EFFECTS OF PROLONGED EXERCISE IN THE HEAT (31°C) AND COOL (18°C) ENVIRONMENTS ON LACTOFERRIN AMONG RECREATIONAL ATHLETES

By

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CERTIFICATE

This is to certify that the dissertation entitled

EFFECTS OF PROLONGED EXERCISE IN THE HEAT (31°C) AND COOL (18°C) ENVIRONMENTS ON LACTOFERRIN AMONG RECREATIONAL ATHLETES

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TABLES OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

EFFECTS OF PROLONGED EXERCISE IN THE HEAT (31°C) AND COOL (18°C) ENVIRONMENTS ON LACTOFERRIN AMONG RECREATIONAL ATHLETES

The present study is a randomised, cross-over study that aims to determine the effects of prolonged exercise in the heat (31°C) and cool (18°C) environments on physiological changes and lactoferrin responses among recreational athletes. Thirteen healthy male recreational athletes (age: 20.9 ± 1.3 years old; body weight: 63.2 ± 7.8 kg) were recruited in this study. Each participant performed 2 separate running trials for 90 min at 60% VO_2max in a random order: running in the heat environment first, followed by running in the cool environment or vice versa. The recovery period between these two trials was one week. To prevent adverse effect of dehydration during exercise, cool plain water (3 $mL.kg^{-1}$ body weights) was given to participants at every 20 min during trial. Participant's nude body weight was measured pre and post exercise during each trial. Heart rate, rate of perceived exertion, oxygen uptake, skin and tympanic temperatures, room temperature, and relative humidity were recorded before warm-up, after warm-up, at every 20 min during trial, and at the end of trial. Blood samples were collected before warm-up, after warm up, at min 30 and 60 during trial, at the end of trial, and 1 h post exercise. Blood samples were used for haematological analysis (hemoglobin concentration). Saliva samples were collected pre and post and 1 h post exercise. From the saliva samples, lactoferrin concentration was measured by ELISA and lactoferrin secretion rate was calculated. The data collected were analysed using two-way ANOVA with repeated measures to measure significant differences between groups and within groups. Results showed that body weight changes, heart rate, skin and tympanic temperatures were

significantly higher in heat compared to cool trials ($p < 0.001$). In addition, prolonged exercise significantly increased ($p < 0.05$) lactoferrin concentration and secretion rate, haemoglobin concentration and plasma volume changes. Based on the results obtained, it can be concluded that in the present study, the physiological parameters had greater changes in the heat trial compared to cool trial whereas, lactoferrin responses (mucosal immunity) were not affected by ambient/room temperatures.

ABSTRAK

KESAN LARIAN BERPANJANGAN DALAM PERSEKITARAN PANAS (31°C) DAN SEJUK (18°C) PADA LAKTOFERIN DALAM KALANGAN ATLET REKREASI

Kajian ini berbentuk berselang secara rawak dan bertujuan untuk menentukan kesan larian berpanjangan dalam persekitaran panas (31°C) dan sejuk (18°C) terhadap perubahan fisiologi dan tindak balas laktoferin dalam kalangan atlet rekreasi. Seramai 13 orang atlet rekreasi lelaki yang sihat (umur: 20.9 ± 1.3 tahun; berat badan: 63.2 ± 7.8 kg) telah terlibat dalam kajian ini. Setiap peserta melakukan 2 ujian larian yang berasingan selama 90 min pada 60% VO_2max dalam turutan yang rawak: sama ada berlari dalam persekitaran panas terlebih dahulu diikuti dengan larian dalam persekitaran sejuk atau sebaliknya. Jarak masa antara kedua-dua ujian adalah selama satu minggu. Untuk mengelakkan kesan sampingan dehidrasi semasa larian, air kosong yang sejuk (3 $mL.kg^{-1}$ berat badan) diberikan kepada peserta pada setiap 20 minit semasa ujian larian. Kadar denyutan jantung, tanggapan daya usaha, pengambilan oksigen, suhu kulit dan telinga, suhu bilik dan kelembapan relatif dicatatkan sebelum pemanasan badan, selepas pemanasan badan, pada setiap 20 minit semasa ujian larian, dan di akhir ujian larian. Sampel darah telah diambil sebelum pemanasan badan, selepas pemanasan badan, pada minit 30 dan 60 semasa ujian larian, di akhir ujian larian, dan 1 jam selepas larian. Sampel darah telah digunakan untuk analisa hematologi (kepekatan hemoglobin). Sampel air liur diambil sebelum, selepas, dan 1 jam selepas ujian larian. Daripada sampel air liur tersebut, kepekatan laktoferin telah dianalisis menggunakan kaedah ELISA dan kadar remebesan laktoferin juga turut dikira. Data yang dikumpul dianalisa menggunakan 'two-way ANOVA with repeated measures' untuk

mengukur perbezaan yang signifikan di antara kumpulan dan di dalam kumpulan. Keputusan menunjukkan bahawa perubahan berat badan, kadar denyutan jantung, dan suhu kulit dan telinga lebih tinggi secara signifikan semasa ujian larian dalam persekitaran panas ($p < 0.001$). Di samping itu, larian berpanjangan telah meningkatkan kepekatan dan kadar rembesan laktoferin, kepekatan hemoglobin, dan perubahan jumlah plasma secara signifikan ($p < 0.05$). Berdasarkan keputusan yang diperolehi, boleh disimpulkan bahawa dalam kajian ini, parameter fisiologi mengalami perubahan yang besar semasa ujian larian dalam persekitaran yang panas berbanding persekitaran yang sejuk namun, tindak balas laktoferin (imuniti mukosa) tidak terjejas oleh suhu bilik/persekitaran.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Exercise is well known as a tool for maintaining individual's health. People practice exercise at every level from recreational to elite level. However, the intensity and duration of exercise may influence the immune system. As reported by numerous studies, moderate intensity exercise enhances the immune function (Nieman *et al.*, 1993; Nieman & Pedersen, 1999; Klentrou *et al.,* 2000) while prolonged, intense exercise may suppress immune function (Nieman, 1994; Nieman and Pedersen, 1999; Gleeson 2000). Enhances immune function may results in decrease risk of getting infection while suppressed immune function may results in high risk of getting infection.

In 1994, Nieman had proposed the "J"-shaped Curve Model (Figure 1.1) to explain the relationship between exercise and susceptibility to infection. This model suggests that, while engaging in moderate intensity exercise may decrease infection risk below average level, high intensity exercise may increase infection risk above average level. Positive effects on immune control and host refuge that come with moderate exercise training are probably related to a summation effect from acute positive changes that occur during each exercise bout (Nieman & Pedersen, 1999).

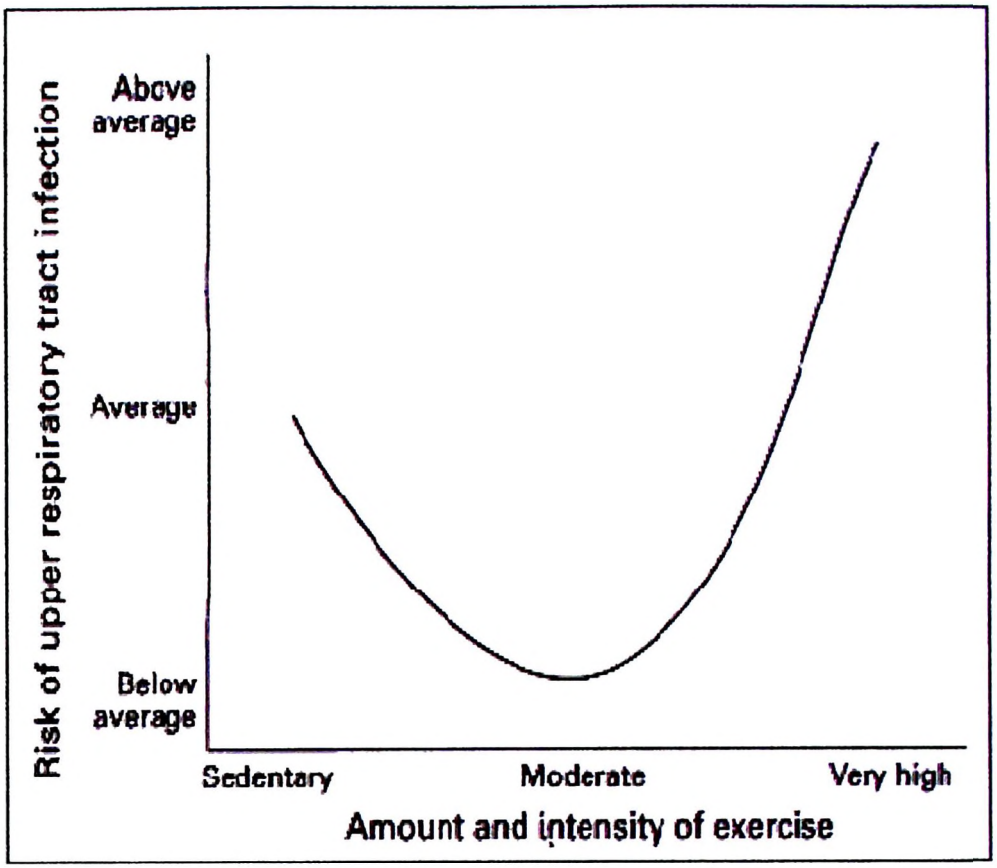

Figure 1.1 J-shaped model of the relationship between amounts and intensity of exercise to the risk of upper respiratory tract infection (Nieman, 1994).

As suppressed immune function is associated with high risk of infection, athletes and coaches are very concern about their training loads. This is because infection may negatively affect athletes' performance (Friman & Ilback, 1998). Infection may affect athletes' focus and energy hence, decreases their performance. This has been demonstrated in the recent Olympic Games where, a UK Olympic gold medallist, Sebastian Coe, who failed to qualify for the team to go to Seoul, because he was having a respiratory infection. However, it was reported that while some individuals can withstand rigorous training and competition schedules without missing a day, others are very susceptible to colds and infections (Fitzgerald, 1988).

Salivary glands produce fluids that contain antimicrobial proteins (AMPs) which include salivary immunoglobulin A (SIgA), lactoferrin, lysozyme, and alpha-amylase. They are components of mucosal immunity which serve as the first line defence against pathogenic microorganisms. Reduced levels of salivary AMPs have been associated with increased risk of upper respiratory tract infection (URTI) (Gleeson *et al.,* 1999; Fahlman and Engels, 2005; West *et al.,* 2006; Neville *et al.,* 2008; Gleeson *et al.,* 2011). To date, studies on the effects of exercise on SIgA are numerous, however studies on lactoferrin is very scarce. In addition, effects of exercise in the heat and cool environments on lactoferrin are tremendously limited.

Lactoferrin is one of the major components of the innate immune system which plays an important role in immune regulation and defense mechanisms against bacteria, fungi and viruses. It is a multifunctional iron binding glycoprotein of the transferrin family that found in most biological fluid (Legrand *et al.*, 2008). Besides act as primary defence factor against mucosal infections, lactoferrin can be considered as polyvalent regulator

which interacts in viral infectious processes. The plausible mechanism for its antiviral activity is by preventing entry of virus in the host cell which lies in the early phase of infection (Berlutti *et al.*, 2011). Hence, it is important to investigate the effects of exercise on lactoferrin and the effects of environmental on lactoferrin.

1.2 Objective of the study

- To determine the effects of prolonged exercise (90 minutes running on treadmill at 60% VO_2max) in the heat (31°C) and cool (18°C) environments on lactoferrin among recreational athletes.

1.2.1 Specific objectives

- To determine the effects of prolonged exercise (90 minutes running on treadmill at 60% VO_2max) in the heat (31°C) and cool (18°C) environments on lactoferrin concentration and responses among recreational athletes.
- To determine the effects of prolonged exercise (90 minutes running on treadmill at 60% VO_2max) in the heat (31°C) and cool (18°C) environments on physiological parameters among recreational athletes.

1.3 Hypotheses

H_{o1}: There are no significant differences in lactoferrin concentration and secretion rate between the prolonged exercise in the heat (31°C) and cool (18°C) environments.

H_{A2}: There are significant differences in lactoferrin concentration and secretion rate between the prolonged exercise in the heat (31°C) and cool (18°C) environments.

H_{o1}: There are no significant differences in physiological parameters between the prolonged exercise in the heat (31°C) and cool (18°C) environments.

H_{A2}: There are significant differences in physiological parameters between the prolonged exercise in the heat (31°C) and cool (18°C) environments.

1.4 Significance of the study

Since relationship between exercise, environmental condition (temperature), and lactoferrin responses has not been established, it is hoped that the results from this study will add knowledge regarding the effects of prolonged exercise in the heat (31°C) and cool (18°C) environments on lactoferrin responses.

CHAPTER 2

LITERATURE REVIEW

2.1 The immune system

Human body depends largely on the immune system as a defense mechanism to have healthy and functioning body. Immune system is a network of cells, tissues, and organs that work together to protect the body from infection (Schindler, 1991). Since human body constantly under attack by viruses, bacteria and parasites, the immune system plays an important role to protect body from these foreign substances.

Immune system can be divided into two divisions: innate (natural or non-specific) and adaptive (acquired or specific) immunity (Gleeson, 2006). Innate immunity acts as the first line of defense because it has an ability to respond quickly. In addition, innate immune cells were important for activating adaptive immunity (Iwasaki & Medzhitov, 2010; Gleeson, 2006; Kasper *et al.*, 2004). The adaptive immune system responds with a proliferation of cells using two mechanism, either attack the invader directly or produce specific defensive proteins, antibodies (also known as immunoglobulin, Ig) which help to counter the pathogen in various way. Both innate and adaptive immunity have the immune system component comprises of cellular and soluble elements (Gleeson, 2006).

The cellular component of innate immune comprise of natural killer cell and phagocytes (neutrophils, eosinophils, basophil, monocyte, macrophages). Meanwhile, the soluble components consist of complement, lactoferrin, lysozymes, cytokine and acute phase protein. On the other hand, the adaptive immunity consists of cellular component

which is B–cells (mature in bone marrow) and T- cells (mature in thymus). The immunoglobulin (Ig) such as Ig A, Ig D, Ig E, Ig G and Ig M are soluble component in adaptive immunity (Gleeson, 2006).

Factors affecting immune functions include exercise, age, gender, psychological stress nutritional, and environmental factors (Gleeson, 2006). It is well known that exercise affects immune system whereby the effects are depending on the intensity and duration of the exercise. In general, regular moderate intensity exercise enhances immune function while prolonged high intensity exercise may depress immune function (Nieman, 1994). During strenuous exercise, the levels of stress hormones were elevated. It is well known that acute bout of exercise causes a temporary depression of various aspect of immune function such as neutrophil oxidative burst, lymphocyte proliferation which last for 3 to 24 h after exercise. These temporary depressions depend on intensity and duration of exercise (Gleeson & Bishop, 1999). This topic will be further discussed later in the following sub-topic.

Aging is associated with a reduced efficiency ("immunosenescence") of both the innate and adaptive immune system (Gomez *et al.*, 2005; Lord et al., 2001). Soon after birth, the ability of thymus gland to produce new (naive) white blood cells (T-lymphocytes, or T cells) begins to decrease (Parham, 2005). This condition happens with a substantial reduction by age 50 and almost complete incapacity by age 60 (Parham, 2005). According to Lord *et al.* (2001) and Miller (1996), the percentages of memory T cells which have been trained to respond to a particular pathogen (respond to a novel invader) were greater compared to naive T cells in older individuals. As a result of these and other

changes, cells of older individuals become less able to respond to both novel and previously encountered infectious agents.

Gender may also affect immune function whereby women are more resistant to viral infection (Beery, 2003) and 2.7 times greater risk than men to acquire an autoimmune disease (Beery, 2003; Jacob *et al.*, 1997). It has been found that immunity is influenced by the menstrual cycle and pregnancy in females whereby estrogen and progesterone modulate the immune function (Haus & Smolensky, 1999). For example, an elevated level of progesterone during pregnancy was found to suppress cell-mediated immune function and Th1 cytokine production (Wilder, 1998). Over 8.5 million American have been diagnosed with autoimmune diseases and generally more common in adults than in children, and are more common in females than in males (Beeson, 1994; Hochberg & Spector, 1990).

High level of psychological stress may also contribute to the increased susceptibility to infectious disease in athletes (MacKinnon, 1997; Sparling, 1993). In one study, it was reported that people who experiencing periods of chronic stress have shown reduction in the number of helper T-lymphocytes (Futterman *et a.l*, 1996), B-lymphocytes (Schaeffer *et al.*, 1985) and SIg-A concentration (Dienzer *et a.l*, 2000). Thus, it can be conclude that psychological stress also can affect the immune function in the body.

Evidence suggests that inadequate or inappropriate nutrition can negatively affect immune function. Dietary deficiencies of protein and specific micronutrients have long been associated with immune dysfunction (Chandra, 2009). The function of immune system can be maintain through an adequate intake of iron, zinc and vitamins A, E, B6

and B12, however excess intakes of some micronutrients can also impair immune function and have other adverse effects on health (Chandra, 2009). Immune system depression has also been associated with an excess intake of fat (Gleeson *et al.*, 2004). Hence, to maintain immune function, athletes should eat a well-balanced diet sufficient to meet their energy requirements.

Another factor that influence the immune system include environmental factor. Extremes of cold, heat and altitude may indirectly influence immune function via their influences on central nervous system and specifically on the hypothalamic-pituitary-adrenal and sympatheticoadrenal-medullary axes, which control the release of adrenal hormones (Jonsdottir, 2000). Since sympathetic nervous system activation appears to be closely related to exercise and exercise associated with changes in salivary markers of mucosal immunity, any additional stimulation of these pathways through exercise in adverse environmental conditions may be expected to cause greater disturbance to these markers of mucosal immune function (Gleeson & Bishop, 2009). It was also claimed that depletion and/or temperature changes in the mucous membrane may alter the cells function responsible for local respiratory tract immunity (Tomasi *et al.*, 1982). This topic will be further discussed later in this chapter.

2.2 Effects of exercise on immune function

Without additional sources of stress, exercise itself has long been known to influence the immune system. According to Nieman (1997) and Shephard & Shek (1998), the outcome of immune responses is depends on the intensity and duration of exercise

whereby moderate intensity exercise results in increased immune function and prolonged high intensity exercise results in immune depression. It has been shown that exercise can affect both the distribution (circulating concentration) and/or the functional activity of the cellular and humoral immune parameters. Athletes and coaches are very concern about their training loads as suppressed immune function is associated with high risk of infection whereby infection may negatively affect athletes' performance (Friman and Ilback, 1998).

2.2.1 Moderate exercise and immune function

A group of 36 mildly obese sedentary women was participated in a study to investigate the relationship between moderate intensity exercise and improvement in cardiorespiratory fitness, changes in natural killer (NK) cell number and activity, and acute upper respiratory tract infection (URTI) symptomatology (Nieman *et al.*, 1990). In this study, each participant have to do brisk walking at 60% heart rate reserve for 45-min per sessions every week for 15 weeks. The results obtained from this study shows that moderate intensity exercise training is associated with elevated NK cell activity, and reduced URTI symptomatology in comparison to the sedentary control group.

In separate study, Nehlsen *et al.* (1991) had a group of 36 sedentary, mildly obese women to investigate the relationship between moderate exercise training and changes in immune system variables and function. They were separate into 2 groups; exercise and non-exercise group. Each participant in exercise group undergoes exercise training (brisk walking at 60 heart rate reserve) 45 min per session, five times per week for 15 weeks. Within exercise group changes were characterised by significant decreases in

number of total lymphocytes and T cell number after 6 week. However there are significant increases in each of the serum immunoglobulins after both 6^{th} and 15^{th} weeks of training. Meanwhile, in non-exercise group, B cell number increased significantly in subjects relative to baseline values at both 6^{th} and 15^{th} weeks, but no significant changes experienced in exercise subjects. In summary, these data suggest that moderate exercise training is not associated with an improvement in lymphocyte function. But it associated with a 20% increase in serum immunoglobulins and several small changes in circulating numbers of immune system variables. These changes were especially apparent after 6 weeks of training, with some attenuation by 15 weeks.

The influence of moderate exercise (45 min at 55% maximal oxygen uptake) on the phagocytic capacity of neutrophils was evaluated in sedentary men by Ortega *et al.* (2005). They had ten healthy male volunteers aged between 20 and 22 years to participate in the study which involved exercised on a cycle ergometer for 45 min at exercise intensity of 120 W (corresponding to 55% of maximal aerobic power). At the end of this study, they noticed that moderate exercise performed by sedentary people stimulates the phagocytic capacity of neutrophils, and the stimulation lasts for at least 24 h. This stimulation was mediates by norepinephrine, although other mechanisms could be involved during the recovery period.

In other study, the effect of moderate acute exercise on immunological salivary parameters over 24 h had been determined (Rosa *et al.*, 2014). Ten male adult were recruited whereby they exercised for 60 min at moderate intensity. The results obtained from this study shows that, moderate intensity exercise does not induce changes immediately after exercise, but after 24 h, it produces an increase in salivary tumour

growth factor beta (TGF-β). In conclusion, there is no change in the immunological parameters of saliva immediately after acute exercise for 60 min at moderate intensity. However, 24 h after exercise, there was an improvement in immunological parameters, which could be modulated by increases in TGF-β but not IL-5 (Rosa *et al.*, 2014).

2.2.2 Prolonged, high intensity exercise and immune function

After prolonged intense exercise, many components of the immune system were altered. This alteration was proposed due to 'open window' event occurred following the prolonged intense exercise (Nieman, 2000). 'Open window' is a condition where, immune system is weak following prolonged intense exercise. During this period, pathogens may easily enter the body hence increase the infection risk. This condition may last up to 3 to 72 hours, depending on the intensity and duration of the exercise (Nieman & Pederson, 1999). Without sufficient recovery following repeated activity such as during heavy periods of training and competition, it may exacerbate the situation that may leads to a chronic depression of several aspects of immune function (e.g., neutrophil respiratory burst, lymphocyte proliferation, monocyte, antigen presentation) (MacKinnon *et al.,* 1991; Gleeson *et al.,* 1995).

Numerous studies have been conducted to study the effects of prolonged or high intensity exercise on immune function. For example, in one study, thirteen semi-endurance elite male runners with an average age of 18 years had volunteered to take part in the study for a period of 14 weeks (12 sessions per week) (Hejazi *et al.*, 2012). This study focused to compare the levels of serum immunoglobulin (IgA, IgM, IgG) in semi-endurance elite

runners during general preparation and competition phase of training. The levels of serum IgM in semi-endurance elite runners after preparation phase reduced significantly, while these levels during the competition phase increased significantly. The levels of serum IgG and IgA also reduced, however not significantly, during both phases. Moreover, after preparation phase, there was no significant change in serum IgA levels; though, these levels reduced, however not significantly, before competition phase. Thus, findings indicated that long and intensive exercises weaken the immune system, while moderate and short drills strengthened this system (Hejazi *et al.*, 2012).

Gillum and colleagues (2013) had investigated the effect of a 50-km trail race on salivary cortisol, IgA, lysozyme, and lactoferrin. In this study, 14 subjects consist of 6 females and 8 males were recruited and had completed the 50 km ultramarathon. It was found that Lactoferrin concentration was higher at 120 min post-race whereas lysozyme concentration was unaffected by the race. In addition, IgA concentration, secretion rate, and IgA/osmolality were lower 120 min post-race. The findings suggests that prolonged exercise significantly decrease IgA responses (supress the immune function) whereby may associated with increase infection risk. However, increase in lactoferrin concentration following the race might overcome the decrease in IgA hence may protect the athletes from suppression of immune function (Gilium *et al.*, 2013).

In 2005, Scharhag and colleagues examined the influence of 4 h cycling at an intensity of 70% of the individual anaerobic threshold on immune cell function. Interleukin-6, C-reactive protein, leukocyte and lymphocyte populations, and activities of NK cells, neutrophils, and monocytes were examined before and after exercise, and also on a control day without exercise. It was found that cycling for 4 h induced a moderate acute

phase response with increases in IL-6 and CRP after exercise. Although absolute numbers of circulating NK cells, monocytes, and neutrophils increased during exercise, on a per cell basis, NK cell activity and neutrophil and monocyte phagocytosis, and monocyte oxidative burst did not significantly change after exercise. It can be concluded that prolonged cycling at moderate intensities does not seems to seriously alter the function of cells of the first line of defence. Therefore, the influence of a prolonged road cycling training session on the immune system is only moderate and appears to be safe from an immunological point of view (Scharhag *et al.*, 2005).

In a separate study, the acute effects of prolonged exercise on salivary immunoglobulin A (SIgA) was determined (Davison *et al.*, 2009). Twelve active men exercised on a cycle ergometer for 2.5 h at approximately 60% of maximal oxygen uptake. It was found that SIgA concentration and secretion rate were unaltered, however there was a significant decrease in SIgA:osmolality ratio following exercise. Besides that, it also found that salivary antibacterial capacity did not change. Thus it can be concluded that prolonged exercise induce decrease in SIgA:osmolality ratio whereby this may suggest depression of mucosal immunity (Davison *et al.*, 2009).

2.3 Mucosal immunity

Mucosal immune system refers to the network of organised structures which protect mucosal surfaces such as those in the gut, uro-genital tract, oral cavity and respiratory system (Gleeson & Pyne, 2000). Mucosal immunity in association with innate

non-specific defence forms the first line of defence against pathogens, allergens and antigens presented at mucosal surfaces (Gleeson & Pyne, 2000).

During inhalation, bacteria are trapped in a mucous gel above the airway epithelium and propelled toward the pharynx by concerted movements of the underlying cilia (Boucher, 2004). The efficacy of this physical clearance is incomplete. The bacteria which unable to remove then penetrating the mucous layer and adhering to the epithelium. Therefore, antibacterial polypeptides are secreted by the surface epithelium and submucosal glands (Ganz, 2002). In human mucosa, peptides and proteins, including lactoferrin, symbolize the bricks of natural non-immune defence against microbial infections (Orsi, 2004). Mucosal secretions protect the oral mucosa via a mechanical washing effect and play an important role in immunity as the first line of defence against potential pathogens invading the oral and nasal cavities (Gleeson & Pyne, 2000).

The influence of intensity and volume of exercise on mucosal immune parameters has been studied, as have some of the confounding factors of physical, environmental and psychological stressors (MacKinnon, 2000). The first research into the effects of exercise on mucosal immune parameters was published by Tomasi *et al.* in 1981, where it was speculated that the temporary antibody deficiency on the mucosal surface, particularly after strenuous exercise, might lead to susceptibility to acquiring viral and bacterial infections.

2.3.1 Salivary lactoferrin

Saliva has defence mechanisms against pathogen microorganisms, in the presence of defence proteins that react in specific (immunoglobulins) or non-specific (lysozyme, peroxydase, cystatins, lactoferrin, hystatins and others) ways, inhibiting microorganisms growth (Amerogen *et al.*, 2004 & Lawrence, 2002). There are two most abundant antimicrobial proteins (AMPs) that produced by epithelial cells and salivary glands, and also localised in granules of neutrophils; lactoferrin and lysozyme (Dubin *et al.*, 2004). Lactoferrin helps to improve immunity by inhibiting iron uptake by microorganisms, thereby reducing bacterial growth (Weinberg, 1992). Meanwhile, lysozyme may enhance protection against gram-positive bacteria (Leitch & Willcox, 1992).

The present study concerned on the effect of prolonged exercise in the heat and cool environments on lactoferrin responses. Lactoferrin is antimicrobial protein that have an ability to hold has iron, hence; it is related to inhibition of microbial growth as well as to modulation of motility, aggregation and biofilm formation of pathogenic bacteria (Berlutti *et al.*, 2011). During exercise, lactoferrin will interacts with microbial, viral and cell surfaces thus inhibiting microbial and viral adhesion and entry into host cells. Lactoferrin can be considered not only as a primary defense factor against mucosal infections, but also a polyvalent regulator which interacts in viral infectious processes.

Its antiviral activity, demonstrated against both enveloped and naked viruses, lies in the early phase of infection, thus preventing entry of virus in the host cell (Berlutti *et al.*, 2011). Furthermore, it was reported that during viral infection, the epithelium can be injured, with the consequence of loss of integrity and protection. As a matter of fact, the

mucosa plays an important role as a protective physical and functional barrier between the external environment and underlying tissues, while the components of its secretions, especially lactoferrin are central elements in the initiation and regulation of innate and adaptive immune responses.

2.3.2 Effects of exercise on lactoferrin

To date, there are limited data exists regarding effects of exercise on salivary lactoferrin. However, one study had conducted an observational study by comparing salivary lactoferrin and lysozyme concentration over 5 months (chronic changes) in elite rowers with sedentary individuals and a graded exercise test to exhaustion (acute changes) with a cohort of elite rowers (West *et. al.*, 2010). As a result, lactoferrin concentration in the observational study was approximately 60% lower in rowers than control subjects at baseline and at the midpoint of the season but the concentration not statistically significant between the groups at the end of the study. There was also a 50% increase in the concentration of lactoferrin from pre-exercise to exhaustion in the graded exercise session. The low concentrations of these proteins may cause by an impairment of innate protection of the upper respiratory tract. Following exhaustive exercise, salivary lactoferrin concentration increased may be due to a temporary activation response that increases protection in the immediate post exercise period. However, these study only conducted by comparing the active and sedentary lifestyle man (West *et. al.*, 2010).

Gillum *et al.* (2013) had conducted a research to study on salivary antimicrobial protein response to prolonged running. This study involved 14 participants which

comprises of 6 females and 8 males completed a 50 km ultramarathon. Saliva was collected pre, immediately after (post) and 1.5 h post-race. As a result, Gillum found that lactoferrin concentration was higher at 1.5 h post-race compared to post-race. At 1.5 h post-race, lactoferrin secretion rate decreased by 36% from pre-race values, but it was not statistically different. The result suggests that prolonged exercise may compromise immune function by decreasing the lactoferrin secretion rate (Peters & Bateman, 1983).

However, a separate study had reported a different finding where serum lactoferrin concentrations increased 3.3-fold after exercise in all groups (Fielding *et al.*, 2000). In this study, twenty-four male volunteers were randomised into three groups (Group C, D and F). Group C performed 10 sets of 10 eccentric contractions of the quadriceps muscles with both legs (100% of the concentric IRM). Meanwhile, Group D and Group F exercised for 2 h at 56% VO_2max on a cycle ergometer followed by a similar bout of eccentric contractions. This inconsistent finding may be attributed to different study protocol employed in both studies.

Another study performed by Inoue (2004) which aim to determine serum lactoferrin concentrations and serum antibacterial activity before and after running exercise had been carried out. This study involve 24 healthy young men where, they were randomly assigned to high, middle, or low intensity of exercise groups (5000 steps running at 180, 130, and 80 steps/min, respectively). Blood samples were collected at baseline and immediately post-, 1 h post- and 4 h post-exercise. The result reveals that serum lactoferrin concentration was increased immediately post-exercise in all groups and may play an antibacterial role in host defences before mobilisation of neutrophils into the circulating pool (Inoue, 2004).

As mentioned earlier, studies on the effects of exercise on lactoferrin responses were limited to date. Hence, more studies are needed to established relationship between exercise intensity and lactoferrin responses.

2.4 Effects of exercise in the heat and cold environment on immune function

Exposure to environmental extremes is also physiologically stressful and can affect immunity. A combination of both exercise and environmental stressors may increase the degree of observed physiological changes above what is observed with either that stressor alone (Lavoy *et al.,* 2011). According to Tyler *et al.* (2013), it is unsurprising that greater effect sizes are observed in performance and capacity tests of longer duration because prolonged exercise in the heat poses a greater threat to thermal homeostasis than shorter bouts of exercise.

An evident from few study shows that, compared with exercise in thermoneutral conditions, exercise in hot conditions is associated with increased core temperature, cardiovascular drift, increased circulating stress hormones and catecholamines and an increased reliance on carbohydrate as a fuel source (Febbraio, 2001; Galbo *et al.,* 1979; Galloway & Maughan 1997). It is important to distinguish between the increase in body temperature because of fever and the increase in body temperature caused by passive heat exposure and vigorous physical activity (Shephard & Shek, 1998). During passive heat exposure or vigorous physical activity, the hypothalamic temperature set point remains the same but problems with heat dissipation causes body temperature to rise (Shephard &

Shek, 1998). It has been shown that, although light exercise even in the presence of extreme environmental conditions might promote a beneficial immune response. Nevertheless, intensive or prolonged exercise and/or heat condition might generally elicit an immunosuppressed response similar to trauma or inflammation. This can, in turn, increase susceptibility to viral infections or URTI (Shephard & Shek, 1998; Gleeson, 2000; Nieman, 2000).

During cold exposure, the body attempts to maintain normal body temperature by increasing heat production and minimising heat loss (Rammsayer, 1993). This is accomplished through involuntary tonic muscular activity, rhythmic muscular activity (shivering), peripheral vasoconstriction, and the suppression of sweat secretion. Although primarily thermoregulatory, these mechanisms may alter immune status by induce the metabolic and hormonal changes. Furthermore, exposure to cold temperatures affects the physical, cellular, and molecular defenses against pathogens in both humans and animals (Emily *et al.*, 2011) because cold temperatures can damage normal physical barriers to infection, such as increased mucus viscosity and decreased ciliary action in the upper respiratory system (Giesbretch, 1995; Shephard & Shek, 1998). Cutaneous barrier function is also disrupted upon cold exposure, even without the occurrence of frostnip or frostbite (Halkier *et al.*, 1995).

Unfortunately, to our knowledge, there are no published studies available in the literature review regarding the effects of exercise in the hot and cool environments on lactoferrin responses to date. However, numerous studies had reported effects of exercise in the hot and cold conditions on other immune parameters. For example, one study had been carried out by Mitchell and McFarlin (2002) to determine the effect of thermal stress

and hydration status on immune function during exercise. Ten trained men completed four cycle ergometer rides at 55% VO_2 peak under the 4 different conditions. Participant need to cycle in normal (22°C, 30% RH) and hot environment (38°C, 45% RH) with and without water intake. The result obtained from this study shows that lymphocytes proliferation was depressed 2 h after exercise in all conditions. Compared with pre-exercise, NK activity was greater at post-exercise in all conditions but not different between conditions. In hot environment, both with and without water intake caused an elevation of leukocyte, lymphocyte, neutrophil, and NK cell at post-exercise and remained elevated 2 h post-exercise. As conclusion, this study found that hydration status did not affect the cell number and function. However, the hot environment caused severe disturbance to immune function compared with neutral environment (Mitchell & McFarlin 2002).

In a separate study, seven healthy active males completed two cycling trials at 70% VO_2max for 90 min in a control (15 °C) and heat (35 °C) environments (Starkie *et al.*, 2005). This study found that exercise decreases the amount of cytokine produced by lipopolysaccharide (LPS)-stimulated monocytes, possibly due to elevated levels of circulating stress hormones. However, heat stress did not augment the suppression in the amount of cytokine produced by circulating monocytes upon stimulation, despite elevated catecholamines (Starkie *et al.*, 2005).

In another study, 11 healthy male subjects performed 2 bouts of exercise (one at 23°C and another one at 40°C) on a cycle ergometer at 50% VO_2max for 30 min with 45 min rest in between (Sever *et al.*, 1996). After performing the 2 bouts of exercise in the thermoneutral and heat condition, the core temperature increased by 0.9°C and 1.6°C

respectively. Immune cells (granulocytes, monocytes and lymphocytes) were significantly higher when exercising at 40°C. Researchers suggest that there is a synergism between heat and exercise exposure due to stress which recruit leukocytes into the peripheral circulation (Sever *et al.*, 1996).

Similarly, another study had been carried out to examine the combined effects of very hot or cold temperatures during exercise in the same group of subjects (MacFarlin and Mitchell, 2003). In this study, there were 10 men who completed 60 min cycling on a cycle ergometer at 60% VO_2peak in 2 different trials: hot (38°C, 45% RH) and cold (8°C, 50% RH). At the end of this study, researchers found that total leukocyte count, neutrophil count and natural killer cell activity (NKCA) was greater at post-exercise and 2 h post-exercise compared to pre-exercise and 24 h post-exercise. Only lymphocyte count was reported to be significantly higher in the heat trial compared to cold trial. Thus researchers concluded that exercise in the heat produce more physiological stress than cold. However, this difference was not manifested in the immune system response. Researchers suggest that heat and cold stress in combination with exercise produce similar disturbances in immunity during recovery from exercise (MacFarlin & Mitchell, 2003).

CHAPTER 3

METHODOLOGY

3.1 Research design and location

A randomized, cross over study design was employed for the present study. Participants performed 2 separate trials exercise in the heat environment followed by exercise in the cool environment or vice versa. Recovery period between these two trials was one week.

This research study was conducted in Sport Science Unit Laboratory, Sport Science Unit, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kelantan, Malaysia. This study has been approved by the Human Research and Ethics Committee, Universiti Sains Malaysia, Health Campus, Kelantan, Malaysia (Appendix A).

3.2 Sample size calculation

Sample size was calculated by using PS Software. The power of the study was set at 80% with 95% confident interval, the standard deviation (σ) observed 37.5 of average power and difference in population mean (δ) was set at 40. The calculated sample size is 11 participants. Considering 10 – 20% participants drop-out rate, 13 participants have been recruited for this study.

3.3 Participants

Thirteen active recreational male athletes were recruited among students of Universiti Sains Malaysia for this study. The probability sampling was done by choosing the participants randomly. The inclusion criteria include healthy male, age ranging between 18 to 30 years old, non-smokers, and physically active (exercised at least three days per week with at least three minute per session). On the other hand, the exclusion criteria include individual who have cold or respiratory tract infection at least 2 weeks prior to the study and on medication. During participation in this study, participants were abstained from taking any supplements that is known to affect immune function such as probiotic, Vitamiv C, Vitamin D, and plant pholipenols like Quercetin.

Before recruitment, participants were given a health questionnaire to assess their overall health status (Appendixes B). Participants were also given the participant's information sheet (Appendixes D) and were asked to fill in the consent form (Appendixes D) when they agreed to participate. Participation in this study was in voluntary basis, thus participants have their right to withdraw from this study at any time during the course of this study. Their participation also may be stopped without their consent if they did not comply with the study procedures.