Murphy et al., 1982) and has been suggested as one of the most promising markers for identifying those athletes more prone to or "at risk of" respiratory illness (Pyne and Gleeson, 1998; Shephard and Shek, 1998; Akimoto et al., 2003b).

2.2 The immune system's responses to physical activity

The immune system is influenced by various types of physiological and psychological stressors (Herbert and Cohen, 1993) including physical activity. The effects of physical activity on the immune system are likely mediated through the interaction

between the nervous, endocrine and immune systems as mention above. As evident by the publication of the symposium "Exercise and Immunology" in Medicine and Science in Sports and Exercise (MSSE) in February 1994 (26:125-194,1994), the field of exercise immunology was considered a sub-discipline of exercise physiology. In addition, the formation of the International Society of Exercise and Immunology (I.S.E.I.) in 1993 recognized the interest in this relatively new area of scientific investigation. Despite the rapid growth of research in this field, a review by Gleeson (2006b) suggests that the influence of exercise on the immune system remains an enigma. There is a general, but scientifically unsubstantiated, belief that while physical fatigue may increase susceptibility to illness on the one hand, regular exercise at moderate intensity may prevent common infections on the other (Mackinnon, 1992). Therefore, literature tends to support a dual effect of exercise on the immune system.

Any attempt to summarize the "exercise effect" on immune function is clouded by the variety of experimental protocols employed. Various permutations and combinations of exercise intensity, duration and mode have been reported, using subjects with a wide variety of fitness levels and training histories (Rowbottom and Green, 2000). In general, the total number of white blood cells (leukocytes) in circulation increases with exercise duration and immediately after exercise (Figure 2.4). For example, six minutes of maximal ergometer rowing in trained rowers was associated with a two-to three-fold increase in circulating lymphocyte quantity immediately post-exercise, with numbers falling to 40% below resting values during recovery (Nielsen et al., 1998). Similar findings have been reported following heavy resistance exercise (Miles et al., 2003). Insufficient recovery between prolonged exercise bouts may also exaggerate the biphasic response: an increased lymphocytosis was observed in response to a 75-minute bout of cycling at $75\%\dot{V}O_{2\,max}$ that was performed with only 3 hours recovery following a similar exercise bout (Ronsen et al., 2001). Peripheral blood lymphocytes consist of the T cell, B cell and NK cell subsets. The circulating concentration of the T cell lymphocytes ($CD3^+$), in response to acute exercise exhibits a biphasic response, with marked increases in T cell number evident during and immediately after exercise and significant falls in number reported during recovery (Figure 2.4); this pattern is

evident for intensive exercise of both short and prolonged duration (Shek et al., 1995). Despite pronounced changes in the concentration of circulating T cells, numbers of circulating B cells did not change significantly from resting values in response to 45 minutes of treadmill running at 80% $\dot{V}O_{2\,max}$ (Nieman et al., 1994). Therefore, it may be that very high intensities of exercise are required to elicit changes in circulating B cell number (Nielsen et al., 1998; Miles et al., 2003).

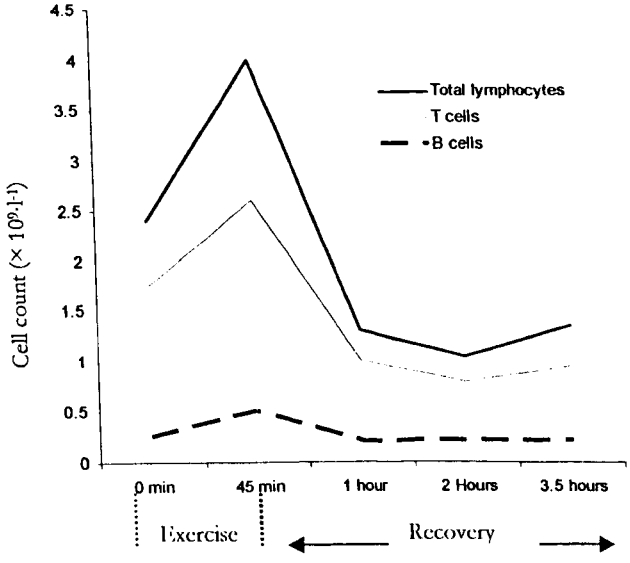

Figure 2. 4 Changes in circulating concentrations of total lymphocytes, T cells and B cells in response to 45-minutes treadmill run at 80% $\dot{V}O_{2\,max}$.

It is not necessarily true that more leukocytes in the circulation are better and therefore interpretable as "immuno-enhancement." From the preceding section, it is clear that the circulating pool of leukocytes represents only one fraction of the total population [e.g., only 0.2% of total lymphocyte mass circulates at any one time (Park and Good, 1974; Gleeson, 2006b)]. While the reported leukocytosis is roughly in proportion to the intensity and duration of the exercise performed (McCarthy and Dale, 1988), it may be increased in higher ambient temperatures (Rhind et al., 1999). Depending on the type and intensity of exercise, training status of the subjects, and even timing of the blood samples, all possible outcomes (increase, decrease, no change) have been reported [many of these reports have been tabulated by Simon (1984), Nieman *et al.*(1991) and Tharp and Preuss (1991)]. In addition, the proper functioning of the immune system depends on many factors such as age, sex, fitness or training status,

sleep, psychological loads, social burdens, hormones, nutrition, diseases, inherited factors and physical stress. Moreover, the small transient changes observed acutely after exercise are often attributable to circadian rhythms or hemo-concentration (Haus et al., 1983; Levi et al., 1985).

Therefore, immunological alterations in response to a number of different exercise protocols are well documented. Functional and enumerative changes in cell populations have been studied in response to one bout of acute exercise or repeated exercise. Investigations of immunological changes in soccer players have only been made in a couple of previous publications (Rebelo et al., 1998; Malm et al., 2004). The effects of playing soccer on mucosal immunity have not been yet investigated.

2.2.1 Carbohydrates and immune system response to exercise

The benefits of consuming a carbohydrate (CHO) beverage immediately before and at frequent intervals during heavy exercise in terms of maintaining exercise capability and performance are well documented (Nieman et al., 2003). Data from several studies suggest that carbohydrate ingestion has emerged as an effective countermeasure and compared with placebo ingestion during sustained and prolonged exertion is associated with an attenuated immunosuppressive effect of exercise (Bishop et al., 1999). Consumption of drinks containing glucose can delay fatigue and prevent hypoglycaemia and dehydration. Hydration status may also influence the incidence of opportunistic communicable disease because drying of the oral and respiratory mucosal may leave it more vulnerable to attack from pathogens. Recent studies have concentrated on the effects of CHO beverages in attenuating the response of blood-born indices of immune function to exercise (e.g. Nieman et al., 2004). The addition of carbohydrate to fluid ingested before and at regular intervals during high-intensity intermittent exercise may attenuate perturbations in immune function (Coyle, 1991; Gleeson et al., 2004b) and decrease the potential for developing symptoms of respiratory tract infections (Nieman and Pedersen, 1999). Carbohydrate ingestion may also attenuate the rise of cortisol concentrations during exercise by maintaining plasma

glucose concentrations (Bishop et al., 1999). Cortisol is a likely mechanism as it has been shown to inhibit transepithelial transport of s-IgA (Sabbadini and Berczi, 1995), to inhibit in vivo B lymphocyte antibody synthesis (Saxon et al., 1978) and has been implicated in the decreased B lymphocyte antibody synthesis after exercise (Nehlsen-Cannarella et al., 1991).

2.3 Susceptibility of athletes to infection illness

A relationship between intense exercise stress and susceptibility to upper respiratory tract infection (URTI) was evident in the early part of the last century. Cowles (1918) reported that virtually all cases of pneumonia at a boy's school occurred in athletes and that respiratory infections seemed to progress toward pneumonia after intense exercise and competitive sport. A survey conducted by the Gatorade Sports Science Institute in 1996 established similar consequences (Mackinnon, 1999). Hence, a majority of elite athletes and their coaches believe that prolonged and intense exertion lowers resistance to upper respiratory tract infection (Tomasi et al., 1982; Fitzgerald, 1991; Mackinnon, 2000; Gleeson, 2006a). Endurance athletes suffer a high incidence of URTIs during intense training and after competition (Nakamura et al., 2006). In general, the paradoxical relationship between exercise and upper respiratory infections risk has been proposed as a "j-Curve" model (Nieman, 1994), suggesting that the risk of URTI may decrease below that of a sedentary individual in moderate exercise, but may rise above average during periods of excessive high-intensity exercise (Figure 2.5). When elite athletes are included in the model, this association was suggested to be "S" shaped (Malm, 2006).

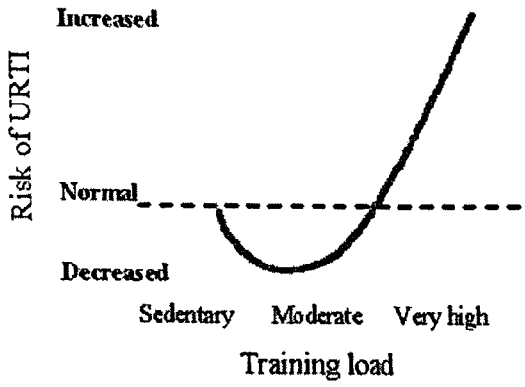

Figure 2. 5 The J-shaped model of the relationship between risk of upper respiratory tract infection and exercise volume.

2.3.1 Saliva composition

Salivary glands are non-excitable effector organs. In response to neural stimulation, an amount of fluid and electrolytes is transferred from the interior of the organism to the outside (Schneyer et al., 1972a; Guyton, 1996). The secretory volume of fluid translocated each day through the salivary glands approaches 750 ml, which represents a rate of approximately 0.5 $ml \cdot min^{-1}$ arising from three major pairs of salivary glands (Dawes, 1974; Guyton, 1996). The relative contributions of salivary glands to total unstimulated saliva secretion are, on average from submandibular glands (65%), parotid glands (23%), minor mucous glands (8%), and sublingual (4%) (Dawes, 1974). Secreted saliva is normally a colourless liquid, dilute with a density ranging from 1002 to 1012 $g \cdot l^{-1}$ (Schneyer et al., 1972b) consisting of inorganic and organic components and water (usually more than 99%)[Garret, 1974; Young and Van Lennep, 1979]. The salivary glands are innervated by both parasympathic and sympathetic nerves (Garret, 1974). The arteries supplying the parotid glands are derived from the external carotid, whereas the submandibular glands are supplied by branches of the facial and lingual arteries. The sublingual glands are supplied by both sublingual and submental arteries (Williams and Wawick, 1980). Parasympathetic stimulation causes a manifest increase in local blood flow to salivary glands by vasodilatation of the vessels. Sympathetic stimulation induces a decrease in saliva volume, which contains high levels of protein and potassium (K^+) [Baum, 1987; Pilardeau et al., 1990; Chicharro et al., 1998].

Salivary IgA is produced in the submucosa of salivary glands and then binds to a receptor (polymeric immunoglobulin receptor, pIgR) located on the mucosal epithelium. This complex is subsequently transported across the mucosal epithelium and released into the saliva as s-IgA (Mostov, 1994; Brandtzaege, 1998). The modification of s-IgA secretion is regulated via the rate of synthesis over days (Goodrich and McGee, 1998; Toellner et al., 1998) or transcytosis over minutes (Kugler, 1999). It has been suggested that, the acute alteration induced by exercise is likely through the modulation of the transepithelial secretory process rather than plasma cells (B lymphocyte) activation (Li and Gleeson, 2004).

2.3.1.1 Salivary IgA responses to exercise

The effect of exercise on salivary immunoglobulin A (s-IgA) may have important clinical implications. Salivary IgA provides a valuable first line of defence against potential pathogens by preventing colonization and replication on the mucosal surfaces of the upper respiratory tract (Mackinnon, 1992), although there is no unanimity (Housh et al., 1991). The first study that reported changes in IgA during intense exercise was carried out on elite male and female members of US national Nordic skiers team (Tomasi et al., 1982). The researchers examined resting IgA concentrations in competitive athletes. The study exhibited a 50% lower resting s-IgA concentration compared with age-matched non-athletes. It was suggested that this low range in IgA may be due to the daily intense training routine and possibly to psychological stress leading up to major competition. A subsequent study from the same laboratory, however, found no difference in resting s-IgA concentration between trained cyclists and age matched sedentary control subjects (Mackinnon et al., 1989). It was suggested that the differences between the two studies were attributed to the status of the athletes; that is, in the study on skiers, elite national team athletes were assessed just before major competition whereas in the study on cyclists, the subjects were not elite and were studied midway through the season and not before competition (Mackinnon, 2000). A study of elite male and female Australian swimmers showed significantly lower resting s-IgA concentrations throughout a six-month season with symptoms of staleness or overtraining compared with those well-trained (Mackinnon and Hooper, 1994). Tomasi and co- workers (1982) and Fitzgerald (1991) believed

that this difference may be related to the perceived high incidence of URTI among elite athletes during intensive training and overtraining. Thus the increased incidence of URTI that has been reported in well-trained athletes during training may be related to decreased s-IgA levels. Various studies have examined how s-IgA concentration or secretion rate is modulated by exercise stress. There are reports demonstrating s-IgA concentrations are suppressed in response to high-intensity exercise (Tomasi et al., 1982; Mackinnon et al., 1987; Tharp and Barnes, 1990; Gleeson and Pyne, 2000), remain either unaltered (McDowell et al., 1991; Walsh et al., 1999) or are elevated in response to moderate-low intensity exercise (Tharp, 1991; Ljungberg et al., 1997; Blannin et al., 1998).

It is likely, however, that both neural and endocrine factors are involved (Korneva et al., 1985; Mackinnon, 1992). High levels of adrenaline, however, have been associated with depressed s-IgA concentrations (McClelland et al., 1980). Furthermore, Mackinnon et al. (1999) reported that s-IgA was suppressed by the emotional stress of competition. These findings suggest that the response of the mucosal secretary immune system is influenced by neuro-endocrine interactions.

2.3.1.2 The association between s-IgA and cortisol

It has been suggested that cortisol plays an important role in inhibiting s-IgA mobilization (Hucklebridge et al., 1998). Wira *et al.* (1990) reported a decline in s-IgA concentration at 24 h after a single injection of dexamethasone, which preceded a rise in serum IgA concentration detected 24 h after the second hormone treatment. They suggested that IgA increased in serum and decreased in salivary secretions due to a redistribution of polymeric IgA from mucosal surfaces to the circulation controlled by glucocorticoids. However, the s-IgA concentration and antigen-specific IgA production after oral antigenic challenge was markedly inhibited. These data suggested that glucocorticoids might impair mucosal IgA synthesis, secretion and function as well as promote bacterial translocation (Moyer et al., 1981). However, Tharp and Barnes (1990) and McDowell et al. (1992) have reported no significant relationship between salivary cortisol and s-IgA.

Cortisol as a stress hormone is utilized to assess the level of physiological or psychological stress (Kindermann et al., 1982; Filaire et al., 1996). Salivary cortisol is a good index of the adrenocortical response to exercise, since salivary cortisol levels closely reflect plasma free cortisol levels, and their measurement represents a methodological advantage over total plasma cortisol measurements (Katz and Shannon, 1964; Port, 1991; Stupnicki and Obminski, 1992). Hence, Vining, et al. (1983) contended that saliva is better than plasma for the measurement of adrenocortical function, since the level of unbound cortisol is reflected more accurately by saliva than serum total cortisol. Moreover, during exercise, salivary and serum levels of cortisol are very similar (Umeda et al., 1981; Stupnicki and Obminski, 1992), and salivary cortisol might therefore be used to evaluate the response of glucocorticoids to exertion (Cook et al., 1987; O'Connor and Corrigan, 1987; Stupnicki and Obminski, 1992). However, salivary cortisol has been questioned as a reliable indicator of glucocorticoid activity at rest, since serum and salivary cortisol values were not correlated (del Corral et al., 1994).

Salivary levels of cortisol increase linearly with exercise intensity and duration (O'Connor and Corrigan, 1987; del Corral et al., 1994; Filaire et al., 1996; Rudolph and McAuley, 1998), but these elevations lose their linearity at certain intensity (Port, 1991). This inflection point in the most subjects coincides with the anaerobic threshold (del Corral et al., 1994). In this regard, it has been suggested that the accumulation of lactate might activate chemoreceptors within the working muscles, which, in turn, could stimulate the pituitary-adrenal system (Few et al., 1980; Farrell et al., 1983).

2.3.2 The s-IgA responses to exercise in extreme environments and sleep deprivation

Athletes have to contend with environmental stressors that adversely affect the immune system like extreme cold or heat, pollution, high altitudes, travel fatigue, jet lag and sleep deprivation in many different local and international settings. Cold weather has been reported to be associated with an increased incidence of URTI.

Acute mountain sickness and high-altitude pulmonary edema are more common in mountain climbers during seasons of lowest ambient temperatures (Giesbrecht, 1995). Altitude training is frequently used by endurance athletes in an attempt to improve sea-level athletic performance. Hypoxia has been associated with marked alterations of the immune system (Pedersen et al., 1994). Living high-training low (LHTL) as a training strategy may be associated with a cumulative negative effect of exercise and hypoxia on secretory IgA levels (Tiollier, et al., 2005).

The impairment in immune function has also been noted after sleep deprivation (Smith and Reilly, 2005). Persistence of inadequate sleep, or successive disrupted sleep has been thought to cause vulnerability to upper respiratory tract infections (Moldofsky, 1994; Boyum, et al. 1996). Therefore, in view of the immune-suppression that occurs after strenuous exercise, a link between the recuperative process of sleep and the immune system has been suggested (Reilly and Ekblom, 2005).

In terms of cold conditions, Walsh et al. (2002) indicated that performing prolonged cycling for 2 h at 70% $\dot{V}O_{2\,max}$ in freezing cold conditions (-6.4 ± 0.1°C) did not influence s-IgA secretion rate compared with prolonged exercise in thermoneutral conditions. Prolonged exercise in the heat is associated with a greater sympathetic activity, compared with prolonged exercise in thermoneutral conditions (Galbo et al. 1979). Laing et al. (2005), despite their expectation of a large decrease in s-IgA secretion rate after prolonged cycling for 2 h at 62 ± 3% $\dot{V}O_{2\,max}$ in the heat (30.3 °C, 76% RH), demonstrated non-significant s-IgA responses to prolonged exercise with *ad libitum* water intake compared with prolonged exercise in thermoneutral conditions. Whilst, various studies have addressed the link between exercise and s-IgA responses, it is clear that little attention has been directed toward the influence of the environmental factors on s-IgA during exercise.

2.4 Soccer-specific intermittent exercise

Intermittent exercise is characterised by high-intensity activity punctuated by short periods of exercise at a lower intensity or by rest (Bishop et al., 1999). Invasive field games, such as soccer are also considered by the variable intensities of low to high intensity activities such as walking, jogging, running, cruising and sprinting (Bangsbo, 1994). The mean relative work rate is around 70% of maximal oxygen uptake ($\dot{V}O_{2\,max}$) [Reilly, 1997]. These intensities can alter any time during a game with more than 1000 changes have been reported (Reilly and Thomas, 1976). In fact, frequent short bouts of these actions are deemed to increase overall exercise intensity and energy expenditure (Bishop et al., 1999). In addition, sweat losses and glycogen depletion at high intensity of exercise during intermittent activity are greater than during continuous exercise over the similar period of time (Shi and Gisolfi, 1998). Thus, intermittent exercise induces higher physiological demands compared with continuous exercise of the same overall work-rate (Palmer et al., 1999).

Coaches, athletes, and medical personnel, who are seeking guidelines on ways to reduce the risk of illness that compromises exercise, training or competitive performance, need to apply the findings of studies in their training and competition schedules. Furthermore, an understanding of related mechanisms will enhance our general knowledge of immune function under a variety of conditions.

Therefore, this thesis was initiated to investigate whether intermittent and continuous exercise protocols at the same work-rate intensity had any different effect on s-IgA responses (Study 1). The effects of an increased frequency of soccer-specific exercise with 48 h between sessions (Study 2) and then repeated soccer-specific exercise in one day (study3) on s-IgA concentrations were examined. The effects of other environmental stresses such as exercising in the heat on s-IgA concentrations were also investigated. Maintenance of exercise performance during soccer-specific intermittent exercise requires adequate hydration and availability of substrate in the

form of carbohydrates. It has also been suggested that CHO supplementation prevents a suppression of s-IgA concentration via a cortisol mediated neuro-endocrine pathway. This hypothesis may be tested by supplementing participants with an experimental CHO sports drink (Study 4). A meta-analysis extended the thesis to library work in order to examine the consensus with respected to effects of exercise on s-IgA by using special software for a comprehensive analysis of previous published papers. The meta-analysis was conducted to highlight overall responses of s-IgA to exercise and clarify the effects of some moderator variables on s-IgA responses. Therefore, it would be possible to compare the findings from the experimental studies with these results.

Chapter 3

General Methods

Chapter 3

General Methods

3.1 Ethical approval

Liverpool John Moores University's Human Ethics Committee issued approval for all the current studies detailed in this thesis. The nature, purpose and risks of the experimental procedures of each study were fully explained orally and by writing to each volunteer before the start of the procedure of experiment. All participants completed informed consent forms (JMU subject consent form EC3, Appendix A) and were made completely aware that they were free to withdraw from the study at any time without any required explanation. Participants were also subjected to a health screen (Appendix B) and completed a physical activity questionnaire (Appendix C) to determine whether they were suitable for doing exercise trials. Prior to experimental sessions, the participants were asked to refrain from strenuous exercise for 48 h prior to and after each exercise trial, drinking of alcohol and caffeine for 24 h and food for 3 h. On arrival at the laboratory, on the day of the experiment trials, the participants gave verbal confirmation of these directions. Participants were again asked to ensure that they were well and fit to participate in the study. In case of the contrary, subject would be asked to postpone his trial until fully recovered. If the participant had any upper respiratory tract infection symptoms such as flu, subject would be asked to withdraw from the study.

3.2 Pre-test assessments

3.2.1 Anthropometric measurements

Each participant's height (m) was determined using a stadiometer (Seca, Germany) and nude body mass (kg) was also measured before, at half-time and after exercise using calibrated precision-weighting scale (Seca, Germany). Height and body mass were used for calculation of body mass index (BMI). The skinfold thickness at 4 sites (biceps, triceps, subscapular and suprailiac) was measured using skinfold calliper

(Harpenden 0120, UK) for calculation of percent of body fat using the skinfold method of Durnin and Womersley (1974).

3.2.2 Maximal oxygen uptake determination ($\dot{V}O_{2\,max}$)

Each participant was assessed for aerobic fitness using an incremental $\dot{V}O_{2\,max}$ test. To avoid the possibility of injury, each session was preceded by a general warm- up period consisting of general body stretching exercises and approximately 5-min of running on the treadmill. The $\dot{V}O_{2\,max}$ test was carried out on a same motorized treadmill in which subjects started running at 10 $km \cdot h^{-1}$, rising by 2 $km \cdot h^{-1}$ every 2 min until 16 $km \cdot h^{-1}$. After 2 min at 16 $km \cdot h^{-1}$, the treadmill was inclined by 2%. The British Association for Sport and Exercise Sciences' (BASES) guidelines (Bird and Davison, 1997) were followed to confirm that that maximal oxygen consumption was reached. Therefore, the test was terminated upon volitional exhaustion and/or attainment of three of the end point criteria ; (1) plateau of oxygen consumption despite rising workload, 2.0 $ml \cdot kg^{-1}$; (2) heart rate within 10 $beats.min^{-1}$ of age- related maximum. (3) respiratory exchange ratio (RER)>1.15. Participants were also verbally encouraged during the final stages of the test to ensure that they reached true exhaustion. Throughout the trial, expired gas volumes (mean expired fractions of oxygen and carbon dioxide) were recorded every 10 seconds using an on-line Metamax gas analyser (Cortex Biophysik, Borsdorph, Germany). The Metamax analyser was calibrated for volume using a 3-litre hydraulic syringe, and for gas using both ambient gas concentrations as well as certificated gases (16% O_2, 4% CO_2). Expired gases collected were expressed in standard terms (Standard Temperature and Pressure Dry). In addition, a Polar Accurex Plus transmitter (Kempele, Finland) was used to measure heart rate continuously. This device was fitted to the chest in the V5 position. The $\dot{V}O_{2\,max}$ was taken as the highest VO_2 value obtained in any 10-s period. Then, all of the data were exported to statistical soft-ware programs (Excel) for later retrieval.

3.3 Heart rate

Heart rate was measured continuously at 5-s intervals by means of a short-rage radio telemetry system (Polar S610i, Polar Electro, Kempele, Finland) during all exercises.

3.4 Rating of perceived exertion (RPE)

Rating of perceived exertion (RPE) was measured using a 6-20 scale (Borg, 1982). The scale that was used is displayed in Table 3.1.

Table 3. 1. Borg's scale used for participants' RPE during exercise.

Rating	Description
6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Some what hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

3.5 The soccer-specific intermittent exercise protocol

Laboratory-based-exercise protocols have been devised to assess the physiological demands of intermittent exercise patterns specific to soccer (Drust, 1997). The protocol chosen consists of the five different exercise intensities (standing, walking, jogging, cruising and sprinting) that are displayed in soccer match-play. Backwards, sideways and actions in contact with the ball cannot be included because of technical

impracticalities when using a motorised treadmill. These actions were included in the next most suitable category i.e. backward jogging was categorised as jogging. After a 15-min intermission, the exercise was continued for a further 45 min to correspond to the duration of a soccer game.

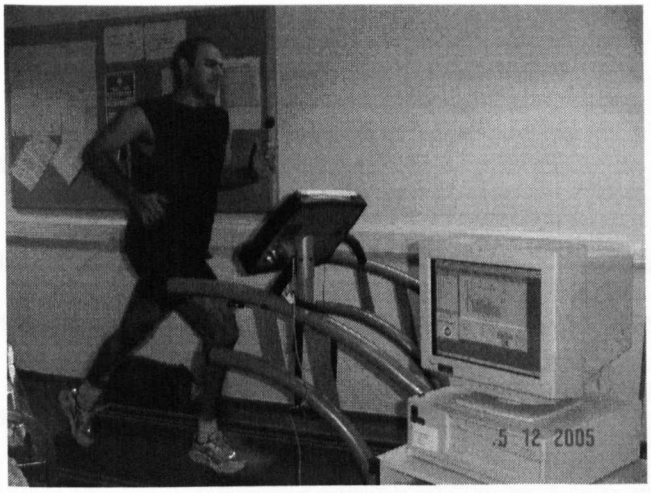

Figure 3. 1. Participant is shown during performance of the exercise protocol on the motor- driven treadmill.

3.6 Saliva sampling and analysis

3.6.1 Saliva collection and treatment

Participants were seated during saliva collections. Initially, participants were required to rinse out their mouths with distilled water to prevent potential sample contamination that might affect IgA and/or cortisol levels (Hooper et al., 1993) . The "passive expectoration method" was used according to the directions given by Navazesh and Christensen (1982). Whole unstimulated saliva was collected by expectoration for 5 min into sterile pre-weighed plastic containers (Sarstedt, UK) with eyes open (Shannon 1972), head tilted slightly forward and making minimal orofacial movement. Saliva was weighed to the nearest *mg* and volume calculated assuming a saliva density of 1.00 $g \cdot ml^{-1}$ (Navazesh and Christensen 1982; Cole and Eastoe 1988). All saliva samples were centrifuged at 5000 $rev \cdot min^{-1}$ for 20 min at room temperature, assayed for osmolality using a freezing point depression Osmometer (Model 3300, Advanced

Instruments Inc ™, Norwood, Massachusetts, USA). Samples were then aliquoted and stored at – 80°C for later analysis. In order to offset the effects of circadian variation and habitual activity known to cause alterations in salivary flow rate, immunoglobulin A and cortisol levels (Dawes, 1974; Dimitriou et al., 2002), saliva samples were collected at the same time of the day.

3.6.2 Determination of saliva flow rate

The saliva flow rate ($\mu l \cdot min^{-1}$) was determined by dividing the volume of saliva by the collection time (5 min).

3.6.3 Measurement of saliva osmolality

Saliva osmolality is the concentration of saliva as a solution. In other words, it is in unit of measurement known as osmoles or miliosmoles per 1000 grams of pure solvent. The unit takes into account the effect of dissociation. An osmole is the weight of the molecules that make up a solute, divided by the number of particles or ions that they separate in a solution. While a solute is dissolved in a solvent, the physical properties of the solvent are altered. In particular, the freezing point is depressed, the boiling point is raised, the osmotic pressure is increased and the vapour pressure is lowered. Hence, measurement of the freezing point of a sample allows for the calculation of salivary concentration. Once a solution is super-cooled several degrees below the freezing point and the sample is frozen, heat of fusion is released causing temperature to rise to a plateau where liquid/ solid equilibrium occurs; this is the freezing point and can be used to determine the concentration of the solvent. The Advanced™ Osmometer (Advanced Instruments Inc., Norwood, Massachusetts, USA) was used for the precise determination of the concentration of solutions by means of the freezing point measurement (Figure 3.2).

Test principle

First, a salivary sample is introduced into the cooling chamber using the 20µl Advanced™ pipette. The sample is automatically rapidly cooled and the display reads "Cooling Sample". When the temperature of the sample reaches 0°C, the display begins counting upwards and the cooling rate slows. After the sample has been sufficiently super-cooled, a mechanical pulse induces the sample to freeze. As the sample freezes, the heat of fusion warms the sample towards the freezing point and the display counts quickly downwards and eventually reaches equilibrium. When the equilibrium has been reached at freezing point, the display reading becomes constant. The display locks in the resultant osmolality, reading, for example, "Osmolality 92 mOsm". The machine after removing the sample from the chamber will be ready for the next sample.

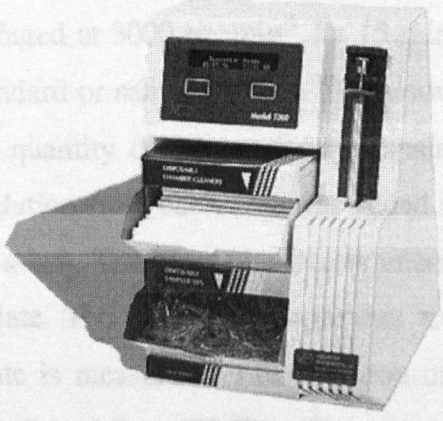

Figure 3. 2 The Advanced Micro-Osmometer Model 3300 used for measuring salivary osmolality.

3.6.4. Determination of solute secretion rate

Solute secretion rate ($\mu Osmol \cdot min^{-1}$) was calculated by multiplying saliva osmolality by saliva flow rate.

3.6.5 Determination of s-IgA concentration and secretion rate

The quantitative measurement of IgA in saliva samples was done by means of the indirect competitive enzyme immunoassay (EIA) kit (Salimetrics, USA).

Test principle

A constant amount of goat anti-human s-IgA conjugated to horseradish peroxidase was added to wells containing specific dilutions of standards or saliva samples vortexed and centrifuged at 3000 rev·min^{-1} for 15 min. The antibody conjugate binds the s-IgA in the standard or saliva samples. The amount of s-IgA present is inversely proportional to the quantity of free antibody remaining. Following incubation and mixing, an equal solution from each tube was added in duplicate to a microtitre plate coated with human s-IgA. Therefore, the free or unbound antibody conjugate binds to the s-IgA on the plate. The unbound components are washed away after incubation and bound conjugate is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). The amount of peroxidase is inversely proportional to the quantity of s-IgA in the samples (Chard, 1990). Optical density was read on a standard automated plate reader at 450 nm (Triturus, IBL, Spain)[Figure 3.3]. The concentration of s-IgA was calculated based on the linear curve fit of the related four parameters. Saliva IgA secretion rate ($\mu g \cdot min^{-1}$) was calculated by multiplying saliva flow rate by IgA concentration.

3.6.6 Determination of salivary total protein and secretion rate

Total protein concentration ($g \cdot l^{-1}$) in saliva was analysed using albumin as standard (Bradford, 1976). Secretion rate of saliva protein ($\mu g \cdot min^{-1}$) was calculated by multiplying saliva flow rate by salivary total protein concentration.

3.6.7 Determination of salivary cortisol concentration

The DRG salivary cortisol ELISA kit (DRG Diagnostics, Germany) was used for the quantification of cortisol ($ng \cdot ml^{-1}$) in saliva samples.

Test principle

A constant amount of goat anti-human salivary cortisol conjugated with horse-radish peroxidase compete is added to wells that containing specific dilutions of standards (0, 2, 5, 10, 20, 40 and 80 $ng \cdot ml^{-1}$ respectively) or saliva samples those thaw completely, vortex and centrifuge at 3000 $rev \cdot min^{-1}$ for 15 minutes before. The antibody conjugate binds to the salivary cortisol in standard or saliva samples. The quantity of salivary cortisol present is inversely proportional to the amount of free antibody remaining. Subsequent to incubation and mixing, an equal volume was added in duplicate, to the microtitre plate coated with human salivary cortisol. Therefore, the free or unbound antibody conjugate binds to the salivary cortisol on the plate. Then unbound components are washed away after incubation. Ultimately, bound conjugate is measured by the reaction of the substrate. The amount of peroxidase is inversely proportional to the quantity of salivary cortisol in attendance in the samples and optical density measured. This reaction produces a blue colour. Following stopping the reaction, a yellow colour is shaped. Finally, optical density is read on a standard plate reader at 450 nm within 10 minutes of adding stop solution.

The open ELISA automation (Triturus, IBL, Spain) was used for accurate sample pipetting, adding reagents, incubation time at appropriate temperature, washing with related buffer, reading and calculating salivary cortisol concentration based on related standard curve (Figure 3.3).

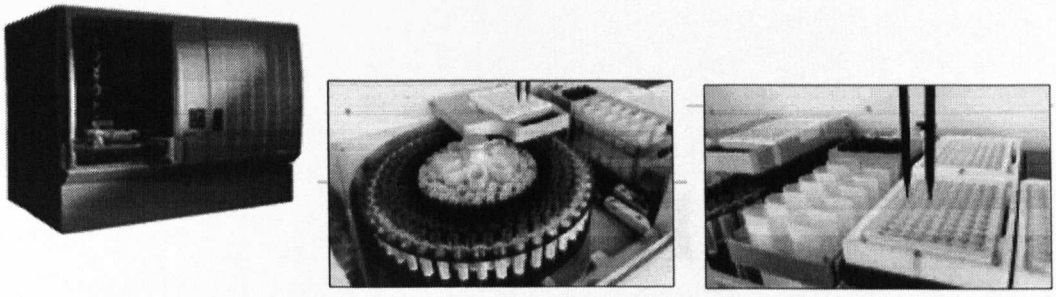

Figure 3. 3 The Triturus, open, fully-automated, multi-test, multi-batch immunoassay system

Chapter 4

Study 1

Salivary IgA Response to Intermittent and Continuous Exercise

Chapter 5

Study 2

The Effects of Repeated Bouts of Soccer-Specific Intermittent Exercise on Salivary IgA

Chapter 6

Study 3

The Effects of Single and Repeated Bouts of Soccer-Specific Exercise on s-IgA

Chapter 6

The study described in this chapter has been accepted as Sari-Sarraf, V., Reilly, T., Doran, D.A., and Atkinson, G. (2007) The effects of single and repeated bouts of soccer-specific exercise on salivary IgA. *Archives of Oral Biology* (In Press).

The effects of single and repeated bouts of soccer-specific exercise on salivary IgA

Performing two bouts of soccer-specific exercise within 48 h did not suppress s-IgA concentration significantly although a small progressive reduction in s-IgA was observed. Soccer players are not always allowed this time for recovery and may undertake two training sessions close together on the same day. Thus, the aim of the present study was to compare the effects of one and two bouts of soccer-specific exercise within one day on s-IgA responses.

6.1 Introduction

Most elite athletes perform more than one exercise session per day. Training twice daily may compromise the ability of individual athletes to recover from the earlier exercise session. Based on the temporary immunosuppressive changes that have been observed for some hours after heavy exercise (Mackinnon, 1997), the "open window" theory has been proposed to explain the transient changes (Nieman, 1994). Where two exercise sessions are completed on the same day, incomplete recovery from the first exercise could result in either quantitatively or qualitatively different responses to the next exercise stress. Several other recent studies have also indicated that a second bout of exercise on the same day induces more pronounced changes in the circulating leukocyte counts and stress hormones compared with a single bout of identical exercise (McFarlin et al., 2003). Soccer players achieve their high levels of fitness mainly during the formal pre-season training period when training twice-a-day is commonly experienced. The sudden change in their daily activity levels at this time

may leave many of them vulnerable also to upper-respiratory tract infections (URTI) due to compromised immune status (Gleeson, 1998).

Mucosal immunity has come under more consideration, with respect to the impact of exercise on salivary immunoglobulin A (s-IgA) concentrations and secretion rates. Salivary IgA provides a major defence against potential pathogens by preventing colonization and replication on the mucosal surfaces of the upper respiratory tract (Mackinnon, 1992). Reduced concentrations or reduction in the release of s-IgA may allow for increased pathogenesis via the mucosal surface (Ostergaard, 1977). Lowered concentrations of s-IgA or chronic s-IgA deficiencies are associated with an increased frequency of URTI episodes, recurrent URTI, or reduced protection against certain infections (Asahi et al., 2002). Changes in s-IgA have also been reported in elite female soccer players during a competition period (Akimoto et al., 2003a) and coincident with or preceding the appearance of URTI in collegiate soccer players (Nakamura et al., 2006). The higher incidence of infection reported in elite athletes may be due, at least in part, to the repetition of training sessions without allowing sufficient time for recovery. However, it has been suggested that, a 3-h rest is adequate for recovery from previous cycling exercise (Li and Gleeson, 2004).

In addition to environmental, dietary, and psychological stress, a possible reason for the variability found in the literature could be the difference in time of day at which the measurements were taken. Most components of the immune system show rhythmic changes. Mucosal immunity is also subject to a diurnal variation. There is some evidence for a morning peak in s-IgA (Dimitriou et al., 2002; Li and Gleeson, 2004) in rested subjects. The hypothalamic pituitary adrenal (HPA) axis is also characterised by a marked circadian pattern. Pulsed ACTH activity is heightened during early morning in day-active individuals. This hypothalamic pituitary pattern is reflected in free cortisol measured in saliva. The normal pattern is a morning peak and evening nadir (Smyth et al., 1997). In sport and clinical research, the persistence of circadian rhythms during exercise conditions could lead to errors in interpreting physiological

responses. It could also invalidate experiments in which measurements were not controlled for time of day. Diurnal variation should therefore be taken into account when determining the effects of exercise on s-IgA and cortisol response.

Data from our laboratory would suggest that in terms of s-IgA responses, when a session of strenuous intermittent exercise specific to soccer is repeated after 48 h of rest, mucosal immunity may be compromised. Many athletes are not allowed even this time for recovery and may undertake two exercise sessions close together on the same day. We hypothesized that salivary variables such as flow rate, osmolality, s-IgA and cortisol concentrations are affected during two bouts of soccer-specific intermittent exercise compared with a single bout of identical exercise in one day. The aim of the present study was to examine the effects of one and two bouts of soccer-specific intermittent exercise within one day on s-IgA and cortisol.

6.2 Methods

Participants

Ten healthy males (Table 6.1) volunteered to participate and provided written informed consent before starting the study, which was approved by the University's Human Ethics Committee. The participants were regularly took part in exercise (moderate level exercise three times a week). They reported no significant oral, dental or other symptoms of infection; no subjects took any medication in their months prior to the experiment and all were non-smokers.

Each participant's height (m) was determined using a stadiometer (Seca, Germany). Nude body mass (kg) was evaluated before and after exercise using calibrated precision-weighing scales (Seca, Germany). The body mass index (BMI) was calculated and percent body fat was estimated using the skinfold method of Durnin and Womersley (1974).

Table 6. 1 Body composition and physiological characteristics of the subjects (mean ± SD) [n=10].

Age (years)	27 ± 5
Height (m)	1.80 ± 0.07
Mass (kg)	75.01 ± 9.10
BMI ($kg \cdot m^{-2}$)	23.1 ± 2.4
Body fat (%)	14.6 ± 1.5
$\dot{V}O_{2\,max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	58.6 ± 8.2
HR rest ($beats \cdot min^{-1}$)	61 ± 4

Experimental procedures

The aerobic fitness of the participants was assessed using an incremental test to determine maximal oxygen consumption ($\dot{V}O_{2\,max}$) was described in Chapter 3 (section 3.2.2).

The participants attended the laboratory on three occasions; temperature and relative humidity (mean ± SD) were 19 ± 1 °C and 45 ± 8 % RH, respectively. The first occasion, for determination of their $\dot{V}O_{2\,max}$, was preceded at least by three days by a visit for familiarisation to ensure that participants were comfortable with the exercise protocol and were able to cope with its demands. After the visit to determine $\dot{V}O_{2\,max}$, participants reported to the laboratory on two occasions, in a counterbalanced order, with one week apart to undertake soccer-specific exercise on the motorized treadmill over 90 min, at an exercise intensity designed to mimic that observed when playing a game (Reilly, 2005). For the "afternoon only" exercise trial (PMEX), participants reported to the laboratory at 14:00 h, and then performed soccer-specific intermittent exercise at 14:30 h. On another occasion, which involved two trials of the same exercise protocol, participants reported to the laboratory at 10:00 h, and again at

14:00h, and performed two segmented bouts of exercise ($AMEX_1$ started at 10:30 h and $PMEX_2$ started at 14:30 h).

The participants were asked to refrain from strenuous exercise for 48 h prior to and after each exercise trial, avoid alcohol and caffeine (Bishop et al., 2006) for 24 h and food for 3 h before exercise. Participants were also asked to empty their bladders before measurement of body mass. On arrival at the laboratory, the participants gave verbal confirmation of their adherence to these directions. Afternoon exercise trials were scheduled for the same time of day to negate the effects of circadian variation. Each session was preceded by a general warm-up consisting of general stretching exercises and approximately 5 min of running on the treadmill. Saliva samples were collected before exercise and immediately post-exercise in the exercise trials. Heart rate was monitored every 5 s during exercise. Subjective ratings of exertion (RPE) were obtained at 15-min intervals using Borg's Scale (1982).

Experimental protocol

Laboratory-based exercise protocols have been devised to replicate the physiological demands of intermittent exercise specific to soccer. The protocol used in this study was that originally designed by Drust et al. (2000). This protocol consisted of five different exercise categories, representing the increasing exercise intensities (standing, walking, jogging, cruising and sprinting) that are observed in soccer match-play. The protocol was composed of six 15-min periods. After the first 45 min and a 15-min "half-time" intermission, the exercise was continued for a further 45 min to correspond to the temporal pattern of a soccer game (as developed in study 2). This protocol has been shown in a previous study to induce physiological responses typical of soccer games played during training sessions.

Participants were allowed to drink water ad libitum before being weighed pre-exercise and after being weighed post-exercise, with the exception of the 10-min period prior to each saliva collection. At half-time each participant was also given 5 $ml.kg^{-1}$ of water.

Analytical methods

Methods of preliminary measurements and saliva collection and analysis were presented in Chapter 3. The coefficients of variation for the analytical methods were 1.7% for osmolality, 1.4% for total protein, 1.6 % for IgA, and 1.9 % for cortisol. The test-retest correlation of the s-IgA, cortisol and total protein procedures in this laboratory are all 0.99.

Statistical analysis

Data are presented as mean ± SD. The data were analysed using a two-factor (3 trials × 2 times) repeated measures analysis of variance (ANOVA). Normality of distribution for all variables was checked with the Shapiro-Wilk test. Assumptions of homogeneity and sphericity in the data were checked and, where appropriate, adjustments in the degree of freedom for the ANOVA were made. Significant F-values from the ANOVA were followed up with the "repeated" specific contrast that is available in SPSS. The effect size was classified according to the system proposed by Cohen (1988), where an effect size of 0.2 represents a small effect, one of 0.5 represents a moderate effect and one of 0.8 or above represents a large effect. Statistical significance was set at an alpha value of $P<0.05$.

6.3 Results

Heart Rate and Rating of Perceived Exertion during exercise

The mean heart rates (HR) for the afternoon intermittent exercise trial (PMEX), and the twice-a-day trials ($AMEX_1$ and $PMEX_2$) were 152 ± 11, 149 ± 11 and 159 ± 11 beats·min^{-1} respectively, with significantly higher HR responses in $PMEX_2$ than $AMEX_1$ ($P=0.001$). The difference between PMEX and $PMEX_2$ was statistically significant ($P<0.05$). The mean heart rate for the PMEX and $AMEX_1$ trials did not differ significantly ($P>0.05$).

Ratings of perceived exertion that were recorded at 15-min intervals averaged 12.5 ± 1.5, 13.0 ± 2.0 and 16.0 ± 1.3 for the PMEX, $AMEX_1$ and $PMEX_2$ sessions, respectively. The ratings of exertion were higher in $PMEX_2$ compared with $AMEX_1$ and PMEX ($P<0.001$). There were no differences in RPE between PMEX and $AMEX_1$ ($P>0.05$).

Changes in body mass

The body mass recorded before, at half-time and after each trial indicated there was a significant main effect of time on body mass ($F_{1.5,\ 13.4}=250.7$, $P=0.001$) [Table 6.2]. There were no differences in loss of body mass between single and repeated bouts of the exercise trials ($P>0.05$). The net losses of body mass during PMEX, $AMEX_1$ and $PMEX_2$ were 1.6 ± 0.3, 1.5 ± 0.3 and 1.1 ± 0.2 kg, respectively.

Table 6. 2 Body mass before, half-time and after soccer-specific intermittent exercise protocols (mean ± SD)[n=10].

Variable		Before Exercise	Half-Time	After Exercise
Mass	PMEX	75.0 ± 9.1	74.3 ± 9.0[a]	73.7 ± 8.9[a,b]
(kg)	$AMEX_1$	75.1 ± 8.9	74.5 ± 9.0[a]	74.0 ± 9.1[a,b]
	$PMEX_2$	74.8 ± 9.0	74.1 ± 8.9[a]	73.5 ± 8.9[a,b]

a: P<0.05. Significant difference compared with pre-exercise. b: P<0.05. Significant difference compared with half-time.

Salivary responses to once and twice daily soccer-specific intermittent exercises

There was a significant main effect of time for saliva flow rate ($F_{1,\ 9}$ = 47.9, P= 0<0.001) which was decreased following exercise in all cases compared with pre-exercise. There were no differences in salivary flow rate between the PMEX session and repeated ($AMEX_1$ and $PMEX_2$) bouts of exercise (Table 6.3).

There was a significant main effect of time ($F_{1,\ 9}$ =28.7, P=0.001) and an interaction between trial and time ($F_{1.8,\ 16}$=4.0, P=0.037) on saliva osmolality. Saliva osmolality increased after completion of each exercise trial compared with pre-exercise. There were no differences in saliva osmolality between the bouts of exercise [Table 6.3], indicating no time-of-day or "repeated-bouts" effect.

The saliva-solute rate of secretion did not change significantly between the exercise sessions. There was not a significant main effect of time for salivary solute secretion rate (Table 6.3).

There was a significant main effect of time ($F_{1,9}$ =47.6, P<0.001) and an interaction between exercise trial and time ($F_{2,18}$=4.5, P=0.026) on saliva protein concentration. Saliva protein concentration increased from 0.81 ± 0.25 $g \cdot l^{-1}$ before PMEX, to 1.60 ± 0.50 $g \cdot l^{-1}$ after exercise, from 0.74 ± 0.4 $g \cdot l^{-1}$ to 1.54 ± 0.44 $g \cdot l^{-1}$ for $AMEX_1$ and from 0.74 ± 0.2 $g \cdot l^{-1}$ to 1.80 ± 0.4 for $PMEX_2$ significantly (P<0.05). Similarly, a significant main effect of time ($F_{1,9}$ =5.3, P<0.05) and an interaction between exercise trial and time ($F_{1.9,17.1}$=6.50, P=0.009) for salivary protein secretion rate were observed [Table 6.3].

Salivary immunoglobulin A (s-IgA)

The main effect of time ($F_{1,9}$ =27.13, P=0.001) and the interaction between trial and time ($F_{1.7,15.6}$=4.2, P=0.03) for s-IgA were significant [Table 6.3]. The s-IgA concentration was increased immediately after $AMEX_1$ was conducted and fell to below the base-line 2.25 h after the exercise trial. There was a rise throughout $PMEX_2$ from 117 ± 64 to 388 ± 187 $mg \cdot l^{-1}$(P<0.05), but this magnitude was not significantly different from $AMEX_1$. The s-IgA concentration was increased immediately after all three experimental trials. Salivary IgA concentration for PMEX, $AMEX_1$ and $PMEX_2$ increased 186%, 57% and 231% compared with pre-exercise, respectively. There were no differences in s-IgA between the single and repeated bouts of exercise (Table 6.3).

The IgA secretion rate peaked after exercise in all cases compared with pre-exercise. The IgA secretion rate increased significantly from 87.12 ± 41.8 $\mu g \cdot min^{-1}$ before PMEX, to 198 ± 113 $\mu g \cdot min^{-1}$ after exercise and from 147 ± 47 to 167 ± 57 $\mu g \cdot min^{-1}$ for $AMEX_1$ and from 85 ± 39 to 175 ± 109 $\mu g \cdot min^{-1}$ for $PMEX_2$ ($F_{1,9}$ =11.3, P= 0.008). There were no differences in s-IgA secretion rate between the single and repeated bouts of exercise (Table 6.2).

There were significant main effects of time ($F_{1,9}$ =9.22, P<0.05) and trial ($F_{1.6,14.6}$=4.5, P=0.03), and interactions between trial and time ($F_{2,18}$=3.8, P=0.04) for the ratio of s-

IgA to osmolality. The ratio of s-IgA to osmolality increased immediately after both the single afternoon trial (PMEX) and the morning exercise trial ($AMEX_1$). A second afternoon trial ($PMEX_2$) pre-exercise ratio that was significantly lower than post-$AMEX_1$, increased again to post-exercise significantly (Fig 4). Similarly, there was a significant main effect of trial ($F_{2, 18}$=6.0, P=0.01) and an interaction between trial and time ($F_{2, 18}$=8.2, P= 0.003) for s-IgA to protein concentrations. However, s-IgA to protein ratio did not differ significantly during exercise (Table 6.3).

Salivary cortisol concentration

The comparison between pre-exercise and post-exercise cortisol concentration neared statistical significant difference (P=0.08). No significant difference between experimental trials (single and repeated) was evident (Table 6.3).

Table 6.3 The effects of once and twice soccer-specific exercise on various salivary parameters (mean ± SD)[n=10].

Variable	Time-point	PMEX	$AMEX_1$	$PMEX_2$	Exercise	Time	Exercise × Time
Saliva flow rate ($\mu l.min^{-1}$)	Pre-exercise	721±207	748±272	860±394	P> 0.05	P< 0.001	P> 0.05
	Post-exercise	574±189[a]	559±240[a]	556±346[a]			
Osmolality ($mOsmol.kg^{-1}$)	Pre-exercise	74.8±12.8	78±18.2	72.6±7.8	P> 0.05	P< 0.001	P= 0.04
	Post-exercise	126.4±44.4[a]	121.3±33.0[a]	146.3±46.7[a]			
Solute secretion rate ($\mu Osmol{\cdot}min^{-1}$)	Pre-exercise	52.8±13.3	56.3±19.4	61.1±23.9	P> 0.05	P= 0.06	P> 0.05
	Post-exercise	68.8±23.6	64.4±25.5	71.5±38.4			
Protein concentration ($g.l^{-1}$)	Pre-exercise	0.81±0.25	0.74±0.37	0.74±0.18	P> 0.05	P< 0.001	P= 0.02
	Post-exercise	1.56±0.5[a]	1.55±0.44[a]	1.8±0.39[a]			
Protein secretion rate ($\mu g.min^{-1}$)	Pre-exercise	121±70	250±158	115±47	P=0.08	P= 0.047	P= 0.009
	Post-exercise	137±94[a]	108±24[a]	92±50[a]			
IgA concentration ($mg.l^{-1}$)	Pre-exercise	125±71	220±108	117±64	P> 0.05	P= 0.001	P= 0.03
	Post-exercise	358±166[a]	346±157[a]	388±187[a]			
IgA Secretion rate ($\mu g.min^{-1}$)	Pre-exercise	87±42	147±47	85±39	P>0.05	P= 0.008	P> 0.05
	Post-exercise	198±113[a]	167±57[a]	175±109[a]			
IgA to osmolality ratio ($\mu g.mOsmol^{-1}$)	Pre-exercise	1.6±0.6	2.7±1.0	1.6±0.8[b]	P= 0.028	P= 0.014	P= 0.043
	Post-exercise	2.8±1.1[a]	2.9±1.2[a]	2.7±1.1[a]			
IgA to protein ratio ($mg.g^{-1}$)	Pre-exercise	163±77	320±104	158±90[b]	P= 0.01	P> 0.05	P= 0.003
	Post-exercise	241±107	221±87	216±107			
Cortisol ($ng.ml^{-1}$)	Pre-exercise	5.8±2.1	9.9±6.1	6.8± 3.5	P= 0.097	P=0.087	P> 0.05
	Post-exercise	8.0±1.9	9.7±4.2	11.1±4.3			

Significant difference compared with pre-exercise: a: P<0.05 Significant difference compared with post-$AMEX_1$: b: P<0.05.

6.4 Discussion

The main findings of the present study were (a) a second exercise bout on the same day induced higher HR and RPE responses than a single bout; (b) performing a soccer-specific intermittent exercise protocol in single and/ or in repeated bouts acutely increased s-IgA concentrations and secretion rate while cortisol concentrations remained unchanged; (c) the second soccer-specific intermittent exercise had similar effects on salivary flow rate, osmolality, total protein, s-IgA concentration, secretion rate, s-IgA to osmolality and protein ratios and salivary cortisol in the short term; (d) s-IgA concentrations and secretion rate as well as cortisol were not affected by the times of day; (e) salivary IgA displayed similar responses to two soccer-specific exercise sessions in one day, at a moderate intensity with a 2.25 h rest in between.

Salivary flow rate was reduced significantly post-$AMEX_1$ compared with pre-exercise and returned to above initial level before $PMEX_2$ commencement. At higher exercise intensities, salivary secretion decreases due to sympathetic stimulation and substantial vasoconstriction (Nieman et al., 2002) and/or concomitant parasympathetic withdrawal (Bosch et al., 2002) which limits secretion rate. Previous studies have also consistently indicated a decrease in salivary flow rate during strenuous exercise (Walsh et al., 2004a). The magnitudes of the decrease in the present study were: PMEX (20.4%), $AMEX_1$ (25.3%) and $PMEX_2$ (35.4%). As the difference between exercise trials was non-significant, these results indicate that salivary flow rate in response to soccer-specific exercise is not necessarily affected by performing more than one bout of intermittent exercise on the same day.

Saliva osmolality rose during each of the exercise trials. These increases are due to a combination of a significant reduction in the volume of saliva secreted (Walsh et al., 1999) and the progressive dehydration present despite the administration of water to participants at half-time and in the time window between the exercise trials (Walsh et al., 2004a).

Mean saliva concentration of IgA and s-IgA secretion rate were increased by the performance of soccer-specific intermittent exercise trials designed to replicate the temporal sequencing experienced in professional settings. Performance of a second bout of soccer-specific exercise ($AMEX_1$ + $PMEX_2$) increased the concentration of s-IgA 200% more than a single session immediately post-$PMEX_2$. Salivary IgA concentration decreased after the 2.25 h rest in between sessions to a value 88% below the initial pre-$AMEX_1$. The lack of statistical significance between single and repeated bouts of soccer-specific exercise is likely attributable to the large intra-individual and inter-individual variation in s-IgA. The overall effect size of 0.14 would not amount to a "small difference" between the three exercise trials, whereas the effect size of 0.48 would indicate a medium difference between the single and the repeated bouts of exercise. It has been demonstrated that s-IgA is stimulated by α-adrenernoreceptors (Proctor et al., 2003) and depressed 24 h after a single injection of glucocorticoid (Wira et al., 1990). The observed increase in s-IgA concentration and secretion rate immediately post-exercise in the present study suggests that cortisol does not affect s-IgA responses. These results are supported by reports that the exercise-induced fall in s-IgA concentration occurs between 2 and 24 h after prolonged strenuous exercise (Mackinnon et al., 1987; Gleeson et al., 2001). The reason for this increase may be partly attributable to higher sympathetic activity and prevention of bacterial adherence and translocation (Alverdy and Aoys, 1991) which play a role in immuno-enhancement in the short term. Further investigations of long-term responses to such experimental exercise interventions are warranted.

Most salivary proteins are secreted via the exocytosis of protein storage granules in salivary acinar cells (Proctor, 1998). Salivary concentrations of total protein increased significantly with exercise in each trial, the increase over pre-exercise values being similar on each occasion. Performing soccer-specific intermittent exercise in the single and in the repeated sessions had similar effects on salivary protein. Exercise is known to increase sympathetic activity and the higher protein concentration in saliva following exercise may be due to increased β-sympathetic activity in the salivary glands (Dawes, 1981). The alteration seemed to stem from the reduction of saliva flow

rate since decreases in salivary protein secretion rate were observed after $AMEX_1$ and $PMEX_2$.

Responses of s-IgA expressed as either the ratio of s-IgA relative to osmolality or s-IgA to protein ratio have been used to correct for the loss of salivary flow rate (Mackinnon and Jenkins, 1993; Blannin et al., 1998). Salivary IgA relative to osmolality showed a significant increase during each of the three exercise sessions. Therefore, both IgA secretion rate and s-IgA to osmolality ratio increased as a consequence of soccer-specific exercise. Regarding the non-significant change of s-IgA to protein ratio during all these exercise trials, it seems that correcting for protein concentration is unsuitable since protein secretion rate itself was increased during the three exercise trials.

Salivary cortisol during all three exercise sessions was increased by an average of 28%, 2% and 39%, respectively immediately after PMEX, $AMEX_1$ and $PMEX_2$. Thus salivary cortisol responses to soccer-specific exercise are not additively affected by two bouts on the same day. It has been demonstrated that exercise at higher than 60% $\dot{V}O_{2\,max}$ increases cortisol concentrations (Galbo et al., 1979). The heart rate and perceived exertion responses suggest that the intensity of exercise may not have exceeded this level. Changes in cortisol exhibit a circadian variation (Reilly et al., 1997) but the 2.25-h time difference between measurements may not have been enough to influence the post-exercise values and allow a diurnal variation to be detected (Dimitriou et al., 2002).

The present study demonstrated that baseline levels of salivary protein secretion rate, s-IgA concentration and secretion rate, IgA to osmolality and protein ratios and salivary cortisol showed peaks in the morning and falls in the afternoon. These trends are consistent with established circadian rhythms (Minors and Waterhouse, 1981).

The completion of the second soccer-specific intermittent exercise ($PMEX_2$) was associated with increases in HR and RPE compared with $AMEX_1$ and PMEX (single bout of exercise). The neuroendocrine changes such as increased levels of adrenaline and noradrenaline in the early afternoon (Minors and Waterhouse, 1981) and insufficient recovery could enhance the physiological and psychophysical states of subjects throughout the second bout of exercise. Heart rate usually reaches a high point of its circadian rhythm around 14:00 h during moderate exercise (Reilly and Brooks, 1990) which may explain why HR during exercise in the afternoon (PMEX) was higher than in the morning (AMEX). The mean heart rate values at pre-start between $AMEX_1$ and $PMEX_2$ were not significantly different. There was a mean difference of 3 units in RPE between PMEX and $PMEX_2$ that cannot be ignored. However, there were no differences in perceived exertion between responses at 10:30 h and 14:30 h on the exercise protocol, in line with observations of Dimitriou et al. (2002). Perceived exertion may not be as sensitive as heart rate to diurnal variation in response to submaximal exercise, since a step increase of one unit on the RPE scale corresponds to an increase in HR of 10 beats·min^{-1}.

Body mass decreased after each exercise trial, in spite of fluid replacement at 5 ml·kg^{1}of body mass during half-time and *ad libitum* between exercise sessions. The reduction in body mass indicates a progressive dehydration, but performing more than one bout of soccer-specific exercise with a limited recovery period did not affect the loss of body mass differentially. The mean difference in body mass pre-start between $AMEX_1$ and $PMEX_2$ was not significant. There was also no significant difference between once-a-day and repeated exercise. Allowing for fluid intake and urination, the sweat rate can be estimated as averaging 1.1 ± 0.2, 1.0 ± 0.2 and 1.1 ± 0.2 l·h^{-1} for PMEX, $AMEX_1$ and $PMEX_2$ respectively. A significant increase in saliva osmolality accompanied this dehydration.

A limitation of this study was that the 2.25-h time window between $AMEX_1$ and $PMEX_2$ may not have been enough to allow a diurnal variation to be detected in this investigation. Furthermore the performance of two soccer-specific intermittent protocols even at this level of intensity in one day restricted the investigator from increasing the exercise intensity for participants in present study.

In conclusion, performing a second bout of soccer-specific exercise in one day with limited time for recovery increased the s-IgA concentration, but did not have a significant carry-over suppressive effect from the first exercise session on s-IgA responses. In terms of s-IgA responses, a cumulative effect of previous exercise when performing a further exercise bout with 2.25 h rest between sessions cannot be ruled out. The salivary cortisol levels are consistent in suggesting that this exercise protocol was not significantly stressful for these participants to demonstrate compromised mucosal immunity. Furthermore the time window between 10:30 and 14:30 h was inadequate for any diurnal variation in salivary responses to exercise to be displayed.

Chapter 7

Study 4

Effects of Carbohydrate Ingestion on s-IgA to Intermittent Exercise in the Heat

Chapter 7

Effects of carbohydrate ingestion on salivary IgA to intermittent exercise in the heat

The findings of the previous studies have demonstrated that not only a single bout of soccer-specific exercise, but also repeated bouts of soccer-specific exercise did not have a significant carry-over suppressive effect on s-IgA responses. These studies were conducted in a thermo-neutral environment. Since soccer matches at major tournaments are regularly played in hot conditions, the effects of soccer-specific exercise in the heat with carbohydrate supplementation on s-IgA responses were the focus of the present study.

7.1 Introduction

Under normal environmental conditions, exercise-induced changes in immune response are relatively small and short-lived, limiting the "open-window" period when the likelihood of incurring an acute infection might be increased (Shephard, 2000). Heat exposure is a form of stress in which elevations in body core temperature occur with concomitant alterations in hormonal and immune responses (Brenner et al., 1995; Cross et al., 1996; Brenner et al., 1998). Exercise in a thermally stressful environment appears to have an additive effect on the hormonal and immune system disturbances compared to heat alone (Shephard, 1998; Shephard et al., 1998; Shephard and Shek, 1999).

There is evidence of an acute increase in the synthesis of immunoglobulins after performing exercise under hot conditions (Brenner *et al.*, 1996). This increase may reflect immediate changes in the proportions of T helper and B cells in the blood (Shephard, 2002). Ford *et al.* (1997) suggested that exercise decreases salivary flow rate, which is further exacerbated by increases in ambient temperature. Regular fluid intake appears to prevent the exercise-induced decrease in flow rate. Athletes

performing prolonged exercise in the heat, with associated increased sympathetic activity and increased fluid losses, might exhibit larger reductions in saliva flow rate and possibly s-IgA secretion rate than when they perform the same exercise in thermoneutral conditions. Fluid can help to keep levels of oral pathogens low and temper the drying of the airways that occurs during exercise, thereby reducing the vulnerability of the upper respiratory mucosal to invading pathogens. Laing *et al.* (2005) showed that a prolonged bout of cycling in the heat evoked a reduction in s-IgA secretion rate, but did not influence s-IgA responses to prolonged exercise when water intake was allowed *ad libitum*.

The addition of carbohydrate to fluid ingested of fluids before and at regular intervals during prolonged and high-intensity intermittent exercise may extend exercise duration (Bishop et al., 1999). It may also attenuate perturbations in immune function (Coyle, 1991; Gleeson et al., 2004b) and decrease the potential for developing symptoms of respiratory tract infections (Nieman and Pedersen, 1999). Carbohydrate ingestion may also attenuate the rise in cortisol concentrations during exercise by maintaining plasma glucose concentrations (Bishop et al., 1999). Cortisol has been shown to inhibit transepithelial transport of s-IgA (Sabbadini and Berczi, 1995), to inhibit in vivo B lymphocyte antibody synthesis (Saxon et al., 1978) and has been implicated in the decreased B lymphocyte antibody synthesis after exercise (Nehlsen-Cannarella et al., 1991). A larger reduction in s-IgA concentration and secretion rate might therefore be expected when prolonged exercise is performed -in hot compared with thermoneutral conditions.

Soccer is a sport that consists of intermittent high-intensity bursts of activity leading to high rates of metabolic heat production. It can be played under hot environmental conditions and fluid replacement is limited during games. Fluid intake before and during the game would reduce the degree of dehydration experienced. The addition of carbohydrate to fluids may supplement the body's limited carbohydrate stores to prevent muscle glycogen depletion and potential adverse changes in immune response.

The aim of the current study was to examine the effects of carbohydrate supplementation to soccer-specific intermittent exercise and heat exposure on s-IgA and cortisol responses.

7.2 Methods

Participants

Ten healthy males (Table 7.1) provided written informed consent for the study, which was approved by the University's Human Ethics Committee. The participants were all recreationally active (moderate level exercise three times a week), were non-smokers, reported no significant oral, dental or other symptoms of infection and were not taking any medication in the month prior to the conduct of the experiment.

Table 7. 1 Physiological and anthropometrics characteristic of the subjects (mean ± SD) [n=10]

Age (years)	26 ± 4
Height (m)	1.77 ± 0.5
Mass (kg)	74.2 ± 5.7
BMI ($kg\ m^{-2}$)	23.4 ± 2
$\dot{V}O_{2\,max}$ ($ml\ kg^{-1}\ min^{-1}$)	62.6 ± 5.6
HR rest ($beats \cdot min^{-1}$)	60 ± 4

Each participant's height (m) was determined using a stadiometer (Seca, Germany). Nude body mass (kg) was also recorded before and after exercise using calibrated precision-weighing scales (Seca, Germany). Whole-body sweat loss was determined by measuring the change in body mass, corrected for fluid intake and urinary losses (Broad et al., 1996).

Experimental procedures

The participants were assessed for aerobic fitness using an incremental test to determine maximal oxygen consumption ($\dot{V}O_{2\,max}$). The procedures for establishing $\dot{V}O_{2\,max}$ were described in Chapter 3 (section 3.2.2).

The participants attended the laboratory on four occasions. The first occasion; was three days prior to determination of $\dot{V}O_{2\,max}$, was for familiarisation to ensure that participants were comfortable with the protocol and was able to cope with its demands. After the second visit to determine $\dot{V}O_{2\,max}$, participants reported to the environmental chamber (30°C, 40 ± 2 % RH) on two more occasions, to undertake soccer-specific intermittent exercise for 90 min on the motorized treadmill (hp cosmos, Germany). These two sessions were separated by seven days. During the week between tests, the participants followed their normal diets and lifestyle pattern.

The participants were asked to avoid strenuous exercise for 48 h prior to and after each exercise trial. No alcohol or caffeine was allowed for 24 h or food for 3 h prior to exercise. After arriving at the laboratory, the participants confirmed orally their compliance with these directions. Each test was scheduled for the same time of day to negate the effects of circadian variation (Reilly and Brooks, 1986). Each session commenced with a general warm-up consisting of general body stretching exercises and about 5 min of running on the treadmill. Saliva samples were collected four times: these were before the exercise trials, immediately after, then 24 and 48 h post-exercise. Short-range radio telemetry was used to monitor heart rate during exercise every 5 s. Participants rated their thermal sensation (Tsens) and perceived exertion (RPE) every 15 min using the thermal sensation (Toner et al., 1986) [Table 7.2] and Borg (Borg, 1982) scales, respectively.

Table 7. 2 Thermal sensation scale of Toner et al. (1986).

Rating	Description
0.0	Unbearably cold
0.5	
1.0	Very Cold
1.5	
2.0	Cold
2.5	
3.0	Cool
3.5	
4.0	Neutral (comfortable)
4.5	
5.0	Warm
5.5	
6.0	Hot
6.5	
7.0	Very hot
7.5	
8.0	Unbearably hot

Experimental protocol

Laboratory-based exercise protocols have been devised to replicate the physiological demands that occur during intermittent exercise specific to soccer. The protocol used in this study was a modified version of that designed by Drust et al. (2000). The protocol (as developed in study 2) consisted of five different exercise categories, representing the increasing exercise intensities (standing "0", walking"4", jogging"10", cruising"13" and sprinting"19") that are observed in soccer match-play. The protocol was composed of six 15-min periods. After the first 45 min and a 15-min "half-time" intermission, the exercise was continued for a further 45 min to correspond to the temporal pattern of a soccer game (Chapter 3).

Carbohydrate administration

All participants were first given either a carbohydrate solution (CHO) or placebo (PLA), the treatment being reversed on the next visit. The study was double blind, placebo controlled, and randomized. The two experimental drinks were a 6% (w/v glucose) orange-flavoured carbohydrate solution or an artificially sweetened orange-

flavoured, carbohydrate-free solution (placebo). Each participant received a supplemental volume based on his body mass (3 $ml \cdot kg^{-1}$ body weight), 5 min before the exercise, at 15, 30 min, at half time, at 60 and 75 min into each exercise trial.

Analytical methods

Methods of preliminary measurements and saliva collection and analysis are presented in Chapter 3. The coefficients of variation of the analytical methods were 1.7% for osmolality, 1.9 % for IgA, and 2.8 % for cortisol.

Statistical analysis

Data are presented as mean ± SD. The data were evaluated using a two-factor (2 trial×4 time measurement) repeated measures analysis of variance (ANOVA) design. Normality of distribution for all variables was checked with the Shapiro-Wilk test. If residuals were found to be skewed or not normally distributed, statistical analysis was performed on the logarithmic transformation of the data. Assumptions of homogeneity and sphericity in the data were checked and, where appropriate, adjustments in the degrees of freedom for the ANOVA were made. Significant differences were analysed using post hoc by t-tests with an appropriate Bonferroni correction factor. The effect size was classified according to the system proposed by Cohen [9], where an effect size of 0.2 represents a small effect, one of 0.5 represents a moderate effect and one of 0.8 or above represents a large effect. Statistical significance was set at an alpha value of $P<0.05$.

7.3 Results

Heart Rate, Rating of Perceived Exertion and Thermal Sensation

Heart rate (main effect of time: $F_{1.5, 13.2}$=48.6, P<0.001), RPE (main effect of time: $F_{2, 17.6}$=38.9, P<0.001) and Tsens ($F_{1.9,17}$=19.6, P<0.001) increased significantly throughout the exercise trials. Heart rate was significantly higher in the CHO trial than when on the placebo (158 ± 7 vs. 155 ± 8 beats·min^{-1}, P<0.05), although the mean difference of 3 beats·min^{-1} should have little practical significance. Mean RPE was 13.7 ± 1.1 and 13.8 ± 1.3 for PLA and CHO, respectively, while the difference between the two treatment protocols was not significant (P>0.05). Thermal sensations (5.7 ± 0.7 and 5.6 ± 0.7 for CHO and PLA respectively) were not significantly different between the two conditions.

Body mass changes

After correction for fluid intake and urine output, body mass decreased significantly throughout the soccer-specific intermittent exercise under both conditions ($F_{1, 9}$=82.5, P<0.001). Values were not significantly different between two treatments (P>0.05). The net loss of body mass was 2.36 ± 0.25 kg during the PLA trial and 2.43 ± 0.6 kg during the CHO treatment. There were no differences in loss of body mass between the two treatments (Table 7.2)

Table 7. 3 Body mass before and after soccer-specific intermittent exercise with carbohydrate (CHO) and placebo (PLA) treatment (mean ± SD) [n=9].

Variable		Before Exercise	After Exercise
Mass (kg)	PLA	74.6 ± 5.5	73.6 ± 5.6[a]
	CHO	74.4 ± 5.9	73.2 ± 5.7[a]

a: P<0.001. Significant difference compared with pre-exercise. b: P<0.05.

Salivary responses

Two participants' data were omitted from the calculation due to deviation from the instructions for collection throughout the 48 h of the study period for both condition.

Salivary flow rate (main effect of time: $F_{1.8, 12.6}$=4.9, P<0.05) increased throughout the PLA treatment and decreased throughout the CHO treatment (interaction: $F_{2.1, 14.9}$=4.4, P<0.05). Difference between treatment conditions neared statistical significance (P=0.055). Whereas, the effect size of 0.2 would suggest a small difference between post-exercise compared with pre-exercise values in the two conditions.

Changes in s-IgA concentration were not significant either between different time points, or between CHO and PLA trials. There was a main effect of time whereby s-IgA secretion rate increased post-exercise by 51% ($F_{2, 14}$ =3.9, P<0.027). Salivary IgA secretion rate showed a greater, albeit non-significant increase post-exercise on CHO treatment compared to the PLA trial (P>0.05)[Table 6.3].

An approximate 30% increase in saliva osmolality after both exercise conditions approached significance (P=0.06). Solute secretion rate (main effect of time: $F_{1.8,}$

$_{12.9}$=5.33, P<0.05) increased throughout the both treatment trial time. Salivary IgA to osmolality ratio (μg·mOsmol^{-1}) demonstrated a non-significant change after PLA and CHO treatment trials (P>0.05) [Table 6.3].

Salivary protein concentration (g·l^{-1}) increased post-exercise and was close to pre-exercise levels by 48 h post-exercise after a sharp reduction 24 h post-exercise (main effect of time: $F_{3, 21}$=11.4, P<0.001). Protein concentrations were significantly higher on the CHO treatment condition compared with the PLA treatment condition immediately post-exercise and 24 h afterward (main effect of exercise: $F_{1, 7}$ =9.5, P=0.018). Salivary protein secretion rate (μg·min^{-1}) was increased after exercise ($F_{3, 21}$ =9.5, P<0.001). Salivary protein secretion rate was significantly higher on the CHO treatment after exercise and 24 h post-exercise than on the PLA treatment condition ($F_{1, 7}$=10.8, p=0.013). Salivary IgA to protein ratio (mg·g^{-1}) decreased post-exercise (main effect of time: $F_{2.3, 16.3}$=4.8, p=0.019). There were no differences in s-IgA to protein ratio between the two treatment conditions (Table 6.3).

Salivary cortisol (ng·ml^{-1}) decreased throughout 24 h after CHO condition (7.5 ± 4.2 to 3.6 ± 0.8 ng·ml^{-1}). Conversely, it increased immediately post-exercise with PLA treatment condition (6.3 ± 3 to 6.5 ± 1.5 ng·ml^{-1}) and decreased 24 h later (main effect of time: $F_{1.7, 11.6}$=4.2, P<0.05). The difference in salivary cortisol between the two conditions neared statistical significance (Table 6.3).

Table 7.4 The effect of ingesting carbohydrate (CHO) and placebo (PLA) on various salivary parameters during a soccer-specific intermittent exercise in the heat (mean ± SD)[n=8].

Variable	Treatment	Pre-exercise	Post- exercise	2 4h after exercise	48 h after exercise	Time	Treatment	Time× Treatment
Saliva flow rate ($\mu l.min^{-1}$)	PLA	520±257	620±244	1097±566	947±488	P=0.016	P=0.055	P=0.015
	CHO	630±413	612±224	805±476	662±265			
Osmolality ($mOsmol.kg^{-1}$)	PLA	74±15	100±28	77±9	87±21	P=0.06	P>0.05	P>0.05
	CHO	81±34	111±48	81±21	80±7			
Solute secretion rate ($\mu Osmol{\cdot}min^{-1}$)	PLA	38.1±15.2	57.3±18.2 [a]	84.0±44.4	82.8±50.0	P=0.012	P>0.05	P=0.04
	CHO	44.6±15.8	60.6±13.2 [a]	63.0±35.5	57.1±22.4			
Protein concentration ($g.l^{-1}$)	PLA	0.87±0.44	1.30±0.46	0.48±0.28 [b]	0.70±0.24 [b]	P=0.001	P=0.018	P=0.07
	CHO	0.93±0.42	1.45±0.42	0.91±0.37 [b]	0.68±0.08 [b]			
Protein secretion rate ($\mu g.min^{-1}$)	PLA	402±139	725±250[a]	450±183	579±157	P=0.001	P=0.013	P>0.05
	CHO	485±142	838±287 [a]	616±251	513±100			
IgA concentration ($mg.l^{-1}$)	PLA	153±42	197±70	147±879	210±123	P=0.09	P>0.05	P>0.05
	CHO	171±125	179±312	137±68	192±68			
IgA Secretion rate ($\mu g.min^{-1}$)	PLA	81.6±46.0	112.0±42.0	148.0±74.0	165.6±77.2 [a]	P=0.027	P>0.05	P>0.05
	CHO	96.4±56.7	156.6±146.7	92.2±41.0	126.4±65.8 [a]			
IgA to osmolality ratio ($\mu g.mOsmol^{-1}$)	PLA	2.11±0.79	1.94±0.66	2.11±0.94	2.41±1.30	P>0.05	P>0.05	P>0.05
	CHO	2.14±0.79	2.40±1.90	1.46±0.36	2.20±0.75			
IgA to protein ratio ($mg.g^{-1}$)	PLA	222±124	159±53	322±164	290±117	P=0.011	P>0.05	P>0.05
	CHO	190±93	187±168	152±39	279±79			
Cortisol ($ng.ml^{-1}$)	PLA	6.3±3.1	6.5±1.6	4.5±0.7 [b]	4.6±1.4	P=0.049	P=0.07	P>0.05
	CHO	7.5±4.2	5.7±1.54	3.6± 0.9[b]	4.6±1.8			

a: P<0.05, Significant difference compared with pre-exercise. b: P<0.05, Significant difference compared with post-exercise.

7.4 Discussion

The aim of the present study was to examine the effects of carbohydrate supplementation during soccer-specific intermittent exercise in the heat on mucosal immunity. Soccer-specific intermittent exercise in the heat has the potential for stimulating the sympathetic nervous system and reducing saliva flow rate, s-IgA concentration and secretion rate to a greater extent than when the exercise is performed in thermoneutral conditions (Laing *et al.*, 2005). It was hypothesised that carbohydrate ingestion before and at regular intervals during the soccer-specific protocol in the heat attenuates the immunosuppressive effects of combined stressors (exercise and heat exposure). The results failed to show that carbohydrate supplementation before and during the soccer-specific intermittent exercise protocol in the heat influenced RPE, Tsens, osmolality, solute secretion rate, s-IgA concentration, s-IgA secretion rate, s-IgA to osmolality ratio or s-IgA to protein ratio or salivary cortisol. Saliva flow rate neared significance and HR was increased with provision of carbohydrate. This increase in HR was deemed to have little practical consequences. These findings are in line with Nassis et al. (1998), but the precise mechanisms are unclear.

Body mass decreased during performance of both the exercises under either conditions, in spite of fluid replacement at 3 $ml \cdot kg^{-1}$of body mass before and at frequent intervals during exercise. The reduction in body mass indicates a progressive dehydration. The sweat rate can be estimated as averaging 1.57 ± 0.2 vs. 1.60 ± 0.4 $l.h^{-1}$ for PLA and CHO treatments, respectively. There was no difference between PLA and CHO conditions as same amount of fluid was ingested on each occasion.

Salivary IgA secretion rate increased immediately after completing the soccer-specific intermittent protocol. The increase in s-IgA secretion rate after exercise is in agreement with findings of others (Schouten et al., 1988; Tharp, 1991; Blannin et al., 1998; Bishop et al., 2000) but contrasts with studies showing no change (Walsh et al.,

1999; Li and Gleeson, 2004), or a decrease in s-IgA secretion rate after exercise (Mackinnon et al., 1993; Steerenberg et al., 1997; Walsh et al., 2002; Laing et al., 2005). The inconsistencies between these findings may be explained by differences in the hydration status of participants before and during the exercise trials (Laing *et al.*, 2005). As such current data suggest that a 1.5 % decline in hydration can be tolerated without imposing a detrimental effect on s-IgA post high intensity activity.

This reverse pattern of change in salivary flow rate after the CHO treatment compared to the PLA condition may have accounted for the corresponding non-significant alteration (46.7% increase post-exercise) in s-IgA concentration ($mg \cdot L^{-1}$) and concurrent increase in s-IgA secretion rate. These changes in salivary flow rate after CHO treatment is agreement with other studies (Ford et al., 1997; Walsh et al., 2004b; Laing et al., 2005). The main fluid constituent of saliva is water, which enters saliva from plasma across acinar cells and dehydration accounts for a 90% decrease in unstimulated saliva flow rate after 24 h without fluid or food (Ship and Fischer, 1997). The decrease in saliva flow rate after exercise was delayed by regular fluid intake irrespective of the type of treatment. Walsh *et al.* (2004) suggested that fluid availability per se has a greater involvement in the change in saliva flow rate during exercise. However, higher ambient temperature per se has been shown to exacerbate the exercise-induced decrease in saliva flow rate. Stimulation of the sympathetic nervous system, for example, by physical or psychological stress, causes vasoconstriction of the blood vessels supplying salivary glands, leading to a reduction in saliva secretion and vasodilatation of vessels by the parasympathetic stimulation resulting in a marked increase in regional blood flow (Chicharro et al., 1998). It has been suggested that sensory stimulation by CHO drinks based on whey with natural aromas (orange) increase salivary flow rate.

Salivary total protein increased after the soccer-specific intermittent exercise protocol on both treatments, likely due to effects of increased β-sympathetic activity on the

salivary glands (Dawes, 1981). In the present study, s-IgA to protein ratio decreased significantly after both treatments, a finding which is in line with Mackinnon and Jenkins (1993). Salivary IgA concentrations relative to total protein and other proteins may need to be corrected for changes in salivary volume occurring due to drying of oral surfaces as a result of exercise (Mackinnon et al., 1993). Salivary protein secretion rate into the salivary ducts also showed an increase after exercise using the present protocol; therefore, expressing s-IgA concentration relative to salivary total protein concentration may be misleading for reporting s-IgA after exercise (Dawes, 1981; Blannin et al., 1998; Walsh et al., 1999; Bishop et al., 2000). Expressing s-IgA relative to osmolality would be a more appropriate correction since the increases were in proportion to the fall in saliva flow rate. However, in the present study, neither osmolality or s-IgA relative to osmolality changed significantly after either trial.

It has been reported that cortisol concentrations are elevated above normal during a soccer match (Carli et al., 1986). Most likely the psychological stress of a real match provides an additional stimulus for cortisol secretion in addition to the physiological stress of exercise. Performing soccer-specific intermittent exercise in hot conditions with elevated saliva cortisol response should result in a decrease in s-IgA concentration, since cortisol has been shown to inhibit transepithelial transport of s-IgA (Sabbadini and Berczi, 1995), and to have a delayed inhibitory effect on in vivo B lymphocyte antibody synthesis (Saxon et al., 1978). Nevertheless, a reduction was not observed in s-IgA concentration after soccer-specific intermittent exercise on either condition. Therefore, a short-term or delayed (Gleeson et al., 2001) effect of salivary cortisol at the concentrations noted may not have an inhibitory effect on the transepithelial transport of s-IgA.

It is difficult to mimic the profiles observed between the soccer-specific intermittent protocol and match-play and this problem causes limitation in this study. These include the inability to recruit soccer players and thereby represent the additional stresses placed on players by competition. These including psychological stress and physical stress of contact with other players, the ball and match-play surface, a lower frequency of activity alterations, the omission of utility movements such as backwards and side-ways walking and jogging, the lack of game skills (e.g. kicking, heading, tackling, dribbling). The exclusion of these components may have resulted in non-significant change in s-IgA responses.

In conclusion, these data show that soccer-specific intermittent exercise results in an increase in s-IgA secretion rate. Additionally these results failed to show that carbohydrate supplementation before and at regular intervals during soccer-specific intermittent exercise in the heat, influenced mucosal immune response significantly in the present subjects.

Chapter 8

The Effects of Exercise on s-IgA Responses

"A Meta-Analysis"

Chapter 8

The effects of exercise on s-IgA responses – a meta-analysis

This chapter contains an account of the attempt to summarise the overall s-IgA responses to exercise in published studies using a more comprehensive and unbiased method of secondary data analysis.

8.1 Introduction

Exercise immunology is a relatively new area of research. The first scientific report on mucosal immunity was published 24 years ago (Tomasi et al., 1982). Although plenty of research findings have been published, the search continues for a comprehensive and generalised understanding of the mucosal immune system responses following exercise. The impact of exercise on mucosal immunity is primarily driven by the perception of a link between exercise-induced immune suppression and common illness, particularly upper respiratory tract infections (URTI). Despite more than two decades of research in this area of interest, there are still missing links in our understanding of the interactions between exercise and mucosal immunity which might emerge with a quantitative research synthesis.

The current approach within this thesis towards understanding the impact of exercise on mucosal immune responses has focused on variations in salivary immunoglobulin A (s-IgA) measured as either absolute concentration or corrected for change in salivary flow rate (secretion rate). Salivary IgA concentrations decrease in response to high-intensity exercise but remain either unaltered or increased in response to moderate-low intensity exercise (Gleeson et al., 2004a). Different descriptions of exercise or training stimuli, subject characteristics, methodological differences in assessment of s-IgA concentration and quality of expression of results are primary limitations in the existing literature. Like many exercise-related studies, in which

research participants are difficult to recruit and retain, sample sizes in the literature on s-IgA responses have generally been small leading to a lack of precision in the magnitude of true response.

The association between mucosal immunity and exercise has also been reviewed previously; most of these reviews have been in the form of a traditional qualitative summary of experimental findings (Gleeson, 2000a, b; Gleeson et al., 2004a). Although this traditional narrative is well accepted, it is usually non-quantitative and often unsystematic, and conclusions are often open to subjectivity (Hunt, 1997). These caveats to traditional methods have led to confusion in the literature with respect to specific and general trends for s-IgA concentration and secretion rate responses to exercise.

A more robust and quantitative approach to research synthesis has been proposed in the form of a meta-analysis of the research findings. This analytical technique minimizes subjectivity by standardizing selection, data pooling, and data analysis to draw conclusions. Although the meta-analytical approach offers a more standardized and unbiased method of data analysis, as compared with a traditional review, it is still confined to the assessment of research available at the time of writing and is still subject to biases such as researchers neglecting to publish small studies with "negative" findings, which are often not published (Egger et al., 2001).

The effect size (ES) proposed by Cohen (1988) and the meta-analysis, popularised by Glass (1977) provide for statistical evaluation of separate but related studies. The ES provides several benefits to researchers. First, it represents a standard unit for measuring and interpreting changes. Second, it allows for comparisons of different exercise or training methods within a single study. The classified effect size values were used in study 2 and 3 in this thesis, when the magnitude of s-IgA changes were

statistically small. Finally, when used as part of a meta-analysis, the ES provides an acceptable method for combining and comparing the treatment effects of related studies.

The purpose of this meta-analysis is two-fold:-firstly to highlight overall responses of s-IgA to exercise and secondly, to clarify the effects that some categorical moderator variables have on s-IgA responses. To the best of available knowledge, this is the first meta-analysis to examine this subject.

8.2 Methods

Data sources

Initial database screening. The search for literature was limited to English language citations to identify articles published between January 1982 and February 2006, and unpublished studies such as PhD theses. This time period is covered by Pub Med database, Ovid, Sport Discus and Medline (National Library of Medicine), related conference proceedings and scrutiny of reference lists from relevant articles. Medical subject heading (MeSh) keywords related to "mucosal immunity", "exercise" and "human" were then combined with the test phrase "salivary IgA". Terms for medical subject headings (MeSH) are contained in the National Library of Medicine's controlled vocabulary used for indexing articles in PubMed. The terminology of MeSH provides a consistent way to retrieve information that may use different terminology for the same concept.

A scan of each title from the PubMed database hits was then carried out. Articles were rejected if they indicated that the study did not involve a measurement of s-IgA, clearly did not involve exercise, or included a treatment such as carbohydrate. The ' acute exercise' classification involved any sport activity like swimming, cycling,

running, soccer and others that were performed only in 'one session', but the 'chronic exercise' classification represented to the same activities carried out over a period of 'one week' or more.

A search was also made of relevant journals, which were not indexed by PubMed. In addition, the reference sections of reviews that focused on s-IgA (Gleeson et al., 2004a) were analysed in a similar manner. The titles of the indexed references were then selected or rejected in accordance with the title scan mentioned previously.

The abstracts of the preliminary citations were then examined for the following criteria: 1) studies had to be published in English; 2) citations found to constitute a roundtable discussion, a letter, or comments were rejected.

Study Inclusion/Exclusion

Subjects. Only studies using subjects who were reported as healthy and non-infected virally were included for analysis.

Research design. If more than one independent study (conducted at different times) was included in a publication, each study met the inclusion criteria, and/or separate data were presented, each was counted as a separate study.

Outcome measure. In studies where the data for s-IgA concentration or secretion rate were presented in graphic form only, an attempt was made to contact the author and acquire the original data for more accurate inclusion in the meta-analysis. Where the data were unattainable, means and standard deviations were approximated from the

graphical presentation of data contained in the actual manuscript with the use of a millimetre ruler.

Data Extraction

Each of the studies that met the inclusion criteria was recorded on a coding sheet. The following characteristics of each study were recorded: Author(s), publication year, originating journal, study title, where the study was found (PubMed, cross-referenced, or hand searched) and exercise mode. A mean test-retest correlation of 0.70 was delimited for s-IgA concentration and secretion rate (Ring et al., 2002). This test-retest correlation was used in the calculation of the SD of the difference score.

Statistical analysis

The major objectives of this study were to synthesise and quantify the effects of exercise on s-IgA concentration and secretion rate. A random-effects model was used because the studies combined were different in many ways (e.g., study population, exposure and outcome assessment methods, follow-up duration and intensity). It was therefore inappropriate to consider all these studies as being drawn from the same population of studies.

Effect size calculation. The calculation of an effect size (ES) was a method of standardizing data for each study. The ES values of s-IgA and secretion rate were calculated in accordance with the method outlined by Deeks et al. (2005) in comprehensive meta-analysis software. An ES is defined as a unitless measure of the efficacy of each supplement centred at zero, if the exercise effect is no different. A scale for ES has been suggested by Cohen (1988), with 0.8 reflecting a large effect, 0.5 a moderate effect, and 0.2 a small effect. Where standard errors (SE) were reported in the original article, they were converted to standard deviations (SD) by multiplying SE

by the square root of the sample size. Results were considered significant if $P \leq 0.05$ was obtained. Confidence intervals (CI) were in all cases reported at the 95% level.

Heterogeneity of study results was statistically examined with the Q-test and I^2 value (Deeks et al., 2005). The presence of significant heterogeneity was followed up with pre-planned sub-group analyses for the categorical moderator variables (Egger et al., 2001). The within subjects SD was used together with the estimation of the test-retest correlation. The hypothesised categorical moderator variables in the sub-group analysis were exercise/training and exercise mode that included swimming, cycling, running, soccer and other sport activities. The outcome variables in the meta-analysis were the changes in mean s-IgA concentration and secretion rate.

All data analysis was completed using Comprehensive Meta-analysis, Version 2 (Biostat, Englewood, NJ). To evaluate publication bias, both Begg's (Begg and Mazumdar, 1994) and Egger's (Egger et al., 1997) tests were performed.

8.3 Results

Overall synthesis of effect sizes

Salivary IgA concentration

One hundred and six studies published since 1982 met the inclusion criteria, providing a total of 2113 research participants for the meta-analysis. The overall weighted mean change in s-IgA concentration was significantly increased post-exercise and post-training ($P<0.001$). The overall standardized difference in s-IgA concentration following both acute exercise and chronic exercise was found to be 0.166 (95% CI = 0.075 to 0.258). Nevertheless, there was evidence of substantial and statistically significant heterogeneity between studies (Q_{105} = 612.1, $P<0.0001$; I^2 = 82.8%).

Therefore, the investigation of possible moderating variables on the heterogeneity of standardized difference is relevant (Figure 8.1).

Salivary IgA secretion rate

Seventy-three studies since 1982 that reported s-IgA secretion rate, providing a total of 1421 research participants, qualified for the inclusion criteria. The overall weighted mean change in s-IgA secretion rate was significantly decreased for post-exercise. The overall standardized difference in s-IgA secretion rate following exercise or training was found to be -0.17 (95% CI = -0.31 to -0.02, P=0.02). Nevertheless, there was evidence of substantial and statistically significant heterogeneity between studies (Q_{72} = 611.8, P<0.0001; I^2 = 88.2%). Consequently, the investigation of possible moderating variables on the heterogeneity of standardized difference is relevant (Figure 8.2).

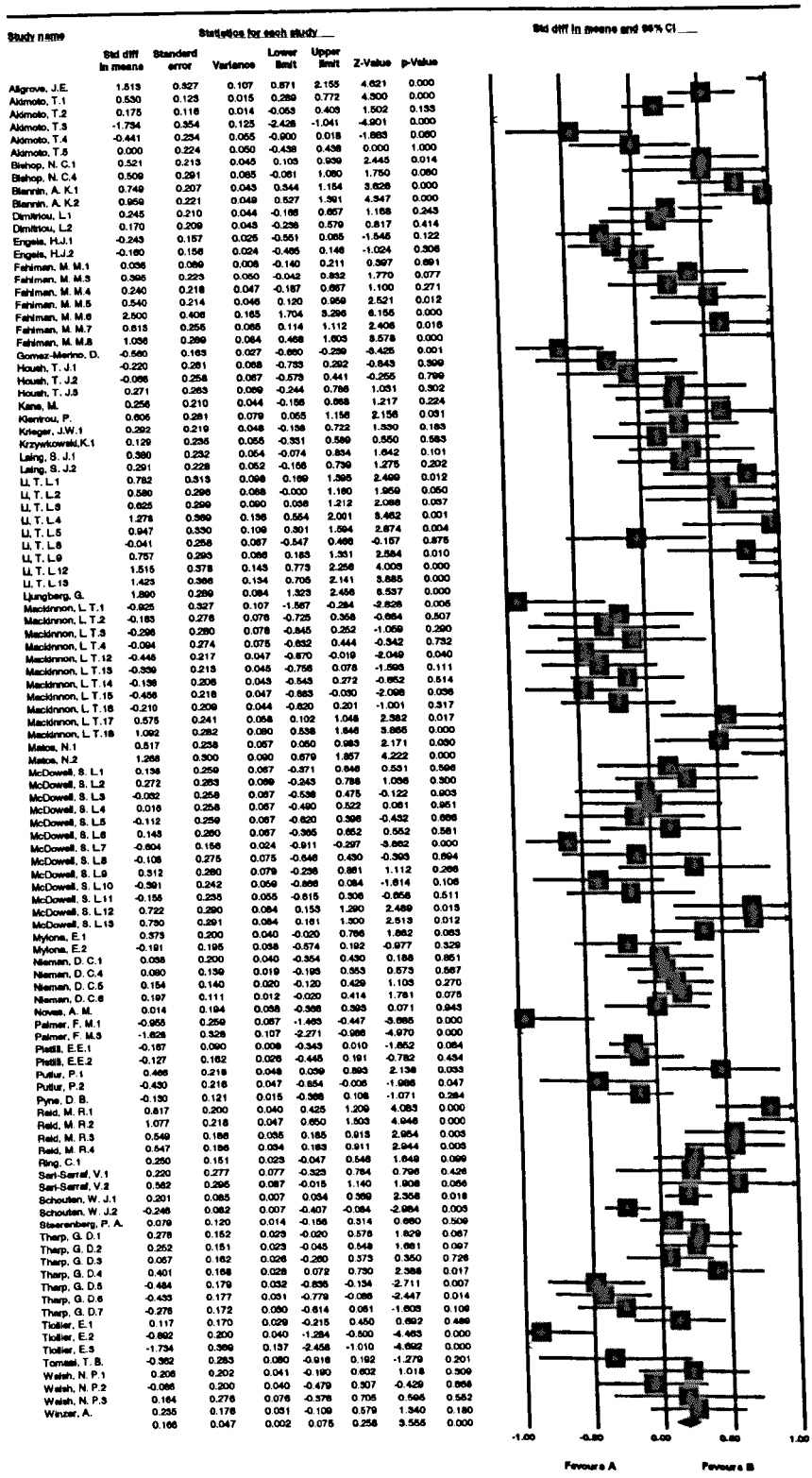

Figure 8.1 Forest plot showing standard difference in means for s-IgA concentrations and 95 % confidence limits for each study. The overall random-effects are shown in the bottom row of the plot.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Allgrove, J.E.	1.181	0.291	0.085	0.610	1.752	4.053	0.000
Akimoto, T.1	0.524	0.123	0.015	0.283	0.766	4.259	0.000
Akimoto, T.2	0.161	0.116	0.014	-0.066	0.389	1.389	0.165
Akimoto, T.3	-7.746	1.245	1.550	-10.186	-5.306	-6.222	0.000
Akimoto, T.4	-2.676	0.479	0.229	-3.614	-1.738	-5.592	0.000
Akimoto, T.5	-1.714	0.351	0.123	-2.403	-1.026	-4.879	0.000
Bishop, N. C.1	-0.238	0.203	0.041	-0.636	0.159	-1.174	0.240
Bishop, N. C.4	0.334	0.281	0.079	-0.218	0.885	1.187	0.235
Blannin, A. K.1	0.140	0.183	0.034	-0.220	0.499	0.762	0.446
Blannin, A. K.2	0.623	0.200	0.040	0.232	1.014	3.123	0.002
Dimitriou, L.1	-0.431	0.216	0.047	-0.855	-0.007	-1.991	0.046
Dimitriou, L.2	-0.527	0.221	0.049	-0.961	-0.094	-2.387	0.017
Engels, H.J.1	-0.436	0.162	0.026	-0.754	-0.118	-2.689	0.007
Engels, H.J.2	-0.272	0.158	0.025	-0.581	0.037	-1.724	0.085
Fahlman, M. M.1	-0.108	0.090	0.008	-0.284	0.067	-1.208	0.227
Fahlman, M. M.2	-0.918	0.181	0.033	-1.273	-0.563	-5.070	0.000
Fahlman, M. M.3	0.262	0.218	0.048	-0.166	0.690	1.199	0.230
Fahlman, M. M.4	0.350	0.221	0.049	-0.084	0.783	1.580	0.114
Fahlman, M. M.5	0.602	0.217	0.047	0.176	1.028	2.770	0.006
Fahlman, M. M.6	0.759	0.227	0.052	0.314	1.204	3.343	0.001
Fahlman, M. M.7	1.470	0.337	0.113	0.810	2.130	4.363	0.000
Fahlman, M. M.8	1.910	0.392	0.154	1.140	2.679	4.866	0.000
Krieger, J.W.1	0.132	0.216	0.047	-0.291	0.555	0.610	0.542
Krzywkowski, K.1	-0.072	0.234	0.055	-0.531	0.386	-0.310	0.757
Laing, S. J.1	-0.620	0.244	0.060	-1.099	-0.142	-2.540	0.011
Laing, S. J.2	-0.434	0.234	0.055	-0.893	0.024	-1.856	0.063
Li, T. L.1	-0.314	0.281	0.079	-0.864	0.235	-1.121	0.262
Li, T. L.2	-0.015	0.274	0.075	-0.552	0.522	-0.054	0.957
Li, T. L.3	0.074	0.274	0.075	-0.464	0.611	0.269	0.788
Li, T. L.8	0.284	0.263	0.069	-0.232	0.800	1.078	0.281
Li, T. L.9	-0.493	0.273	0.075	-1.029	0.043	-1.804	0.071
Li, T. L.12	-1.317	0.353	0.124	-2.008	-0.625	-3.733	0.000
Li, T. L.13	-1.146	0.332	0.110	-1.797	-0.494	-3.448	0.001
Ljungberg, G.	2.564	0.359	0.129	1.861	3.267	7.150	0.000
Mackinnon, L. T.2	-0.557	0.294	0.087	-1.134	0.020	-1.892	0.059
Mackinnon, L. T.3	-0.325	0.281	0.079	-0.876	0.226	-1.156	0.248
Mackinnon, L. T.4	-0.494	0.290	0.084	-1.063	0.074	-1.704	0.088
Mackinnon, L. T.5	-0.411	0.255	0.065	-0.910	0.089	-1.610	0.108
Mackinnon, L. T.6	-0.398	0.254	0.065	-0.897	0.101	-1.563	0.118
Mackinnon, L. T.7	-0.051	0.274	0.075	-0.486	0.588	0.186	0.852
Mackinnon, L. T.8	-0.299	0.280	0.078	-0.848	0.249	-1.069	0.285
Mackinnon, L. T.9	-0.332	0.301	0.090	-0.921	0.257	-1.104	0.270
Mackinnon, L. T.10	-0.927	0.350	0.123	-1.614	-0.241	-2.649	0.008
Mackinnon, L. T.11	-0.624	0.320	0.102	-1.251	0.003	-1.949	0.051
Matos, N.1	0.462	0.235	0.055	0.001	0.923	1.964	0.050
Matos, N.2	0.664	0.247	0.061	0.180	1.148	2.688	0.007
Nieman, D. C.1	-0.179	0.202	0.041	-0.574	0.217	-0.886	0.376
Nieman, D. C.2	-1.014	0.246	0.061	-1.496	-0.531	-4.119	0.000
Nieman, D. C.4	-0.742	0.157	0.025	-1.050	-0.434	-4.724	0.000
Nieman, D. C.5	-1.188	0.182	0.033	-1.544	-0.832	-6.538	0.000
Nieman, D. C.6	-0.889	0.129	0.017	-1.142	-0.635	-6.870	0.000
Novas, A. M.	-0.408	0.202	0.041	-0.803	-0.013	-2.025	0.043
Palmer, F. M.1	-0.842	0.250	0.063	-1.332	-0.352	-3.368	0.001
Palmer, F. M.3	-1.272	0.289	0.083	-1.838	-0.705	-4.402	0.000
Pistilli, E.E.1	-0.834	0.104	0.011	-1.037	-0.630	-8.032	0.000
Pistilli, E.E.2	-0.630	0.177	0.031	-0.977	-0.284	-3.563	0.000
Reid, M. R.1	0.490	0.183	0.034	0.131	0.849	2.673	0.008
Reid, M. R.2	0.051	0.173	0.030	-0.288	0.391	0.296	0.767
Reid, M. R.3	0.199	0.175	0.031	-0.144	0.541	1.136	0.256
Reid, M. R.4	0.066	0.173	0.030	-0.273	0.406	0.383	0.702
Ring, C.1	0.337	0.153	0.023	0.037	0.638	2.202	0.028
Sari-Sarraf, V.1	0.432	0.286	0.082	-0.129	0.993	1.508	0.131
Sari-Sarraf, V.2	0.351	0.282	0.080	-0.202	0.904	1.243	0.214
Thatcher, R.1	-0.259	0.321	0.103	-0.889	0.371	-0.806	0.420
Thatcher, R.2	-0.152	0.318	0.101	-0.776	0.471	-0.478	0.632
Thatcher, R.3	0.239	0.321	0.103	-0.390	0.867	0.744	0.457
Thatcher, R.4	-0.415	0.330	0.109	-1.061	0.231	-1.258	0.208
Thatcher, R.5	-0.435	0.331	0.109	-1.084	0.213	-1.315	0.188
Thatcher, R.7	0.125	0.317	0.101	-0.497	0.747	0.394	0.694
Walsh, N. P.1	-0.278	0.204	0.042	-0.678	0.121	-1.365	0.172
Walsh, N. P.2	-0.244	0.203	0.041	-0.641	0.154	-1.201	0.230
Walsh, N. P.3	-0.153	0.275	0.076	-0.693	0.386	-0.557	0.578
Winzer, A.	0.268	0.176	0.031	-0.077	0.614	1.522	0.128
	-0.169	0.074	0.006	-0.315	-0.023	-2.274	0.023

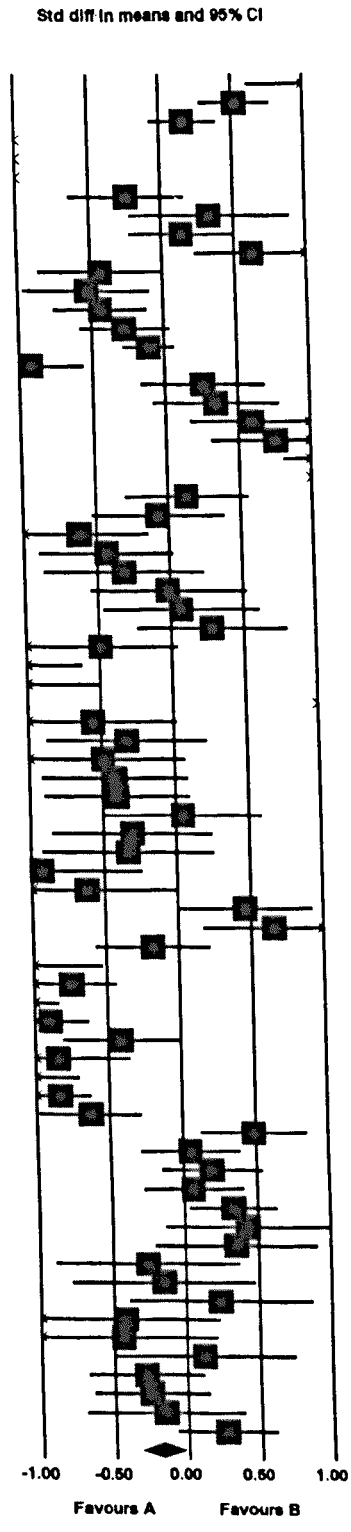

Figure 8.2 Forest plot showing standard difference in means for s-IgA secretion rates and 95% confidence limits for each study. The overall random-effects are shown in the bottom row of the plot.

Sub-group analysis:

Acute or chronic exercise effects on s-IgA concentration

The number of studies involving acute exercise and chronic exercise was 73 and 33, respectively. The pooled effect sizes (95% CI) for the studies involving acute exercise and chronic exercise demonstrated that the effect size for acute exercise was larger than for chronic exercise (0.2 vs. 0.07) [Figure 8.3]. The difference in pooled effect size for studies involving acute exercise was statistically significant (Q_{72} = 373.1, P<0.001). A subgroup analysis revealed that the acute exercise groups had a significant (P<0.001) increase in s-IgA compared with pre-exercise, and this increase was significantly greater than chronic exercise groups (Q_2 = 18.42, P = 0.0001).

Acute and chronic exercise effects on s-IgA secretion rate

Sixty-one and twelve studies were found that involve s-IgA secretion rate response to acute exercise and chronic exercise, respectively. The pooled standardized difference (95% CI) for the studies involving acute exercise (-0.31) and chronic exercise (0.37) demonstrated that the effect size for both moderators was significant. The overall weighted mean change in s-IgA secretion rate increased following chronic exercise (P=0.01), whereas it decreased significantly after acute exercise (P<0.01) [Figure 8.3]. The difference in pooled standardized difference for studies involving both moderators, acute exercise (Q_{60} = 433.3, P<0.01) and chronic exercise (Q_{11} = 80.04, P<0.01) was statistically significant. The acute exercise triggered a significant (P<0.01) suppression of s-IgA secretion rate. However, subgroup analysis revealed that chronic exercise groups had a significant (P = 0.01) increase for s-IgA secretion rate following chronic exercise. The difference between acute exercise and chronic exercise effects on s-IgA secretion rate was significant (Q_2 = 37.44, P < 0.0001).

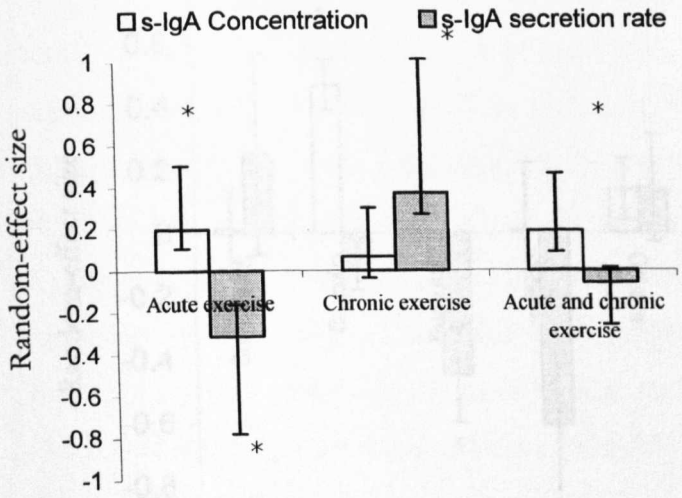

Figure 8. 3 The random-effect size for exercise, training and exercise +training on s-IgA concentration (n=106) and secretion rate (n=73). Data are means ± SEM. * Significant effect (P<0.05).

Exercise mode and s-IgA concentration

The number of studies involving swimming, cycling, running, soccer and other sports was 14, 31, 27, 8 and 26, respectively. The pooled standardized difference range (95% CI) for the studies involving swimming, cycling, running and soccer confirmed that cycling has a large effect size compared with swimming, running, and soccer. The pooled effect size range at 95 % CI for the studies involving other sports events was not significant (Figure 8.4). Whereas, the swimming (P>0.05), cycling (P<0.001), soccer (P>0.05) and other sports (P>0.05) revealed an increase in s-IgA concentration compared with pre-experiments, the running experiments showed a decrease post-exercise (P>0.05). The difference in pooled effect size for these subgroups was statistically significant (Q_4= 22.42, P=0.0002).

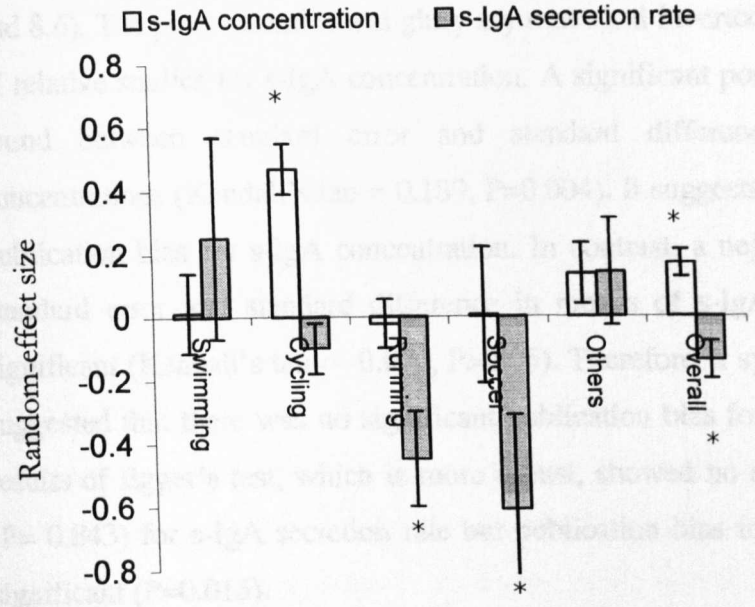

Figure 8. 4 The random-effect size for mode of exercise or training, on s-IgA concentration (n=106) and secretion rate (n=73). Data are means ± SEM. * Significant effect (P<0.05).

Exercise mode and s-IgA secretion rate

The quantity of the combined studies involving swimming, cycling, running, soccer and other sports was 5, 27, 16, 12 and 13, respectively. The pooled effect size (95% CI) for the studies involving swimming, cycling, running and soccer confirmed that running and soccer had the larger effect size compared with other sport events. The pooled effect size at 95 % CI for the studies involving other sports events was not significant (Figure 8.4). Running (P<0.01), cycling (P>0.05), soccer (P = 0.04) and other sports (P>0.05) caused a decrease in s-IgA secretion rate compared with pre-exercise, but s-IgA secretion rate increased immediately after swimming (P.0.05) and other sport conditions (P>0.05). The difference in pooled standardized difference between exercise modes was statistically significant (Q_4= 11.03, P=0.026).

Publication bias:

Begg's funnel plot to evaluate publication bias is a graphic display of standard error and standard difference in means of s-IgA concentrations and secretion rate (Fig 8.5 and 8.6). The plots resemble a slightly asymmetrical inverted funnel in the distribution of relative studies for s-IgA concentration. A significant positive correlation was also found between standard error and standard difference in means of s-IgA concentrations (Kendall's tau = 0.189, P=0.004). It suggests that there was significant publication bias for s-IgA concentration. In contrast, a negative correlation between standard error and standard difference in means of s-IgA secretion rates was not significant (Kendall's tau = -0.070, P>0.05). Therefore, a symmetrical inverted funnel suggested that there was no significant publication bias for s-IgA secretion rate. The results of Egger's test, which is more robust, showed no significant publication bias (P= 0.843) for s-IgA secretion rate but publication bias for s-IgA concentration was significant (P=0.015).

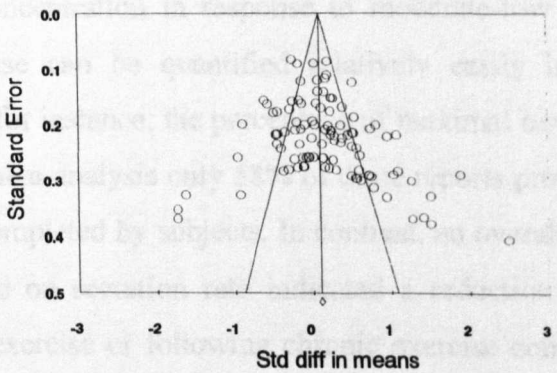

Figure 8. 5 Funnel plot of standard difference in means and standard error for s-IgA concentration.

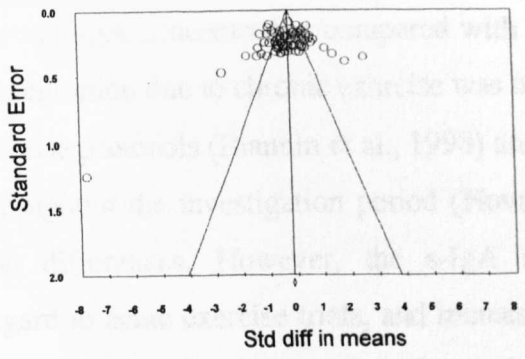

Figure 8. 6 Funnel plot of standard difference in means and standard error for s-IgA secretion rate.

8.4 Discussion

This analysis examined the s-IgA responses to acute exercise sessions and chronic exercise. The results of the present meta-analysis demonstrated an overall elevation in s-IgA concentration immediately following acute exercise trials or chronic exercise compared with pre-exercise or before chronic exercise. The significant effect size for s-IgA from the combined data set resulted from the acute effects of exercise rather than chronic adaptation. This finding supports those studies that reported typically increased s-IgA concentration in response to moderate-low intensity exercise. The intensity of exercise can be quantified relatively easily in controlled laboratory experiments using for instance, the percentage of maximal oxygen uptake and/or peak heart rate. In this meta-analysis only 58% of these reports provided details of exercise or training loads completed by subjects. In contrast, an overall effect of acute exercise or chronic exercise on secretion rate indicated a reduction in s-IgA secretion rate immediately post-exercise or following chronic exercise compared with pre-exercise or before chronic exercise. This evidence from a methodological viewpoint, indicates that monitoring s-IgA as a secretion rate is the best option (Fahlman and Engels, 2005). However, in about 31% of existing studies, data were published with inappropriate correction for definition of s-IgA. The increase in s-IgA concentration after each acute exercise trial seemed to result at least in part from the reduction of saliva flow rate.

Examination of subgroups with regard to acute exercise revealed a significant post-exercise trial increase in s-IgA concentration compared with pre-exercise. The overall increase of s-IgA concentration due to chronic exercise was not significant. The higher intensity of acute exercise protocols (Blannin et al., 1998) and adaptation with chronic exercise intensity throughout the investigation period (Novas et al., 2003) may have contributed to these differences. However, the s-IgA secretion rate decreased significantly with regard to acute exercise trials, and increased with regard to chronic exercise. The common trend due to acute exercise trials and chronic exercise for s-IgA secretion rate rather than absolute s-Ig concentration lends further support for correction of s-IgA in experimental reports (Fahlman and Engels, 2005).

The majority of acute exercise trials or chronic exercise protocols involved in studies included swimming (13.2%), cycling (29.2%), running-based (25.5%) and other sports (24.6%) conditioning and very few studies have examined soccer (7.5%). The quality of exercise modality such as cycling and running can be controlled and manipulated relatively easily in laboratory experiments compared with other sports in the field. In terms of s-IgA concentration, cycling was a unique exercise and training mode that indicated a significant increase post acute exercise. However, following correction as secretory IgA, a significant decline was demonstrated after running and even after playing soccer, although soccer comprised a considerably smaller number of studies compared with other sport events. This response may be due to specificity of exercise, running vs. cycling. In other words, when a subject engages in heavy exercise workloads (e.g. overtraining, a competitive endurance race event like marathon, or even heavy match schedules), lower s-IgA as a marker of infection risk should be evident (Nieman and Bishop, 2006).

Publication bias

Even though both Begg's and Egger's tests suggested that there was a presence of bias for s-IgA concentration in published studies, it is likely that high standard errors (small studies) associated with large effect sizes and studies with positive results were preferentially published. However, studies published about s-IgA secretion rate showed an absence of bias.

Limitations

This analysis, like others, is prone to certain limitations. First and foremost, a meta-analysis inherits the limitations of the individual studies it is composed of. A second potential positive bias could be extracted from nonreporting of negative findings. Meta-analyses are at risk for bias, because nonreporting of negative studies is, unfortunately, all too common. Authors are more likely to report studies with positive results in the belief that editors are unlikely to accept manuscripts with negative findings (Nissen and Sharp, 2003). Such bias can lead to false optimistic meta-analyses about a new protocol or a new treatment. Finally, another important statistical limitation is that meta-analysis cannot determine the difference between specific subgroups.

In conclusion, the meta-analysis revealed that in comparison to chronic exercise, acute exercise elevates s-IgA concentration and reduces s-IgA secretion rate immediately after acute exercise. A bias for s-IgA concentration in published studies supports using the s-IgA secretion rate for correction of absolute s-IgA concentration. Therefore, acute exercise has a suppressive effect on s-IgA responses as a marker of mucosal immunity in contrast to the effect of chronic exercise.

Chapter 9

Synthesis of Findings

Chapter 9

Synthesis of findings

The purpose of this chapter is to interpret and integrate the findings obtained from all of the studies within this thesis. The realisation of the aims of the thesis will be confirmed prior to reviewing the original hypotheses. Within the general discussion that follows, the main findings of previous chapters will be reviewed and interpreted with respect to the immunological aspects of soccer-specific intermittent exercise. The outcomes of these studies will be presented prior to arriving at an overall conclusion. Finally, some recommendations derived from the findings of this thesis are given that may help athletes to minimise the impact of intermittent exercise on their mucosal immunity and inform researchers of directions that future investigations might take.

9.1 Realisation of aims

An intermittent exercise protocol based on soccer match-play work-rate was applied to evaluate the s-IgA responses to soccer-specific intermittent exercise (aim 1). The s-IgA responses to soccer-specific intermittent exercise performance were not significantly different from responses to continuous exercise performed at the same average work-rate. The effects of successive soccer-specific intermittent exercise on s-IgA responses were investigated in laboratory conditions for fulfilment of aims 2 and 3. The effects of two identical bouts of soccer-specific intermittent exercise with 48-h rest in between on s-IgA responses were compared through 48 h post-exercise (aim 2). The pattern of response for s-IgA concentration following each exercise bout was similar on both occasions. In the subsequent study, performing a second bout of soccer-specific exercise in one day with 2.25 h for recovery (aim 3) increased the s-IgA concentration, but did not have a significant carry-over suppressive effect from the first exercise session on s-IgA responses. The effect of thermal strain on s-IgA responses with carbohydrate treatment (aim 4) suggests that carbohydrate supplementation whilst exercising in the heat, did not have a significant influence on s-IgA responses compared with placebo.

In order to place these results in perspective, a quantitative analysis of the related literature was conducted. A comprehensive meta-analysis on the effect of acute exercise on s-IgA responses revealed an overall elevation in s-IgA concentration after acute exercise. In terms of overall s-IgA secretion rate, post acute exercise and following chronic exercise a significant decline compared with pre acute and chronic exercise was indicated. There was a bias for s-IgA concentration in publications.

9.2 Review of hypotheses

A series of hypothesis were formulated prior to conducting the studies described. The findings of the proposed hypotheses are as follows.

Hypothesis 1: The s-IgA responses associated with soccer-specific intermittent exercise is different from responses to continuous exercise that is performed at the same average work-rate.

This hypothesis was rejected. Although physiological responses to intermittent exercise conforming to the activity pattern of soccer match-play were similar to those for continuous exercise at the same average work-rate, perceived exertion during intermittent exercise was higher than during continuous exercise. Changes in s-IgA did not differ between exercise types during or post-exercise, or throughout the 48 h afterwards. Therefore, an s-IgA response to both intermittent and continuous exercise at the same average work-rate was similar.

Hypothesis 2: The s-IgA responses associated with a second bout of soccer-specific exercise are greater than for a first bout of exercise 48 h earlier.

This hypothesis was rejected. Performing two soccer-specific intermittent exercise protocols with 48 h between sessions was not sufficiently stressful to suppress the s-IgA responses. The second soccer-specific intermittent exercise trial placed a similar aggregate demand on the heart and perception of effort as the first exercise trial performed according to the same protocol exercise. Thus, the effect of a second bout of intermittent exercise after 48 h on s-IgA responses was not greater than for the first bout.

Hypothesis 3: The s-IgA responses associated with one bout of soccer-specific intermittent-exercise in one day are lower than for a second bout of exercise performed after 2.25 h recovery.

This hypothesis was rejected. Performing a second bout of soccer-specific exercise in one day with 2.25 h recovery in between was associated with increases in HR and RPE compared with $AMEX_1$ and PMEX (single bout of exercise), increased the s-IgA concentration, but did not have a significant carry-over effect from the first exercise session on s-IgA responses. Consequently, performing a second bout of exercise after 2.25 h recovery did not alter the s-IgA responses.

Hypothesis 4: The s-IgA responses to soccer-specific intermittent exercise in the heat are attenuated with carbohydrate (CHO) supplementation compared with placebo (PLA).

This hypothesis was rejected. The carbohydrate supplementation before and during the soccer-specific intermittent exercise protocol conducted in the heat had no effect on s-IgA responses. In addition, carbohydrate supplementation before and during the soccer-specific intermittent exercise protocol completed in the heat did not influence

RPE or thermal sensations (Tsens), but HR was increased compared to placebo. Hence, administration of CHO or placebo during the intermittent exercise protocol in the heat had a similar influence on s-IgA responses.

Traditional data-analysis methods for summarising experimental findings of a non-quantitative nature and their conclusions are often open to subjectivity and therefore, have led to confusion in the literature. The aim of this meta-analysis that was to highlight consistent findings in overall s-IgA responses to exercise. The present comprehensive meta-analysis revealed an overall elevation in s-IgA concentration after both acute and chronic exercise trials and significant a decline in s-IgA secretion rate. However, the s-IgA responses depend on acute exercise dimensions or chronic exercise modality.

9.3 General discussion

Soccer players must train hard and may also be at increased risk of URTI and compromised immune function (Nieman and Bishop, 2006). Changes in s-IgA have been coincident with or preceded the appearance of URTI in collegiate soccer players (Nakamura et al., 2006). However, methodological problems restrict the direct information that can be collected in competitive match situations due to the changeable and irregular nature of the work-rate profile that makes control of experimental conditions difficult. An emphasis must therefore be placed on laboratory simulations to supplement the data provided during games. The intermittent protocol performed on the motorised treadmill was a model of match-play. Technical restrictions of the protocol also used in the current investigations placed limitations on the re-creation of the activity profile observed in a soccer match. The first idea within this thesis was to compare two major training forms that are used by athletes (intermittent and continuous exercise) and their effect on s-IgA responses. Intermittent exercise is characterised by brief high-intensity activity punctuated by periods of exercise at a lower intensity or by rest. Soccer is also characterised by the variable

intensities of low to high intensities activities (Bangsbo, 1994). Hence, the main aims of the other sections of the thesis focused on some aspects of stress experienced by soccer players during the season, such as matches twice per week with 48 h rest in between or successive training sessions on one day. They have also to train or compete sometimes exposed to hot conditions. In order to examine the effect of physiological and psychological stress imposed by such situations on s-IgA a series of experiments were conducted in laboratory. The overall effect of exercise on s-IgA according to previous studies was explored by using a comprehensive meta-analysis which comprised the final section of this collection of studies.

There are reports demonstrating that s-IgA concentrations are suppressed in response to high-intensity exercise, remain either unaltered or are elevated in response to moderate-low intensity exercise. The primary finding of the first investigation (study 1 in chapter 4) was that the pattern of change in s-IgA responses, and salivary cortisol did not differ significantly between the soccer-specific and continuous exercise at the same work-rate intensity. The literature suggests that there is an intensity-dependent effect of acute exercise on s-IgA concentrations. Since the average intensity of both exercise protocols was below 60% $\dot{V}O_{2\,max}$, it is suggested that moderate intensity exercise, whether intermittent or continuous, is not stressful enough to induce significant perturbations in s-IgA and cortisol response.

Soccer players may be particularly vulnerable to a reduction in s-IgA concentrations and subsequent upper respiratory tract infections (URTI), since they have little opportunity to avoid repeated stress when competitive matches are scheduled close together without sufficient recovery between them. Mackinnon and Hooper (1992) reported a progressive decrease in s-IgA secretion rates after 90 min running at 75 % $\dot{V}O_2$peak performed on three successive days. They also suggested a similar cumulative effect of intense daily exercise on IgA concentrations, at least during competition. Our results (study 2 in chapter 5) demonstrated that the s-IgA

concentration increased immediately following exercise in both trials and result at least in part from the reduction of saliva flow rate that associated with an increased β-adrenergic activity of autonomic nervous system, dehydration or evaporation of saliva through hyperventilation. In addition, exercise-induced increased in vasopressin (antidiuretic hormone; ADH) might also decrease salivary flow rate (Lavelle, 1988). The data obtained suggest a consistent trend towards a reduction in s-IgA responses (albeit non-significant), attributed to performance of the second bout of exercise within the 48-hour period. The IgA secretion rate and cortisol did not differ between the time-points. These inconsistent results may be due to the intensity of the present exercise protocol and/or the fitness level of subjects. Mackinnon (1996) suggested that differences in subject fitness may account for the range of s-IgA responses reported in the literature.

Soccer players may frequently participate in hard training sessions on successive days and may sometimes undertake two exercise sessions close together on the same day with brief time for recovery between them. Data from the present research study (study 2 in chapter 5) would suggest that in term of s-IgA responses, when a session of strenuous intermittent exercise specific to soccer is repeated after 48 h of rest, s-IgA may be compromised. Therefore, it was hypothesized that s-IgA responses and salivary cortisol concentrations are affected during two bouts of soccer-specific intermittent exercise compared with a single bout of identical exercise in one day (study 3 in chapter 6). Performance of a second soccer-specific exercise bout did not appear to suppress s-IgA outcomes. Performing a second bout of soccer-specific exercise increased the concentration of s-IgA more than a single session (albeit non-significant) immediately post-exercise and decreased after the 2.25 h rest in between sessions to below the initial pre-exercise. The lack of statistical significance between two trials 48 h apart (study 2) and also single and repeated bouts of soccer-specific exercise in one day (study 3) are likely attributable to the large intra-individual and inter-individual variation in s-IgA. There was no time-of-day effect on s-IgA concentration and secretion rate or salivary cortisol in the short term when performing soccer-specific exercise.

Soccer is sometimes played under hot environmental conditions and opportunities for fluid replacement are limited during games. Although fluid intake before and during a game would reduce the degree of dehydration experienced, carbohydrate (CHO) ingestion during exercise stress and imposition of a thermal load may attenuate exercised-induced immune responses and decrease the potential for developing symptoms of respiratory tract infections. The attenuation of cortisol response to the exercise is probably part of the underlying mechanism (Bishop et al., 1999). The addition of carbohydrate to fluids may also supplement the body's limited carbohydrate stores, mitigate potential adverse changes in s-IgA responses. Therefore, it was hypothesised that carbohydrate ingestion before and at regular intervals during soccer-specific exercise in the heat (study 4 in chapter 7) attenuates the immunosuppressive effects of combined stressors (exercise and heat exposure). However, the main findings of this investigation were that the salivary immunoglobulin-A (s-IgA) concentration was not significantly affected by the carbohydrate treatment or differed at any time-point post-exercise, perhaps because the overall severity of the physiological strain and subjective stress associated with the laboratory setting in the heat was lower than in match-play. The increase in salivary cortisol that is known to inhibit transepithelial transport of s-IgA after prolonged exercise in the heat was not evident in these investigations. Therefore, the alterations in s-IgA concentrations are not necessarily related to the increase in salivary cortisol which occurs during exercise. The large intra-individual and inter-individual variation in s-IgA is likely the reason for the non-significant difference between CHO and PLA trials.

The entire results concerning the effect of soccer-specific exercise on s-IgA responses are summarised in Table 9.1. A similarity in salivary responses to the soccer-specific protocol in all studies, except in the case of s-IgA secretion rate, s-IgA to osmolality or protein ratios and also total protein responses, is demonstrated.

Table 9. 1 The comparison of salivary responses between continuous and intermittent exercise (study 1), first and second bout of exercise with 48 h apart (study 2), single and twice exercise in one day (study 3), CHO and PLA in the heat (study 4).

Variable	Continuous vs. Intermittent	First vs. Second bout of exercise	Single vs. two sessions in one day	CHO vs.PLA
Saliva flow rate	↔	↔	↔	↔
Osmolality	↔	↔	↔	↔
Solute secretion	↔	↔	↔	↔
Protein Concentration	NA	↔	↔	P=0.018
Protein Secretion rate	NA	↔	P=0.08	P=0.013
IgA concentration	↔	↔	↔	↔
IgA secretion rate	↔	↔	↔	↔
IgA to osmolality ratio	↔	↔	P=0.028	↔
IgA to protein ratio	NA	↔	P=0.01	↔
Cortisol	↔	↔	↔	↔

↔ no significant difference between trials; NA: Not applicable

The meta-analytical approach is a more standardized and nonbiased method of data analysis (chapter 8) and the meta-analysis conducted as part of this thesis was the first to examine this subject. It was demonstrated that an overall elevation in s-IgA concentration occurs immediately following exercise compared with pre-exercise. In contrast an overall effect of exercise on secretion rate indicated a reduction (although small but significant) in s-IgA secretion rate immediately post-exercise compared with pre-exercise. In terms of s-IgA concentration, the results of the present experiments are in line with this finding, but s-IgA secretion rate increased immediately post-exercise in the present studies. Thirty-two percent of studies included in the meta-analysis have indicated similar results. The disparity may arise from differences in the exercise protocols used in these experiments. For instance, cycling was a unique exercise mode that induced significant increases in s-IgA concentration post-exercise, possibly due to its non impact nature and ease at which exercise and sampling can be conducted. In addition, investigations involving soccer comprised a considerably smaller number of studies compared with other sport events.

9.4 Conclusions

The intermittent and continuous exercise protocols that were performed at the same average work-rate demonstrated that moderate intensity exercise, whether intermittent or continuous, is not stressful enough to induce significant perturbations in s-IgA and cortisol responses.

Two bouts of soccer-specific intermittent exercise performed 48 h apart were designed to provide a repeatable physiological stress comparable to a game of soccer. The results indicated that responses of s-IgA and its associated markers were variable, and the exercise intensity was lower than typically associated with competitive soccer match-play. However, a cumulative effect of successive competitive games undertaken within 48 h cannot be excluded.

Two 90-min exercise sessions performed at a moderate intensity with a 2.25 h rest in between did not necessarily have adverse effects on s-IgA responses. Therefore, if the training programme consists of more than one session of exercise per day, a recovery interval might be enough for mucosal immunocompetence in subsequent competition (48 h) or training session (at least 2.25 h), but might not be enough for other physiological system.

Carbohydrate ingestion before and at regular intervals during soccer-specific intermittent exercise in the heat did not influence s-IgA or salivary cortisol responses. Thus, hydration without the inclusion of carbohydrate before and at frequent intervals during exercise may be more important, although current data suggest that a 1.5 % decline in body weight can be tolerated without imposing a detrimental effect on s-IgA

secretion rate following high-intensity activity. In addition, CHO supplementation might be relevant to other markers of immune function.

The schematic model in Figure 9.1 incorporates the likely factors involved in the responses of salivary flow rate, s-IgA concentration and secretion rate. The soccer-specific protocols were not significantly stressful for these participants throughout the studies and hence mucosal immunosuppression was not observed. The diagram does highlight the possibility that exercise at higher intensity could compromise mucosal immunity.

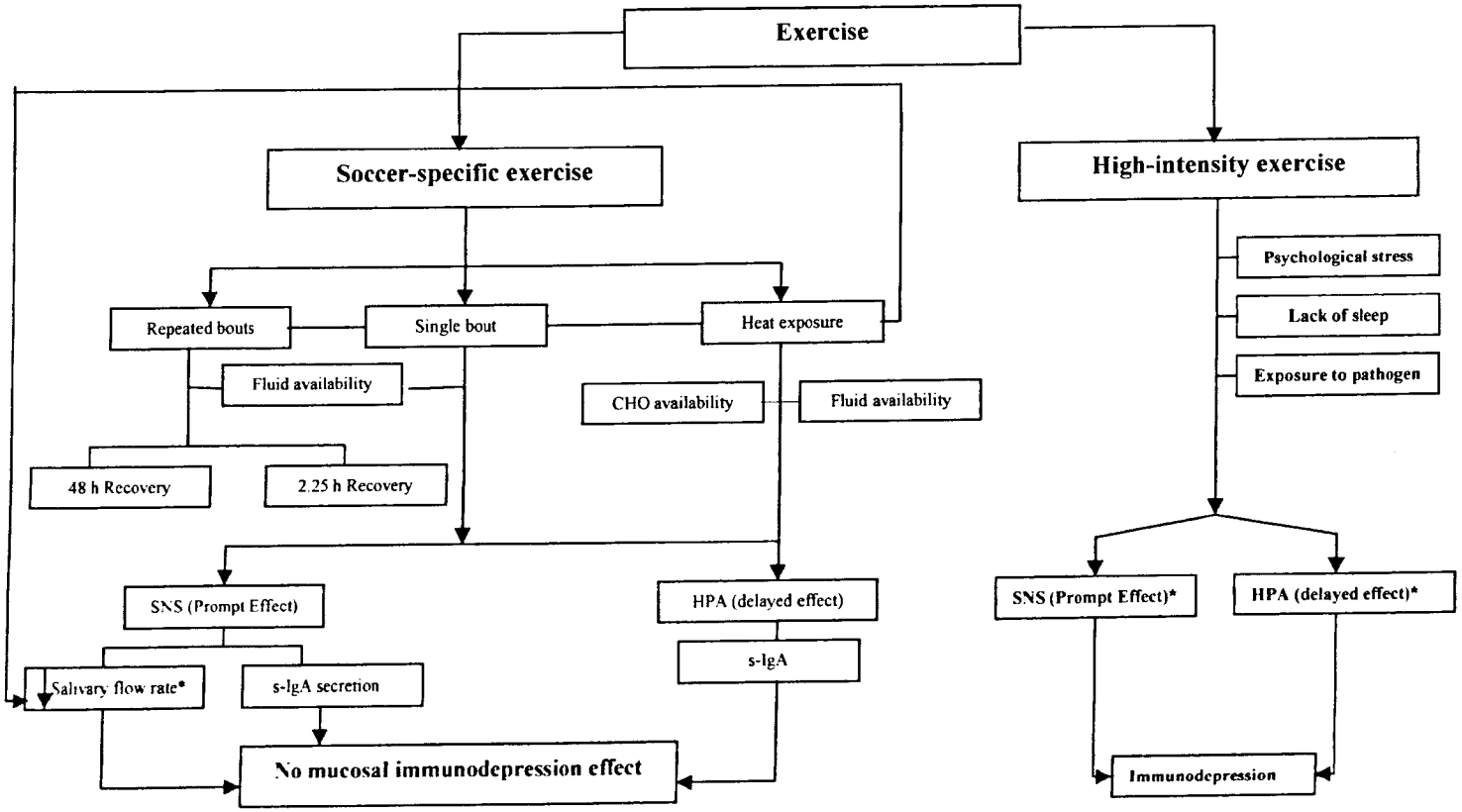

Figure 9. 1 Mucosal immunity during and following soccer-specific exercise and intensitive exercise
* Significant changes

Meta-analysis revealed an overall elevation in s-IgA concentration post acute exercise significantly, but the increase following chronic exercise was not significant. In contrast, in terms of s-IgA secretion rate, an overall significant decline was found post acute exercise, whereas, an overall significant increase was observed following chronic exercise. However, the presence of bias in published studies for s-IgA concentration was revealed. Although the evidence from a methodological viewpoint indicates that monitoring s-IgA as a secretion rate is the best option, about 31% of existing data from published studies were without an appropriate correction for definition of s-IgA.

Comparisons between studies of s-IgA responses are confounded by the way that data are expressed by different research groups. In the present studies, although the s-IgA concentrations were expressed as secretion rate and also were corrected for changes in osmolality and total protein, the evidence from a methodological viewpoint indicates that monitoring s-IgA, as a secretion rate is the best option.

9.6 Recommendations for future research

The studies completed within this thesis provided an overview of the s-IgA responses to soccer. In achieving this culmination, some issues have arisen and certain findings have prompted the formulation of recommendations for future research.

The average intensity of the soccer-specific intermittent exercise protocol that has been used was moderate and not as stressful as competitive match-play. Therefore, the exercise protocol could be adjusted by modifying the exercise intensity categories and reducing the recovery periods to correspond more closely to competitive stress.

The lack of statistical significance in the current studies is likely attributable to the large intra-individual and inter-individual variation in s-IgA. The small effect size in these studies suggests that with this magnitude of change in s-IgA concentrations, more than ten subjects would be needed to detect a significant difference between either exercise trials or time-points with higher statistical power.

The inclusion of some specific match actions i.e. dribbling, passing, shooting, heading and other utility movements in the soccer-specific protocol, may lead to the simulated protocols possessing an even greater validity to soccer match-play, thus improving the relevance of the data to the practical setting. This change might require the validation of a field-based protocol broadly similar in format to the laboratory-based model employed in the present study.

The s-IgA responses to field studies such as soccer training sessions and match-play at home or away fixtures and at different levels should also be investigated. Such information would lead to links between laboratory-based research and field-based work being established.

There are several possible mechanisms to explain exercise-induced changes in immunoglobulins. Activation and regulation of immunoglobulins are complex and involve blood-borne variables such as B cells and cytokines [e.g. interleukin 6 (IL-6)]. It is suggested that the relationship between B cells, IL-6 and s-IgA should be investigated during soccer-specific exercise, representative of the intensity used by soccer players both in training and during match-play.

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Appendices

Appendix A

Participants Consent form

EC3

FORM OF CONSENT TO TAKE PART AS A SUBJECT IN A MAJOR PROCEDURE OR RESEARCH PROJECT

Title of project:

The effect of soccer-specific intermittent exercise on s-IgA responses

Name of Researcher: Vahid Sari Sarraf

Director of studies: Professor Tom Reilly

I, ...agree to take part in
(**Subject's** full name)*

the above named project/procedure, the details of which have been fully explained to me and

described in writing.

Signed ..... Date .....
(Subject full name*)

I,VAHID SARI-SARRAF **(Investigator)** certify that the details of this project/procedure have been fully explained and described in writing to the subject named above and have been understood by him.

Signed ..... Date .....
(Investigator)

I, ...certify that the details of this
(**Witness** full name*)

project/procedure have been fully explained and described in writing to the subject named above and

have been understood by him/her.

Signed ..... **Date** .....
(Witness)

NB The witness must be an independent third party.
* Please print in block capitals

Appendix B

Participants' health Screen

Health Status Questionnaires

(Based on Kibler B W (1990) *The Sport Preparticipation Fitness Examination*)

As you are to be a subject in this study, would you complete the attachments questionnaires, please? Your cooperation in this greatly appreciated.

Any information contained herein will be treated as confidential

Thank you for your time

Schedule
Could you please put your rather time during the week for participation in this study.

Monday Tuesday Wednesday Thursday Friday Saturday Sunday

Address: _____

_____

Tell No: _____
Mobile No: _____

Email: _____
Occupation:

Name: Date of Birth: Nationality:
Sex: Height (cm): Weight (kg):

BMI: Weight (kg)/ Height (m^2) = _____ /_____ =_____

Skinfold Data

				Average
Biceps	_____	_____	_____	_____
Triceps	_____	_____	_____	_____
Sub scapula	_____	_____	_____	_____
Supre iliac	_____	_____	_____	_____

Sum of Skinfolds: _____

%Body fat: _____

Heart Rate

Before exercise _____
During exercise _____
After exercise _____
Recovery _____

Blood Pressure:

Before Exercise:

Systole: _____

Diastole: _____

After Resistance Exercise:

Systole: _____

Diastole: _____

Muscle Soreness rating

Prior to exercise _____
Immediately post exercise _____
6 hours after exercise _____
24 hours after exercise _____
48 hours after exercise _____

Personal Medical History Questionnaire

Have you personally had any of the following medical problems?

	Yes	No	Comments
Allergies	___	___	_____
Asthma	___	___	_____
Chronic cough	___	___	_____
Pneumonia	___	___	_____
Tuberculosis	___	___	_____
Heart disease	___	___	_____
Heart murmur	___	___	_____
High blood pressure	___	___	_____
Chest pains	___	___	_____
Shortness of breath	___	___	_____
Epilepsy	___	___	_____
Fainting spells	___	___	_____
Diabetes	___	___	_____
Cancer	___	___	_____
Hernia	___	___	_____
Kidney disease	___	___	_____
Emotional illness	___	___	_____
Excessive weight loss	___	___	_____
Mononucleosis	___	___	_____
Concussions	___	___	_____
Vision problems	___	___	_____
Hearing problems	___	___	_____
Operations	___	___	_____
Heat intolerance	___	___	_____
Frequent headaches	___	___	_____
Blood clotting disorders	___	___	_____
Irregular menstrual periods	___	___	_____

Family Medical History Questionnaire

Has anyone in your immediate family (father, Mother, grandparents, brothers, or sisters) had any of the following?

Death under age 40 from heart disease	___	___	_____
Heart disease	___	___	_____
High blood pressure	___	___	_____
Diabetes	___	___	_____
Cancer	___	___	_____
Tuberculosis	___	___	_____
Alcohol, smoke or drug abuse	___	___	_____
Fainting spells	___	___	_____
Asthma	___	___	_____

Orthopedic History Questionnaires

Have you personally had any of the following orthopedic problems?

Neck injury	___	___	_____
Back injury	___	___	_____
Shoulder injury	___	___	_____
Elbow injury	___	___	_____
Wrist injury	___	___	_____
Hand injury	___	___	_____
Other arm injury	___	___	_____
Rib injury	___	___	_____
Hip injury	___	___	_____
Knee injury	___	___	_____
Ankle injury	___	___	_____
Foot injury	___	___	_____
Other leg injury	___	___	_____

Exercise-Induced Bronchospasm (EIB) Questionnaire

Instructions: For "yes" answers, indicate whether you have experienced the symptom recently, whether you experience it with a cold or infection, and whether it occurs related to exercise.

	Yes	No	Recent (within past2 months)	With colds/ infection	Exercise (before, during, or after?)
1. Eyes					
A. Itchy	___	___	_____	_____	_____
B. Watery	___	___	_____	_____	_____
C. Puffy	___	___	_____	_____	_____
2. Nose					
A. Itchy	___	___	_____	_____	_____
B. Stuffy	___	___	_____	_____	_____
C. Sneezing	___	___	_____	_____	_____
D. Runny	___	___	_____	_____	_____
E. Hay fever	___	___	_____	_____	_____
F. Postnasal drip	___	___	_____	_____	_____
3. Sinus Infection					
A. Yellow/ green nasal discharge	___	___	_____	_____	_____
B. Tender sinuses/ headache	___	___	_____	_____	_____
4. Ears					
A. Popping sensation	___	___	_____	_____	_____
B. Ears feel full/ congested	___	___	_____	_____	_____
C. Sensation of being in rising elevator	___	___	_____	_____	_____
5. Chest (after exercise)					
A. Cough	___	___	_____	_____	_____
B. Wheezing	___	___	_____	_____	_____
D. Noisy breathing	___	___	_____	_____	_____
E. Chest tightness	___	___	_____	_____	_____
F. Unable to get deep breath	___	___	_____	_____	_____
G. Asthma	___	___	_____	_____	_____

6. Skin Reactions
   A. Hives
   B. Itchy skin
   C. Dry Skin
   D. Swelling of skin
   E. Eczema
   F. Atopic dermatitis
7. Contact Dermatitis
   (skin reacts to things that touch the skin)
   A. Underwrap
   B. Tape
   C. Sweat bands
   D. Deodorants
   E. After-shave lotion
   F. Elastic
   G. Other

	Yes	No	(within past2 months)	With colds/ infection	(before, during, or after?)
8. Medication and Food					
A. Aspirin					
B. Penicillin					
C. Sulfa Drug					
D. Other medication					
E. Food allergy					
9.Allergy or sensitivity to the following:					
A. Dust					
B. Animals/pets					
C. Mold/mildew					
D. Pollen/grass					
E. Air pollution					
10. Life-threatening (systemic) reactions requiring hospital treatment					
A. Shock from bee sting					
B. Difficulty breathing					
11. If you ran 1 mile and rested 15 minutes:					
A. Would your chest feel tighter?					
B. Would you experience coughing?					
If yes to A or B above, are you more likely to have these sensation in					
A. Cold weather?					
B. Certain seasons of year?					
C. Periods of air pollution?					
12. Have you had skin tests for allergies in the past?					
13. Have you had allergy shots? (Immunotherapy)					
14. Recent cold/ chest colds					
15. Headache after exercise					
16. Stomachache after exercise					

17. Have you taken any of the following medications?

A. Antihistamine	___	___	_____	_____	_____
B. Decongestant/cold medicine	___	___	_____	_____	_____
C. Antibiotics	___	___	_____	_____	_____
D. Bronchodilators (to open breathing passages)	___	___	_____	_____	_____
E. Other	___	___	_____	_____	_____
F. Are you taking any of these medications now?	___	___	_____	_____	_____

18. Have you ever been hospitalised for the following?

A. Pneumonia	___	___	_____
B. Asthma	___	___	_____
C. Brochitis/bronchiolitis	___	___	_____
D. Other lung problem	___	___	_____

19. Is there any thing to your knowledge that may prevent you from successfully completing the test that have been outlined to you?

___ ___

20. Do you currently smoke cigarettes or cigars?

___ ___

21. Do you drink alcoholic beverages? ___ ___

22. Do you feel faint when you have your blood drawn?

___ ___

Participant signature and date: _____

Investigator signature: _____

Appendix C

Physical Activity Questionnaire

Physical Activity Readiness Questionnaire

Name ..... Date .....

PURPOSE

The purpose of this lab is to help you determine your physical readiness for participation in a program of regular exercise.

PROCEDURE

1. Read the directions on the "PAR-Q & You" form shown on the next page.
2. Answer each of the seven questions on the form.
3. If you answered "yes" to one or more of the questions, follow the directions just below the PAR-Q questions regarding medical consultation.
4. If you answered "no" to all seven questions, follow the directions on the following page.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES NO

1. ☐ ☐ Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
2. ☐ ☐ Do you feel pain in your chest when you do physical activity?
3. ☐ ☐ In the past month, have you had chest pain when you were not doing physical activity?
4. ☐ ☐ Do you lose your balance because of dizziness or do you ever lose consciousness?
5. ☐ ☐ Do you have a bone or joint problem that could be made worse by a change in your physical activity?
6. ☐ ☐ Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
7. ☐ ☐ Do you know of any other reason why you should not do physical activity?

If you answered:

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want - as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those that are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- Start becoming much more physically active - begin slowly and build up gradually. This is the safest and easiest way to go.
- Take part in a fitness appraisal - this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively.

DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of a temporary illness such as a cold or a fever - wait until you feel better; or
- If you are or may be pregnant - talk to your doctor before you start becoming more active.

Please note: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Appendix D

Subjects' information sheets

Research Institute for Sport and Exercise Science

Participant Information Sheet

Name of experimenter:

Director of Studies: Professor Tom Reilly

Project title:

Salivary IgA responses to laboratory-based soccer specific intermittent and continuous exercise

Purpose of study:

The present study is designed to determine the effect of soccer-specific-intermittent and continuous exercise on Salivary content

Dear Participant

You are invited to participate as a volunteer in a research project designed to determine the effects of soccer-play on the immune system. Below you can find a brief description of the study protocol and test procedures. Information obtained during the course of the study may help us to understand better the body's internal resistance to the stress of exercise.

Study protocol and testing procedures:

Prior to the commencement of the main study, you are asked to report to the laboratory to undertake a familiarisation session in the laboratory and determine your maximal physiological capacity during running on a treadmill. You will be asked to provide a salivary sample before doing the treadmill run. Thereafter (usually 3 days after the first visit) , you will be asked to report to the laboratory for further sessions running on the treadmill on two occasions each over 90

minutes will entail. One session entails soccer-specific-exercise and another session after a week involves continuous exercise (The order of these sessions will be reversed for half of the subjects). You will have 15 minutes half-time rest between each 45-minute exercise bout. To avoid the possibility of injury, each session will be preceded by a general warm- up period consisting of general body stretching exercises and approximately 5-min of running on the treadmill. You will asked to provide a saliva sample five times: before exercise, immediately after exercise and 6 hours, 24 hours and 48 hours post-exercise.

During exercise your heart rate will be monitored by means of a device worn on your chest. This device should not hinder you in any way.

Also during exercise you will be asked to rate the perceived severity of perceived by pointing to a number on a scale. Your muscle soreness will also be rated at the time a salivary sample is obtained.

You will have access to a shower after the exercise. You should bring your own sport wear for the sessions.

All the information collected about you during the course of the research will be kept strictly confidential. Any published report of the research will not identify you and the records will be destroyed when no longer needed.

Take time to decide whether or not you wish to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. Also, if you decide to take part you are still free to withdraw at any time without any required explanation.

Research Institute for Sport and Exercise Science

Name of experimenter:

Director of Studies: Professor Tom Reilly
Supervisor II : Mr. Dominic Doran

Project title:

Immune system responses to two consecutive laboratory-based soccer specific intermittent exercises.

Purpose of study:

The present study is designed to determine the effect of two consecutive soccer-specific-intermittent on immune system

Dear Participant

You are invited to participate as a volunteer in a research project designed to determine the effects of soccer-play on the immune system. Below you can find a brief description of the study protocol and test procedures. Information obtained during the course of the study may help us to understand better the body's immune responses to the stress of exercise, in particular soccer.

Study protocol and testing procedures:

Prior to the commencement of the main study, you are asked to report to the laboratory to undertake a familiarisation session in the laboratory and determine your physiological capacity during running on a treadmill. You will be asked to provide a saliva sample before doing the treadmill run. Thereafter (usually 3 days after the first visit), you will be asked to report to the laboratory for further

sessions, running on the treadmill on two occasions each over 90 minutes to mimic the exercise intensity of playing a game. One session entails exercise at various speeds and another session after 48 hours involves the same exercise. You will have 15 minutes half-time rest between each 45-minute exercise bout. Each session will be preceded by a general warm-up period consisting of general body stretching exercises and approximately 5 minutes of jogging on the treadmill. You will asked to provide a saliva sample five times: before exercise, immediately after exercise and 24 hours and 48 hours post-exercise.

During exercise your heart rate will be monitored by means of a device worn on your chest. This device should not hinder you in any way.

Throughout exercise you will be asked to rate the perceived severity of exercise by pointing to a number on a scale.

You will have access to a shower after exercise. You should bring your own sports-wear for the sessions.

All the information collected about you during the course of the research will be kept strictly confidential. The information from the maximal test will be fed back to you as it indicates your current aerobic fitness level. Any published report of the research will not identify you and the records will be destroyed when no longer needed.

Take time to decide whether or not you wish to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign the standard University's consent form. Also, if you decide to take part you are still free to withdraw from the study at any time without any required explanation.

I thank you for your co-operation.

Vahid Sari-Sarraf

Research Institute for Sport and Exercise Science

Participant Information Sheet

Name of experimenter:

Director of Studies: Professor Tom Reilly
Supervisor II : Mr. Dominic Doran
Supervisor III : Professor Greg Atkinson

Project title:

The effects of soccer-specific exercise performed once or twice a day on mucosal immunity

Purpose of study:

The present study is designed to compare salivary IgA and Cortisol responses to single bout of soccer-specific exercise at different times of day and to a second bout at the same intensity on the same day.

Dear Participant

You are invited to participate as a volunteer in a research project designed to determine the effects of soccer-play on the immune system. Below you can find a brief description of the study protocol and test procedures. Information obtained during the course of the study may help us to understand better the body's immune responses to the stress of exercise, in particular soccer.

Study protocol and testing procedures:

Prior to the commencement of the main study, you are asked to report to the laboratory to undertake a familiarisation session in the laboratory and determine your physiological capacity during running on a treadmill. Thereafter (usually 3 days after the first visit), you will be asked to report to the laboratory for further sessions, running on the treadmill on three occasions each over 90 minutes to mimic the exercise intensity of playing a game or a home training session. One session entails exercise for 90 minutes at various speeds. One more session which involves two trials of the same exercise (EX1 start at 10:30 h and EX2 start at 14:30 h). You will have 15 minutes half-time rest between each 45-minute exercise bout. Each session will be preceded by a general warm-up period consisting of general body stretching exercises and approximately 5 minutes of jogging on the treadmill. You will asked to provide a saliva sample before exercise and immediately after each exercise trial. The two days of exercise will be separated by one week.

During exercise your heart rate will be monitored by means of a device worn on your chest. This device should not hinder you in any way.

Throughout exercise you will be asked to rate the perceived severity of exercise by pointing to a number on a scale.

You will have access to a shower after exercise. You should bring your own sports-wear for the sessions.

All the information collected about you during the course of the research will be kept strictly confidential. The information from the maximal test will be fed back to you as it indicates your current aerobic fitness level. Any published report of the research will not identify you and the records will be destroyed when no longer needed.

Take time to decide whether or not you wish to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign the standard University's consent form. Also, if you decide to take part you are still free to withdraw from the study at any time without any required explanation.

I thank you for your co-operation.

Vahid Sari-Sarraf

Research Institute for Sport and Exercise Science

Participant Information Sheet

Name of experimenter:

Director of Studies: Professor Tom Reilly
Supervisor II : Mr. Dominic Doran

Project title:

The effects of carbohydrate supplementation on mucosal immunity to soccer-specific intermittent exercise in the heat

Purpose of study:

The present study is designed to determine the effect of consuming sports drinks in the heat (30°C) on the salivary IgA of a simulated soccer match on a motorised treadmill.

Dear Participant

You are invited to participate as a volunteer in a research project designed to determine the effects of soccer-play on the immune system. Below you can find a brief description of the study protocol and test procedures. Information obtained during the course of the study may help us to understand better the body's immune responses to the stress of exercise, in particular soccer.

Study protocol and testing procedures:

Prior to the commencement of the main study, you are asked to report to the laboratory to undertake a familiarisation session in the laboratory and determine your physiological capacity during running on a treadmill. You will be asked to provide a saliva sample before doing the treadmill run. Thereafter (usually 3 days after the first visit), you will be asked to report to the laboratory for further

sessions, running on the treadmill on two occasions each over 90 minutes in the environmental chamber at 30°C to mimic the exercise intensity of playing a game. One session entails exercise at various speeds and another session after one week involves the same exercise. You will have 15 minutes half-time rest between each 45-minute exercise bout. During each session you will drink either flavoured water (Placebo) or sports drink (CHO).

Each session will be preceded by a general warm-up period consisting of general body stretching exercises and approximately 5 minutes of jogging on the treadmill. You will ask to provide a saliva sample five times: before exercise, immediately after exercise and 24 hours and 48 hours post-exercise.

During exercise your heart rate will be monitored by means of a device worn on your chest. This device should not hinder you in any way.

Throughout exercise you will be asked to rate the perceived severity of exercise by pointing to a number on a scale.

You will have access to a shower after exercise. You should bring your own sports-wear for the sessions.

All the information collected about you during the course of the research will be kept strictly confidential. The information from the maximal test will be fed back to you as it indicates your current aerobic fitness level. Any published report of the research will not identify you and the records will be destroyed when no longer needed.

Take time to decide whether or not you wish to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign the standard University's consent form. Also, if you decide to take part you are still free to withdraw from the study at any time without any required explanation.

I thank you for your co-operation.

Vahid Sari-Sarraf