The most exciting phrase to hear in science, the one that heralds the most discoveries, is not "Eureka!" (I found it!) but "That's funny..." ~Isaac Asimov

University of Alberta

Comparison of T cell subsets and cytokine production in response to endurance exercise in trained and untrained females

by

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Abstract

Th1 and Th2 immunity was examined following 60 minutes of exercise in 3 different conditions: (UT) untrained females cycled at an intensity equivalent to 20watts below their (VT); (TE1) trained females cycled at the same intensity as the untrained females (90watts) and; (TE2) the trained females cycled at 20watts below their own VT. Blood samples were taken at rest, post-exercise, and after 2-hours of recovery. Th2 cells decreased and IL-12 and IFN-γ concentrations increased following TE1 and TE2 exercise conditions ($P<0.05$). IL-2 increased in the TE2 and decreased in the UT. IL-10 concentrations were lower in the TE1 compared to the UT ($P<0.05$). The findings indicate that Th1 immunity was elicited in the trained women, while Th2 immunity was elicited in the untrained women following endurance exercise. In conclusion, Th1 and Th2 immunity is altered in females following acute endurance exercise and this alteration is influenced by the individual's fitness level.

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Nomenclature and Abbreviations

WBC	White Blood Cell
RBC	Red Blood Cell
CD	Clusters of Differential
CD3	T Cells
CD3+CD4+/ Th	Helper T Cells
CD3+CD8+/ Tc/s	Suppressor or Cytotoxic T Cells
Th1	Helper T Cell type 1
Th2	Helper T Cell type 2
$CRTH_2$	Cellular marker for Type 2 T cells
CD3-CD16+CD56+/ NK	Natural Killer Cell
CD20+CD19+	B cells
APC	Antigen Presenting Cell
Ig	Immunoglobins
IF	Immunofluorescence
MAb	Monoclonal Antibodies
IL	Interleukin
IFN	Interferon
TNF	Tumor Necrosis Factor
TGF	Transforming Growth Factor
IGF	Insulin-like Growth Factor
PHA	Phytoaemagglutinn mitogen
LPS	Lippolysccaride mitogen

URTI	Upper Respiratory Tract Infection
CNS	Central Nervous System
CRH	Corticotropin Releasing Hormone
ACTH	Adrenocorticotropic Hormone
$V0_{2max}$	Maximal Oxygen Consumption
VT	Ventilatory Threshold
RER	Respiratory Exchange Ratio
HR	Heart Rate
PO	Power Output
OC	Oral Contraceptives
T0	1^{st} resting blood sample
T60	2^{nd} blood sample immediately post exercise
T180	3^{rd} blood sample after 2 hours of recovery

Chapter 1

Introduction

1.1 Introduction

Exercise immunology is a new and developing area of exercise physiology in which advances and discoveries in the understanding of the immune system enable researchers to develop new theories as to how exercise influences immunity. In particular, it has been recently discovered that there are cellular subsets of Helper T cells (Th), known as Th0, Th1, Th2 and Th3 (De Carli et al., 1994; Doug & Flavell, 2001; Romagnani, 1995; Yates et al., 2000). These Th cells can be identified by the cytokines they produce. Cytokines are polypeptides that are secreted by various nucleated cells. They play a central role in the regulation of the immune system and in the inflammatory response. Cytokines provide communication between various immune cells and to other cells outside the immune system. Generally they act locally, in a paracrine or autocrine manner, although they do travel through the vascular system. Th1 cells normally secret interleukin (IL)-2, interferon (IFN)-γ, tumor necrosis factor (TNF) -α and TNF-β. Th2 cells predominantly secrete IL-4, IL-5, IL-6, IL-10 and IL-13 (Doug & Flavell, 2001; Romagnami, 1995). Th0 is considered a precursor to these two cells and is found to secrete many of these various cytokines. Th3 is believed to be the only cell that secretes transforming growth factor (TGF)-β (Cerwenka and Swain, 1999). Th1 cytokines support the development of cellular-mediated immune response, which is important for defense against intracellular pathogens. Th2 response supports the humoral-mediated immune

response, which helps to defend against certain parasites and free-living bacteria (Romagnani, 1995).

Research has shown that Th1 and Th2 regulate each other, which creates a homeostasis within the adaptive immune system (Morel & Oriss, 1998). Depending on the type of infection, either Th1 or Th2 will differentiate to control the portion of the immune system most suitable to combat the infection. Eventually, the adaptive immune system will return to homeostasis through cross-regulation between the two cell's cytokines (Morel & Oriss, 1998). However, if either Th1 or Th2 is chronically favoured, this may leave the other component of the immune system suppressed, increasing the susceptibility to various pathogens or certain chronic diseases that may be allowed to develop, based on which Th cell line is influenced (Spellberg & Edwards, 2001).

There is a growing interest in understanding how the immune system responds to various physiological stressors, such as exercise. It has been shown that marathon running can cause a decrease in both immune cell numbers and function, creating an "open window" of susceptibility to disease (Nieman, 2000). It has been shown that various stress hormones, such as cortisol and epinephrine, increase in response to strenuous endurance exercise and may influence this immune response (Pedersen et al., 1997). Interestingly, both cortisol and epinephrine have been shown to inhibit the release of pro-inflammatory cytokines, and the cytokines produced by Th1 cells (IL-2, IL-6, INF-γ, TNF-α, and TNF-β). As well, it has been shown that IL-4 and IL-10 secretion is stimulated by stress hormones (Elenkov & Chrousos, 1999; Rhind et al., 1995). It has also been suggested that cytokines released during endurance exercise may also modulate the immune response. IL-6, which stimulates Th2 cell differentiation, increases during

and after endurance exercise (Moldoveanu et al., 2001; Northoff et al., 1994; Pedersen 2000). The exact mechanism for this exercise-stimulated increase in IL-6 still remains unclear. This increase may be due to stimulation by either an increase in IL-1 or in cortisol, or in both (Moldoveanu et al., 2001; Pedersen, 2000). It has also been suggested that the muscle fibers produce IL-6 in response to muscle damage (Pedersen, 2000; Pedersen & Toft, 2000).

Research suggests that training at a moderate intensity can benefit the immune system, although this has not been extensively examined (Mackinnon, 2000). The decreased IL-2 production and lymphocyte proliferation may be attenuation in trained individuals, post-submaximal endurance exercise (Moldoveanu et al, 2001; Rhind et al., 1996). The majority of this research is based on male participants. Trained females may in fact show a different immune response after acute strenuous endurance exercise as studies have shown that resting cortisol levels do not change with endurance training in females (Filaire et al., 1998). However, other studies have reported that females, compared to males, may be hypocortisolic during endurance and during combined strength and endurance training programs (Bell et al., 1997; Horne et al., 1997; Tsai et al., 1991). Females are often excluded from research studies due to the problem of coordinating the study with the menstrual cycle in each participant. This gap in the literature leaves many unanswered questions about immune system function in response to various types of exercise in women.

1.2 Purpose and Hypothesis

The purpose of this study was to investigate the immune response in trained and untrained females following an acute strenuous endurance exercise bout. The immune

response was defined by changes in various immune cells numbers and lymphocyte cytokine production. It was hypothesized that acute strenuous endurance exercise would cause a shift between Th1 and Th2 type response in females, whereby Th2 cell numbers and Th2 cytokine production would be favoured. To fully examine the immune response to endurance exercise in trained and untrained females, two exercise intensities were compared. One exercise condition allowed for a comparison between trained and untrained women performing the same amount of work. The second comparison was made after participants performed different amounts of work, but at the same intensity relative to fitness level in both groups. It was also hypothesized that there would be a difference in the cytokine production of Th1 and Th2 cells in trained and untrained females following the different exercise conditions.

1.3 Significance

Literature has shown that individuals suffering from certain diseases such as, multiple sclerosis, rheumatoid arthritis, type 1 diabetes and other autoimmune diseases have an over production of Th1 cytokines. Individuals with conditions such as allergies, asthma, systemic lupus, and systemic sclerosis have an overproduction of Th2 cytokines and in the case with those who have human immunodeficiency virus (HIV) there is a suppression of Th1 cytokines. This has led to the suggestion that there is an imbalance between Th1 and Th2 cells which may contribute to the severity or the progression of these diseases (Spellberg & Edwards, 2001). It has also been suggested that the conditions of these diseases may be improved by stabilizing the balance between Th1 and Th2 immunity (Liblau et al., 1995; McGurik & Mills, 2002; Morel & Oriss, 1998; Spellberg & Edwards, 2001).

Exercise has been shown to affect various components of the immune system (Mackinnon, 1999; Nieman, 1997). More specifically, acute strenuous endurance exercise has been shown to suppress peripheral T cell counts, as well as lymphocyte proliferation (Mackinnon, 1999; Nieman, 1997; Pedersen, 2000). Cytokine concentrations have been found to change after acute strenuous endurance exercise (Mackinnon, 1999; Moldoveanu et al., 2001; Pedersen, 2000; Smith, 2003). Depending on the specific cytokine examined, concentrations have been either elevated or decreased. Additionally, training has been linked with an adaptive response in T cells, lymphocyte proliferation and cytokine production (Mackinnon, 1999, 2000; Moldoveanu et al., 2001; Pedersen & Toft, 2000). Consequently, it was hypothesized that acute and chronic exercise may influence the balance of Th cell subsets and their cytokine production.

1.4 Delimitations

For this study, healthy trained and untrained females were recruited. Both progesterone and estrogens influence components of the innate and adaptive immune system (Beagley and Gockel, 2003). Therefore, changes with the female menstrual cycle will have an effect on he immune system (Northern et al., 1994; Pehlivanoglu et al., 2001). To control for this, each participant's menstrual cycle was monitored by questionnaire, and participants were required to be taking oral contraceptives (OC) during and four months prior to the study. The exercise tests were conducted during the first seven days of the OC cycle, which mimics the early follicular phase of the menstrual cycle. Individuals with severe allergies or immune disorders were excluded from the study, due to unforeseen implications on the immune response. Participants were also be asked to refrain from taking anti-inflammatory or anti-histamine medication three days

prior to testing, as such medication has been shown to affect the level of cytokine production (Rhind et al., 2002).

1.5 Limitations

Participants were volunteers from Edmonton and the surrounding community. They were not randomly selected from a greater population or randomly assigned to the two groups, since it was a cross-sectional study. Additionally, only peripheral blood was analyzed for changes in immune response. It must be kept in mind that blood represents only 2% of the lymphocyte population in the body. Finally, the findings from this research can only be claimed to occur during the first seven days of the pharmaceutical contraceptive cycle. Though it is highly probable that similar findings would occur during other phases of the OC and the menstrual cycle, this cannot be stated with confidence, as female sex hormones do influence the immune system.

1.6 References

Beagley, K.W. & Gockel, C.M. (2003). Regulation of innate and adaptive immunity by the female sex hormones oestradiol and progesterone. *Immunology and Medical Microbiology, 38*, 13-22.

Bell, G., Syrotuik, D., Socha, T., Maclean, I., & Quinney, H. A. (1997). Effect of strength training and concurrent strength and endurance training on strength and testosterone, and cortisol. *Journal of Strength and Conditioning Research, 11*(1), 57-64.

Cerwenka, A., & Swain, S. L. (1999). TGF-beta1: Immunosuppressant and viability factor for T lymphocytes. *Microbes and Infection, 1*(15), 1291-1296.

De Carli, M., D'Elios, M. M., Zancuoghi, G., Romagnani, S., & Del Prete, G. (1994). Human Th1 and Th2 cells: Functional properties, regulation of development and role in autoimmunity. *Autoimmunity, 18*(4), 301-308.

Doug, C., & Flavell, R. A. (2001). Th1 and Th2 cells. *Current Opinion in Hematology, 8,* 47-51.

Elenkov, I. J., & Chrousos, G. P. (1999). Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. *Trends in Endocrinology and Metabolism, 10*(9), 359-368.

Filaire, E., Duche, P., & Lac, G. (1998). Effects of amount of training on the saliva concentrations of cortisol, dehydoepiandrosterone and on the dehydroepiandrosterone: Cortisol concentration ratio in women over 16 weeks of training. *European Journal of Applied Physiology, 78,* 466-471.

Horne, L., Bell. G., Fisher, B., Warren, S., & Janowaks-Wiezoek, J. (1997). Interaction between cortisol and tumor necrosis factor with concurrent resistance and endurance training. *Clinical Journal of Sports Medicine, 7*(4), 247-251.

Liblau, R. S., Singer, S. M., & McDevitt, H. O. (1995). Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunology Today, 16*(1), 34-38.

Mackinnon, L. T. (1999). *Advances in exercise immunology.* Champaign, IL: Human Kinetics.

Mackinnon, L. T. (2000). Chronic exercise training effects on immune function. *Medicine and Science in Sports and Exercise, 32*(7), S369-S376.

McGurik, P., & Mills, K. H. G. (2002). Pathogen-specific regulatory T cells provoke a shift in the Th1/Th2 paradigm in immunity to infectious diseases. *Trends in Immunology, 23*(9), 450-455.

Moldoveanu, A. I., Shepard, R. J., & Shek, P. N. (2001). The cytokine response to physical activity and training. *Sports Medicine, 31*(2), 115-144.

Morel, P. A., & Oriss, T. B. (1998). Crossregulation between Th1 and Th2 cells. *Critical Reviews in Immunology, 18,* 275-303.

Nieman, D (1997). Exercise immunology: Practical applications. *International Journal of Sports of Medicine, 18*(S1), 91-100.

Nieman, D. (2000). Is infection risk linked to workload? *Medicine and Science in Sports and Exercise, 32*(7), S406-S411.

Northern, A. L. D., Rutter, S. M., & Petersen, C. M. (1994). Cyclic changes in the concentrations of peripheral blood immune cells during the normal menstrual cycle. *Proceedings of the Society for Experimental Biology and Medicine, 207,* 81-88.

Northoff, H., Weinstock, C., & Berg, A. (1994). The cytokine response to strenuous exercise. *International Journal of Sports Medicine, 15*, S167-S171.

Pedersen, B. K. (2000). Exercise and cytokines. *Immunology and Cell Biology, 78*, 532-535.

Pedersen, B. K., Bruunsgard, H., Koller, M., Kappel, M., MacLean, D. A., Nielsen, H. B., Rohde. T., Ullum, H., & Zacho, M. (1997). Exercise-induced immunomodulation: Possible roles of neuroendocrine and metabolic factors. *International Journal of Sports Medicine, 18*(S1), 2-7.

Pedersen, B. K., & Toft, A. D. (2000). Effects of exercise on lymphocytes and cytokines. *British Journal of Sports Medicine, 34*, 246-251.

Pehlivanoglu, B., Balkaner, Z. D., Ridvanagaoglu, A. Y., Durmazlar, N. Ozturk, G., Erbas, D., & Okur, H. (2001). Impact of stress, gender and menstrual cycle on immune system: Possible role nitric oxide. *Archives of Physiology and Biochemistry, 109*(4), 383-387.

Rhind, S. G., Gannon, G. A., Shepard, R. J., & Shek, P. N. (2002). Indomethacin modulates circulating cytokine responses to strenuous exercise in humans. *Cytokine, 19*(3), 153-158.

Rhind, S. G., Shek, P. N., & Shephard, R. J. (1995). The impact of exercise on cytokine and receptor expression. *Exercise Immunology Review, 1*, 97-148.

Rhind, S. G., Shek, P. N., Shinkai, S., & Shepard, R. J. (1996). Effect s of moderate endurance exercise and training on in vitro lymphocyte proliferation, interleukin-2 (IL-2) production and IL-2 receptor expression. *European Journal of Applied Physiology, 74,* 348-360.

Romagnani, S. (1995). Biology of human Th1 and Th2 cells. *Journal of Clinical Immunology, 15*(3), 121-129.

Smith, L. L. (2003). Overtraining, excessive exercise and alerted immunity: Is this a T helper-1 versus T helper-2 lymphocyte response? *Sports Medicine, 33*(5), 347-364.

Spellberg, B., & Edwards, J. E. (2001). Type1/Type 2 immunity in infectious diseases. *Clinical Infectious Diseases, 32,* 76-102.

Tsai, L., Johansson, C., Pousette, A., Tegelman, R., Carlstrom, K., & Hemmingsson, P. (1991). Cortisol and androgen concentrations in female and male elite endurance athletes in relation to physical activity. *European Journal of Applied Physiology, 63,* 308-311.

Yates, A., Bergmann, C., Van Hemmen, J. L., Stark, J., & Callard, R. (2000). Cytokine-modulated regulation of helper-T cell populations. *Journal of Theoretical Biology, 206,* 539-560

Chapter 2

Literature Review

2.1 Immune System: General

The immune system includes the tissue, cells and molecules involved with the prevention and elimination of any invading pathogen that could potentially harm the body (Janeway et al., 2001). Examples of pathogens are viruses, bacteria, fungi, allergens and parasites. The immune system also defends the body against tumors and aids in the repair of damaged tissue.

Our immune system is comprised of two main components, the innate and the adaptive immune systems (Janeway et al., 2001). Innate immunity is composed of nonspecific mechanisms that provide the first line of defense against an infection (Goldsby et al., 2000). The cells of the innate immune system are readily available throughout the body and found mainly in the blood stream. Other components that play an important role in the innate immune system include complement, which contributes to inflammation, and mucosal membranes, which trap invading pathogens so they can be removed from body. Response from the innate immune system is rapid and does not rely on antigen recognition in order to defeat a pathogen (Janeway et al., 2001). Once a pathogen has entered the body, the innate immune system attempts to destroy it before it evolves into an infection (Goldsby et al., 2000). However, when the pathogen has overwhelmed the first line of defense, the adaptive immune system will be signaled.

Adaptive immunity is the result of the adaptations of the immune system to an infection with that particular pathogen (Janeway et al., 2001). The adaptive immune

system has specificity and diversity, meaning that its cells can recognize and respond to any particular pathogen that possibly exists (Goldsby et al., 2000). This is possible because every pathogen has a unique antigen molecule, which allows it to be recognizable. Due to this diversity, the immune system also has the ability to "remember" a particular antigen that has invaded the body. This memory allows the immune system to react promptly and efficiently if the host encounters that particular pathogen again. This is possible to the capacity of clonal expansion of the immune cells in response to the second infection. The final key feature of the adaptive immune system is that these cells are able to recognize the body's own molecules and cells. Failure to do so could lead to an autoimmune disorder, such as Type 1 diabetes (Goldsby et al., 2000).

The adaptive immune system is further sub-divided into the humoral-mediated component and the cellular-mediated component (Goldsby et al., 2000). Humoral-mediated immunity involves the production of antibodies, also known as immunoglobins (Ig). Immunoglobins are soluble proteins, produced by B cells, which circulate in the blood and the lymphatic system during an infection. They recognize the particular infectious antigen, which they will bind to, neutralize and destroy by a variety of responses (Goldsby et al., 2000). Cellular-mediated immunity involves the actual attack of cells, either by attacking the pathogen directly or by destroying the cell that is host to the pathogen through lysing or causing apoptosis (Goldsby et al., 2000). It must be kept in mind that the cellular-mediated, humoral-mediated and the innate immune system communicate with each other, helping to stimulate and regulate the entire immune system response (Janeway et al., 2001).

2.2 Immune System: The Cells

White blood cells (WBC), or leukocytes, are the cellular components of the immune system. All leukocytes originate from hematopoietic stem cells in the bone marrow. Many of the cells mature in the marrow, while others migrate and mature elsewhere in the body (Janeway et al., 2001). Eventually, all of the leukocytes migrate to peripheral tissue, circulating in blood or the lymphatic system, where they help to fight invading pathogens.

Leukocytes can be divided into one of the three subclasses: granulocytes, monocytes and lymphocytes (Janeway et al., 2001; Turgeon, 1993). Within these subclasses there are many different leukocytes; however, those that are most commonly found circulating in the blood will be discussed.

There are three types of granulocytes: neutrophils, eosinophils and basophils. All three are rather short-lived and are heavily recruited during an innate immune response. Neutrophils are phagocytotic, meaning they ingest invading pathogens. These cells are the most numerous in the blood and most important for the innate immune system response against bacteria (Janeway et al., 2001). Eosinophils are the chief defense against parasites and play a role in allergy and asthmatic responses. The role of basophils is still unclear, but it has been suggested that they also play a role with in allergies and the release of histamine (Janeway et al., 2001).

Monocytes are immature macrophages. Hematopoietic growth factors in tissue or blood influence monocytes to develop into macrophages (Janeway et al., 2001). Macrophages are also phagocytotic, traveling to the area of infection to consume the invading pathogens. These cells are part of the innate immune response, although they

are heavily involved with cellular-mediated immunity. They are regulated by cytokines that are released through the adaptive immune response and, in turn, can initiate the adaptive immune system through presentation of the antigen to T and B cells (Janeway et al., 2001).

Lymphocytes are small, inactive cells in a healthy individual (Turgeon, 1993). These cells are comprised of T cells, B cells, and natural killer (NK) cells. T cells and B cells have antigen receptors that allow them to recognize specific antigens on pathogens. This makes them the key components of the adaptive immune response. Once activated by their particular antigen, T cells and B cells will differentiate and proliferate, which means they divide into their respective roles and multiply. T cells, depending on their role, can attack and kill invading pathogens, regulate other components of the immune system, and establish a memory of the invading pathogen (Goldsby et al., 2000; Janeway et al., 2001). B cells release Ig, which are designed to seek out the specific antigens and destroy them. NK cells do not have antigen receptors and are therefore a part of the innate immune system. They kill the pathogen by lysing any cells that are host to the pathogen (Janeway et al., 2001).

2.3 T Cells

T cells are the primary part of the cellular mediated component of the adaptive immune system. They are comprised of two main subsets, Helper T (Th) cells and Cytotoxic/Suppressor T (Tc/Ts) cells. These are often classified according to antigen markers on their surface, referred to as clusters of differential (CD). Helper T cells have CD4+ marker while Tc/s cells have CD8+ marker.

Helper T cells are among the most important cells in the immune system. They help with regulation and communicate between cells of the adaptive and the innate immune systems (Goldsby et al., 2000). The Th cells activate and regulate Tc/Ts cells, B cells, NK cells, macrophages, and many other immune cells (Janeway et al., 2001). Recently it has been discovered that there are further subsets to the Th cells. These Th cell subsets are Th0, Th1, Th2 and Th3 and are classified according to the various cytokines that they release (Dong & Flavell, 2001; Romagnani, 1995; Weiner, 2001). These subsets differentiate from activated Th cells (Romagnani, 1995; Weiner, 2001). A Th cell is activated when it comes in contact with the particular antigen that it recognizes, either through the pathogen itself or through an antigen presenting cell (APC), such as a macrophage or dendritic cells (Janeway et al., 2001). Upon activation, the Th cell becomes a Th0 cell and will further differentiate into Th1, Th2 or Th3 cells. The exact mechanism behind this differentiation is yet to be determined. It has been speculated that the APC releases cytokines, which in turn stimulate either Th1 or Th2 differentiation. Interleukin 6 (IL-6) is thought to stimulate Th cells to differentiate into Th2, while IL-12 may stimulate Th1 (see Figure 2.1) (Dong & Flavell, 2001; Rincon et al., 1997; Spellberg & Edwards, 2001).

Cytokines are polypeptides that are produced by cells such as lymphocytes and macrophages; they affect the behaviour of cells in the immune system, as well as the cells that originally produced it (Janeway et al., 2001; Thomson, 1994). Cytokines bind to surface receptors and alter the pattern of gene expression on their target cells. Usually their actions alter the rate of cell proliferation and affect the differentiation of the cells (Thomson, 1994). By secreting cytokines, Th cells can communicate, stimulate and

regulate other cells, such macrophages, B- cells, and Tc/s cells. Cytokines circulate in the blood; however, they mostly act locally, in a paracrine or autocrine manner. Paracrine interactions occur when the secreting/producing cells and the target cells are in close proximity to each other. Autocrine interaction occurs when the cytokine affects the cells that it originally came from, resulting in self-stimulation of that particular cell. There are some key characteristics of cytokines: ambiguity, redundancy, synergy, and antagonism (Goldsby et al., 2000; Thomson, 1994). Ambiguity means that cytokines have multiple target cells and multiple actions, and there is redundancy of these actions between the various cytokines. Also, for a particular action to occur, a cell many require more than one cytokine; this is known as synergy. Finally, many cytokines are antagonistic to each other, inhibiting the secretion or actions of other cytokines.

There are many types of cytokines, such as the interleukins (IL) the interferons (IFN), and the tumor necrosis factors (TNF). The ILs are primarily secreted by lymphocytes, dendritic cells and macrophages, and primarily affect the growth and differentiation of various immune cells (Goldsby et al., 2000). The IFNs are proteins that are able to interfere with viral replication and help prevent the spread of viruses from infected to non-infected cells. They also aid in the regulation of many immune responses (Janeway et al., 2001; Goldsby et al., 2000). TNFs are proteins that are cytotoxic to tumor cells and play a role in inflammatory responses (Goldsby et al., 2000).

Th1, Th2 and Th3 regulate different aspects of the immune system and, therefore, secrete different cytokines. Th1 secretes IL-2, TNF-α, TNF-β, and IFN-γ, communicating primarily with the cellular-mediated component of the immune system (Dong & Flavell, 2001; Goldsby et al., 2000; Romagnani, 1995; Spellberg & Edwards, 2001). Th2 cells,

however, secrete IL-4, IL-5, IL-6, IL-10, and IL-13, which communicate with humoral immunity (Dong & Flavell, 2001; Romagnani, 1995; Spellberg & Edwards, 2001). It is not fully understood what exact role Th3 cells play in the adaptive immune response, but they are the only T cells that secret TGF-β (Weiner, 2001). The specific roles of each of these cytokines will be discussed in detail later.

When the immune system is not responding to a pathogen/antigen challenge, there is homeostasis. This homeostasis also exists between Th1 and Th2 cells and their cytokines. This regulates the immune system, as many of the cytokines produced by Th1 and Th2 are antagonistic to each other (Janeway et al., 2001; Morel & Oriss, 1998; Spellberg & Edwards, 2001). For example, many of the cytokines that Th1 produce are also pro-inflammatory cytokines, while many of the cytokines Th2 cells produce are anti-inflammatory cytokines. If an infection occurs, there is often an inflammatory response, which helps to eliminate the invading pathogen. However, this response can become detrimental to the host if not regulated. Therefore, Th2 cells will differentiate and release cytokines that inhibit this inflammation, allowing the body to return to homeostatic state.

An activated Th cell will differentiate into Th1 or Th2, depending on the type of pathogen that is challenging the host. Th1 cells react against viruses, mycobacteria (bacteria that inhabit cells), yeasts, and parasitic protozoan. Th2 cells react against free-living bacteria and parasitic worms (Janeway et al., 2001; McGuirk & Mills, 2002; Morel & Oriss, 1998). The common cold is generally the result of a viral infection. The viral antigen is presented to the T cell by an APC. This will stimulate the Th cell to differentiate into Th1. Fever, malaise, soreness and inflammation are due to the response of cellular-mediated immunity (Janeway et al., 2001). Once the infection is cleared, Th2

will be stimulated to differentiate and release the cytokines that will inhibit the Th1 reaction and allow the body to return to homeostasis (Morel & Oriss, 1998). The opposite will occur in response to extra-cellular pathogen, in which the humoral immune system will combat the infection.

If Th1 or Th2 type response is chronically favoured, the immune system may have difficulty regulating itself and leave the body more susceptible to particular infections. It has been shown that in several diseases, such as human immunodeficiency virus (HIV), Type 1 diabetes, rheumatoid arthritis, multiple sclerosis, lupus, allergies and with asthma, one particular T cell subset is favoured, allowing over production of the various cytokines (Clerici & Shearer, 1993; Karol, 2002; Liblau et al., 1995; Spellberg & Edwards, 2001). With the organ specific autoimmune disorders, the combination of Th1 cells and their cytokines appears to be dominant. For HIV/AIDS, systemic autoimmune disorders, allergies, and asthma, the opposite occurs, with dominance of Th2 cytokines. Even with aging, Th2 cell differentiation and cytokine production is favored (Sandmand et al., 2002). This has led many to theorize that this imbalance may have an effect on the severity and progress of the disease (Clerici & Shearer, 1993; Karol, 2002; Liblau et al., 1995; Spellberg & Edwards, 2001). For example, although Th2 cells are produced more than Th1 cells in individuals with HIV, in a few of these individuals this does not occur. These particular individuals experience a slower progression to developing AIDS, and in some cases they do not fully develop the disease (Clerici & Shearer, 1993).

Despite the evidence of differentiation in Th cells and their regulation of the adaptive immune system, it must be stressed that Th cells are not the primary control of the immune system. Many different cells, not just Th cells, release cytokines. Therefore,

regulation and communication of the immune system are not entirely dependent on Th1 and Th2 cells. It must be kept in mind that the immune response to stress or infection is complex and multi-dimensional, involving many different systems and cells. Examining the differentiation of Th1 and Th2 and their cytokines represents only a portion of this large, complex and integrated system.

2.4 Stress Hormones and the Immune System

When the body is experiencing physiological stress, such as exercise, a number of hormones are released. These hormones affect many physiological responses of the body, including the immune system and its function. In response to stress, the central nervous system (CNS) responds in two ways. First, it activates the sympathetic nervous system, which then stimulates the release epinephrine and norepinephrine from the adrenal medulla (Elenkov & Chrousos, 1999; Nagatomi et al., 2000; Webster et al., 2002). Epinephrine and norepinephrine are hormones responsible for the "flight or fight" response. The second response to stress by the CNS is the release of corticotropin releasing hormone (CRH) by the hypothalamus. This hormone will stimulate the anterior pituitary gland to release adrenocorticotropic hormone (ACTH) (Elenkov & Chrousos, 1999; Rook et al., 1994; Webster et al., 2002). ACTH will further stimulate the adrenal cortex to secrete glucocorticords, such as cortisol (Elenkov & Chrousos, 1999; Rook et al., 1994; Webster et al., 2002).

Epinephrine and cortisol are stress hormones that affect the immune system (Chryssikopoulos, 1997; Elenkov & Chrousos, 1999). Both hormones modulate the secretion of many cytokines, decreasing pro-inflammatory cytokines and cytokines produced by Th1 cells, IL-1, IL-2, IL-6, IL-8, IL-11, IL-12, TNF-α, and INF-γ, while

stimulating anti-inflammatory cytokines such as IL-4, IL-10, and IL-13 (Chryssikopoulos, 1997; Elenkov & Chrousos, 1999). These changes in the production of these various cytokines will down regulate inflammation and also affect the function of many immune cells, such as macrophages, neutrophils, and lymphocytes (Elenkov & Chrousos, 1999). Due to the suppression of IL-2, IL-12 and INF-γ and the stimulation of IL-4 and IL-10, there is a shift in Th1 and Th2 immunity, where Th2 appears to be favored. Since Th2 cells primarily control humoral immunity, antibody and B-cell activity increases during stress. Th1 cells also appear to have β-2 receptors, suggesting that these cells are also regulated by the epinephrine and norepinephrine released by the CNS in response to stress (Elenkov & Chrousos, 1999; Nagatomi et al., 2000).

Cytokines in turn regulate the endocrine and nervous system. IL-1 and IL-6 can also increase the secretion of ACTH and consequently the release of cortisol (Pedersen & Hoffman-Goetz, 2000; Webster et al., 2002). This could possible create a negative-feedback loop between the immune, endocrine and nervous system, which most likely serves as a protective mechanism against inflammation.

Sex hormones can also affect the immune system. Both estrogens and testosterone have been shown to increase serum levels of IL-6 (Chryssikopoulos, 1997; Pedersen & Hoffman-Goetz, 2000). Estradiol also increases levels of the hormone prolactin (Vera-Lastra et al., 2002; White-Welkley et al., 1996). Receptors for prolactin, progesterone and estradiol have been found on lymphocytes, suggesting that these hormons may indirectly contribute to the regulation of these cells (Chryssikopoulos, 1997; Vera-Lastra et al., 2002). Therefore, the function of the immune system will be altered as these hormones

increase and decrease during the different phases of the menstrual cycle (Beagley and Gockel, 2003).

2.5 Acute Exercise and the Immune System

Exercise is a physiological stress placed on the body, and research has shown that strenuous exercise may compromise certain aspects of the immune system (Mackinnon 2000; Nieman et al., 1997, 2000). During acute strenuous exercise, cortisol, epinephrine, and nor-epinephrine and even prolactin levels increase (Borer 2003; Kjaer et al., 1985; Pedersen and Hoffman-Goetz, 2000; Shinkai et al., 1996; Starkie et al., 2001). It also appears that the increase with cortisol and epinephrine levels is linear, up to a set point, to the duration of the exercise (Petersen & Hoffman-Goetz, 2000), suggesting that endurance exercise places large amounts of stress on the body. The acute exercise-induced immune responses of Th1 and Th2 cells and their specific cytokines will be further discussed.

The majority of the literature has demonstrated that Th (CD4) cell numbers increase during prolonged intense bouts of exercise, and then fall below baseline during recovery. This suppression can last anywhere between 2 and 72 hours, depending on the intensity and duration of the exercise (Mackinnon, 2000). Lymphocyte proliferation also appears to be suppressed during and following an intense bout of exercise (Mackinnon, 1999; Nieman 1997; Pedersen & Toft, 2000). This suggests that there is suppression in T cell numbers and function in response to a strenuous endurance exercise bout.

Currently there are few studies that have examined Th1 and Th2 cell numbers and their cytokines. It is difficult to identify Th1 and Th2 cells, as they are defined by their function rather than physical characteristics. However, a few studies have directly

measured T cells for Th1 and Th2 cytokine pattern through the use of immunofluorescence (IF). Steensberg et al. (2001) found that, with nine male endurance runners tested during and following a 2. 5 hour run at 75% of their $V0_{2max}$, the percentage of CD4 and CD8 cells producing IFN-γ and IL-2 decreased. The percentages of CD4 and CD8 cells producing IL-4 did not change significantly. This suggested that Th1 cells were suppressed, allowing Th2 cells to become dominant following the exercise (Steendberg et al., 2001). Ibfelt et al. (2002) found that, in seven healthy males who ran for 1. 5 hours downhill at 75% of $V0_{2max}$, the percentage of CD8 cells producing IFN-γ decreased significantly following the run. Similarly, IL-4 producing CD8 cells did not change in response to the exercise. A third study (Starkie et al., 2001) examined the effects of an adrenergic blocker on T cell (CD3) IL-2 and IFN-γ production. The results showed that, following a strenuous 20-minute bout of exercise (10 minutes at 82% of $V0_{2max}$ and 10 minutes at 78% of $V0_{2max}$), the percentages of IL-2 and IFN-γ producing T cells decreased significantly in six endurance runners.

Although there is limited research specifically investigating the Th1 and Th2 response to strenuous endurance exercise, similar responses have also been demonstrated in cases of severe physiological stress, such as surgery, where Th1 cytokine concentrations decrease following aortic surgery or carotid endarterectomy (Berguer et al., 1999; Spellberg & Edwards, 2001). Studies examining psychological stress have also found that the percentage of Th cells producing Th1 cytokines decreased following a psychologically stressful event, while Th2 cytokines remained unaffected (Kang & Fox, 2001; Paik et al., 2000). This suggests that Th1 differentiation may be suppressed during a different stressful event, such as a severe bout of exercise.

The majority of the studies examining cytokine response to acute strenuous exercise have focused on plasma concentrations and lymphocyte stimulated production as markers for change. These types of measurements can help to indicate if there is a change in the cytokine profile in response to exercise. However, due to differences in biochemical measures and methodological procedures conducted in different laboratories, there have been contradictory findings in the literature with respect to certain cytokines (Moldoveanu et al., 2001). Despite this, the common findings in the literature will be discussed along with these various cytokines.

Interleukin (IL)-2

Th1 cells primarily release IL-2, which in turn stimulates Th cells to proliferate and grow. Therefore, IL-2 is vital for both humoral and cellular-mediated aspects of the immune system (Janeway et al., 2001; Thomas, 1994). Interluekin-2 stimulates Tc/s cell growth and proliferation, and to a lesser extent B cells, and NK cells.

Two studies have found no changes in plasma/serum IL-2 concentration and lymphocyte production after strenuous endurance exercise (Haahr et al., 1991; Weinstock et al., 1997). However, the majority of the literature that has examined IL-2 after a strenuous endurance bout of exercise found a decrease in plasma concentrations (Espersen, et al., 1990; Suzuki, et al., 2000). When the mitogen phytohaemagglutinin (PHA) is used for lymphocyte stimulation, IL-2 concentrations have also been shown to decrease post exercise (Fu et al., 2003; Lewicki et al., 1988; Rhind et al., 1996; Tvede et al., 1993). In all the studies, IL-2 concentrations returned to baseline after 1-2 hours of recovery.

Interferon (IFN)-γ

Interferon-γ is also released by Th1 cells. It stimulates macrophages, activates NK cells, and increases the major histocompatibility complex (MHC) I and II, which help immature T cells to determine if they are Th cells or Tc/s cells (Spellberg & Edwards, 2001; Thomas, 1994). Most importantly, IFN-γ inhibits Th2 cytokine and is often considered the cytokine that represents Th1 immunity (Janeway et al., 2001; Thomas, 1994).

There is inconsistency in the literature regarding the response of IFN-γ to strenuous endurance exercise using plasma/serum and using lymphocyte stimulated biochemical measures. Some studies have found no significant changes in IFN-γ production and stimulation post exercise (Haahr et al., 1991; Nieman et al., 2001; Suzuki et al., 1999, 2000; Weinstock et al., 1997); increases in IFN-γ (Braum et al. 1997; Moyna et al., 1996; Mueller et al., 2000; Viti et al., 1985); as well as decreases after exercise (Baum et al., 1997; Henson et al., 2000; Surkina et al., 1994; Weinstock et al., 1997). This inconsistency may be due to differences in the exercise intensity and duration or differences in biochemical techniques. For example, Weinstock et al. (1997) examined both IFN-γ concentrations in plasma and with lipopolysaccharide (LPS) stimulated lymphocytes in 15 trained male runners following a strenuous exercise test. No changes were found in the plasma; however, there were deceases in IFN-γ in the LPS stimulated lymphocytes. As well, IFN-γ degrades quite quickly, which means that the timing of the blood samples can be crucial in attaining reliable results (Moldoveanu et al., 2001).

Interleukin (IL)-4

Th2 cells produce IL-4, which in turn promotes Th2 cells to continue growing and proliferating. It stimulates B cells to become activated and grow. IL-4 also inhibits macrophages and increases the growth of mast cells, and orchestrates asthmatic and allergic responses (Janeway et al., 2001; Speelberg & Edwards, 2001; Thomas, 1994). The majority of literature has found no changes in IL-4 production or stimulation after exercise (Moyna et al., 1996; Mueller et al., 2000; Nieman et al., 2001; Suzuki et al., 2000), suggesting IL-4 production is unaffected by exercise stress.

Interleukin (IL)-10

Th2 cells produce IL-10, which inhibits the cytokines that Th1 cells secrete. As well, it inhibits macrophages and stimulates mast cell growth (Janeway, 2001; Speelberg & Edwards, 2001). IL-10 is also a key anti-inflammatory cytokine (Thomas, 1994).

Studies have found IL-10 to increase in plasma following strenuous exercise, and to return eventually to baseline during recovery (Bishop et al., 2001; Nieman et al., 2001; Ostrowski et al., 1999; Suzuki et al., 2000). However, Mueller et al. (2000) and Gannon et al. (1997) found that no changes occurred in IL-10 post exercise with lymphocyte stimulation or in plasma concentrations. Insulin like growth factor (IGF)-1 has been shown to increase IL-10 concentration in PHA stimulated cells, as well as in serum (Warzecha et al., 2003). Circulating concentrations of IGF-1 have been shown to rise due to exercise (McCarty, 1997). Therefore, this may be one of the possible mechanisms behind the changes of IL-10 as a result of endurance exercise.

Interleukin (IL)-6

IL-6 stimulates Th2 production and IL-4 secretion. Th2 cells also release IL-6 that in turn stimulates other activated Th cells to differentiate into Th2 (Rincon et al., 1997; Speelberg & Edwards, 2001). IL-6 is often considered a pro-inflammatory cytokine; however, it has many anti-inflammatory properties as well (Thomas, 1994). It has been found to increase plasma IL-1ra, IL-10 (anti-inflammatory cytokines) and cortisol (Steensberg et al., 2003). It is released by antigen presenting cells (APC) in response to stress or infection, and can also be produced by muscle tissue during strenuous exercise (Pedersen & Toft, 2000; Rincon et al., 1997).

Numerous studies have shown that strenuous exercise will increase the plasma concentrations of IL-6 during and following exercise (Moldoveanu, 2001; Nieman et al., 2001; Northoff et al., 1994; Ostrowski et al., 1999). Eventually the levels of IL-6 return to pre-exercise concentrations; however, this depends on the intensity and the duration of the exercise, as well as on adequate recovery time. Brenner et al. (1999) found that exercise at 60% of VO_{2max} for 120 minutes increased IL-6 levels, but that exercising at 90% of $V0_{2max}$ for 5 minutes did not alter IL-6 levels. This suggests that exercise duration may be a key factor affecting IL-6 production. The increase in IL-6 during exercise has been found to precede the changes with other cytokines (Ostrowski et al., 1998). This suggests IL-6 plays a central role in the cytokine response to exercise (Steensberg et al., 2003).

Interleukin (IL)-12

IL-12 stimulates Th1 cell differentiation from an activated Th cell (Rincon et al., 1997). Macrophages or other APCs usually secrete IL-12. Rhind et al. (2002) conducted

an acute 60-minute exercise bout of 60% of $V0_{2max}$ and found a rise in IL-12 plasma concentrations in ten healthy male participants. However, these levels returned to base line immediately after exercise. Many other studies have attempted to analyze IL-12 changes after strenuous endurance exercise but were not successful, as concentrations were below the detection limit of the immunoassay kits (Gannon et al., 1997; Steensberg et al., 2001; Suzuki et al., 2000).

Despite the controversies in the literature with certain cytokines, some conclusions can be drawn. It is well documented that IL-6 increases and remains elevated after prolonged strenuous exercise. As stated earlier, IL-6 stimulates Th2 cytokine production. Though IL-4 does not change, IL-10 has been found to increase in response to exercise. This suggests that exercise does not inhibit Th2 response and may in fact promote it. As well, it appears that strenuous endurance exercise decreases many Th1 cytokines. IL-2 concentration and production decreases post-exercise, and despite the controversies with IFN-γ, the studies that have used immunofluorescence for biochemical analysis have all found IFN-γ to decrease as well. This suggests that strenuous exercise may induce a shift of Th1 and Th2 cells, favoring Th2 immunity.

It has also been suggested that these changes in cytokine patterns may not be related to Th1 and Th2 immunity but may be more representative of shift between pro/anti-inflammatory responses (Gannon et al., 1997). Most likely the inflammatory and Th response are interrelated. The shift from pro- to anti-inflammatory cytokines may be a protective mechanism in response to stress. In order to protect the body from excess inflammation, perhaps Th1 cells and their cytokines must be compromised. This shift between Th1 and Th2 may be another explanation as to why athletes are more susceptible

to upper respiratory tract infection (URTI) (Smith, 2003). URTIs are usually in the form of a virus which is controlled and attacked by the cellular-mediated defenses, initiated by Th1 cells. If these Th1 cells are inhibited or suppressed following a competition, the athlete may be more susceptible to specific viral/ bacterial infection.

2.6 Comparing Trained and Untrained Individuals

Attempting to understand how the immune system responds to an acute bout of exercise is very important for therapeutic and clinical reasons. Examining how the immune system is altered by chronic exercise or regular training can lead to further insight into how the immune response adapts to physiological stress. It can also provide an indication of the overall health of individuals in various populations. There is evidence that intensive exercise training, without adequate recovery, can lead to a chronic suppression of the immune system (Bruunsgaard et al., 1997; Mackinnon, 2000; Nieman, 1997), causing training athletes to be more susceptible to URTIs. However, the effect of moderate exercise training on the immune system is still not fully understood. Despite anecdotal claims that moderate endurance training will benefit the immune system, there is limited research to support this, particularly in females. Currently, no known studies have examined the effects of exercise training, either moderate or intense, on the Th1 and Th2 cytokine profile in humans. Cross-sectional, training and animal studies have provided some evidence that regular exercise may affect these cytokines, especially in response to a stimulus such as a strenuous exercise bout.

Cross-Sectional Studies

The majority of cross-sectional studies have only compared resting immune measures between athletes and sedentary individuals. Although some studies have found

no differences between trained and untrained individuals (Eliakim et al., 1997; Henson et al., 2001; Nieman et al., 1994, 1995), others have found differences with respect to lymphocyte cell counts and proliferation, and in various cytokine concentrations (Mueller et al. 2000; Nieman et al. 2000; Shinkai et al., 1995; Yan et al., 2001). Nieman et al. (2000), comparing marathon runners and sedentary controls, found that in resting levels lymphocyte proliferation was higher in the athletes, while IL-6 was higher in the sedentary controls. Mueller et al. (2000) found higher plasma IFN-γ levels in recreational cross-country skiers, compared to elite cross-country skiers and sedentary controls. It was also found that there were no differences in plasma IL-4 and IL-10 concentrations between the three groups. In another study that compared older male runners (ages 65 +) to sedentary aged matched controls, T cell proliferation, IL-2, IFN-γ and IL-4 LPS stimulated concentrations were found to be higher in the runners (Shinkai et al., 1995). This was an important finding, as Th2 type response becomes more dominant in the elderly. In this case, the runners seemed to have a higher Th1 cytokine profile than their sedentary controls.

Finally, a large cross-sectional study examined young, middle-aged, and elderly moderately active and sedentary males and found that B cell counts were higher in the sedentary individuals, and even more so in the elderly (Yan et al., 2001). This finding suggests that Th2 type response, known to control humoral immunity, is more dominant in the aged and that perhaps moderate exercise can alleviate this dominance.

Further research is needed to understand fully the immune system differences between trained and untrained individuals. It would also be beneficial to examine changes in the immune system, between these two populations, after a strenuous

endurance exercise bout. There is a growing belief that resting immune cell measures may not adequately test for differences between trained and untrained individuals (Shephard & Shek, 1998). In healthy individuals, the immune system is in homeostasis (Janeway et al., 2001) and remains homeostatic unless stimulated by a stressor such as an invading pathogen, or by an increase in environmental, psychological or physiological stress. Therefore, a response to acute strenuous endurance exercise may test for differences in the immune system and its function between trained and untrained individuals (Shephard & Shek, 1998). Understanding these differences will allow further insight into the immune response to exercise training.

Training Studies

Many studies have examined changes in the immune system before and after training. Nehlsen-Cannarella et al. (1991) conducted a study examining pre-menopausal women before and after a 15-week walking program (5 days/week, 45 minutes at 60% of heart rate reserve). They found that resting lymphocyte percentages, particularly T cell, decreased after 6 weeks of training. However, this decrease was attenuated at 15 weeks, suggesting that an adaptive response to training occurred. Other studies have found increases (Host et al., 1995; Woods et al., 1999;), decreases (Kajiura et al., 1995; Pizza et al., 1995; Scanga et al., 1998), or no change (Ferrandez et al. 1996; Nieman et al. 1993, 1998) in resting T cells (CD3) and their subsets (CD4 and CD8). This discrepancy may be largely due to differences in the training program, length of the study, and differences in biochemical measures. Most studies solely examined values before and after training, but if an adaptive response is to be observed, blood samples (at rest and in response to acute exercise) may need to be taken periodically throughout the training program.

Rebelo et al. (1998) investigated the effect of an 11-month training program and participation in soccer matches on circulating leukocytes and lymphocytes in male players. It was found that in resting samples, naïve T cell (CD4+CD45RA+) numbers increased, while CD8+CD57+ decreased during the preseason. These numbers returned to baseline halfway through the season.

Rhind et al. (1996) examined the effects of 12 weeks of moderate training on pre- and post-immune cell responses to acute exercise in males. It was found that resting B cells decreased with training and plasma Il-2 increased with training. Interestingly, the decreases in IL-2, IL-2 receptors and lymphocyte proliferation after acute exercise were significantly less after training than before the training program. This again suggests an adaptive response of the immune system.

Nieman et al. (1993) examined the effect of a 12-week walking program on sedentary elderly women (67-85 years of age). Although there were no immune changes after the training program, the number of URTIs that the women in the exercise group reported was significantly less than those reported by the control group. As stated earlier, cellular-mediated immunity initiates the response against URTIs. Therefore, this research suggests that moderate exercise may compromise the cellular-mediated response.

Animal Studies

Moriguchi et al. (1998) randomized ten lean and ten obese female rats into exercise training and control groups. After 40 weeks of training, there was a smaller decrease in lymphocyte proliferation in the obese rats. Kohut et al. (2001) compared young and old male mice and found that with 8 weeks of exercise training, IL-2 and IFN-γ (LPS stimulate spleen cells) increased, more so in the older mice, while IL-10 was not

affected by the exercise training. The authors suggested that the training program seemed to have an effect on Th1 cytokines, while not affecting Th2. Another study, with similar conditions, also found that IL-2 increased (Con-A stimulated spleen cells) in mice after a 22-month training program (Nieman et al., 1994).

2.7 Gender

Gender differences are not often studied in the field of exercise immunology, as the majority of research has been done with male participants only. This is largely due to the challenges posed by the effects of the female menstrual cycle on the immune system. Changes in the circulating hormones that occur during the menstrual cycle affect how the immune system responds to various stressors. Pehlivanoglu et al. (2001) compared lymphocyte cell numbers during the follicular and luteal phase, before and after acute mental stress. During the luteal phase, CD4 and CD8 numbers were higher. As well, after acute mental stress, there was a greater suppression of the CD4:CD8 ratio during the luteal phase. Therefore, it may be ideal to test female participants during the same phase of the menstrual cycle to control for this. At this time, the few studies that have examined immune response to exercise in females have controlled for the phase of menstrual cycle.

Females are more likely to use a Th1 type response rather than a Th2 type response (Goldsby et al., 2000). The exact mechanism for these gender differences and changes throughout the menstrual cycle is still unclear, although it is believed that the estrogens may be responsible (Ahmed et al., 1985; Goldsby et al., 2000). During pregnancy, when the ratio of estrogens to progesterone is high, there is a shift in the Th1, Th2 and Th3 balance to favor Th2 and Th3 responses (Goldsby et al., 2000; Raghupathy, 2001). This is a protective mechanism to prevent the rejection of the fetus, which is a

semiallogenic graft (Ahmed et al., 1985). Estradiol also regulates the release of prolactin, which is not a sex hormone, although it is in far greater concentrations in females than in males (Vera-Lastra et al., 2002). Receptors for prolactin have been found on T cells and B cells, suggesting that it also plays a role in the regulation of the immune system (Goldsby et al., 2000).

Finally, females may have a different immune response to exercise than males. It has been demonstrated in a few studies that females, compared to males, had higher resting cortisol levels during endurance and concurrent (endurance and resistance) training (Bell et al. 1997; Horne et al., 1997; Tsai et al., 1991). Tegelman et al. (1990) found that, when compared to sedentary controls, elite female athletes had higher cortisol levels as well. With elevation in levels of circulating cortisol, a different immune response to stress may be observed in trained females when compared to sedentary controls or to males.

Understanding the female's immune response to exercise, especially concerning the Th1 and Th2 cells and their cytokines, is important. Females are 2-3 times more susceptible to autoimmune diseases than males (Bijlsma et al., 2002; Whitacre, 2001). It would be beneficial to understand the physiological differences in response to exercise between females and males. This knowledge can perhaps lead to further research concerning the role that regular exercise plays in people's lives and the significance of these immune responses.

2.8 Summary

It is well known that strenuous endurance exercise will modulate various components of the immune system. Although examination of the Th1 and Th2 response

is relatively new in exercise immunology, it has been demonstrated that there may be a suppression of Th1 type response following strenuous endurance exercise. As well, it has been shown that with training there may be an adaptation in the immune system. Further research is needed to understand this adaptation, as well as the differences that may exist between trained and untrained individuals. At this time there are no known studies that have compared the Th1 and Th2 response and their cytokines to strenuous endurance exercise in trained and untrained individuals. Finally, the majority of the research in exercise immunology has primarily examined males; thus there is a need to examine the response to exercise of women's immune system.

2.9 References

Ahmed, S. A., Penhale, W. J., & Talal, N. (1985). Sex hormones, immune responses and autoimmune disease: Mechanism of sex hormone action. *American Journal of Pathology, 121,* 531-551.

Bell, G., Syrotuik, D., Socha, T., Maclean, I., & Quinney, H. A. (1997). Effect of strength training and concurrent strength and endurance training on strength and testosterone, and cortisol. *Journal of Strength and Conditioning Research, 11*(1), 57-64.

Berguer, R., Bravo, N., Bowyer, M., Egan, C., Knolmayer, T., & Ferrick, D. (1999). Major surgery suppresses maximal production of helper T-cell type 1 cytokines without potentiating the release of helper T cell type 2 cytokines. *Archives of Surgery, 134,* 540-544.

Bijlsma, J.W.J., Straub, R.H., Masi, A.T., Lahita, R.G., Cutolo, M. (2002). Neuroendocrine immune mechanisms in rheumatic diseases. *Trends in Immunology, 23,* 59-61.

Bishop, N., Walsh, N., Haines, D., Richards, E., & Gleeson, M. (2001). Pre-exercise carbohydrate status and immune responses to prolonged cycling: II. Effect on plasma cytokine concentration. *International Journal of Sports Nutrition and Exercise Metabolism, 11,* 503-512.

Borer, K. T. (2003). *Exercise endocrinology.* Windsor, ON: Human Kinetics.

Braum, M., Muller-Steinhardt, M., Liesen, H., & Kirchner, H. (1997). Moderate and exhaustive endurance exercise influences the interferon-gamma levels I whole blood culture supernatants. *European Journal of Applied Physiology, 76,* 165-169.

Brenner, I. K. M., Natale, V. M., Vasiliou, P., Moldoveanu, A. I., Shek, P. N., & Shepard, R. J. (1999). Impact of three different types of exercise on components of the inflammatory response. *European Journal of Applied Physiology, 80,* 452-460.

Bruunsgaard, H., Hartkopp, A., Mohr, T., Konradsen, H., Heron, I., Mordhorst, C. H., & Pedersen, B. K. (1997). In vivo cell-mediated immunity and vaccination response following prolonged, intense exercise. *Medicine and Science in Sports and Exercise, 29*(9), 1176-1181.

Chryssikopoulos, A. (1997). The relationship between the immune and endocrine systems. *Annals New York Academy of Sciences, 816,* 83-93.

Clerici, M., & Shearer, G. M. (1993). A Th1/ Th2 switch is a critical step in the etiology of HIV infection. *Immunology Today, 14*(3), 107-111.

Consitt, L. A., Copeland, J. L., & Tremblay, M. S. (2002). Endogenous anabolic hormone responses to endurance versus resistance exercise and training in women. *Sports Medicine, 32*(1), 1-22.

Doug, C., & Flavell, R. A. (2001). Th1 and Th2 cells. *Current Opinion In Hematology, 8,* 47-51.

Elenkov, I. J., & Chrousos, G. P. (1999). Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. *Trends in Endocrinology and Metabolism, 10*(9), 359-368.

Eliakim, A., Wolach, B., Kodesh, E., Gavrieli, R., Radnay, J., Ben-Tovim, T., Yarom, Y., & Falk, B. (1997). Cellular and humoral immune response to exercise among gymnasts and untrained girls. *International Journal of Sports Medicine, 18*(3), 208-212.

Espersen, G. T., Elbaek, A., Ernst, E., Toft, E., Kaalund, S., Jersild, C., & Grunnet, N. (1990). Effect of physical exercise on cytokines and lymphocyte subpopulations in human peripheral blood. *APMIS, 98,* 395-400.

Ferrandez, M. D., Maynar, M., & De la Fuente, M. (1996). Effects of a long-term training program of increasing intensity on the immune function of indoor Olympic cyclists. *International Journal of Sports Medicine, 17*(8), 592-596.

Fu, S. C., Qin, L., Leung, C. K., Chan, B. P. & Chan, K. M. (2003). Regular moderate exercise training prevents decrease CD4+ T-lymphocytes induced by a single bout

of strenuous exercise in mince. *Canadian Journal of Applied Physiology, 28*(3), 370-381.

Gannon, G. A., Rhind, S. G., Suzui, M., Shek, P. N., & Shephard. R. J. (1997). Circulating levels of peripheral blood leucocytes and cytokines following competitive cycling. *Canadian Journal of Applied Physiology, 22*(2), 133-147.

Goldsby, R. A., Kindt, T. J., & Osborne, B. A. (2000). *Kuby immunology* (4th ed.). New York: W. H. Freeman.

Haahr, P. A., Pedersen, B. K., Fomsgaard, A., Tvede, N., Diamant, M., Klarlund, K., Halkjaer-Kristensen, J., & Bendtzeb, K. (1991). Effect of physical exercise on in vitro production of interleukin 1, interleukin 6, Tumor Necrosis Factor- alpha, Interleukin 2, and Interferon-gamma. *International Journal of Sports Medicine, 12,* 223-227.

Henson, D. A., Nieman, D. C., Kernodle, M. W., Sonnenfeld, G., Morton, D., & Thompson, M. M. (2001). Immune function in adolescent tennis athletes and controls. *Sports Medicine, Training and Rehabilitation, 10*, 235-246.

Horne, L., Bell. G., Fisher, B., Warren, S., & Janowaks-Wiezoek, J. (1997). Interaction between cortisol and tumor necrosis factor with concurrent resistance and endurance training. *Clinical Journal of Sports Medicine, 7*(4), 247-251.

Host, C. R., Norton, K. I, Olds, T. S., & Lowe (1995). The effects of altered exercise distribution on lymphocyte subpopulations. *European Journal of Applied Physiology, 72*, 157-164.

Janeway, C. A., Travers, P., Walport, M., & Shlomchik, M., (2001). *Immuno biology: The immune system in health and disease* (5th ed.). New York: Garland.

Kajiura, J. S., MacDougall, J. D., Ernst, P. E., & Younglai, E. V. (1995). Immune response to changes in training intensity and volume in runners. *Medicine and Science in Sports and Exercise, 27*(8), 1111-1117.

Kang, D., & Fox, C. (2001). Th1 and Th2 cytokine responses to academic stress. *Research in Nursing and Health, 24,* 245-257.

Karol, M. H. (2002). Respiratory allergy: What are the uncertainties? *Toxicology, 181*(2), 305-310.

Kjaer, M., Christensen, N. J., Soone, B., Richter, E. A., & Galbo, H. (1985). Effect of exercise on epinephrine turnover in trained and untrained male subjects. *Journal of Applied Physiology, 59*(4), 1061-1067.

Kohut, M. L., Boehm, G. W., & Moynihan, J. A. (2001). Moderate exercise is associated with enhanced antigen-specific cytokine, but not IgM antibody production in aged mice. *Mechanisms of Ageing and Development, 122,* 1135-1150.

Kohut, M. L., Boehm, G. W., & Moynihan, J. A. (2001). Prolonged exercise suppresses antigen-specific cytokine response to upper respiratory infection. *Journal of Applied Physiology, 90,* 678-684.

Lewicki, R. Tchorewski, I. I., Majewska, E., Nowak, Z., & Baj, Z. (1988). Effect of maximal physical exercise on T-lymphocytes subpopulations and on interleukin 1 (IL-1) and interleukin 2 (IL2) production in vivo. *International Journal of Sports Medicine, 9,* 114-117.

Liblau, R. S., Singer, S. M., & McDevitt, H. O. (1995). Th1 and Th2 CD4+ T cells in the pathogenesis of organ- specific autoimmune diseases. *Immunology Today, 16*(1), 34-38.

Mackinnon, L. T. (1999). *Advances in exercise immunology.* Champaign, IL: Human Kinetics.

Mackinnon, L. T. (2000). Chronic exercise training effects on immune function. *Medicine and Science in Sports and Exercise, 32*(7), S369-S376.

McCarty, M. F. (1997) Up-regulation of IGF binding protein-1 as an anticarcinogenic strategy: relevance to caloric restriction, exercise, and insulin sensitivity. *Medical Hypotheses, 48*(4), 297-308.

McGurik, P., & Mills, K. H. G. (2002). Pathogen-specific regulatory T cells provoke a shift in the Th1/Th2 paradigm in immunity to infectious diseases. *Trends in Immunology, 23*(9), 450-455.

Moldoveanu, A. I., Shepard, R. J., & Shek, P. N. (2001). The cytokine response to physical activity and training. *Sports Medicine, 31*(2), 115-144.

Morel, P. A., & Oriss, T. B. (1998). Crossregulation between Th1 and Th2 cells. *Critical Reviews in Immunology, 18,* 275-303.

Moriguchi, S., Kato, M., Sakai, K., Yamamoto, S., & Shimizu, E. (1998). Exercise training restores decreased cellular immune functions in obese Zuker rats. *Journal of Applied Physiology, 84*(1), 311-317.

Moyna, N. M., Acker, G. R., Fulton, J. R., Weber, K., Goss, F. L., Robertson, R. J., Tollerud, D. J., & Rabin, B. S. (1996). Lymphocyte function and cytokine production during incremental exercise in active and sedentary males and females. *International Journal of Sports Medicine, 17*(8), 585-591.

Mueller, O., Villiger, B., O'Callaghan, B., & Simon, H. U. (2000). Immunological effects of competitive versus recreational sports in cross-country skiing. *International Journal Sports Medicine, 22,* 52-59.

Nagatomi, R. Kaifu, T., Okutsu, M., Zhang, X., Kanemi, O., & Ohmori, H. (2000). Modulation of the immune system by the autonomic nervous system and its implication in the immunological changes after training. *Exercise Immunology Review, 6,* 54-74.

Nehlsen-Cannarella, S. L., Nieman, D. C., Balk-Lamberton, A. J., Markoff, P. A., Chritton, D. B., Gusewitch, G., & Lee, J. W. (1991). The effects of moderate exercise training on immune response. *Medicine & Science in Sports & Exercise, 23*(1),64-70.

Nieman, D. (1997). Exercise immunology: Practical applications. *International Journal of Sports Medicine, 18*(S1), 91-100.

Nieman, D. C., Brendle, D., Henson, D. A., Suttles, J., Cook, V. D., Warren, B. J., Butterworth, D. E., Fagoaga, O. R., & Nehlsen-Cannarella, S. L. (1995). Immune function in athletes versus nonathletes. *International Journal of Sports Medicine, 16*(5), 329-333.

Nieman, D. C., Buckley, K. S., Henson, D. A., Warren, B. J., Suttles, J., Ahle, J. C., Simandle, S., Fagoaga, O. R., & Nehlsen-Cannarella, S. L. (1995). Immune function in marathon runners versus sedentary controls. *Medicine and Science in Sports and Exercise*, 27(7), 986-992.

Nieman, D. C., & Henson, D. A. (1994). Role of endurance exercise in immune senescence. *Medicine and Science in Sports and Exercise, 26*(2), 172-181.

Nieman, D. C., Henson, D. Garner, E., Butterworth, D., Warren, B., Utter, A., Davis, J., Fagoaga, O., & Nehlsen-Cannarella, S. L. (1997). Carbohydrate affects natural killer cell redistribution but not activity after running. *Medicine and Science in Sports and Exercise, 29*(10), 1318-1324.

Nieman, D. C., Henson, D. A., Gusewitch, G., Warren, B. J., Dotson, R. C., Butterworth, D. E., & Nehlsen-Cannarella, S. L. (1993). Physical activity and immune function in elderly women. *Medicine and Science in Sports and Exercise, 25*(7), 823-831.

Nieman, D. C., Miller, A. R., Henson, D. A., Smith, L. L., Utter, A. C., Vinci, D. M., Davis, J. M., Kaminsky, D. E., & Shute, M. (2001). Cytokine changes after and marathon race. *Journal of Applied Physiology. 91,* 109-114.

Nieman, D. C., Miller, A. R., Henson, D. A., Warren, B. J., Gusewitch, G., Johnson, R. L., Davis, J. M., Butterworth, D. E., Herrings, J. L., & Nehlsen-Cannarella, S. L. (1994). Effect of high-versus moderate intensity exercise on lymphocyte

subpopulations and proliferative response. *International Journal of Sports Medicine, 15*(4), 199-206.

Nieman, D. C., Nehlsen-Cannarella, S. L., Fagoaga, O. R., Henson, D. A., Shannon, M., Hjertman, J. M. E., Schmitt, R. L., Bolton, M. R., Austin, M. D., Schilling, B. K., & Thrope, R. (2000). Immune Function in female elite rowers and non-athletes. *British Journal of Sports Medicine, 34,* 181-187.

Nieman, D. C., Nehlsen-Cannarell, S. L., Henson, D. A., Koch, A. J., Bitterworth, D. E., Fagoaga, O. R., & Utter, A. (1998). Immune response to exercise training and/or energy restriction in obese women. *Medicine and Science in Sports and Exercise, 30*(5), 679-686.

Nieman, D. C., Simandle, S., Henson, D. A., Warren, B. J., Suttles, J., Davis, J. M., Buckley, K. S., Ahle, J. S., Bitterworth, D. E., Fagoaga, O. R., & Nehlsen-Cannarella, S. L. (1995). Lymphocyte proliferation response to 2. 5 hours of running. *International Journal of Sports Medicine, 16*(6), 404-408.

Northoff, H., Weinstock, C., & Berg, A. (1994). The cytokine response to strenuous exercise. *International Journal of Sports Medicine, 15,* S167-S171.

Ostrowski, K., Rohde, T., Asp, S., Schjerlong, P., & Pedersen, B. K (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *Journal of Physiology, 515*(1), 287-291.

Paik, I., Toh, K., Lee, C., Jung-jin, K., & Lee, S. (2000). Psychological stress may induce increased humoral and decreased cellular immunity. *Behavioral Medicine, 26*(3), 139-142.

Pedersen, B. K., & Hoffman-Goetz, L. (2000). Exercise and the immune system: Regulation, integration and adaptation. *Physiological Reviews, 80*(3), 1055-1081.

Pedersen, B. K., & Toft, A. D. (2000). Effects of exercise on lymphocytes and cytokines. *British Journal of Sports Medicine, 34,* 246-251.

Pehlivanoglu, B., Balkaner, Z. D., Ridvanagaoglu, A. Y., Durmazlar, N. Ozturk, G., Erbas, D., & Okur, H. (2001). Impact of stress, gender and menstrual cycle on immune system: Possible role nitric oxide. *Archives of Physiology and Biochemistry, 109*(4), 383-387.

Pizza, F. X., Flynn, M. G. Sawyer, T., Brolinson, P. G., Starling, R. D., & Andres, F. F. (1995). Run training versus cross training: Effect of increased training on circulating leukocyte subsets. *Medicine and Science in Sports and Exercise, 16*(3), 180-184.

Raghupathy, R. (2001). Pregnancy: Success and failure within the th1/Th2/Th3 paradigm. *Seminars in Immunology, 1394,* 219-227.

Rebelo, A. N., Candelas, J. R., Fraga, M. M., Duarte, J. A. R., Soares, J. M. C., Magalhaes, C., & Torrinha, J. A. (1998). The impact of soccer training on the immune system. *Journal of Sports Medicine and Physical Fitness, 38,* 258-261.

Rhind, S. G., Shek, P. N., Shinkai, S., & Shepard, R. J. (1996). Effect s of moderate endurance exercise and training on in vitro lymphocyte proliferation, interleukin-2 (IL-2) production and IL-2 receptor expression. *European Journal of Applied Physiology, 74,* 348-360.

Rincon, M., Anguita, J., Nakarmura, T., Fikrig, E., & Flavell, R. A. (1997). Interleukin (IL)-6 directs the differentiation of IL-4-producting CD4+ T cells. *Journal of Experimental Medicine, 185*(3), 461-469.

Romagnani, S. (1995). Biology of human Th1 and Th2 cells. *Journal of Clinical Immunology, 15*(3), 121-129.

Rook G. A. W., Hernandez-Pando, R., & Lightman, S. L. (1994). Hormones, peripherally activated prohormones and regulation of the Th1/Th2 balance. *Immunology Today, 15*(7), 301-303.

Scanga, C. B., Verde, T. J., Paolone, A. M., Andersen, R. E., & Wadden, T. A. (1998). Effects of weight loss and exercise training on natural killer cell activity in obese women. *Medicine and Science in Sports and Exercise, 30*(12), 1666-1671.

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

Shinkai, S., Kohno, H., Kimura, K., Komura, T., Asai, H., Inai, R., Oka, K., Kurokawa, Y., & Shepard, R. J. (1995). Physical activity and immune senescence in men. *Medicine and Science in Sports and Exercise, 27*(11), 1516-1526.

Shinkai, S., Watanabe, S., Asai, H., & Shek, P. N. (1996). Cortisol response to exercise and post-exercise suppression of blood lymphocyte subset counts. *International Journal of Sports Medicine, 17*(8), 597-603.

Smith, L. L. (2003). Overtraining, excessive exercise and alerted immunity: Is this a T helper-1 versus T helper-2 lymphocyte response? *Sports Medicine, 33*(5), 347-364.

Spellberg, B., & Edwards, J. E. (2001). Type1/Type 2 immunity in infectious diseases. *Clinical Infectious Diseases, 32,* 76-102.

Starkie, R. L. . Rolland, J., & Febbraio, M. A. (2001). Effect of adrenergic blockade on lymphocyte cytokine production at rest and during exercise. *American Journal of Physiology- Cell Physiology, 281,* C1233-C1240.

Steendberg, A., Fischer, C. P., Keller, C., Moller, K., & Pedersen, B. K. (2003). IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *American Journal of Physiology-Endocrinology and Metabolism, 285*, E433-E437.

Steendberg, A., Toft, A. D., Bruunsgaard, H., Sandmand, M., Halkjaer-Kristensen, J., & Pedersen, B. K. (2001). Strenuous exercise decreases the percentage of type 1 T cells in the circulation. *Journal of Applied Physiology, 91,* 1708-1712.

Surkina, I. D., Danilenko, S. V., Dudov, N. S., et al., (1994). The role of the immune system in processes of adaptations to stress in sports-men. *Clinical Sciences, 87*(S), 22-27.

Suzuki, K., Totsuka, M., Nakaji, S., Yamada, M., Kudoh, S., Liu, Q., Sugawara, K., Yamaya, K., & Sato, K. (1999). Endurance exercise causes interaction among stress hormones, cytokines neutrophils dynamics and muscle damage. *Journal of Applied Physiology, 87*(4), 1360-1367.

Suzuki, K., Yamada, M., Kurakake, S., Okamura, N., Yamaya, K., Liu, Q., Kudoh, S., Kowatari, K., Nakaji, S., & Sugawara, K. (2000). Circulating cytokines and hormones with immunosuppressive but neutrophil-priming potential rise after endurance exercise in humans. *European Journal of Applied Physiology, 81,* 281-287.

Tegelman, R., Johansson, C., Hemmingsson, P., Eklof, R., Carlstrom, K., & Pousette, A. (1990). Endogenous anabolic and catabolic steroid hormones in male and female athletes during off-season. *International Journal of Sports Medicine, 11*(2), 103-106.

Thomson, A. (1994). *The cytokine handbook* (2nd ed.). London, UK: Harcourt Brace.

Tsai, L., Johansson, C., Pousette, A., Tegelman, R., Carlstrom, K., & Hemmingsson, P. (1991). Cortisol and androgen concentrations in female and male elite endurance athletes in relation to physical activity. *European Journal of Applied Physiology, 63,* 308-311.

Turgeon, M. L., (1993). *Clinical hematology: Theory and procedures* (2nd ed.). Toronto, ON: Little, Brown.

Tvede, N., Kappel, M., Halkjaer-Kristensen, J., Galbo, H., & Pedersen, B. K. (1993). The effect of light, moderate and severe bicycle exercise on lymphocyte subsets, natural and lymphokine activated killer cells, lymphocyte proliferative response

and interleukin 2 production. *International Journal of Sports Medicine, 1495,* 275-282.

Vera-Lastra, O., Jara, L. J., & Espinoza, L. R. (2002). Prolactin and autoimmunity. *Autoimmunity Reviews, 1,* 360-364.

Viti, M., Muscatella, M., Paulescu, L. Bocci, V., & Almi, A. (1985). Effect of exercise on plasma interferon levels. *Journal of Applied Physiology, 59,* 426-428.

Warzecha, Z., Dembinski, A., Ceranowicz, P., Konturek, S. J., Tomaszewska, R., Stachura, J., & Konturek, P. C. (2003). IGF-1 stimulates production of interleukin-10 and inhibits development of caerulein-induced pancreatitis. *Journal of Physiology & Pharmacology, 54*(4), 575-90.

Webster, J. L., Tonelli, L., & Sternberg, E. M. (2002). Neuroendocrine regulation of immunity. *Annual Reviews- Immunology, 20*(1), 125-150.

Weiner, H. L. (2001). Induction and mechanism of action of transforming growth factor-beta-secreting Th3 regulatory cells. *Immunology Review, 182,* 207-214.

Weinstock, C., Konig, D., Harnischmacher, R., Keul, J., Berg, A., & Northoff, H. (1997). Effect of exhaustive exercise stress on the cytokine response. *Medicine and Science in Sports and Exercise, 29*(3), 345-354.

Whitacre, C. C. (2001) Sex differences in autoimmune disease. *Nature Immunology, 2*(9), 777-780.

Woods, J. A., Ceddia, M. A., Wolters, B. W., Evans, J. K., Lu, Q., & McAuley, E. (1999). Effects of 6 months of moderate aerobic exercise training on immune function in the elderly. *Mechanisms of Ageing and Development, 109,* 1-19.

Yan, H., Kuroiwa, A., Tanaka, H., Shindo, M., Kiyonaga, A., & Nagayanna, A. (2001). Effects of moderate exercise on immune senescence in men. *European Journal of Applied Physiology, 86,* 105-111.

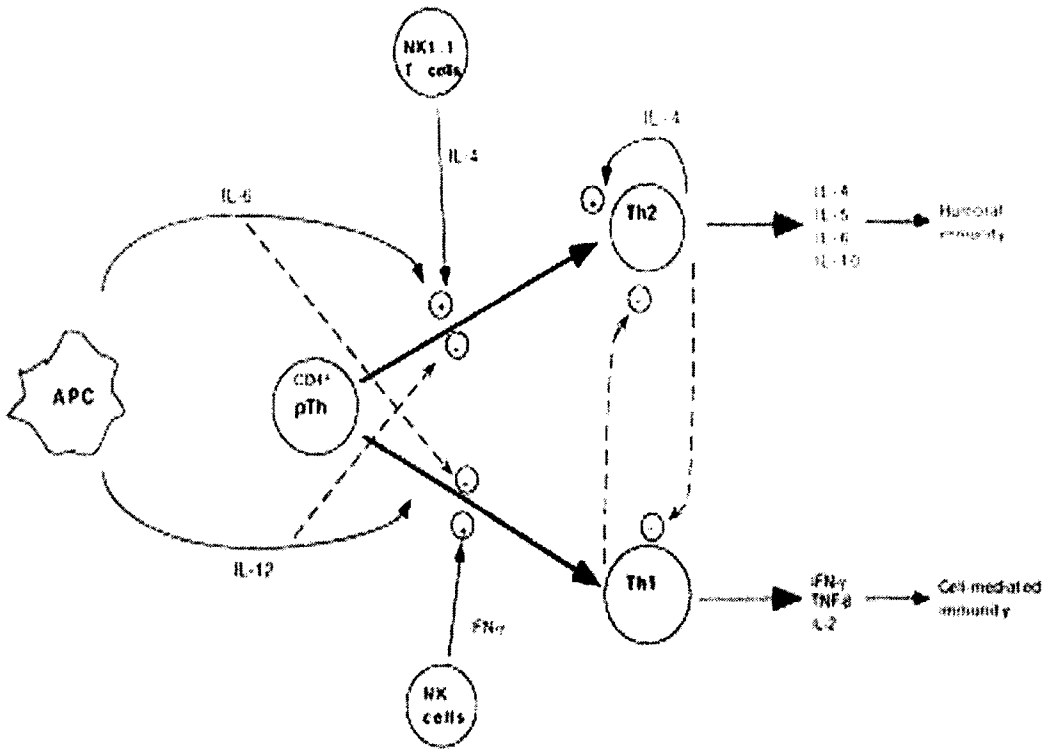


Figure 2.1. Schematic Schematic model for the differentiation of precursor Th cells (pTh) into effector Th1 and Th2 cells.[1]

[1] Note: From "Interleukin (IL)-6 directs the differentiation of IL-4-producting CD4+ T cells," by M. Rincon et al., 1997, *Journal of Experimental Medicine,* 185(3), p. 467. Reprinted with permission.

Chapter 3

Comparison of T Cell Subsets and Cytokine Production in Response to Strenuous Endurance Exercise in Trained and Untrained Females

3.1 General Introduction

Acute strenuous exercise has been suggested as a possible model with which to examine the physiological changes that occur with acute stress (Shephard & Shek, 1998; Shephard, 2001). It has been demonstrated that there are transient changes with the immune system in regard to cell numbers and their function following acute strenuous endurance exercise (Mackinnon, 1999, 2000; Nieman, 1997, 2000; Pedersen et al., 1998; Pedersen, 2000). Cytokine concentrations have also been shown to change following acute strenuous endurance exercise, whereby an inhibition of pro-inflammatory cytokines and a stimulation of anti-inflammatory cytokines have been reported (Bishop et al., 2001; Brenneret et al., 1999; Fu et al., 2003; Henson et al., 2004; Smith, 2003; Steensberge et al., 2001; Suzuki et al., 2000). It has been suggested that a type 2 helper T cell (Th2) response is favored and that a type 1 helper T cell (Th1) is suppressed following such exercise (Elenkov & Chrousos, 1999; Rhind et al., 1995; Steensberge et al., 2001).

Aerobic training can potentially benefit the immune system (Mackinnon, 2000). Therefore, the immune system in aerobically fit individuals may respond to the physiological stress of exercise differently than in those individuals who are sedentary. There is limited research supporting this claim (Mueller et al., 2000; Nieman et al., 2000; Shinkai et al., 1995; Yan et al., 2001). As well, the majority of available research involves male participants. Females may in fact show a different immune response after

acute strenuous endurance exercise, due to different circulating levels of reproduction hormones. Another limitation with many of the present cross-sectional studies is that only resting measurements are compared; despite similar resting levels, there can be dramatic changes in the immune responses as a result of acute exercise. Therefore, the immune response to physiological stress has not been fully examined.

The purpose of this study was to examine immune responses, specifically T cell subsets and cytokine concentrations in healthy trained and untrained females following acute endurance exercise conditions. It was hypothesized that there would be a difference between the Th1 and Th2 type responses, whereby Th2 cells numbers and cytokine production would be increased following 60 minutes of endurance exercise in females. This immune response was examined in untrained women and aerobically trained women. The trained females completed two exercise conditions at 2 different intensities to allow for a relative and absolute intensity comparison to the untrained females. The first exercise bout allowed for a comparison between trained and untrained women performing the same amount of work; the second comparison was made after both groups of participants performed different amounts of work but at the same intensity relative to fitness level. It was also hypothesized that there would be a difference in the shift between cytokine production of Th1 and Th2 cells in trained and untrained females following the different exercise bouts.

3.2 Methods

Participants

Eleven trained and 14 untrained females between the ages of 18 and 36 were recruited for this study from Edmonton and the surrounding community. An untrained

individual was defined as a person who had not participated in formal exercise training for at least a year, who may have participated in recreational exercise no more than two times per week, and who had a $V0_{2max}$ less than 35ml/kg/min determined on a cycle ergometer. A trained individual was defined as a person who had been participating in formal endurance exercise training three or more times per week for the two months previous to the study, and who had a $V0_{2max}$ greater than 45ml/kg/min measured using a cycle ergometer.

Participants were required to complete a Physical Activity Readiness Questionnaire (PAR-Q) and a menstrual/general health questionnaire (see Appendix B). Inclusion criteria were that participants must be 1) healthy and free of certain conditions that could affect their performance or the immune system, such as allergies, asthma, and other immunological/hormonal disorders; 2) non-smokers; 3) currently not using medication that can affect immune function or inflammation; 4) abstaining from using vitamin or mineral supplements in excess of their recommended daily intake; or 5) taking oral contraceptives (OC) for at least four months prior to and for the duration of the study.

Height and weight were recorded for all participants. The sum of 6 skinfolds were taken at the following sites: tricep, subscapular, iliac crest, abdominal, and front and rear thigh. The mean of two skinfolds, within 4mm, was taken at each of these sites. The Yuhasez formula was used to calculate body fat percentage [% fat = (sum of 6 skinfolds x 0.217) – 4.47].

Exercise Testing

Maximal aerobic power (VO_{2max}) and ventilatory threshold (VT) were measured in each participant, using a graded exercise test to exhaustion on a Monark Ergomedic cycle (Varberg, Sweden) while being monitored with a calibrated metabolic cart (Parvomed True Max 2400, Utah). Time of test was done set to the participant's convenience, usually one week prior to the 60-minute endurance exercise test. This test protocol followed the American College of Sports Medicine (ACSM) guidelines for a graded cycle exercise test. Participants were allowed to warm up for approximately 5 minutes. Each participant began the test at power output (PO) of 40 watts (W), with an increase of 20W every 2 minutes. The criteria for determining VO_{2max} were two of the following: a plateau in oxygen consumption with continued effort, respiratory exchange ratio (RER) greater than 1. 1, or the participant reaching volitional exhaustion. Heart rate was also recorded every minute using a heart rate monitor (Polar USA, CT). Ventilatory threshold (VT) was determined using the "V-slope" method, according to the methodology of Wasserman et al., 1999. Oxygen consumption and power output at $V0_{2max}$ and VT were used as dependent variables.

The 60-minute endurance exercise test on a Monark cycle ergometer took place during days 1-7 of the OC cycle. For the untrained group, the intensity of the test was performed at 20W just below the PO that elicited VT. The trained group were asked to perform two endurance exercise tests. The first exercise condition was conducted at the mean absolute intensity achieved by the untrained group (approximately 90 watts). This allowed for a comparison of the trained and untrained participants at the same absolute intensity of exercise. The second condition was done at 20W just below the PO of their

own VT. This allowed a comparison of the same relative intensity between the groups. Similar exercise intensity and duration have been shown in previous literature to elicit an immune response (Brenner et al., 1999; Rhind et al., 2002; Tvede et al., 1993; Vassilakopoulos et al., 2002). Heart rate was also measured throughout the endurance tests in both groups, as described previously.

Participants were asked to refrain from participating in any physical exercise, consuming alcohol, and ingesting any anti-inflammatory/histamine (including pain or allergy relief) medication for 36 hours prior to the day of the testing. Subjects arrived in the morning (7:00am) following a 12-hour overnight fast. Upon arrival, participants were provided with a meal (CLIF bar, Berkley CA) standardized to their body mass. The standardized amount was equivalent to1 gram (g) of carbohydrate, 0. 13g of fat, and 0. 24g of protein per 1 kilogram (kg) of body mass. The amount of carbohydrate followed the guidelines outlined by Sherman (1989) for pre-event meals. See Appendix C for nutritional information on the CLIF bars. Two hours were given before the exercise test to allow for digestion of the breakfast.

Participants were required to drink a minimum of 500 ml of water during the two hours before the test, 125 ml every 15 minutes during the exercise challenge, and an additional 500 ml during the two hours of recovery. Body mass was taken pre- and post-exercise to determine fluid loss. In incidences where there was fluid loss, the participants were required to drink that volume of water in addition to the 500 ml consumed after the test.

Blood Collection

Blood samples were collected by a trained phlebotomist, at rest (T0), immediately following the exercise test (T60), and again after two hours of recovery (T180). Venipuncture of the anti-cubital vein was used for sample collection. Two 10 ml blood samples were collected in vacutainer tubes at each time point. One tube was coated with tripotassium ethylene diamine tetraacetic acid (K_3EDTA), and the other with sodium heparin, both anti-coagulants. Two anti-coagulants were used because K_3EDTA inhibits cellular function and therefore could alter cytokine production.

Blood Analysis

Whole blood was analyzed for hematocrit, hemoglobin, plasma cortisol, plasma cytokine concentration (IL-6, IL-12), lymphocyte phenotyping, lymphocyte stimulated cytokine production (IL-2, IL4, IL-6, IL-10, and IFNγ), and total leukocyte numbers.

Total blood cell count, white blood cell differentiation (granulocytes, monocytes and lymphocytes), hemoglobin, and hematocrit were determined using an automated Coulter counter (Beckman Coulter AcT 5diff Cap Pierce Hematology Analyzer, Miami FL), which follows the electrical principle of counting cells. The sample passes through an aperture where an electrical current is passed between two electrodes. Blood cells are poor conductors of electricity, and so they increase the electrical resistance of the sample. Each cell alters the flow of resistance differently and this resistance is measured to count and differentiate between specific cells.

Lymphocyte Isolation

The lymphocytes were isolated from the blood to be stimulated for cytokine production. Whole blood samples were centrifuged at 700 x gravity (x g) for 10 minutes.

The plasma was transferred into microcentrifuge tubes and stored at -80°C until analysis. Four ml of sterile buffer solution was added to the remaining sample [red blood cell (RBC) pellet and buffy coat] and re-suspended gently. Histopaque 1077 (Sigma Chemical Co., St. Louis, MO) was gently added to the sample in a 1:1 ratio (4 ml: 4ml). The sample was spun at 400 x g for 30 minutes at room temperature. The lymphocyte band was removed, and 10 g·L-1 of bovine serum albumin (BSA) in phosphate buffer saline (PBS) was added to the isolated lymphocytes; this was further centrifuged at 250 x g for 10 minutes at 4°C.

Excess fluid was disposed, and 1 ml of complete culture medium (CCM) was added to the cell pellet. The number of lymphocytes in the sample were counted using a hemocytometer, and the sample was further diluted to a concentration of 1×10^6 cells·ml^{-1} for the lymphocyte stimulated cytokine analysis.

Lymphocyte Cytokine Production

The samples were stimulated with Phytohaemagglutinn (PHA), a mitogen known to activate T cells. The mitogen was added to an isolated lymphocyte sample to a concentration of 1×10^6 cells·ml^{-1}, and 2 ml of complete culture medium (CCM). The samples were incubated for 48 hours at 37°C in a controlled atmosphere (5% CO_2, 95% relative humidity) to allow for cytokine proliferation. After the incubation, the samples were spun in the centrifuge for 10 min at 200 x g at 4°C. The supernatant was removed from the sample, transferred to microcentrifuge tubes, and stored at –80°C for later determination of cytokine production.

Immunofluorescence

The lymphocyte phenotypes were identified by direct incubation with labeled monoclonal antibodies (mAb) specific to surface antigens. This labeling emits a particular fluorescence that can be read by a flow-cytometer (Becton Dickinson, Sunnyvale, CA). Data are presented as the percentage of total mononuclear cells. The peripheral concentration of each immune subtype was determined by calculating the percentages from the immunofluorescence analysis with the differential WBC count.

The plate used for immunofluorescence was preconditioned with 200µl of 4% fetal calf serum (FCS) in PBS and allowed to incubate at room temperature for approximately 30-minutes. Whole blood from the 10 ml K_3EDTA tube was centrifuged at 700 x g for 10 minutes. The serum was discarded, and RBCs were lysed with lysis buffer. This was allowed to incubate for 10 minutes at 37°C. The tube was similarly centrifuged again, excess lysis buffer was withdrawn, and these steps were repeated until the RBC pellet was gone. One µl of 4% FCS in PCS buffer was added to the tube, and the white blood cell pellet was re-suspended. One hundred µl of the sample was added to each well of the microliter plate. The plate was then centrifuged for 2 minutes to pellet the cells, was vortexed, and the wells were washed with 200µl of the 4% FCS in PBS buffer, and then centrifuged again for 2 minutes. The wells were labeled with 10µl of each particular monoclonal antibody (mAb; Sigma Chemical Co., St. Louis, MO and BD Pharmigne, Mississauga, ON) labeled with fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), quantum red (QR) or biotin (B). The following clusters of differentiation (CD) were examined: CD3+ (T cells), CD3+CD4+ (Th cells), CD3+CD8+ (Tc/s cells), CRTH2+CD3+, CRTH2+CD4+ (Th2 cells), CRTH2+CD8+ (Tc/s2) CD20+

CD19+ (B cells), CD3-CD16+CD56+ (NK cells), and CD25+CD3+ (activated T cells), CD25+CD4+ (activated Th cells), CD25+CD8+ (activated Tc/s cells), and CD25+CRTH2+ (activated CRTH2 cells). Refer to Appendix C for specific mAbs added to each well.

After the mABs were added, the plate was incubated in the refrigerator (4°C) for 30 minutes. Following incubation, 10µl of quantum red (QR) strept-avidin was added to each biotin labeled well, and the plate was incubated for an additional 30 minutes at 4°C. Each well was washed again with 200µl of 4% FCS in PBS buffer. Finally 200µl of cell fixative (PBS with sodium azide) was added. The plate was covered and stored in a refrigerator at 4°C until analysis. Samples were acquired with FasCalibur (BD Bioscience, San Jose, CA). A minimum of 6000 gated cells were acquired and analyzed with CellQuest Pro (BD Bioscience, San Jose, CA) for relative percentage of lymphocyte populations.

Plasma Cytokines and Lymphocyte Stimulated Cytokines (ELISA)

Extra-cellular cytokines IL-6 and IL-12 concentrations were measured in plasma. The concentrations of IL-2, IL-4, IL-6, IL-10, and IFN-γ were examined on unstimulated culture media, as well as the PHA stimulated culture media. Concentrations were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (OptEIA from BD Pharmigen, ON). Procedure was followed according to the company's specifications. The ranges of standard curves for each of the cytokines are as follow: IL-2, 7.8 – 1000 $pg \cdot ml^{-1}$; IL-4, 7.8 – 500 $pg \cdot ml^{-1}$; IL-6, 4.7 - 600 $pg \cdot ml^{-1}$; IL-10, 4.7 - 600 $pg \cdot ml^{-1}$; IL-12, 15.7 - 2000 $pg \cdot ml^{-1}$; IFN-γ, 7.8 - 600 $pg \cdot ml^{-1}$. Samples were analyzed in duplicate. If the co-efficient of variance (CV) was over 15 %, the sample was

re-analyzed. The average CV for each of the cytokines were the following: IL-2 - *6. 7%*; IL-6 - *4.6%*; IL-10 - *4.5%*; IL-12 - *9.9%*; IFN-γ - *7.24%*.

Samples were added to antigen-coated micro-well plates, in which the specific cytokines will bind to the antigen. Excess sample was washed away with wash buffer, and a second enzyme-conjugated antigen was added that binds to the cytokines. The excess was again washed away, and a substrate for the enzyme was added to produce the colour. The colour absorption was read on a Spectra Max 190 plate reader (Molecular Devices, Sunnyvale, CA) at wavelength of 450-570 nm and concentrations determined against a standard curve.

Hormones

Cortisol was measured in plasma with commercially available radioimmunoassay (RIA) kits (Oxoid, Stillwater, MN) in duplicate. The procedure was followed according to the company's specifications. RIA operates similarly to the ELISA procedure; however, in this case the antigen is labeled with a radioactive label (^{125}I) instead of an enzyme. Ten µl of each sample were added to the GammaCoat tubes, in duplicate. The ^{125}I was added, following 45 minutes of incubation in a water bath at 37°C. Samples were decanted and counted on a 1470 Wizard Gamma Counter (Wallace, Turku, Finland). The samples were compared to a standard curve (1-60 $ug \cdot dl^{-1}$) to determine plasma cortisol concentrations. The average CV for the assay was 8.7%.

3.3 Statistical Analysis

Means and standard deviations were calculated for all blood measures taken before, during, and after the strenuous endurance exercise test. All measures were examined for normal distribution using Kurtosis and Skewness techniques. In cases

where the results were not normally distributed, concentrations were converted to logarithms before analysis. Separate two-way analysis of variance (ANOVA) procedures with repeated measures were used to determine any significant differences in the immune and hormonal measures at three different times (T0, T60, and T180) and between the exercise conditions. Three separate comparisons were performed. First, the untrained group exercise condition (UT) and the trained group's first exercise condition at the same absolute intensity (TE1) were compared. The second comparison examined the "relative" exercise intensity comparison between the UT exercise condition and the second higher intensity exercise condition of the trained group (TE2). Finally, the third repeated measure analysis compared the trained group's two exercise conditions (TE1 vs. TE2). Any significant F-ratios determined by ANOVA were further analyzed with a Tukey multiple comparison procedure. A standard independent t-test was used to determine any significant differences between group characteristics and exercise performance. An alpha of $p<0.05$ was considered significant.

3.4 Results

Participant Characteristics

There were no significant differences with regard to participant characteristics (age, height, weight, body percent fat) between the untrained and trained participants (Table 3. 1). Participants in the trained group exercised 5 times per week (range 4-7 days/week). These participants were involved in a variety of different endurance sports and training, including synchronized swimming, competitive and recreational cycling, recreational and cross-country running, and triathlon.

Different pharmaceutical contraceptives were taken by the participants. In the untrained group, 5 participants were taking *Ortho Tri-Cyclen*, 3 were taking *Tri-phasil*, 2 were taking *Ortho-Cyclen*, 3 taking *Allesse*, and 1 was taking *Diane*. In the trained group, 2 were taking *Tri-phasil*, 3 were taking *Allesse,* 2 were taking *Ortho Tri-Cyclen*, and 1 participant each was taking *Marvelon, Lo Estrin*, or *Diane*. One individual was using *Ortho-Evra*, a dermal pharmaceutical contraceptive patch. Refer to Appendix C for composition of these pharmaceutical contraceptives.

Two of the participants in the trained group were unable to complete both 60-minute exercise conditions during days 1-7 of the same OC cycle. Therefore, they were tested approximately one month later, during days 1-7 of their following OC cycle.

Performance Measurements

There were significant differences between the groups with regard to all performance measures (Table 3. 1). The trained group had a significantly higher $V0_{2max,}$ power output (PO) at $V0_{2max}$ test, PO at VT, percentage of $V0_{2max}$ at VT, and $V0_2$ at VT.

Blood Measures

Hemoglobin and Hematocrit

There was a statistically significant main effect of time for hemoglobin (Hb), resulting in a significant increase from T0 to T60 between UT and TE1 exercise conditions (Table 3. 2). However, Hb concentrations at T180 returned to baseline and were significantly lower than T60 for all exercise conditions (Table 3. 2). There was a significant interaction effect between the TE1 and TE2 exercise conditions. Hemoglobin concentration was significantly elevated from T0 to T60, and significantly decreased

from T60 to T180, returning to baseline with TE2, but not with the lower intensity exercise condition (TE1).

There was a significant time main effect for hematocrit (Hct) indicating that T180 had decreased significantly from T60, returning to or just below baseline values for all 3 exercise conditions. A significant increase between T0 to T60 occurred for both the UT and TE1 exercise conditions (Table 3. 2).

White Blood Cells

There was a significant main effect of time that showed circulating white blood cell (WBC) concentrations increased from T0 to T60 and again from T60 to T180 for all 3 exercise conditions (Table 3. 3). There was also a significant main effect for exercise condition between the UT and TE1 comparison, which indicated that WBC concentrations were significantly lower for the TE1 compared to UT exercise condition.

A significant main effect for time occurred with circulating neutrophil concentrations exhibiting an elevation from T0 to T60, and again after 2 hours (T180) with all 3 exercise conditions. A significant main effect of exercise condition occurred between UT and TE1, indicating that TE1 had a significantly lower neutrophil concentration compared to the UT group (Table 3. 3).

There was a significant main effect of time for circulating lymphocyte concentrations. These cells increased post exercise from rest and returned to baeline levels after two hours (T180) of recovery (Table 3. 3).

There was a significant main effect of time where circulating monocyte concentration increased at rest and remained elevated at T60 and T180 (Table 3. 3).

Eosinophils and Basophils

There was a significant main effect of time for circulating eosinophil concentrations indicating a decrease just below baseline from T60 to T180 after both the UT and TE2 exercise conditions (Table 3. 4).

There was a main effect of time where circulating basophil concentrations increased significantly from T0 to T60, and remained significantly elevated from rest at T180 for both the UT and TE2 and the TE1 and TE2 comparisons. There was a significant interaction effect that revealed a significant elevation in basophils from T0 to T60 with the TE2 exercise condition, but not in the TE1 exercise condition (Table 3. 4).

Percentage of Lymphocyte Subsets- Immunofluorescence

The percentage of circulating $CRTH_2$+CD4+ (Th2) cells was not normally distributed, and therefore it was converted to logarithm before statistical analysis. There were no significant changes in the relative percentage of circulating CD3+ (T) cells, CD3+CD8+ (Tc/s) cells, $CRTH_2$+CD8+ (Tc/s2) in any of the exercise conditions (Table 3. 5). However, the proportion of CD3+CD4+ cells was affected by group.

There was a significant main effect of exercise condition indicating that TE2 had a higher percentage of CD3+CD4+ (Th) cells than UT across all time points. There was a significant main effect of time indicating that the percentage of circulating CD3+CD4+ cells significantly increased from T60 to T180 for both the TE1 and TE2 exercise conditions (Table 3. 5).

The percentages of CD3-CD56+CD16+ (NK) circulating cells were not normally distributed, and so they were converted to logarithm before statistical analysis. There was

a main effect of time that showed that the proportion of circulating CD3-CD56+CD16+ cells was significantly decreased just below base line values from T60 to T180 in the UT and TE2 exercise conditions (Table 3. 5).

There was a significant main effect of time for the percentage of circulating CD20+CD19+ (B) cells resulting in an elevation just above baseline values from T60 to T180 (Table 3. 5).

There was a significant main effect of time for the percentage of total circulating $CRTH_2$ cells, exhibiting an elevation, close to baseline values, from T60 to T180 for both the UT and TE1 and the TE1 and TE2 exercise conditions (Table 3. 6).

Absolute Cell Numbers of Lymphocyte Subsets

To calculate the absolute cell number/concentration for lymphocyte subsets, the percentage of gated cells was multiplied by the lymphocyte concentration. Circulating cell numbers for CD3+, CD3+CD4+, CD3+CD8+, Total $CRTH_2$, $CRTH_2$+CD4+, $CRTH_2$+CD8+, CD3-CD16+CD56+ and CD20+CD19+ were calculated. There were no significant differences with the cell concentration with CD3+CD4+, CD3+CD8+ and with CD20+CD19+ cells.

A main effect of time was found for circulating CD3+ cells numbers, illustrating a significant increase from rest to T60 with both the UT and TE1, and the TE1 and TE2 exercise conditions. Cell numbers also decreased significantly from T60 to T180 for the TE1 and TE2 comparison (Table 3. 7).

There was a main effect of time for CD3-CD16+CD56+ resulting in a significant decrease of circulating cell numbers from T60 to T180 for both the UT and TE1 and with UT and TE2 exercise conditions (Table 3. 7).

There was a significant interaction effect for $CRTH_2$ resulting in an elevation of these circulating cell numbers from T0 to T60 in the UT exercise condition, but not in TE1. There was a significant main effect of exercise condition showing that TE2 had a significantly lower number of $CRTH_2$ cells than UT. There was also a main effect of time where cell numbers significantly increased from rest to T60 for both the UT and TE2 exercise comparisons (Table 3. 8).

There was a significant interaction in $CRTH_2$+CD4+ circulating cell numbers with the UT and TE1 exercise conditions; however, there were no further significant findings when analyzed with the Tukey multiple comparison procedure. There was a significant main effect of exercise conditions where TE2 had lower circulating cell numbers than the UT exercise condition (Table 3. 8).

There was a main effect of time for CRTH2+CD8+ cell numbers indicating a significant decrease back to baseline values from T60 to T180 with both the UT and TE1 exercise comparisons. There was an additional main effect of time for these cells resulting in a significant elevation from rest to T60 with the UT and TE2 exercise conditions (Table 3. 8).

Percentage T Cell Subsets expressing the IL-2 α Receptor (CD25 Activation Marker)

There was a main effect of time whereby the percentage of circulating CD25+CD3+ cells had significantly elevated from rest to T180 in the UT and TE1 exercise conditions. There was also a significant main effect of time when comparing the UT and TE2 exercise conditions; however, there were no further significant differences observed after analysis with the Tukey multiple comparisons procedure (Table 3. 9).

There was a main effect of time with the percentage of circulating CD25+CD4+ cells resulting in a significant increase at T180 from both T0 and T60 with both the UT and TE1 exercise conditions. These cells also significantly increased from T60 in comparison of the UT and TE2 exercise conditions (Table 3. 9). There was also a main effect of exercise condition, where TE2 had a significantly lower percentage of circulating cells than TE1.

There was a main effect of time for the percentage of circulating $CRTH_2$+CD25+ cells, indicating a significant increase above resting values from T60 to T180 with the UT and TE1 and the UT and TE2 exercise conditions (Table 3. 9). There was also a main effect of exercise condition, where TE2 had a significantly lower percentage of $CRTH_2$+CD25+ cells than TE1. There was no significant difference between exercise conditions at any of the specific time points.

Proportion of Th2 cells to Total CD4+ population.

There was a significant decrease from T60 following recovery in TE1 when compared to UT (Figure 3. 10). There was also a significant interaction effect between

TE2 and UT, however analysis with the Tukey multiple comparison procedure did not reveal any further significant findings.

Cytokines: PHA Stimulated Cytokine Production

There were no significant differences observed with any of the cytokine concentrations between the UT and TE1 exercise condition. There were no significant differences found with Interleukin (IL)-6 with any of the exercise comparisons (Table 3. 11 and Figure 3. 3). IL-4 concentrations were below the detection limit of the ELISA kit and therefore were not reported.

A significant interaction effect was found with the IL-2 logarithm conversions between the UT and TE2 exercise conditions (Table 3. 11 and Figure 3. 1). However, the Tukey multiple comparison procedure revealed no further significant differences.

A significant main effect of exercise condition was found indicating that the TE2 had significantly higher IL-10 concentration than TE1 (Table 3. 11 and Figure 3. 4). There were no significant differences with IL-10 concentration between any of the time points.

There was a significant main effect of time, where IFN-γ concentrations were significantly elevated at T180 from resting levels when comparing the TE1 and TE2 exercise conditions (Table 3. 11 and Figure 3. 2).

Cytokines: Plasma Concentrations

Plasma IL-6 concentrations were below the detection limit of the assay and therefore were not reported. IL-12 concentrations were below the detection limit of the

assay for some of the samples; as a result, half of the lowest detection limit was inserted for statistical analysis. IL-12 concentrations were not normally distributed, and the concentrations were converted to logarithm before analysis. There was a significant main effect of time, where the IL-12 was significantly elevated at T180 from rest and T60. This was found for the comparison of the UT and TE2 and the TE1 and TE2 exercise conditions. A significant interaction effect was found with the IL-12 concentrations between the UT and TE2 exercise condition (Table 3. 12 and Figure 3. 5). However, the Tukey multiple comparison procedure revealed no further significant differences.

Cytokines: Unstimulated

Random samples of unstimulated lymphocytes were selected and also analyzed for IL-2, IL-4, IL-6, IL-10 and IFN-γ. All cytokine concentrations were close to or below the detection limit of the ELISA kits, except for IL-6. Only 11 samples were selected across all exercise conditions. Therefore, the three exercise conditions were combined for statistical analysis. There were significant increases in IL-6 concentration post exercise (T60) and significantly decreases below baseline values from T60 to T180 (Table 3. 13).

Stress Markers

Neutrophil: Lymphocyte Ratio

There was a significant increase in the ratio of neutrophils to lymphocytes at T180 compared to both rest and T60 in all exercise conditions, but this did not differ between groups (Figure 3. 6).

Cortisol

There was a significant main effect of time for plasma cortisol concentrations indicating a significant decrease below resting values from T60 to T180, with the UT and TE2 exercise conditions. There was a significant main effect of exercise conditions, where TE2 had significantly higher plasma cortisol concentrations than TE1. A significant interaction effect was also found indicating that TE2 plasma cortisol concentrations were significantly elevated at T60 from resting values and had significantly decreased close to resting levels at T180. Finally, TE2 was significantly higher than TE1 at T60 (Table 3. 14).

CD4:CD8 Ratio

There was no significant difference between the exercise conditions or over time with the ratio of CD3+CD4+ to CD3+CD8+ cells (Table 3. 15).

3.5 Discussion

This study was designed to examine the immune response following 3 different exercise conditions in healthy women. The purpose of this investigation was two fold: to examine the Th1 and Th2 immune response following endurance exercise in women, and to compare this immune response between exercise conditions that differed in exercise intensities. It was hypothesized that all three exercise conditions would elicit a Th2 immune response whereby Th2 cell numbers would increase and there would be an elevation of Th2 cytokine production and a decrease in Th1 cytokine production. It was also hypothesized that there would be a difference with this immune response between trained and untrained women.

The findings of this study showed changes with circulating immune cells following all three exercise conditions. The majority, but not all, of the results are consistent with previous findings (Mackinnoon, 1999; Nieman, 1997; Pedersen and Hoffman-Goetz; 2000). The inconsistenties were likely due to the duration and intensity chosen in this study (Nieman, 1997; Pedersen and Hoffman- Goetz, 2000). The results of this study did not completely support the hypothesis with regard to changes related to Th1 and Th2 immunity. Following the exercise in the untrained women, Th2 immunity appeared to be favoured, as indicated by the decrease in stimulated IL-2 production, and an elevation with Th2 cells. This was not the case, however, in the same (TE1) or higher (TE2) exercise intensities in the trained women, where Th2 cells numbers decreased, and Th1 cytokines were elevated. These results indicate that there was a stronger Th1 immune response observed in the trained women, and a stronger Th2 immune response observed in the untrained women following endurance exercise.

General Immune Changes Following Endurance Exercise in Healthy Untrained and Aerobically Trained Women

The results of this study demonstrated a leukocytosis response to exercise, indicated by an elevated concentration of circulating WBC (neutrophils, and lymphocytes) immediately after exercise and following recovery. This is consistent with previous reports (Mackinnon, 1999; Nieman et al., 1995, 1997; Shinkai et al., 1996). It has been suggested that the increase in WBC concentrations is a result of the recruitment and trafficking of immune cells by catecholamines, cytokines, chemokins, and alterations with adhesion molecules (Mackinnon 1999; Shephard, 2003; Woods et al., 1998). Since

endurance exercise alters the production of catecholamines, chemokines, cytokines and adhesion molecules, this leads to the redistribution of immune cells (Mackinnon, 1999; Nieman et al., 1997; Pedersen & Hoffman-Goetz, 2000). These results indicate that the exercise conditions used in this study were effective in altering the distribution and trafficking of circulating immune cells.

The elevation of neutrophil concentration was similar following the exercise in the untrained women (UT) and the trained women at the higher intensity (TE2) but was lower following exercise in the trained women at the same intensity (TE1). Therefore, it appears that aerobically trained women must work at a higher intensity of exercise in order to elicit a similar elevation of neutrophil concentration in comparison to untrained women. Although this difference is not a consistent finding in all cross-sectional studies, similar results have been reported by researchers (Eliakim et al., 1997; Oshida et al., 1988). Suzuki et al. (1999) reported that untrained males showed a decrease in neutrophil dynamics following 90 minutes of endurance exercise at 90 watts after 3 consecutive workouts on separate days. It was suggested that the increase in neutrophil concentrations following an exercise bout after repeated exercise is attenuated. Since in the present study there was a difference between trained and untrained neutrophil concentrations following 60 minutes of exercise at the same intensity, it can be suggested that aerobic training may lead to attenuation with neutrophil redistribution following acute endurance exercise.

The lymphocyte changes showed a typical biphasic response following all 3 exercise conditions (Mackinnoon, 1999). After 2 hours of recovery, however, the lymphocyte cell concentrations did not significantly decrease below resting values. This finding is inconsistent with the literature (Mackinnon, 1999; 2000; Nieman et al., 1995,

1997; Pedersen & Hoffman-Goetz, 2000; Tvede et al., 1993). Previous studies have examined lymphocyte changes following marathon races, exercise bouts of high intensity, and exercise bouts lasting 2 hours or longer. The duration of the exercise conditions in this study was 60 minutes at a moderate intensity (Nehlsen-Cannarella, 1998); and may not have been strenuous enough to elicit a compromise in circulating lymphocyte numbers, compared to other research using longer and more intense exercise bouts (Nieman et al., 1999).

Further analysis of the lymphocyte response revealed no changes with B (CD19+CD20+) cell concentrations following any of the exercise conditions. This result is also consistent with previous findings, which reported little to no change with B cells following moderate endurance exercise (Mackinnon, 1999; Nieman et al., 1994, 1999; Shek et al., 1995). Thus, it appears that the acute endurance exercise used in this study does not influence the redistribution and trafficking of B cells, which play a pivotal role in humoral immunity and immunoglobulin production.

There was an increase with circulating NK (CD3-CD16+CD56+) cells immediately following the exercise in the untrained women and with the trained women's higher intensity exercise condition. This is consistent with previous research (Mackinnon, 1999; Moyna et al., 1996; Pedersen & Hoffman-Goetz, 2000; Nieman et al., 1994; Wood et al., 1998). However, this increase in NK cells was not observed in the trained women at the lower exercise intensity, suggesting that the NK cell distribution may be dependent on the intensity of the exercise and an individual's fitness level (Mackinnon, 1999).

T cell concentrations significantly increased after 60 minutes in both the untrained and trained women, regardless of exercise condition. Despite these changes, Th

(CD3+CD4+) and Tc/s (CD3+CD8+) cell concentrations did not significantly change following the endurance exercise. This is contrary to some previous research (Nieman et al., 1995; Pedersen and Hoffman-Goetz, 2000; Shek et al., 1995; Shinkai et al., 1996; Steensberg et al., 2001). Previous research has reported an elevation of Th and Tc/s cell concentrations immediately post exercise, followed by a decrease in concentrations reaching levels below resting values. In support of this study, however, Nieman et al. (1994) found no changes with Th and Tc/s cell numbers after 45 minutes of exercise bout at 50% of $V0_{2max}$ in men, but did find significant changes at 80% of $V0_{2max}$. In another study, Nieman et al. (1999) also found no changes with Th and Tc/s cells following 2.5 hrs of rowing at approximately 57% of $V0_{2max}$ in female rowers. The untrained participants in this study exercised at approximately 55% of $V0_{2max}$ while the trained exercised at 44% of $V0_{2max}$ in their first exercise condition and at 65% of $V0_{2max}$ in the second exercise condition. It may be that the intensities used in this study were not high enough to elicit significant changes in the Th and Tc/s cells. Thus it may be concluded that 60 minutes of moderate endurance exercise did not affect the recruitment of lymphocytes, specifically Th and Tc/s cells, to the circulation.

Interestingly, there were changes with activated T (CD3+CD25+) and Th (CD4+CD25+) cells following all three exercise conditions. These changes indicated that the acute endurance exercise used in this study was able to alter the distribution and trafficking of these particular cells. Since activated Th cells produce many of the Th1 and Th2 cytokines, an increase in activated Th cells following the endurance exercise may influence the Th1 and Th2 responses. Previous research has shown that activated T cells and Th cells both increase (Rhind et al., 1996) and decrease (Sellar et al., 2003; Shore et

al., 1999) following endurance exercise. More research is needed to examine the physiological implications of changes that occur with this marker in the post exercise condition.

Physiological Stress of Exercise

The changes that occur in the concentration of the particular immune cells observed following exercise have been used to indicate the level of physiological stress placed on the immune system. For example, the neutrophil to lymphocyte ratio (NE:LYM) has previously used as one such indictor. Nieman et al. (1995) found a positive correlation between cortisol levels and the NE:LYM ratio following 2.5 hours of running in endurance trained males. However, in this study there was also no correlation found between NE:LYM ratio to cortisol ($r = -0.057$). This ratio increased after exercise and during recovery. In this study, the NE:LYM ratio increased after two hours of recovery, compared to rest, and immediately post in all 3 exercise conditions. This seems to be a typical response due to the elevation of neutrophil concentrations and the decrease of lymphocyte concentrations following exercise (Nieman et al., 1995, 1999; Sellar et al., 2003). Another common indicator of exercise induced immune stress is the ratio of Th cells and Tc/s cells (CD4:CD8). Typically there is an increase with both CD3+CD4+ and CD3+CD8+ cells following endurance exercise (Mackinnoon, 1999; Nieman, 1997, 1999; Pedersen and Hoffman-Goetz; 2000). The increase in CD3+CD8+ cells is usually greater than in CD3+CD4+ cells, and causes the CD4:CD8 ratio to decrease following exercise (Kajiura et al., 1995; Mackinnon, 1999; Moyna et al., 1996; Pedersen & Hoffman-Goetz, 2000; Shinkai et al., 1996). With regard to this study, the exercise intensity was likely not great enough to elicit significant changes in Th and Tc/s cells,

and therefore the ratio did not significantly change. It has been reported that ratios below 1.5, the immune system could be considered impaired and there may be an increase risk of infection (Fitzgerald, 1991). In the current study, the ratio was below 1.5 only in the untrained women immediately after exercise, indicating that this population may be slightly more prone to infection at this time point. However, this was only temporary, and the ratio increased above 1.5 following the two hours of recovery.

Increases in plasma and serum cortisol concentrations have been reported immediately following endurance exercise (Consitt et al., 2002; Duclos et al., 2003; Mackinnon, 2000; Nieman et al., 1995, 2001; Steensberg et al., 2001). The present findings do not support this, as a significant increase of cortisol was not observed after exercise, although there was significant decrease following two hours of recovery in the untrained and in trained women's second exercise conditions. Studies reporting little or no significant change in cortisol concentrations have typically examined changes elicited by moderate endurance exercise (50-70% of $V0_{2max}$, 90 watts), and exercise extending for 90 minutes or less in duration (Baum et al., 1997; Henson et al., 2000; Mitchell, et al., 2002; Moyna et al., 1996; Pedersen and Hoffman-Goetz, 2000; Suzuki et al., 1999). Since the exercise conditions of this study would be considered moderately intense, this may explain the lack of elevation in plasma cortisol levels post exercise. Other factors may have contributed to the cortisol values obtained in the study. Anticipation of exercise has been known to elicit a normal cortisol levels to rise, therefore resting values may have already been elevated. As well, the exercise tests were conducted in the morning, were, according to the circadian rhythm cortisol concentrations are at the highest.

Plasma and serum IL-6 exhibit a consistent increase following endurance exercise, and these concentrations remain elevated for several hours (Brenner et al., 1999; Moldoveanu et al., 2001; Nieman et al., 2001; Ostrowski et al., 1999; Rhind et al., 1995; Suzuki et al., 1999; Weinstock et al., 1997). However, in all 3 exercise conditions in this study, plasma IL-6 levels were below the detection limit of the assay; therefore, the data from this study do not agree with other research (Pedersen and Hoffman-Goetz, 2000; Pedersen, 2000; Rhind et al, 1995; Weinstock et al., 1997). It has been theorized that skeletal muscle damage due to exercise may stimulate IL-6 production, either from the tissue directly or from localized monocytes (Ostrowski et al., 1998; Pedersen et al., 2003; Rincon et al., 1997). Studies examining eccentric exercise have shown far greater increases in plasma IL-6 concentrations than those with concentric muscle contractions (Bruunsgaard et al., 1997; Pedersen et al., 2003). It is unlikely that the intensity and mode of exercise in this current study were sufficient to cause muscle damage to elicit this type of IL-6 response.

In summary, the level of intensity achieved during exercise in this study is considered low to moderate compared to that used in other research (Nehlsen-Cannarella, 1998; Nieman et al., 1994). Therefore, it is likely that the level of intensity and the duration with the 3 exercise conditions may have influenced the Th1 and Th2 immune responses observed in this study. Previous research has found suppression in Th1 immunity following *strenuous* endurance exercise (Ibelt et al., 2002; Steensberg et al., 2001). This level of intensity (1. 5 hours of running at 5% downhill grade, at 75% of $V0_{2max}$; 2. 5 hours of running at 75% of $V0_{2max}$) may explain the Th1 response observed in the TE1 and TE2 exercise conditions. Interestingly, despite the similar intensity, the

untrained women exhibited a favored Th2 response. This may indicate that, regardless of the intensity and physiological stress, the immune system of trained women responded differently to endurance exercise than that of untrained women.

Th1 and Th2 Response Following 60 Minutes of Endurance Exercise in Trained and Untrained Women

One of the purposes of this investigation was to examine the changes in Th1 and Th2 cell concentrations and their respective Th1 and Th2 cytokine production. The antigen marker $CRTH_2$ has been used to determine the proportion of circulating Th2 cells, since it is found on T cells that secrete Th2 cytokines (Cosmi et al., 2000). Currently there are no known reported studies that have examined the changes with $CRTH_2$ in response to endurance exercise. Due to the lack of a reliable marker for Th1 cells, only the changes of Th2 cells can be reported. This study found that changes with total $CRTH_2$, Th2 ($CRTH_2$ + CD4+) and Tc/s2 ($CRTH_2$ + CD8+) cell concentrations occurred in all 3 exercise conditions. It is recommended that further research be performed to verify these findings and to further evaluate the physiological significance of these changes.

Th1 and Th2 changes following endurance exercise with untrained and trained women at the same absolute exercise intensity

As previously discussed, there was a redistribution of immune cells following 60 minutes of endurance exercise in both untrained and trained women at the lower intensity of exercise in this study. However, the redistribution of Th2 cells appeared to be different between these two groups. The concentration of Th2 ($CRTH_2$+CD4+) cells decreased immediately after exercise in the trained women, while cell concentration was elevated in

the untrained women following exercise. Circulating Tc/s2 ($CRTH_2$+CD8+) cell numbers were slightly elevated post exercise and significantly decreased at T180 with both of the exercise conditions.

The proportion of Th2 cells to the total CD4 population followed a similar response. Additionally, there were no changes in the concentration of Th or Tc/s cells following the exercise. Both these findings indicate that the redistribution of Th2 and Tc/s2 in the circulation was specific to these cells alone, and not due to changes in the general Th or Tc/s population. According to the results of this study, endurance exercise at an absolute power output of 90 watts elicited different changes with $CRTH_2$ cell recruitment in trained and untrained women. This alteration in the proportion and population of these cells in the circulation may have affected the Th1 and Th2 response. A greater number of $CRTH_2$ cells circulating in the blood can indicate a Th2 response, while a decrease in these cells can indicate that Th2 immunity may be suppressed.

Activated $CRTH_2$ cells were elevated during recovery when comparing the untrained women and the trained women's lower intensity exercise condition. This change was likely due to the untrained women's exercise condition, as no significant changes were found over time when comparing the trained group's two different intensity exercise conditions. This increased percentage of activated $CRTH_2$ cells also suggests that Th2 immunity may be favored following endurance exercise in untrained women.

Despite the changes with circulating Th2 cell concentration, there were no significant changes of Th1 cytokines (IL-2 and IFN-γ) or Th2 cytokines (IL-4, IL-6, IL-10) following exercise or between the untrained women and trained women's first exercise condition. IL-2 has been commonly found to decrease following endurance

exercise (Fu et al., 2003; Lewicki et al., 1988; Rhind et al., 1996; Tvede et al., 1993). These inconsistencies may be due to the intensity of the exercise conditions. Additionally, the individual participants samples of this study had a large degree of variability that decreased the opportunity to observe a significant difference over time or between the exercise conditions. Some research has also reported no significant changes in IFN-γ production and stimulation after exercise (Nieman et al., 2001; Suzuki et al., 1999, 2000; Weinstock et al., 1997). However, previous research examining changes with IFN-γ post exercise is inconsistent. It is also difficult to compare the IFN-γ response because of the different exercise protocols used, and because these studies examined IFN-γ in plasma, serum, and with PHA or LPS stimulated lymphocytes. Previous results have also been inconsistent with IL-10. Some researchers have reported no changes in IL-10 either in plasma or in stimulated lymphocytes (Mueller et al., 2000; Gannon et al., 1997). Others have found IL-10 concentrations to increase following endurance exercise (Nieman et al., 2001; Ostrowski et al., 1999; Suzuki et al., 2000). According to the results of this study, endurance exercise of the intensity and duration used does not affect IL-10 production in trained and untrained women.

IL-6 was produced by PHA stimulated lymphocytes, a finding that has not received much attention in the exercise immunology literature. There were no significant changes in IL-6 either between trained and untrained women in the 3 exercise conditions or over time. It may be concluded that stimulated lymphocyte production of IL-6 was not affected by moderate endurance exercise. Interestingly, there was an increase in IL-6 in the unstimulated condition, after 60 minutes of exercise that returned to resting levels after 2 hours of recovery. This cytokine is produced by Th2 cell but originates primarily

from monocytes (Morel & Oriss, 1998; Rincon et al, 1997). IL-6 can stimulate Th2 cell differentiation and proliferation (Rhind et al., 1995; Rincon et al., 1997; Steensberg et al., 2003) and is considered to be an anti-inflammatory cytokine (Duclos et al., 2003; Morel & Oriss, 1998; Pedersen et al., 2003). It is possible that endurance exercise may not influence T cell production of IL-6 (measured with PHA stimulation) but may affect the production of IL-6 from other cells, such as monocytes.

Finally, the results found IL-4 to be below the detection limit of the assay in all three exercise conditions. This is consistent with previous research (Moyna et al., 1996; Mueller et al., 2000; Nieman et al., 2001; Suzuki et al., 2000). Therefore, IL-4 did not appear to be influenced by endurance exercise in healthy women.

A comparison between the untrained and trained women at the same exercise intensity exhibited a difference in $CRTH_2$ and the related subset (Th2 and Tc/a2) recruitment to the circulation. This difference indicated that the Th1 and Th2 response following exercise may depend on an individual's aerobic fitness level. Endurance exercise may have promoted a Th2 immune response with untrained women, while the opposite may have occurred with trained females.

Th1 and Th2 changes following endurance exercise with untrained and trained women at the same relative exercise intensity

Similar changes were found with $CRTH_2$, Th2, and Tc/s2 cell numbers following 60 minutes of exercise in the untrained women and the higher exercise intensity in the trained women. Thus, endurance exercise at the same relative intensity (i.e., 20 watts below VT) was sufficient to elevate $CRTH_2$ and Tc/s2 cell numbers and the percentage of activated $CRTH_2$ cells immediately post exercise in both trained and untrained women.

Additionally, there were no changes over time with Th2 cell numbers. These results suggest that trained women need to exercise at a higher PO than untrained women to elicit similar changes with these cells.

Despite the lack of change in Th2 cells following exercise, there was a significant lower concentration of these cells in the trained women's second exercise condition when compared to the untrained women. The reason for this lower concentration may be that the trained women performed their second higher intensity exercise condition 1-2 days after their first. Any change with Th1 or Th2 phenotype occurs within the first 48 hours following stimulation (Morel & Oriss, 1998). The first exercise condition (TE1) appeared to decrease Th2 cell numbers; this response may have influenced the second exercise condition, lowering the overall Th2 cell number in the circulation of the trained women.

As seen with the comparison between untrained and trained women at the same absolute intensity, there were no changes with PHA stimulated IL-6, IL-10 or IFN-y. However, a significant interaction with IL-2 was found in the untrained and the trained higher intensity exercise conditions. Further analysis did not reveal any differences between time points or exercise conditions. However, examining the trends (Figure 3. 1), it appears IL-2 decreased below resting levels at T60 in the untrained group, while concentrations were elevated above resting levels in the TE2 exercise condition. This may indicate that there was a different production of IL-2 at the same relative exercise intensity in trained women compared to untrained women. However, caution is advised with this interpretation, since these changes were not statistically significant. It was hypothesized that IL-2 would decrease following the exercise conditions, as has been reported shown in the literature (Espersen et al., 1990; Kohut, et al., 2001; Steensberg et

al., 2001; Suzuki et al., 2000). However, conflicting results exist in the literature. Tevde et al. (1993) found that the intensity of the endurance exercise does influence PHA stimulated IL-2 production. These researchers examined sedentary males who cycled at 25%, 50%, and 75% of their $V0_2max$. The higher intensity exercise showed a significant decrease in IL-2 concentration, while the exercise bout at 50% did not. In addition, Rhind et al. (1996) reported an attenuated suppression of PHA stimulated IL-2 in response to an exercise bout following a 12-week training program. According to the literature and the results of this study, it is possible that aerobic training may lead to increased production of IL-2 following endurance exercise.

IL-2 can be produced by activated Th1 cells and activates and stimulates proliferation of other Th1 cells (Janeway et al., 2001; Kuby et al., 2000; Morel & Oriss, 1998). It also stimulates B cells, NK cells and macrophages. IL-2 is very important for cellular-mediated immunity, and a chronic decreased level of IL-2 is considered immunosuppressive (Elenkov & Chrouos, 1999). The results of this study indicate that Th1 immunity and proliferation may be promoted in the trained women. This conclusion is supported by the elevation of IL-12 in the plasma following the two hours of recovery. IL-12 is produced by antigen presenting cells (APC), such as macrophages. This increase with IL-12 may be linked to the increase with circulating monocytes following the exercise conditions. IL-12 is also important for the generation of Th1 cells. It stimulates Th0 cells to differentiate into Th1 cells and leads to proliferation of Th1 one cells by stimulating IL-2 production (Morel & Oriss, 1998; Rhind et al., 1995). The increase in IL-12 could also be associated with the increase in IL-2 production and percentage of activated T cells and Th cells seen in the trained women's higher intensity exercise

condition. The results of this study demonstrate the numerous variables that can influence the Th1 and Th2 responses. The nature of the inter-connection between cytokines and Th1 and Th2 cells requires further research.

To summarize, the results of this study did not reveal changes with $CRTH_2$, Th2 and Tc/s2 cell numbers in trained and untrained women following an endurance exercise set at a relative intensity determined by the individual's own fitness level. It also appears that trained women may have a greater Th1 response following endurance exercise, as evidenced by the increase in IL-2 production. In the untrained women, IL-2 decreased following exercise, suggesting that Th1 immunity may be suppressed. This suppression may allow Th2 immunity to be favored. The most profound finding indicating that Th1 immunity may be favored in trained women was the elevation of plasma IL-12, which promotes Th1 differentiation. These results suggest that untrained and trained women have similar changes in $CRTH_2$, Th2 and Tc/s cell numbers, but they may have different Th1 and Th2 responses.

Th1 and Th2 changes following endurance exercise with trained women at the different exercise intensities

There were no changes in $CRTH_2$, Th2 and Tc/s2 cell numbers and the percentage of activated $CRTH_2$ cells following exercise in trained women. This lack of change suggests that intensity of exercise does not have a major impact on the redistribution of these cells in trained women.

Interestingly, the concentration of PHA stimulated IFN-γ significantly increased following recovery in both of the exercise conditions. IFN-γ is a pro-inflammatory cytokine and is important for the cellular-mediated immune response. Th1 and NK cells

produce IFN-γ. This cytokine stimulates the activation of macrophages and NK cells and is cross-regulated with Th2 cells, inhibiting their proliferation and production of Th2 cytokines (Morel & Oriss, 1998). The majority of exercise immunology research has found little or no change in either plasma or lymphocyte stimulated IFN-γ production following endurance exercise (Haahr et al., 1991; Nieman et al., 2001; Moldoveanu et al., 2001; Suzuki et al., 1999, 2000). Despite these reports, the results from this study appear to suggest that trained women have greater IFN-γ concentrations following endurance exercise, thus promoting a Th1 response.

The concentration of IL-12 in plasma also increased in both exercise conditions for the trained women. IL-12 and IFN-γ are linked, since IL-12 stimulates Th1 and NK cells to produce IFN- γ, while, IFN- γ stimulates monocytes to produce IL-12 (Morel & Oriss et al., 1998; Rhind et al., 1995). This cross-stimulation between these two cytokines may explain the elevation in both plasma IL-12 and lymphocyte stimulated IFN-γ following exercise. Regardless of intensity, it appears that acute endurance exercise promotes a Th1 immune response in trained women.

IL-10 concentration did not significantly change over time, although it was significantly lower in the lower intensity exercise comparison compared to the higher intensity exercise condition. This suggests that exercise intensity may influence IL-10 production. IL-10 is produced by Th2 cells and is strongly associated with immunosuppression, inhibiting cellular-mediated and inflammatory responses. It inhibits the secretion of IFN-γ by T cells and NK cells, and reduces the nitric oxide production and cytotoxicity of macrophages (Kuby et al., 2001; Moldveanu et al., 2001; Morel & Oriss, 1998). An examination of the trends (Figure 3. 3) indicates that there was a

decrease in IL-10 production following the lower intensity exercise condition that was not seen in the higher intensity exercise condition. This decrease in IL-10 production may be due to the decrease in the percentage of activated $CRTH_2$ cells, as well as the increase in IFN-γ seen in the trained participants. While this result could indicate that Th2 immunity may be suppressed following the lower intensity exercise condition, caution is advised since this is based on a trend only.

It appears that trained women may have a predominant Th1 immune response following endurance exercise. This response may be slightly affected by the intensity of exercise, where lower intensity exercise may lead to a temporary suppression of Th2 immunity. These findings are contrary to the hypothesis, and further research will be needed to determine the physiological implications of this conclusion.

Summary

Th2 immunity may be favoured following endurance exercise in untrained women. Th2 cytokines and circulating $CRTH_2$ cells and their subsets were unaffected by the endurance exercise in untrained women, and there was a decrease IL-2 production by stimulated lymphocytes post exercise. Following the trained women's lower intensity exercise condition, there was a favoured Th1 immune response. This was concluded based on elevated plasma IL-12 concentrations and the increase in IFN-γ production following the exercise. The production of the Th2 cytokine IL-10 was lower in this condition compared to the higher intensity exercise condition, suggesting a suppressed Th2/anti-inflammatory response when challenged. In addition, there was a decrease in $CRTH_2$ cell numbers and their subsets immediately following exercise when compared to the untrained women. Both the lower $CRTH_2$ cell numbers and the reduced IL-10

production after stimulation suggest a temporary suppression of Th2 immunity in the circulation, thereby allowing Th1 immunity to become more prominent.

Examination of the trained women's higher intensity exercise condition shows that the immune response also favored Th1 immunity. Following the exercise bout plasma IL-12 was elevated, and after stimulation with a T cell mitogen, IL-2 and IFN-γ increased. All of these changes suggest Th1 immunity. There was a decrease in $CRTH_2$ cells and their subsets, again suggesting lower concentration of Th2 cells in the blood. Despite this, Th2 cytokines after stimulation were not affected by the endurance exercise. Therefore, the suppression of Th2 immunity may not be as physiological significant in the trained women's lower intensity exercise condition.

It can be suggested that higher intensity endurance exercise favours a Th2 immune response in the untrained women. However, Th2 immunity may be suppressed, however, at the same relative intensity in trained women. This Th2 immune suppression may even be greater following the same absolute exercise intensity in aerobically trained women. It has been shown that there is a suppression of Th1 immunity following strenuous endurance exercise (Ibelt et al., 2002; Smith, 2003; Steensberg et al., 2000). This suppression has been suggested to be a protective mechanism against inflammation (Northoff et al., 1998; Morel, Oriss et al., 1998; Pedersen & Hoffman-Goetz, 2000). This temporary suppression, however, could increase the risk for an URTI (Mackinnon, 1999; Nieman, 1998; Northoff et al., 1998). According to the findings of the current study, untrained women may be at greater susceptible to URTI following endurance exercise, while trained women may be protected, since their Th1 immunity under the stimulating conditions used, does not appear to be suppressed. Perhaps aerobic training leads to an

adaptation with the immune response whereby Th1 immunity is favoured following endurance exercise.

3.6 Limitations of the Study

One limitation of this study relates to the choices that had to be made to control for the reproductive cycle. All participants were using pharmaceutical contraceptives (OC). While all the OC contained estrogen and progesterone supplements, the amount of each differed between brands (refer to Appendix C). Estrogens and progesterone have been shown to regulate some aspects of the immune system (Goldsby et al., 2000; Pehlivanoglu et al., 2001; Vera-Lastra et al., 2002). These different compositions may have increased the variability of the present results. The exercise challenges were done on days 1-7 of the OC cycle (representing the follicular phase of the menstrual cycle). Due to this control, the results of this study can only be claimed to occur during this particular phase. Changes during the later phase of the menstrual cycle may not differ greatly, but this cannot be stated with confidence and requires further investigation (Burrows et al., 2002). In addition, these results cannot be generalized to women who are not using OC. A future comparative study that examines women both on and off OC may explain any influence that these supplemental hormones have on the immune system.

The complexity and integration of the immune system makes it difficult to evaluate fully. Only certain immune and hormonal parameters related to Th1 and Th2 immunity were chosen for this study. For reasons of cost and time, only a select few of the Th1 and Th2 cytokines were examined. Further analysis with other Th1 and Th2 cytokines, such as TNF-α, -β,IL-5 and IL-13 might have provided better support and understanding of the immune response that occurred post exercise. Analysis of the

antigen marker $CRTH_2$ has not been reported in exercise immunology literature; therefore this study's findings cannot be compared to previous research. Laboratory techniques and procedures for acquiring and analyzing these cells were novel. Further research is needed to validate these findings and laboratory techniques. At this time there is not a reliable marker for Th1 cells. Thus only Th2 cell concentration could be analyzed, and any change related to Th1 cells remains unknown.

Unlike animal studies, where immune parameters can be measured elsewhere such as in the spleen, lymph nodes and muscles, studies examining humans are usually limited to blood. Circulating lymphocytes only represent approximately 2% of the total lymphocyte population in the body (Nehlsen-Cannarella, 1998). Different immune responses to endurance exercise may be occurring elsewhere in the body, such as in the muscles and lymph nodes. Thus lymphocytes in the circulation may not adequately reflect the rest of the immune system. These results can only be claimed to have occurred in the circulation; changes elsewhere in the body are only speculated. As well, cytokine production was measured in controlled conditions, and stimulated with a T cell mitogen that favours Th1 immunity. Therefore, there is limitations with using invirto assessment of cytokine production.

Finally, the means and standard deviations of some the PHA stimulate cytokines may not fully represent the changes resulting from endurance exercise. For example, lymphocyte stimulated IL-6 production did not change over the three time points in all three exercise conditions. However, examination of the individual IL-6 concentrations following exercise (Figure E. 4) indicates that IL-6 may have changed in response to

endurance exercise. These changes were not found to be significant due to the large variability and different levels of concentrations between samples.

3.7 Conclusion

The results from this study indicated that several immune changes occurred in both aerobically trained and untrained women following an hour of cycling exercise. Since the trained participants completed two different exercise bouts, one at the mean PO of the untrained group (90 watts) and the second at 20 watts below their own individual ventilatory threshold, the immune responses between untrained and trained women could be compared in greater depth.

There were some atypical findings following the exercise conditions. Usually Th and Tc/s cells decrease following exercise (Mackinnon, 1999), but this decrease was not significant in any of the exercise conditions. Consequently, the CD4:CD8 ratio did not significantly change. Cortisol levels also did not increase significantly following the exercise bouts; this was perhaps due to the influence of the circadian rhythm, as well as, the intensity and duration of the exercise conditions. Another atypical finding in this investigation was the low concentration of plasma IL-6. Typically plasma IL-6 concentrations are elevated above resting levels following exercise (Moldoveanu et al., 2001; Pedersen & Hoffman-Goetz, 2000). The lack of change in all these measures indicates that the intensity of the exercise challenge was inadequate to stimulate such changes. Despite this, typical changes occurred with the differential WBC counts. A decrease in T cells and NK cells occurred, which is commonly reported in the literature (Mackinnon, 1999; Pedersen & Hoffman-Goetz, 2000). In addition, the percentage of

activated T cells, Th and Tc/s cells increased following the exercise, which has previously been found (Rhind et al., 1996).

Trained women had a more pronounced Th1 response following 60 minutes of endurance exercise. There was also a decrease in circulating Th2 cell numbers immediately following both exercise conditions. Furthermore, there was a lower IL-10 production after stimulation with the TE1 exercise condition. Both findings suggested Th2 immunity suppression. This suppression was contrary to the hypothesis of this study and to the literature that has examined these immune measures. The untrained women, however, did show a dominant Th2 response following their exercise condition. As a result, the immune system in healthy women responds to moderate endurance exercise differently depending on the individual fitness level.

Further research, examining similar immune measures in women, is needed establish consistent findings. In addition, investigation is needed concerning any gender differences in this immune response. The influences of OC and the menstrual cycle need to be taken into account, as the role they play with the immune response to exercise has not yet been fully established. Exercise immunology is still a relatively new area. Consistent findings with these aspects of the immune system need to be established before physiological relevance can be claimed.

3.8 References

Bishop, N., Walsh, N., Haines, D., Richards, E., & Gleeson, M. (2001). Pre-exercise carbohydrate status and immune responses to prolonged cycling: II. Effect on plasma cytokine concentration. *International Journal of Sports Nutrition and Exercise Metabolism, 11,* 503-512.

Braum, M., Muller-Steinhardt, M., Liesen, H., & Kirchner, H. (1997). Moderate and exhaustive endurance exercise influences the interferon-gamma levels I whole blood culture supernatants. *European Journal of Applied Physiology, 76,* 165-169.

Brenner, I. K. M., Natale, V. M., Vasiliou, P., Moldoveanu, A. I., Shek, P. N., & Shepard, R. J. (1999). Impact of three different types of exercise on components of the inflammatory response. *European Journal of Applied Physiology, 80,* 452-460.

Bruunsgaard, H., Hartkopp, A., Mohr, T., Konradsen, H., Heron, I., Mordhorst, C. H., & Pedersen, B. K. (1997). In vivo cell-mediated immunity and vaccination response following prolonged, intense exercise. *Medicine and Science in Sports and Exercise, 29*(9), 1176-1181.

Burrows, M., Bird, S. R. & Bishop, N. (2002). The menstrual cycle and its effect on the immune status of female endurance runners. *Journal of Sports Sciences, 20,* 339-344.

Consitt, L. A., Copeland, J. L., & Tremblay, M. S. (2002). Endogenous anabolic hormone responses to endurance versus resistance exercise and training in women. *Sports Medicine, 32*(1), 1-22.

Cosmi, L., Annunziato, F., Iwasaki, M., Galli, G., Maggi, R. M. E., Nagata, K., & Romagnani, S. (2000). CRTH2 is the most reliable marker for the detection of circulating human type 2 Th and type 2 T cytotoxic cells in health and disease. *European Journal of Immunology, 30,* 2972-2979.

Duclos, M., Gouarne, C., & Bonnemaison, D. (2003). Acute and chronic effect of exercise on tissue sensitivity to glucocorticords. *Journal of Applied Physiology, 94,* 869-875.

Elenkov, I. J., & Chrousos, G. P. (1999). Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. *Trends in Endocrinology and Metabolism, 10(*9), 359-368.

Eliakim, A., Wolach, B., Kodesh, E., Gavrieli, R., Radnay, J., Ben-Tovim, T., Yarom, Y., & Falk, B. (1997). Cellular and humoral immune response to exercise among

gymnasts and untrained girls. *International Journal of Sports Medicine, 18*(3), 208-212.

Espersen, G. T., Elbaek, A., Ernst, E., Toft, E., Kaalund, S., Jersild, C., & Grunnet, N. (1990). Effect of physical exercise on cytokines and lymphocyte subpopulations in human peripheral blood. *APMIS, 98*, 395-400.

Fitzgerald, L. (1991). Overtraining increases the susceptibility to infection. *International Journal of Sports Medicine, 12*(S1), S5-S8.

Fu, S. C., Qin, L., Leung, C. K., Chan, B. P. & Chan, K. M. (2003). Regular moderate exercise training prevents decrease CD4+ T-lymphocytes induced by a single bout of strenuous exercise in mince. *Canadian Journal of Applied Physiology, 28*(3), 370-381.

Gannon, G. A., Rhind, S. G., Suzui, M., Shek, P. N., & Shephard. R. J. (1997). Circulating levels of peripheral blood leucocytes and cytokines following competitive cycling. *Canadian Journal of Applied Physiology, 22*(2), 133-147.

Goldsby, R. A., Kindt, T. J., & Osborne, B. A. (2000). *Kuby immunology* (4th ed.). New York: W. H. Freeman.

Haahr, P. A., Pedersen, B. K., Fomsgaard, A., Tvede, N., Diamant, M., Klarlund, K., Halkjaer-Kristensen, J., & Bendtzeb, K. (1991). Effect of physical exercise on in vitro production of interleukin 1, interleukin 6, Tumor Necrosis Factor- alpha, Interleukin 2, and Interferon-gamma. *International Journal of Sports Medicine, 12,* 223-227.

Henson, D., Nieman, D., Nehlsen-Cannarella, S., Fagoaga, O., Shannon, M., Bolton, M., Davis, M., Gaffney, c., Kelln, W., Austin, M., Hjertman, J., & Schilling, B. (2000). Influence of carbohydrate on cytokine and phagocytic response to 2 h of rowing. *Medicine and Science in Sports and Exercise, 32*(8), 1384-1389.

Ibfelt, T., Petersen, E. W., Bruunsgaard, H., Sandman, M., & Pedersen, B. K. (2002). Exercise-induced change in type 1 cytokine-producing CD8+ T cells is related to a decrease in memory T cells. *Journal of Applied Physiology, 93,* 645-648.

Janeway, C. A., Travers, P., Walport, M., & Shlomchik, M., (2001). *Immuno biology: The immune system in health and disease* (5th ed.). New York: Garland.

Kjaer, M., Christensen, N. J., Soone, B., Richter, E. A., & Galbo, H. (1995). Effect of exercise on epinephrine turnover in trained and untrained male subjects. *Journal of Applied Physiology, 59*(4), 1061-1067.

Kohut, M. L., Boehm, G. W., & Moynihan, J. A. (2001). Moderate exercise is associated with enhanced antigen-specific cytokine, but not IgM antibody production in aged mice. *Mechanisms of Ageing and Development, 122,* 1135-1150.

Kohut, M. L., Boehm, G. W., & Moynihan, J. A. (2001). Prolonged exercise suppresses antigen-specific cytokine response to upper respiratory infection. *Journal of Applied Physiology, 90,* 678-684.

Kuby, J. (2000). *Immunology.* New York: W. H. Freeman.

Lewicki, R. Tchorewski, I. I., Majewska, E., Nowak, Z., & Baj, Z. (1988). Effect of maximal physical exercise on T-lymphocytes subpopulations and on interleukin 1 (IL-1) and interleukin 2 (IL2) production in vivo. *International Journal of Sports Medicine, 9, 114*-117.

Mackinnon, L. T. (1999). *Advances in exercise immunology.* Champaign, IL: Human Kinetics.

Mackinnon, L. T. (2000). Chronic exercise training effects on immune function. *Medicine and Science in Sports and Exercise, 32*(7), S369-S376.

Mitchell, J. B., Dugas, J. P., McFarlin, B. K., & Nelson, M. J. (2002). Effect of exercise, heat stress, and hydration on immune cell number and function. *Medicine and Science in Sports and Exercise, 34*(12), 1941-1950.

Moldoveanu, A. I., Shepard, R. J., & Shek, P. N. (2001). The cytokine response to physical activity and training. *Sports Medicine, 31*(2), 115-144.

Morel, P. A., & Oriss, T. B. (1998). Crossregulation between Th1 and Th2 cells. *Critical Reviews in Immunology, 18,* 275-303.

Moyna, N. M., Acker, G. R., Fulton, J. R., Weber, K., Goss, F. L., Robertson, R. J., Tollerud, D. J., & Rabin, B. S. (1996). Lymphocyte function and cytokine production during incremental exercise in active and sedentary males and females. *International Journal of Sports Medicine, 17*(8), 585-591.

Mueller, O., Villiger, B., O'Callaghan, B., & Simon, H. U. (2000). Immunological effects of competitive versus recreational sports in cross-country skiing. *International Journal of Sports Medicine, 22,* 52-59.

Nehlsen-Cannarella, S. L. (1998). Cellular response to moderate and heavy exercise. *Canadian Journal of Physiology and Pharmacology, 76,* 485-489.

Nieman, D. C. (1997). Exercise immunology: Practical applications. *International Journal of Sports Medicine, 18(*S1), 91-100.

Nieman, D. C. (1998). Exercise and resistance to infection. *Canadian Journal of Physiology and Pharmacology, 76,* 573-580.

Nieman, D. C. (2000). Is infection risk linked to workload? *Medicine and Science in Sports and Exercise, 32*(7), S406-S411.

Nieman, D. C., Brendle, D., Henson, D. A., Suttles, J., Cook, V. D., Warren, B. J., Butterworth, D. E., Fagoaga, O. R., & Nehlsen-Cannarella, S. L. (1995). Immune function in athletes versus nonathletes. *International Journal of Sports Medicine, 16*(5), 329-333.

Nieman, D. C., Buckley, K. S., Henson, D. A., Warren, B. J., Suttles, J., Ahle, J. C., Simandle, S., Fagoaga, O. R., & Nehlsen-Cannarella, S. L. (1995). Immune function in marathon runners versus sedentary controls. *Medicine and Science in Sports and Exercise, 27*(7), 986-992.

Nieman, D. C., Henson, D. Garner, E., Butterworth, D., Warren, B., Utter, A., Davis, J., Fagoaga, O., & Nehlsen-Cannarella, S. L. (1997). Carbohydrate affects natural killer cell redistribution but not activity after running. *Medicine and Science in Sports and Exercise, 29*(10), 1318-1324.

Nieman, D. C., Miller, A. R., Henson, D. A., Smith, L. L., Utter, A. C., Vinci, D. M., Davis, J. M., Kaminsky, D. E., & Shute, M. (2001). Cytokine changes before and after marathon race. *Journal of Applied Physiology, 91,* 109-114.

Nieman, D. C., Miller, A. R., Henson, D. A., Warren, B. J., Gusewitch, G., Johnson, R. L., Davis, J. M., Butterworth, D. E., Herrings, J. L., & Nehlsen-Cannarella, S. L. (1994). Effect of high-versus moderate intensity exercise on lymphocyte subpopulations and proliferative response. *International Journal of Sports Medicine, 15*(4), 199-206.

Nieman, D. C., Nehlsen-Cannarella, S. L., Fagoaga, O. R., Henson, D. A., Shannon, M., Davis, J. M., Austin, M. D., Hisey, C. L., Holbeck, J. C., Hjertman, M. E., Bolton, M. R., & Schilling, B. K. (1999). Immune response to two hours of rowing in elite female rowers. *International Journal Sports Medicine, 20,* 476-481.

Nieman, D. C., Nehlsen-Cannarella, S. L., Fagoaga, O. R., Henson, D. A., Shannon, M., Hjertman, J. M. E., Schmitt, R. L., Bolton, M. R., Austin, M. D., Schilling, B. K., & Thrope, R. (2000). Immune Function in female elite rowers and non-athletes. *British Journal of Sports Medicine, 34,* 181-187.

Nieman, D. C., Simandle, S., Henson, D. A., Warren, B. J., Suttles, J., Davis, J. M., Buckley, K. S., Ahle, J. S., Bitterworth, D. E., Fagoaga, O. R., & Nehlsen-Cannarella, S. L. (1995). Lymphocyte proliferation response to 2.5 hours of running. *International Journal of Sports Medicine, 16*(6), 404-408.

Northoff, H., Berg, A., & Weinstock, C. (1998). Similarities and differences of the immune response to exercise and trauma: the IFN-γ concept. *Canadian Journal of Physiology and Pharmacology, 76,* 497-504.

Oshida, Y., Yamanouchi, K., Hayamizu, S., & Sato, Y. (1988). Effect of acute physical exercise on lymphocyte subpopulations in trained and untrained subjects. *International Journal of Sports Medicine, 9,* 137-140.

Ostrowski, K., Rohde, T., Asp, S., Schjerlong, P., & Pedersen, B. K (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *Journal of Physiology, 515*(1), 287-291.

Pedersen, B. K. (2000). Exercise and cytokines. *Immunology and Cell Biology, 78,* 532-535.

Pedersen, B. K., & Hoffman-Goetz, L. (2000). Exercise and the immune system: Regulation, integration and adaptation. *Physiological Reviews, 80*(3), 1055-1081.

Pedersen, B. K., Ostrowski, K., Rohde, T., & Bruunsgaard, H. (1998). The cytokine response to strenuous exercise. *Canadian Journal of Physiology and Pharmacology, 76*(5), 505-11.

Pedersen, B. K., Steensberg, A., Fischer, C., Keller, C., Keller, P., Plomgaard, P., Febbraio, M., & Saltin, B. (2003). Searching for the exercise factor: Is IL-6 a candidate? *Journal of Muscle Research and Cell Motility, 24,*113-119.

Pehlivanoglu, B., Balkaner, Z. D., Ridvanagaoglu, A. Y., Durmazlar, N. Ozturk, G., Erbas, D., & Okur, H. (2001). Impact of stress, gender and menstrual cycle on immune system: Possible role nitric oxide. *Archives of Physiology and Biochemistry, 109(*4), 383-387.

Rhind, S. G., Gannon, G. A., Shepard, R. J., & Shek, P. N. (2002). Indomethacin modulates circulating cytokine responses to strenuous exercise in humans. *Cytokine, 19*(3), 153-158.

Rhind, S. G., Shek, P. N., & Shephard, R. J. (1995). The impact of exercise on cytokine and receptor expression. *Exercise Immunology Review, 1,* 97-148.

Rhind, S. G., Shek, P. N., Shinkai, S., & Shepard, R. J. (1996). Effect s of moderate endurance exercise and training on in vitro lymphocyte proliferation, interleukin-2 (IL-2) production and IL-2 receptor expression. *European Journal of Applied Physiology, 74, 348*-360.

Rincon, M., Anguita, J., Nakarmura, T., Fikrig, E., & Flavell, R. A. (1997). Interleukin (IL)-6 directs the differentiation of IL-4-producting CD4+ T cells. *Journal of Experimental Medicine, 185*(3), 461-469.

Sellar, C. M. (2003). *The effects of rowing exercise and carbohydrate supplementation on the immune system.* Unpublished Master's thesis, University of Alberta, Edmonton, Alberta, Canada.

Shek, P. N., Sabiston, B. H., Buguet, A. & Radomski, M. W. (1995). Strenuous exercise and immunological changes: A multiple-time-point analysis of leukocyte subsets, CD4/CD8 ratio, immunoglobulin production and NK cell response. *International Journal of Sports Medicine, 16*(7), 466-474.

Shephard, R. J. (2001). Exercise in the heat: A double threat to the immune system? *The Physician and Sportsmedicine, 29*(6), 1-11.

Shephard, R. J. (2003). Sepsis and mechanisms of inflammatory response: Is exercise a good model? *British Journal of Sports Medicine, 35,* 223-230.

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

Sherman, W. (1989). Pre-event nutrition. *Sport Science Exchange, 1*(12), 12.

Shinkai, S., Kohno, H., Kimura, K., Komura, T., Asai, H., Inai, R., Oka, K., Kurokawa, Y., & Shepard, R. J. (1995). Physical activity and immune senescence in men. *Medicine and Science in Sports and Exercise, 27*(11), 1516-1526.

Shinkai, S., Watanabe, S., Asai, H., & Shek, P. N. (1996). Cortisol response to exercise and post-exercise suppression of blood lymphocyte subset counts. *International Journal of Sports Medicine, 17*(8), 597-603.

Shore, S., Shinkai, S., Rhind, S. & Shephard, R. J. (1999). Immune responses to training: how critical is training volume? *Journal of Sports Medicine and Physical Fitness, 39,* 1-11.

Smith, L. L. (2003). Overtraining, excessive exercise and alerted immunity: Is this a T helper-1 versus T helper-2 lymphocyte response? *Sports Medicine, 33*(5), 347-364.

Steendberg, A., Fischer, C. P., Keller, C., Moller, K., & Pedersen, B. K. (2003). IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *American Journal of Physiology-Endocrinology and Metabolism, 285,* E433-E437.

Steendberg, A., Toft, A. D., Bruunsgaard, H., Sandmand, M., Halkjaer-Kristensen, J., & Pedersen, B. K. (2001). Strenuous exercise decreases the percentage of type 1 T cells in the circulation. *Journal of Applied Physiology, 91,* 1708-1712.

Suzuki, K., Totsuka, M., Nakaji, S., Yamada, M., Kudoh, S., Liu, Q., Sugawara, K., Yamaya, K., & Sato, K. (1999). Endurance exercise causes interaction among stress hormones, cytokines nuetrophils dynamics and muscle damage. *Journal of Applied Physiology, 87*(4), 1360-1367.

Suzuki, K., Yamada, M., Kurakake, S., Okamura, N., Yamaya, K., Liu, Q., Kudoh, S., Kowatari, K., Nakaji, S., & Sugawara, K. (2000). Circulating cytokines and hormones with immunosuppressive but neutrophil-priming potential rise after endurance exercise in humans. *European Journal of Applied Physiology, 81,* 281-287.

Tvede, N., Kappel, M., Halkjaer-Kristensen, J., Galbo, H., & Pedersen, B. K. (1993). The effect of light, moderate and severe bicycle exercise on lymphocyte subsets, natural and lymphokine activated killer cells, lymphocyte proliferative response and interleukin 2 production. *International Journal of Sports Medicine, 1495,* 275-282.

Vassilakopoulos, T., Karatza, M. H., Katsaounou, P., Kollintza, A., Zakynthinos, S., & Roussos, C. (2003). Antioxidants attenuate the plasma cytokine response to exercise in humans. *Journal of Applied Physiology, 94,* 1025-1032.

Vera-Lastra, O., Jara, L. J., & Espinoza, L. R. (2002). Prolactin and autoimmunity. *Autoimmunity Reviews, 1,* 360-364.

Wasserman, K. (1987). Determinants and detection of anaerobic threshold and consequences of exercise above it. *Circulation, 76*(S5), S29-S39.

Weinstock, C., Konig, D., Harnischmacher, R., Keul, J., Berg, A., & Northoff, H. (1997). Effect of exhaustive exercise stress on the cytokine response. *Medicine and Science in Sports and Exercise, 29*(3), 345-354.

Woods, J. A., Ceddia, M. A., Wolters, B. W., Evans, J. K., Lu, Q., & McAuley, E. (1999). Effects of 6 months of moderate aerobic exercise training on immune function in the elderly. *Mechanisms of Ageing and Development, 109,* 1-19.

Yan, H., Kuroiwa, A., Tanaka, H., Shindo, M., Kiyonaga, A., & Nagayanna, A. (2001). Effects of moderate exercise on immune senescence in men. *European Journal of Applied Physiology, 86,* 105-111.

Table 3.1. Subject Characteristics and Exercise Performance Measurements.

Characteristics				
	Age (years)	Weight (kg)	Height (cm)	Body fat (%)
UT mean	26	63. 3	163. 7	24
± S. D	4	12. 7	5. 2	7
T mean	24	61. 5	166. 8	19
± S. D	3	6. 6	5. 3	6

Performance					
	$V0_2$max (ml/kg/min)	Max PO (watts)	PO at VT (watts)	% of max at VT	$V0_2$ at VT (ml/kg/min)
UT mean	31. 9	163. 2	101. 9	65. 9	21. 5
± S. D	4. 1	27. 1	23. 3	6. 3	3. 9
T mean	49. 4 [a]	254. 6 [a]	175. 9 [a]	71. 4 [a]	35. 0 [a]
± S. D	3. 6	41. 5	28. 9	1. 77	3. 78

UT= untrained group
T= trained group
PO= power output
VT= ventilatory threshold

a= significantly different from untrained group, $p < 0.05$.

Table 3.2. Changes with Hemoglobin Concentration and Changes with Hematocrit in Response to 60 minutes of Endurance Exercise.

		T0	T60 [a]	T180 [b]
Hemoglobin ($g \cdot L^{-1}$)	UT mean	134. 4	137. 4	134. 5
	± S. D	6. 7	8. 1	7. 6
	TE1 mean	134. 5	136. 1	132. 7
	± S. D	10. 9	11. 9	11. 7
	TE2 mean	132. 5	138. 1 [d]	131. 7 [e]
	± S. D	11. 8	12. 2	10. 5
		T0	T60 [a]	T180 [b]
Hematocrit ($L \cdot L^{-1}$)	UT mean	0. 41	0. 42	0. 41
	± S. D	0. 02	0. 05	0. 03
	TE1 mean	0. 41	0. 40	0. 39
	± S. D	0. 06	0. 03	0. 04
	TE2 mean	0. 39	0. 41	0. 39
	± S. D	0. 04	0. 04	0. 03

UT= untrained group exercise condition
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.

a= significantly different from T0 in groups UT and TE1; time main effect, $p < 0.05$
b=significantly different from T60 in all groups; time main effect, $p < 0.05$
d= significantly different from T0; interaction effect, $p < 0.05$
e= significantly different from T60; interaction effect, $p < 0.05$

Table 3.3. Changes in Circulating White Blood Cell (WBC), Neutrophil, Lymphocyte, and Monocyte Concentrations in Response to 60 minutes of Endurance Exercise.

		T0	T60[b]	T180[c]
WBC	UT mean	6. 1	7. 9	9. 6
10^9 cells·L^{-1}	± S. D	1. 2	1. 1	2. 5
	[a]TE1 mean	5. 1	6. 3	8. 0
	± S. D	1. 0	0. 8	1. 8
	TE2 mean	4. 7	7. 2	8. 8
	± S. D	1. 0	1. 9	1. 8
		T0	T60[b]	T180[c]
Neutrophils	UT mean	3. 4	4. 5	6. 7
10^9 cells·L^{-1}	± S. D	1. 0	0. 9	2. 2
	[a]TE1 mean	2. 7	3. 5	5. 4[f]
	± S. D	0. 7	0. 5	1. 5
	TE2 mean	2. 5	4. 3[e]	6. 2[f]
	± S. D	0. 7	1. 3	1. 4
		T0	T60[b]	T180[d]
Lymphocytes	UT mean	1. 9	2. 5	2. 1
10^9 cells·L^{-1}	± S. D	0. 5	0. 6	0. 7
	TE1 mean	1. 7	2. 1	1. 9
	± S. D	0. 5	0. 6	0. 5
	TE2 mean	1. 7	2. 1	1. 8
	± S. D	0. 4	0. 6	0. 5
		T0	T60[b]	T180[b]
Monocytes	UT mean	0. 4	0. 5	0. 5
10^9 cells·L^{-1}	± S. D	0. 1	0. 1	0. 1
	TE1 mean	0. 3	0. 4	0. 4
	± S. D	0. 1	0. 1	0. 1
	TE2 mean	0. 3	0. 4	0. 4
	± S. D	0. 1	0. 1	0. 1

UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.

a = significantly different from UT at all time points; group main effect, $p < 0.05$
b= significantly different from T0 in all groups; time main effect, $p < 0.05$
c= significantly different from T0 and T60 in all groups; time main effect, $p < 0.05$
d= T180 significantly different from T60 in all groups; time main effect, $p < 0.05$
e= significantly different from T0; interaction effect, $p < 0.05$
f= significantly different from T0 and T60; interaction effect, $p < 0.05$

Table 3.4. Changes in Circulating Eosinophils and Basophils Concentrations in Response to 60 minutes of Endurance Exercise.

		T0	T60	T180[b]
Eosinophils	UT mean	0. 25	0. 27	0. 21
10^9 cells·L^{-1}	± S. D	0. 09	0. 10	0. 08
	TE1 mean	0. 25	0. 27	0. 24
	± S. D	0. 21	0. 20	0. 13
	TE2 mean	0. 22	0. 26	0. 21
	± S. D	0. 20	0. 21	0. 14
		T0	T60[a c]	T180[ac]
Basophils	UT mean	0. 09	0. 12	0. 12
10^9 cells·L^{-1}	± S. D	0. 05	0. 05	0. 07
	TE1 mean	0. 13	0. 14	0. 15
	± S. D	0. 06	0. 06	0. 06
	TE2 mean	0. 06	0. 13[d]	0. 13
	± S. D	0. 03	0. 08	0. 06

UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.

a= significantly different from T0 in groups UT vs. TE2; time main effect, $p < 0.05$
b=significantly different from T60 in groups UT vs. TE2; time main effect, $p < 0.05$
c= significantly different from T0 in groups TE1 vs. TE2; time main effect, $p < 0.05$
d= significantly different from T0; interaction effect, $p < 0.05$

Table 3.5. Percentage of Gated Lymphocyte Subsets in Response to 60 minutes of Endurance Exercise.

		T0	T60	T180
Total CD3+	UT mean	80	74	74
(T cells)	± S. D	10	9	23
	TE1 mean	76	74	73
	± S. D	11	15	25
	TE2 mean	77	75	80
	± S. D	7	10	5
		T0	T60	T180[b]
CD3+/CD4+	UT mean	26	23	22
(Th cells)	± S. D	26	23	25
	TE1 mean	43	37	41
	± S. D	20	18	23
	[a]TE2 mean	41	36	43
	± S. D	18	17	21
		T0	T60	T180
CD3+/CD8+	UT mean	21	19	27
(Tc/s cells)	± S. D	14	12	17
	TE1 mean	20	20	20
	± S. D	8	11	11
	TE2 mean	27	21	23
	± S. D	14	9	6
		T0	T60	T180[c]
CD3-/CD16+/CD56+	UT mean	4	4	3
(NK cells)*	± S. D	4	3	4
	TE1 mean			
	± S. D	4	3	3
		3	3	3
	TE1 mean	3	4	3
	± S. D	4	4	2
		T0	T60	T180[d]
CD20+/CD19+	UT mean	9	8	10
(B cells)	± S. D	6	3	6
	TE1 mean	11	7	11
	± S. D	6	4	6
	TE2 mean	10	8	11
	± S. D	4	4	4

UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.
* = converted to logarithms before statistical analysis.

a= significantly different than UT; group main effect, $p < 0.05$
b= significantly different from T60 in groups TE1 vs. TE2; time main effect, $p < 0.05$
c= significantly different from T60 in UT vs TE2; time main effect, $P < 0.05$
d= significantly different from T60 in all groups; time main effect, $p < 0.05$

Table 3.6. Percentage of Gated CRTH2 Cells and Subsets in Response to 60 minutes of Endurance Exercise.

		T0	T60	T180 [a]
Total $CRTH_2$	UT mean	22	24	27
	± S. D	18	18	18
	TE1 mean	22	15 [b]	21
	± S. D	13	9	14
	TE2 mean	15	12	14
	± S. D	9	8	9
		T0	T60	T180
$CRTH_2$+CD4+	UT mean	9	10	10
(Th2 cells) *	± S. D	12	10	12
	TE1 mean	10	5	9
	± S. D	9	7	13
	TE2 mean	4	3	4
	± S. D	4	2	4
		T0	T60	T180
$CRTH_2$+/CD8+	UT mean	4	5	4
(Tc/s2 cells)	± S. D	4	5	4
	TE1 mean	5	4	3
	± S. D	4	4	2
	TE2 mean	3	3	2
	± S. D	2	4	2

UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.
* = converted to logarithms before statistical analysis.

a= significantly different from T60 in groups UT vs. TE1 and TE1 vs. TE2; time main effect, $p < 0.05$
b= significantly different from T0; interaction effect, $p < 0.05$

Table 3.7. Absolute Cell Numbers of Lymphocyte Subsets in Response to 60 minutes of Endurance Exercise.

		T0	T60 [ab]	T180[c]
Total CD3+	UT mean	1. 45	1. 73	1. 53
(T cells)	± S. D	0. 34	0. 46	0. 79
10^9 cells·L^{-1}	TE1 mean	1. 31	1. 49	1. 32
	± S. D	0. 47	0. 51	0. 54
	TE2 mean	1. 29	1. 59	1. 39
	± S. D	0. 29	0. 53	0. 39
		T0	T60	T180
CD3+/CD4+	UT mean	0. 44	0. 51	0. 42
(Th cells)	± S. D	0. 45	0. 49	0. 45
10^9 cells·L^{-1}	TE1 mean	0. 71	0. 74	0. 73
	± S. D	0. 32	0. 45	0. 46
	TE2 mean	0. 64	0. 71	0. 67
	± S. D	0. 32	0. 42	0. 38
		T0	T60	T180
CD3+/CD8+	UT mean	0. 38	0. 45	0. 53
(Tc/s cells)	± S. D	0. 26	0. 3	0. 41
10^9 cells·L^{-1}	TE1 mean	0. 34	0. 42	0. 37
	± S. D	0. 17	0. 29	0. 21
	TE2 mean	0. 47	0. 46	0. 40
	± S. D	0. 33	0. 29	0. 18
		T0	T60[a]	T180[d]
CD3-/CD16+/CD56+	UT mean	0. 06	0. 1	0. 06
(NK cells)*	± S. D	0. 07	0. 08	0. 08
10^9 cells·L^{-1}	[e]TE1 mean			
	± S. D	0. 05	0. 06	0. 03
		0. 04	0. 05	0. 02
	[e]TE2 mean	0. 06	0. 09	0. 05
	± S. D	0. 10	0. 10	0. 04
		T0	T60	T180
CD20+/CD19+	UT mean	0. 16	0. 19	0. 19
(B cells)	± S. D	0. 09	0. 09	0. 12
10^9 cells·L^{-1}	TE1 mean	0. 19	0. 16	0. 2
	± S. D	0. 12	0. 12	0. 14
	TE2 mean	0. 18	0. 19	0. 2
	± S. D	0. 08	0. 11	0. 09

UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.
* = converted to logarithms before statistical analysis.

a= significantly different from T0 with UT vs. TE2; main effect for time, $p < 0.05$
b= significantly different from T0 with TE1 vs. TE2; main effect for time, $p < 0.05$
c= significantly different from T60 with TE1 vs. TE2; main effect for time, $p < 0.05$
d= significantly different from T60 with UT vs. TE1 and UT vs. TE2; main effect for time, $p < 0.05$
e= significant main effect for time with TE1 vs. TE2, $p < 0.05$

Table 3.8. Absolute Cell Numbers of CRTH2 and Subsets in Response to 60 minutes of Endurance Exercise.

		T0	T60[d]	T180
Total $CRTH_2$	UT mean	0. 40	0. 57[a]	0. 52
10^9 cells·L^{-1}	± S. D	0. 29	0. 40	0. 29
	TE1 mean	0. 40	0. 31	0. 39
	± S. D	0. 25	0. 20	0. 23
	[e]TE2 mean	0. 25	0. 27	0. 24
	± S. D	0. 15	0. 2	0. 17
		T0	T60	T180
$CRTH_2$+/CD4+	[b]UT mean	0. 16	0. 23	0. 18
(Th2 cells)	± S. D	0. 2	0. 21	0. 19
10^9 cells·L^{-1}	[b]TE1 mean	0. 18	0. 10	0. 15
	± S. D	0. 14	0. 12	0. 21
	[e]TE2 mean	0. 07	0. 06	0. 07
	± S. D	0. 05	0. 05	0. 05
		T0	T60[d]	T180[c]
$CRTH_2$+/CD8+	UT mean	0. 07	0. 12	0. 07
(Tc/s2 cells)	± S. D	0. 08	0. 13	0. 07
10^9 cells·L^{-1}	TE1 mean	0. 08	0. 08	0. 04
	± S. D	0. 06	0. 07	0. 03
	TE2 mean	0. 04	0. 07	0. 04
	± S. D	0. 03	0. 1	0. 04

UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.

a= significantly different than T0; interaction effect with UT vs. TE1, $p < 0.05$
b= significant interaction effect with UT vs. TE1, $p < 0.05$
c= significantly different from T60 with UT vs. TE1; main effect for time, $p < 0.05$
d= significantly different from T0 with UT vs. TE2; main effect for time, $p < 0.05$
e= significantly different from UT; main effect for group, $p < 0.05$

Table 3.9. Percentage of Gated T Cell Subsets with and without CD25 Activation/IL-2 Receptor Marker in Response to 60 minutes of Endurance Exercise.

		T0	T60	T180[a]
CD25+/CD3+	[b]UT mean	4	9	7
	± S. D	4	5	6
	TE1 mean	7	6	9
	± S. D	5	4	5
	[b]TE2 mean	6	6	6
	± S. D	4	4	4
		T0	T60	T180[cd]
CD25+/CD4+	UT mean	9	7	10
	± S. D	5	5	7
	TE1 mean	7	7	10
	± S. D	4	5	6
	[e]TE2 mean	6	6	6
	± S. D	6	4	5
		T0	T60	T180
CD25+/CD8+	UT mean	1	1	1
	± S. D	2	1	1
	TE1 mean	1	2	1
	± S. D	1	3	1
	TE2 mean	1	1	1
	± S. D	1	1	1
		T0	T60	T180[f]
CRTH2+/CD25+	UT mean	4	3	4
	± S. D	3	3	3
	TE1 mean	4	3	4
	± S. D	3	2	4
	[g]TE2 mean	2	2	3
	± S. D	2	1	3

UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.

a= significantly different than T0 in groups UT vs. TE1; time main effect, $p < 0.05$
b= significant time main effect in groups UT vs. TE2 $p < 0.05$
c=significantly different than T0 and T60 in groups UT vs. TE1; time main effect UT vs. TE1, $p < 0.05$
d= significantly different than T60 in groups UT vs. TE2; time main effect, $p < 0.05$
e= significantly different than T0 in groups UT vs. TE1; time main effect, $p < 0.05$
f= significantly different than T60 in groups UT vs TE1 and UT vs. TE2; time main effect, $p < 0.05$
g= significantly different from TE1; group main effect, $p < 0.05$

Table 3.10. Changes in the proportion of Th2 cells to Total CD4+ cells in Response to 60 minutes of Endurance Exercise.

		T0	T60	T180
Proportion	[b]UT mean	16	21	19
(nmol·L^{-1})	± S. D	18	20	19
	TE1 mean	20	18	15[a]
	± S. D	16	25	18
	[b]TE2 mean	27	25	28
	± S. D	36	37	37

UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.

a= significantly different than T60; interaction effect with UT vs. TE1, $p < 0.05$.
b= significant interaction effect found between UT and TE2 groups, $p < 0.5$.

Table 3.11. Changes with Phytohaemagglutinin (PHA) Stimulated Cytokine Concentrations in Response to 60 minutes of Endurance Exercise.

		T0	T60	T180[b]
IFN-γ	UT mean	1. 8	2. 6	1. 7
$ng \cdot ml^{-1}$	± S. D	2. 6	3. 0	2. 2
	TE1 mean	1. 8	1. 9	3. 1
	± S. D	2. 0	1. 9	3. 2
	TE2 mean	1. 2	1. 8	1. 5
	± S. D	2. 0	2. 6	2. 4
		T0	T60	T180
IL-10	UT mean	0. 8	0. 8	0. 7
$ng \cdot ml^{-1}$	± S. D	0. 7	0. 6	0. 7
	TE1 mean	0. 9	0. 3	0. 9
	± S. D	0. 6	0. 3	0. 5
	[a]TE2 mean	0. 9	1. 0	1.0
	± S. D	0. 5	0. 5	0. 6
		T0	T60	T180
IL-6	UT mean	3. 7	3. 4	4. 1
$ng \cdot ml^{-1}$	± S. D	3. 3	2. 4	3. 5
	TE1 mean	3. 9	4. 1	4. 0
	± S. D	3. 2	2. 7	3. 5
	TE2 mean	2. 8	3. 9	3. 7
	± S. D	2. 4	2. 7	2. 0
		T0	T60	T180
IL-2	[c]UT mean	249. 5	71. 9	24. 8
$pg \cdot ml^{-1}$ *	± S. D	443. 0	123. 5	31. 5
	TE1 mean	115. 4	57. 2	252. 4
	± S. D	231. 6	50. 1	297. 8
	[c]TE2 mean	69. 1	149. 5	91. 9
	± S. D	114. 6	203. 1	190. 6

UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.
IFN= interferon.
IL= interleukin
* = converted to logarithms before statistical analysis.

a= significant difference from TE1; group main effect, $p < 0.05$
b= significantly different from T0 with TE1 vs. TE2; time main effect, $p < 0.05$
c= significant interaction effect found between UT and TE2 groups, $p < 0.05$

Table 3.12. Changes in IL-12 Cytokine Concentrations in Plasma (pg·ml-1 converted to logarithms) in Response to 60 minutes of Endurance Exercise.

		T0	T60	T180[a]
IL-12	UT mean	34. 1	31. 9	33. 4
$pg \cdot ml^{-1}$ *	± S. D	66. 7	60. 0	56. 8
	TE1 mean	17. 9	18. 2	34. 6
	± S. D	15. 7	15. 3	33. 2
	TE2 mean	20. 5	23. 0	35. 9[b]
	± S. D	18. 5	20. 9	24. 8

UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.
IL= interleukin.
* = converted to logarithms before statistical analysis.

a= significant difference from T0 and T60 with groups UT vs. TE2 and with TE2 vs. TE2; time main effect, $p < 0.05$
b= significant difference from T0 and T60 with TE2; interaction effect with comparison between UT vs. TE2, $p < 0.05$

Table 3.13. Changes with Unstimulated IL-6 Cytokine Concentrations in Response to 60 minutes of Endurance Exercise (n=11).

		T0	T60	T180
IL-6	mean	994. 2	1407.1	826. 5
$ng \cdot ml^{-1}$	± S. D	779. 7	931. 7	802. 7

T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.
IL= interleukin.

a= significantly different from T60, $p < 0.05$

Table 3.14. Changes in Plasma Cortisol Concentrations in Response to 60 minutes of Endurance Exercise.

		T0	T60	T180[a]
Cortisol	UT mean	718. 3	773. 5	630. 6
($nmol \cdot L^{-1}$)	± S. D	220. 9	373. 7	293. 9
	[b]TE1 mean	718. 6	723. 6[d]	635. 9
	± S. D	131. 5	113. 3	141. 0
	TE2 mean	728. 6[c]	881. 6	720. 1[c]
	± S. D	92. 08	165. 3	199. 5

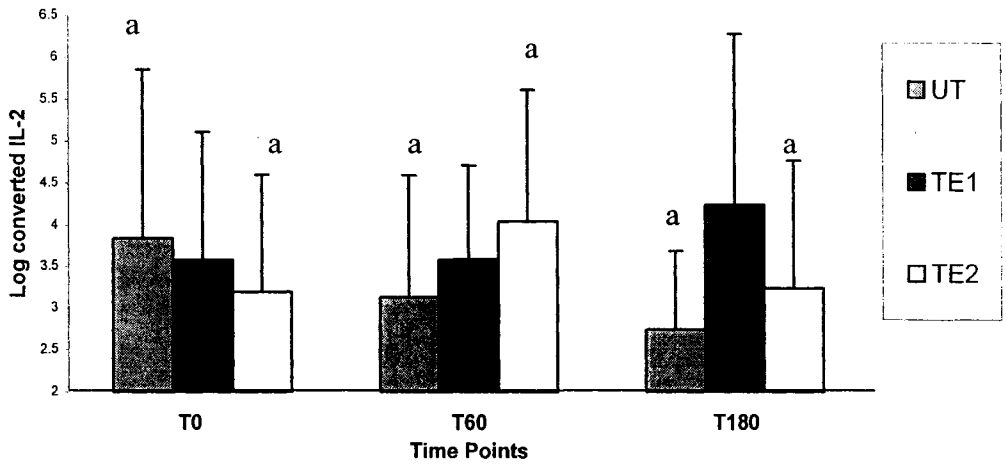
UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.

a= significant difference from T60, with UT vs. TE2; time main effect, $p < 0.05$
b=significant difference from TE2; group main effect, $p < 0.05$
c= significant difference from T60; interaction effect with TE1 vs. TE2 comparison, $p < 0.05$
d=significant difference from TE2 at this specific time point; interaction effect with TE1 vs. TE2 comparison, $p < 0.05$

Table 3.15. Changes with CD4:CD8 Ratio in Response to 60 minutes of Endurance Exercise.

		T0	T60	T180
CD4:CD8	UT mean	1. 97	1. 45	2. 25
	± S. D	1. 82	1. 18	3. 88
	TE1 mean	2. 09	1. 87	2. 05
	± S. D	0. 86	0. 70	1. 09
	TE2 mean	1. 62	1. 53	2. 01
	± S. D	0. 96	0. 98	1. 28

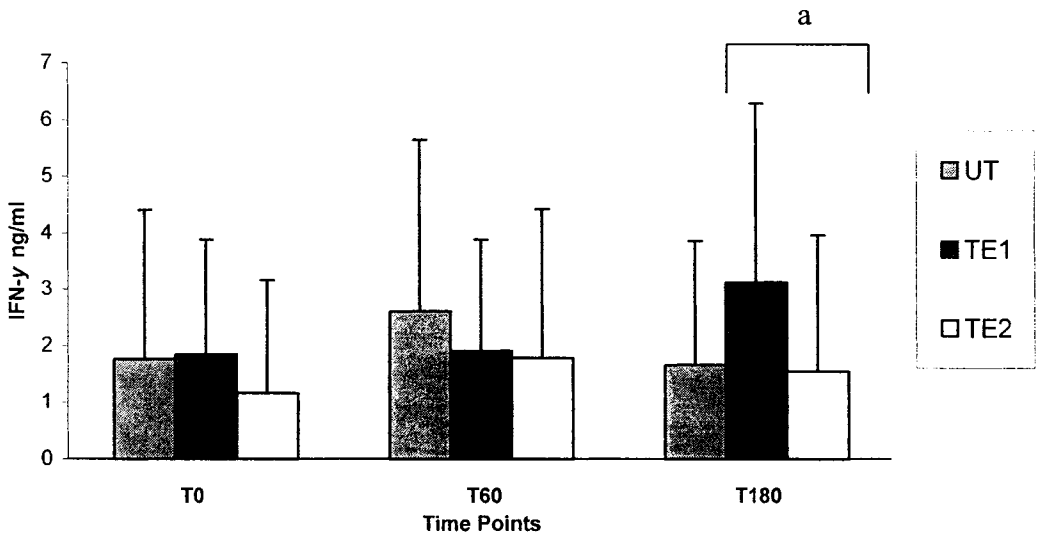
UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.



UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.

a= significant interaction effect found between UT and TE2 groups, $p < 0.05$

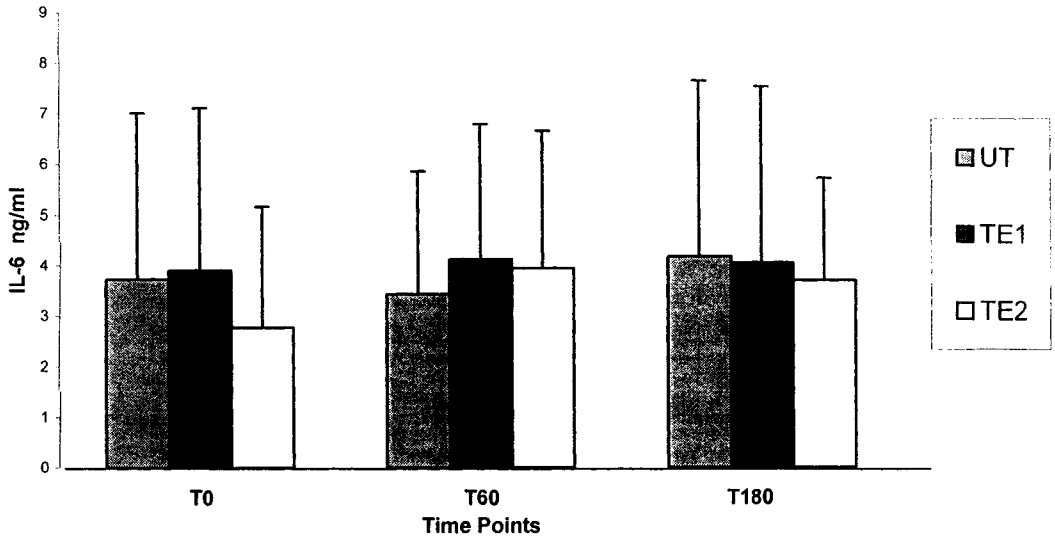
Figure 3.1. Changes with PHA stimulated Interleukin-2 production in response to 60 minutes of endurance exercise.



UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.

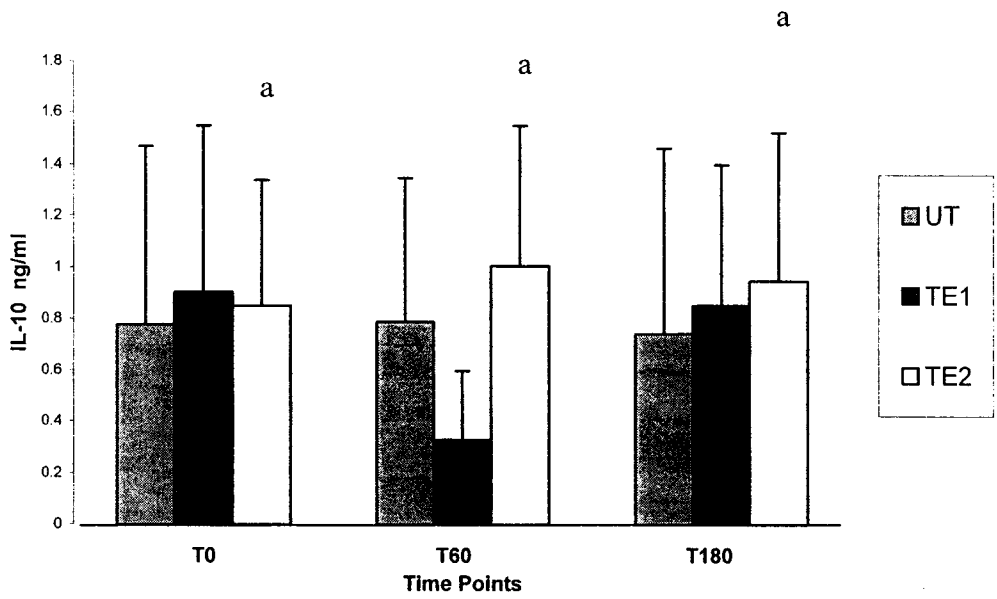
a= T180 significantly different from T0 with TE1 vs. TE2; time main effect, $p < 0.05$

Figure 3.2. Changes with PHA stimulated Interferon-gamma production in response to 60 minutes of endurance exercise.



UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.

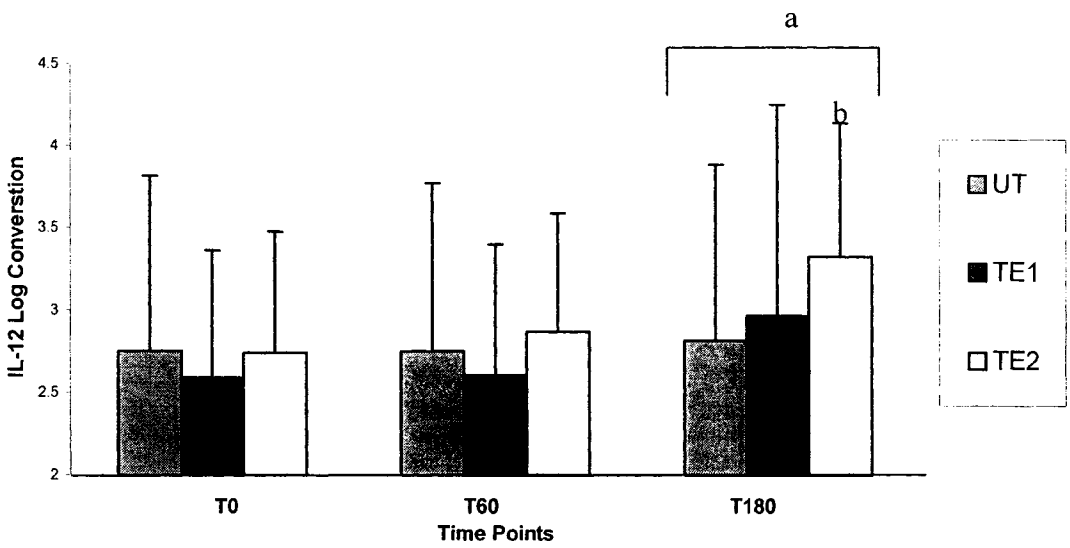
Figure 3.3. Changes with PHA stimulated Interleukin-6 production in response to 60 minutes of endurance exercise.



UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.

a= TE2 significant difference from TE1; group main effect, $p < 0.05$.

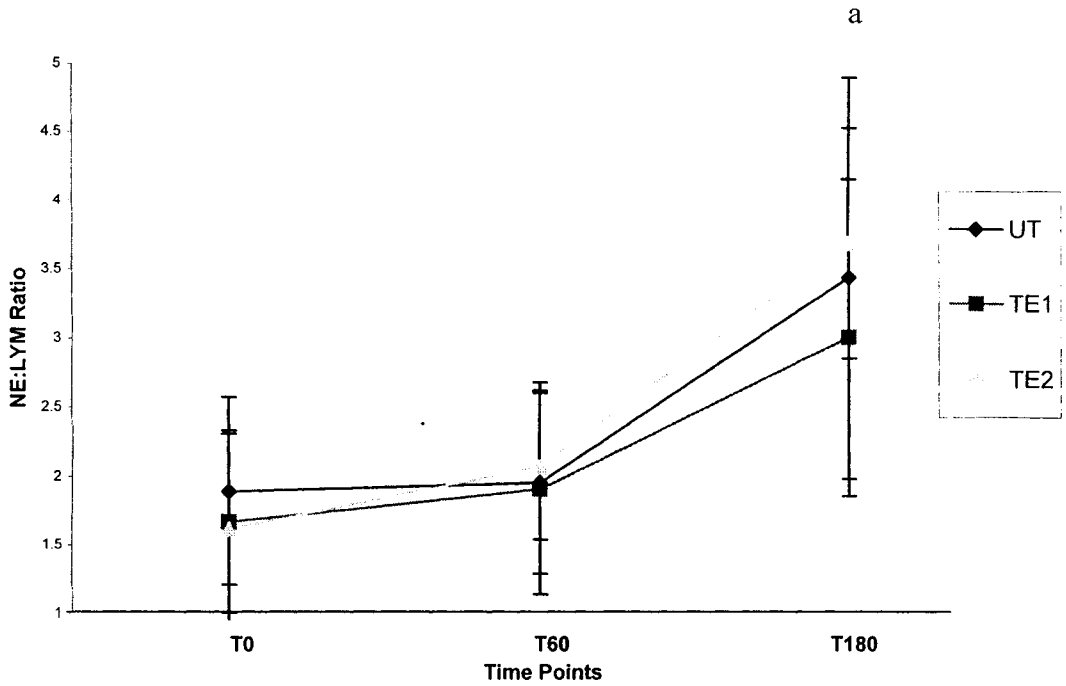
Figure 3.4. Changes with PHA stimulated Interleukin-10 production in response to 60 minutes of endurance exercise.



UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.

a= T180 significant difference from T0 and T60 with groups UT vs. TE2 and with TE2 vs. TE2; time main effect, $p < 0.05$.
b= T180 significant difference from T0 and T60 with TE2; interaction effect with comparison between UT vs. TE2, $p < 0.05$.

Figure 3.5. Changes with plasma Interleukin-12 in response to 60 minutes of endurance exercise.



UT= untrained group exercise condition.
TE1= trained group exercise condition matched in intensity to UT.
TE2= trained group exercise condition at higher relative intensity.
T0= resting sample before exercise.
T60= sample immediately post the 60-minutes of exercise.
T180= sample two hours post exercise.

a= T180 significant difference from T0 and T60 with all three exercise conditions; time main effect, $p < 0.05$.

Figure 3.6. Changes with the neutrophil:lymphocyte ratio in response to 60 minutes of endurance exercise.

Chapter 4

General Discussion and Conclusion

4.1 Discussion

The immune system is a highly complex, multi-layered and multi-integrated system. It is comprised of many different cells and molecules that work together to protect the body. The immune system can be self-regulated though cytokines, chemokines and other proteins released from cells and tissue. It is also cross-regulated with the endocrine and nervous systems. Therefore, studying the immune system can be an overwhelming task due to the different parameters and complex mechanisms involved. Since acute exercise places a physiological demand on the body, it is not surprising that exercise alters the parameters and mechanisms of the immune system. It has been consistently reported in the exercise immunology literature that acute endurance exercise alters the distribution and function of circulating immune cells (Mackinnon, 1999; Nieman et al., 1995, 1997). This alteration is dependent on the intensity and duration of the exercise bout.

Recent research has reported that there are cellular subsets of Helper T cells, referred to as Th0, Th1, Th2 and Th3 (De Carli et al., 1994; Doug & Flavell, 2001; Romagnani, 1995; Yates et al., 2000). These cells regulate different aspects of the immune system and can be identified by the cytokines they secrete. Th1 cells normally secret interleukin (IL)-2, interferon (IFN)-γ, tumor necrosis factor (TNF)-α and TNF-β. These cytokines stimulate the cellular-mediated component of the adaptive immune system and are also known as pro-inflammatory cytokines (Doug and Flavell, 2001;

Morel and Oriss, 1998). Th2 cytokines are considered to be anti-inflammatory and control the humoral-mediated component of the immune system. Th2 secretes IL-4, IL-5, IL-6, IL-10 and IL-13 (Doug and Flavell, 2001; Romagnami, 1995). Th0 is considered a precursor to these two cells, and is found to secrete many of the Th1 and Th2 cytokines. Th3 is the only cell that secretes transforming growth factor (TGF)-β whose role has yet to be clearly defined (Cerwenka, 1999). Through the production of their specific cytokines, Th1 and Th2 control and regulate each other creating a homeostasis in the adaptive immune system (Morel & Oriss, 1998). The physiological stress of exercise can alter the function and distribution of cells within the immune system, including Th1 and Th2 cells. Previous research has found that Th1 cytokines decrease following strenuous endurance exercise, while Th2 cytokines either remain unchanged or become elevated (Ibfelt et al., 2002; Smith, 2003; Steensberg et al, 2001). It was, therefore, hypothesized that acute endurance exercise may lead to a temporary suppression of Th1 immunity while Th2 immunity is favoured.

The primary purpose of this study examined the Th1 and Th2 immune response to acute endurance exercise in trained and untrained females. To fully examine this immune response in greater depth, three exercise conditions were compared. The first exercise condition was completed by the untrained women who cycled for an hour at 20 watts below their VT (mean PO = 90W). The second exercise condition was completed by the trained women, who cycled for an hour at 90 watts, which was the average PO of the untrained women. For the third exercise condition, the trained women also cycled at 20 watts below their own VT (mean PO = 155W). Blood samples were taken at rest (T0),

immediately post the 60 minutes of exercise (T60) and following two hours of recovery (T180) for each of the exercise conditions.

The intriguing finding with this study was the different Th1 and Th2 immune responses observed between trained and untrained women after exercise. Th1 immunity was favoured while Th2 immunity appeared to be temporarily suppressed following the endurance exercise in trained women. This conclusion was based on the decrease concentration of Th2 cells and the increase of IFN-γ and IL-12 concentrations following both exercise conditions. There was also an increase in IL-2 production following the trained women's higher intensity exercise condition, as well as a decrease in IL-10 after mitogen stimulation in cells of these participants with the lower intensity exercise condition. This change with Th2 cells and cytokine production was not observed in the untrained women. The concentration of Th2 cells, IL-10 and IFN-γ production after stimulation were unaffected by the endurance exercise in untrained women. There was a decrease with IL-2 production post exercise in the untrained women, suggesting that Th1 immunity was temporarily suppressed. Therefore, it can be suggested that Th2 immunity was enhanced in the untrained women following endurance exercise.

Following exercise there is an immediate and general inflammatory response. The extent of this response is dependent on the intensity, duration and extent of musculoskeletal damage caused by the exercise (Mackinnon, 1999; Pedersen and Hoffman-Goetz, 2000). Inflammation is a part of the healing process, though it can lead to extensive damage if not controlled. In order for inflammation to be regulated it needs to be suppressed. This in turn can lead to suppression of the cellular-mediated or the Th1 component of the immune system (Brenner et al., 1994; Northoff et al., 1998; Pedersen

and Hoffman-Goetz, 2000; Smith, 2003). Once the inflammation has subsided, the immune system will counter-regulate itself, retuning to homeostasis. Therefore, the temporary suppression of the cellular-mediated or Th1 immunity can be viewed as a protective mechanism (Northoff et al., 1998; Morel and Oriss et al., 1998; Pedersen and Hoffman-Goetz, 2000). The concern for athletes is how long the cellular-mediated component remains suppressed, as this can "open a window" for the risk of infection (Nieman et al., 1997; Nieman, 1998). It has been suggest that IFN-γ may play a central role in endotoxin tolerance, which is the mechanism that counter-regulates immunosuppression (Northoff et al., 1998). An increase with IFN-γ production at T180 was observed in both the trained women's exercise conditions, providing evidence that immunosuppression was counter regulated in this cohort. It seems likely that endurance training attenuates the inflammatory response and initiates the endotoxin tolerance more quickly.

According to these findings the immune system responds differently in trained and untrained women, regardless of the intensity of the endurance exercise. These findings indicate that there has been an adaptation as a result of endurance training that influences the Th1 and Th2 immune response to the physiological stress of exercise. With repeated exposure to physiological stress and inflammation caused by endurance exercise, the immune system can "learn" and adapt to minimize the effect on the body (Northoff et al., 1998; Pedersen and Hoffman-Goetz, 2000). This adaptation to exercise is not entirely surprising, as the immune system has evolved to constantly adapt and change to combat pathogens and other challenges the host faces.

The results of this investigation do not completely support the original hypothesis and in some regards are inconsistent with previous research. It is likely that the intensity chosen for the exercise conditions played a central role in the discrepancy in these findings. The majority of previous research has examined exercise that is of higher intensity (>65% of $V0_{2max}$) and longer duration (> 2 hours) (Baum et al., 1997; Henson et al., 2000; Mitchell et al., 2002; Moyna et al., 1996; Pedersen and Hoffman-Goetz, 2000; Suzuki et al., 1999). All 3 exercise conditions in this study were 60 minutes and would be considered to be of moderate intensity (calculated to be 55% of $V0_{2max}$ for the untrained's, 44% of $V0_{2max}$ for the lower trained's first and 65% of $V0_{2max}$ for the trained's second exercise condition) (Nehlsen-Cannarella, 1998; Nieman et al., 1994). Additionally, cortisol levels were only significantly elevated in the trained women at the higher intensity exercise condition. Since cortisol was involved with anti-inflammatory regulation, the lack if change indicates that there was not an extensive inflammatory response post exercise (Chryssikopoulos, 1997; Elenkov & Chrousos, 1999; Steensberg et al, 2003). The undetectable concentration of plasma IL-6 also indicates that inflammation due to musculoskeletal damage was not extensive in these exercise condition (Pedersen and Hoffman-Goetz, 2000; Pedersen et al., 2003). Changes in the immune system with this study were slightly different to previous research (due to the difference in intensity). Since most individuals who exercise regularly for health and recreational purposes do so at a low to moderate intensity and similar duration, more research examining this level of exercise may be beneficial and contribute to the exercise immunology literature.

Another possible contributing factor to the some of the differences of this study to other research may be due to gender differences in immune system responses. The majority of literature has been based on the exercise immune response of males. Since this study examined females, it is difficult to compare these findings directly with the literature. As well, there may be gender differences with the immune response to endurance exercise. Females tend to be hypercortisolic compared to males (Bell et al., 1997; Horne et al., 1997; Tsai et al., 1991), which can influence the sensitivity to of immune cells to glucocorticords (Dohi et al, 2003; Duclos et al., 2003; Kam et al., 1993; Rohleder et al., 2001). Receptors for estradiol and prolactin have been found on lymphocytes, indicating that reproductive hormones may also regulate aspects of the immune system (Goldsby et al., 2000; Maurer, 1982; Vera-Lastra et al., 2002). Both of these hormones are in higher concentration in women.

Hormonal changes during the menstrual cycle can also influence certain parameters of the immune response to exercise (Chryssikopoulos, 1997; Northern et al., 1994; Pehlivanoglu et al., 2001). This present study controlled for these possible changes by testing the participants during the first 7 days of their pharmaceutical contraceptive cycle, which is similar to the follicular phase. During this particular phase reproductive hormones are at lower concentrations, thus limiting the effect they may have on the immune system (Northern, et al., 1994). Only a limited number of studies have executed this control of the menstrual cycle. Future studies are encouraged to control for the menstrual cycle, and thereby decreasing the variability in the immune response.

Females also tend to initiate a cellular-mediated immune response against pathogens, while males tend to initiate a humoral response (Goldsby et al., 2000) and

these responses may be reflected in the immune system changes following endurance exercise. Finally, women are 2 to 3 times more susceptible than men to autoimmune diseases (Bijlsma, et al., 2002; Whitcare, 2001). The role that these factors play in the immune response to exercise has not been established and further investigation with gender differences is needed.

4.2 Conclusion

It can be concluded from this present study that Th1 and Th2 immunity in women was altered following acute endurance exercise at a moderate intensity. The findings in this study also demonstrated a dominant Th1 response in trained women and dominant Th2 response in untrained women following 60 minutes of cycling. Thus, an individual's fitness level and likely a result of adaptation to endurance training influenced the immune response to the acute stress of endurance exercise.

Acute endurance exercise has been used as a model to examine the effects of physiological stress on the body. Following events such as surgery, trauma, septic shock and psychological stress there are similar changes with immune cell numbers, function and cytokine and hormonal production as seen following strenuous endurance exercise (Berguer et al., 1999; Kang and Fox, 2001; Paik et al., 2000; Pehlivanoglu et al., 2001; Shephard, 2001). The findings in this study may indicate that the immune system in trained women can tolerate the acute stress of endurance exercise better than those who are untrained. Therefore, it is likely that the immune system in trained women can also tolerate other acute stressful challenges. However, at this time it is difficult to claim that these adaptations with the Th1 and Th2 response based on the observations in trained women of this study influence immunocompetence and resistance to chronic disease.

Further investigation of Th1 and Th2 response to a variety of stress in consideration of gender, the menstrual cycle and with different intensities and durations before claims can be made that trained women are "healthier" than those who are untrained in terms of their immune response to stress.

4.3 References

Baum, M., Muller-Steinhardt, M., Liesen, H., & Kirchner, H. (1997). Moderate and exhaustive endurance exercise influences the interferon-gamma levels I whole blood culture supernatants. *European Journal of Applied Physiology, 76,* 165-169.

Bell, G., Syrotuik, D., Socha, T., Maclean, I., & Quinney, H. A. (1997). Effect of strength training and concurrent strength and endurance training on strength and testosterone, and cortisol. *Journal of Strength and Conditioning Research, 11*(1), 57-64.

Berguer, R., Bravo, N., Bowyer, M., Egan, C., Knolmayer, T., & Ferrick, D. (1999). Major surgery suppresses maximal production of helper T-cell type 1 cytokines without potentiating the release of helper T cell type 2 cytokines. *Archives of Surgery, 134,* 540-544.

Bijlsma, J.W.J., Straub, R.H., Masi, A.T., Lahita, R.G., Cutolo, M. (2002). Neuroendocrine immune mechanisms in rheumatic diseases. *Trends in Immunology, 23,* 59-61.

Brenner, I. K. M., Natale, V. M., Vasiliou, P., Moldoveanu, A. I., Shek, P. N., & Shepard, R. J. (1999). Impact of three different types of exercise on components of the inflammatory response. *European Journal of Applied Physiology, 80,* 452-460.

Chryssikopoulos, A. (1997). The relationship between the immune and endocrine systems. *Annals New York Academy of Sciences, 816,* 83-93.

Cerwenka, A., & Swain, S. L. (1999). TGF-beta1: Immunosuppressant and viability factor for T lymphocytes. *Microbes and Infection, 1*(15), 1291-1296.

De Carli, M., D'Elios, M. M., Zancuoghi, G., Romagnani, S., & Del Prete, G. (1994). Human Th1 and Th2 cells: Functional properties, regulation of development and role in autoimmunity. *Autoimmunity, 18*(4), 301-308.

Dohi, K., Kraemer, W. J., & Mastro, A. M. (2003). Exercise increases prolactin-receptor expression on human lymphocytes. *Journal of Applied Physiology, 94,* 518-524.

Doug, C., & Flavell, R. A. (2001). Th1 and Th2 cells. *Current Opinion In Hematology, 8,* 47-51.

Duclos, M., Gouarne, C., & Bonnemaison, D. (2003). Acute and chronic effect of exercise on tissue sensitivity to glucocorticords. *Journal of Applied Physiology, 94,* 869-875.

Elenkov, I. J., & Chrousos, G. P. (1999). Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. *Trends in Endocrinology and Metabolism, 10*(9), 359-368.

Goldsby, R. A., Kindt, T. J., & Osborne, B. A. (2000). *Kuby immunology* (4th ed.). New York: W. H. Freeman.

Henson, D., Nieman, D., Nehlsen-Cannarella, S., Fagoaga, O., Shannon, M., Bolton, M., Davis, M., Gaffney, C., Kelln, W., Austin, M., Hjertman, J., & Schilling, B. (2000). Influence of carbohydrate on cytokine and phagocytic response to 2 h of rowing. *Medicine and Science in Sports and Exercise, 32*(8), 1384-1389.

Ibfelt, T., Petersen, E. W., Bruunsgaard, H., Sandman, M., & Pedersen, B. K. (2002). Exercise-induced change in type 1 cytokine-producing CD8+ T cells is related to a decrease in memory T cells. *Journal of Applied Physiology, 93,* 645-648.

Horne, L., Bell. G., Fisher, B., Warren, S., & Janowaks-Wiezoek, J. (1997). Interaction between cortisol and tumor necrosis factor with concurrent resistance and endurance training. *Clinical Journal of Sports Medicine, 7*(4), 247-251.

Kam, J. C., Szefler, S. J., Surs, W., Sher, E. R., & Leung, D. Y. M. (1993). Combination IL-2 and Il-4 reduces glucocorticoid receptor-binding affinity and T cell response to glucocoticords. *Journal of Immmunology, 151*(7), 3460-3466.

Kang, D., & Fox, C. (2001). Th1 and Th2 cytokine responses to academic stress. *Research in Nursing and Health, 24,* 245-257.

Mackinnon, L. T. (1999). *Advances in exercise immunology.* Champaign, IL: Human Kinetics.

Mitchell, J. B., Dugas, J. P., McFarlin, B. K., & Nelson, M. J. (2002). Effect of exercise, heat stress, and hydration on immune cell number and function. *Medicine and Science in Sports and Exercise, 34*(12), 1941-1950.

Morel, P. A., & Oriss, T. B. (1998). Crossregulation between Th1 and Th2 cells. *Critical Reviews in Immunology, 18,* 275-303.

Moyna, N. M., Acker, G. R., Fulton, J. R., Weber, K., Goss, F. L., Robertson, R. J., Tollerud, D. J., & Rabin, B. S. (1996). Lymphocyte function and cytokine production during incremental exercise in active and sedentary males and females. *International Journal of Sports Medicine, 17*(8), 585-591.

Mitchell, J. B., Dugas, J. P., McFarlin, B. K., & Nelson, M. J. (2002). Effect of exercise, heat stress, and hydration on immune cell number and function. *Medicine and Science in Sports and Exercise, 34*(12), 1941-1950.

Nieman, D.C. (1998). Exercise and resistance to infection. *Canadian Journal of Physiology and Pharmacology, 76,* 573-580.

Nieman, D. C., Henson, D. Garner, E., Butterworth, D., Warren, B., Utter, A., Davis, J., Fagoaga, O., & Nehlsen-Cannarella, S. L. (1997). Carbohydrate affects natural killer cell redistribution but not activity after running. *Medicine and Science in Sports and Exercise, 29*(10), 1318-1324.

Nieman, D. C., Simandle, S., Henson, D. A., Warren, B. J., Suttles, J., Davis, J. M., Buckley, K. S., Ahle, J. S., Bitterworth, D. E., Fagoaga, O. R., & Nehlsen-Cannarella, S. L. (1995). Lymphocyte proliferation response to 2.5 hours of running. *International Journal of Sports Medicine, 16*(6), 404-408.

Northern, A. L. D., Rutter, S. M., & Petersen, C. M. (1994). Cyclic changes in the concentrations of peripheral blood immune cells during the normal menstrual cycle. *Proceedings of the Society for Experimental Biology and Medicine, 207,* 81-88.

Northoff, H., Berg, A., & Weinstock, C. (1998). Similarities and differences of the immune response to exercise and trauma: The IFN-γ concept. *Canadian Journal of Physiology and Pharmacology, 76,* 497-504.

Paik, I., Toh, K., Lee, C., Jung-jin, K., & Lee, S. (2000). Psychological stress may induce increased humoral and decreased cellular immunity. *Behavioral Medicine, 26*(3), 139-142.

Pedersen, B. K., & Hoffman-Goetz, L. (2000). Exercise and the immune system: Regulation, integration and adaptation. *Physiological Reviews, 80*(3),1055-1081.

Pedersen, B. K., Steensberg, A., Fischer, C., Keller, C., Keller, P., Plomgaard, P., Febbraio, M., & Saltin, B. (2003). Searching for the exercise factor: Is IL-6 a candidate? *Journal of Muscle Research and Cell Motility, 24,*113-119.

Pehlivanoglu, B., Balkaner, Z. D., Ridvanagaoglu, A. Y., Durmazlar, N. Ozturk, G., Erbas, D., & Okur, H. (2001). Impact of stress, gender and menstrual cycle on

immune system: Possible role nitric oxide. *Archives of Physiology and Biochemistry, 109*(4), 383-387.

Rohleder, N., schommer, N. C., Hellhammer, D. H., Engel, R., & Kirschbaum, C. (2001). Sex differences in glucocorticords sensitivity of proinflammatory cytokine production after psychological stress. *Psychosomatic Medicine, 63,* 966-972.

Romagnani, S. (1995). Biology of human Th1 and Th2 cells. *Journal of Clinical Immunology, 15(*3), 121-129.

Shephard, R. J. (2001). Exercise in the heat: A double threat to the immune system? *The Physician and Sportsmedicine, 29(*6), 1-11.

Smith, L. L. (2003). Overtraining, excessive exercise and alerted immunity: Is this a T helper-1 versus T helper-2 lymphocyte response? *Sports Medicine, 33*(5), 347-364.

Steendberg, A., Fischer, C. P., Keller, C., Moller, K., & Pedersen, B. K. (2003). IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *American Journal of Physiology-Endocrinology and Metabolism, 285,* E433-E437.

Steendberg, A., Toft, A. D., Bruunsgaard, H., Sandmand, M., Halkjaer-Kristensen, J., & Pedersen, B. K. (2001). Strenuous exercise decreases the percentage of type 1 T cells in the circulation. *Journal of Applied Physiology, 91,* 1708-1712.

Suzuki, K., Totsuka, M., Nakaji, S., Yamada, M., Kudoh, S., Liu, Q., Sugawara, K., Yamaya, K., & Sato, K. (1999). Endurance exercise causes interaction among stress hormones, cytokines neutrophils dynamics and muscle damage. Journal of *Applied Physiology, 87(*4), 1360-1367.

Tsai, L., Johansson, C., Pousette, A., Tegelman, R., Carlstrom, K., & Hemmingsson, P. (1991). Cortisol and androgen concentrations in female and male elite endurance athletes in relation to physical activity. *European Journal of Applied Physiology, 63,* 308-311.

Whitacre, C. C. (2001) Sex differences in autoimmune disease. *Nature Immunology, 2*(9), 777-780.

Vera-Lastra, O., Jara, L. J., & Espinoza, L. R. (2002). Prolactin and autoimmunity. *Autoimmunity Reviews, 1,* 360-364.

Yates, A., Bergmann, C., Van Hemmen, J. L., Stark, J., & Callard, R. (2000). Cytokine-modulated regulation of helper-T cell populations. *Journal of Theoretical Biology, 206,* 539-560.

Measurement of Aerobic Fitness

Aerobic fitness will be assessed with a combined ventilatory threshold (VT)/maximal oxygen consumption (VO_2max) test. This is measured during a progressive, incremental exercise test to exhaustion (until the person says that you cannot continue). Exercise intensity is quite light at the beginning of the test and becomes more difficult every minute or two. The actual test usually lasts for about 12 to 15 minutes, with an additional 5 to 10 minutes of warm-up and cool-down exercise before and after. During the test, expired gases are collected using a special breathing apparatus. Heart rate is monitored continuously with a heart rate monitor. You will do this test on a Monark ergocycle ergometer (stationary bicycle). This is a standard exercise test protocol for assessing aerobic fitness levels.

Body Composition Measurement

Body composition will include measuring your height, weight, and skin-fold thicknesses. The skin-fold measurements involve taking pinches of skin and the underlying layer of fat with a device (caliper) at various sites on your body and adding these together to calculate a sum. These sites are on the back of the upper arm (triceps), underneath the shoulder blade (subscapular), at the side of the waist (iliac crest), beside the belly button (abdominal), the front of the upper leg (front thigh) and the rear of the upper leg (rear thigh).

60 Minute Exercise Challenge

The exercise bout is 60 minutes long and is required to be completed during days 1-7 of the oral contraceptive cycle. We will determine this timing with you. The test will be completed on a stationary cycle (Monark Ergocycle) and involves cycling continuously for a 60-minute time period, with an additional 5 minute warm-up and cool-down. The intensity is classified as moderate that will be equivalent to an effort just lower than what is called the "ventilatory threshold," a level at which everyone should be able to exercise for about 60 minutes. This is based on your own individual aerobic fitness test, described above, so that we are confident that you will be able to finish the total 60 minutes. Your heart rate will be monitored continuously using a heart rate monitor.

We will also collect blood samples from you in order to examine changes in immune system. We will require three samples, one to be taken before the exercise (at rest), one immediately after exercise, and one after two hours of recovery. An individual trained in taking blood will take the blood samples from an arm vein under sterile conditions. The amount of blood taken for each individual sample will be 20 ml (10ml is about the size of a teaspoon), and this amount poses no problem to your health.

As mentioned before, if you are in the trained group, you will be asked to perform two 60-minute endurance exercise bouts on two different days. We will provide you with adequate recovery time between each of these exercise bouts. The only difference between the two 60 minute bouts will be the intensity or effort of exercise. You will be required to perform one of the 60-minute bouts at the same mean intensity that the normally trained group completed. This should represent a "lower-moderate" intensity for an endurance-trained individual. The other 60-minute bout will be performed at a

moderate intensity (equivalent to the intensity just below your individual ventilatory threshold).

We also need to make sure all our subjects have had a similar diet on the day of the 60-minute exercise bout. To do this, each participant will be asked to arrive early in the morning (7 a.m.) after an overnight fast (10 hours) of no food or caffeine, but water is acceptable. You will be provided with a standard breakfast that consists of a flavorful nutrition bar and water that is to be consumed before the exercise test. The 60-minute exercise bout will begin 2 hours after breakfast to allow for digestion. During the exercise challenge you will be asked to drink 125ml of water every fifteen minutes. As well, during recovery you will be asked to consume a certain amount of water to allow yourself to be re-hydrated. We will determine this amount by how much fluid you lost (through sweat and breathing) during the 60 minutes of exercise.

Benefits:

There are no direct benefits; however you will receive a written report that includes your own physical fitness and the "health" of your immune system. We will also provide you with a consultation concerning these matters if you wish.

Risks:

The 60 minute exercise bout and the aerobic fitness test (VT/V02max) require maximal effort in order to go to exhaustion and/or to perform to each person's fullest capabilities. With this type of exercise there may be some health risk. During and after the tests, it is possible to experience symptoms such as abnormal blood pressure, fainting, lightheadedness, muscle cramps or strain, nausea, and in very rare cases (0.5 per 10,000 in testing facilities such as exercise laboratories, hospitals and physicians' offices), heart rhythm disturbances or heart attack. While serious risk to healthy participants is highly unlikely, it must be acknowledged, and participants willingly assume the risks associated with very hard exercise.

The exercise test will be administered by qualified personnel under the supervision of Dr. Gordon Bell. Personnel are trained to handle identifiable risks and emergencies, and have certification in CPR. Certifications can be produced if requested.

The laboratory staff will stop the exercise protocols if they are concerned about your safety. You can also stop any of the research procedures at any time. Please inform the researchers of any symptoms such as pain, illness, or unusual fatigue experienced during or after the exercise challenges.

There is some risk of bruising and/or infection at the blood collection site where the skin is punctured. These risks are minimized by having the blood collected by trained personnel who follow rigorous laboratory procedures for sterility and puncture site care.

Though it is not a physical risk, we understand that discussing your menstrual health/cycle may be an uncomfortable topic for you. Please be aware that we will be asking questions concerning menstrual health/cycle and type of "pill" you use. All information you provide will be held in the strictest of confidence.

Time Commitment: *How long will it take?*

<u>V02max/VT and body composition testing:</u> approximately 60 minutes.

Appendix B

Health Questionnaire

Please answer the following questions as accurately as possible.

Name: _____ Date of Birth: _____

Phone: _____ Email: _____

1. List any prescribed medication or over-the-counter medication/herbs/vitamins that you regularly take:

   _____

   _____

   _____

2. Please list any medical conditions/ chronic diseases that you may have:

   _____

   _____

   _____

3. Do you have any of the following conditions?

   a. Diabetes Yes_____ No_____

   b. Asthma Yes_____ No_____

   c. Severe Allergies Yes_____ No_____

   d. Multiple sclerosis Yes_____ No_____

   e. Rheumatoid arthritis Yes_____ No_____

   f. Lupus Yes_____ No_____

   g. Cancer Yes_____ No_____

   h. Thyroid disease Yes_____ No_____

4. At what age did you have your first menstrual period? _____ (years)
5. Are you taking oral contraceptives (the "pill")? Yes _____No _____

   "YES" - answer the questions below:

   i. Please provide the name of the brand you use:

   _____

   ii. Is it a 21 or 28 day cycle? _____

   iii. How long have you been taking them? _____

   iv. Do you have regular menstruation (i.e., do you have a period each oral contraceptive cycle)? _____

   v. When do you start your next cycle of oral contraceptives (i.e., new package)? _____

6. Do you smoke? Yes_____ No_____
7. Are you involved in a regular routine of physical activity? Yes_____ No_____

   "YES" - please answer questions below:

   i. How many days a week do you participate in your routine?

   _____

   ii. What is the range of minutes per session?

   _____

   iii. How long have you been doing this routine for?

   _____

8. Please list and describe ALL of the physical activities that you are involved in:

ACTIVITY	DURATION (minutes/session)	FREQUENCY (sessions/week)	INTENSITY (see below)
E.g. Running	45	3	1 2 3 **4** 5
			1 2 3 4 5
			1 2 3 4 5
			1 2 3 4 5
			1 2 3 4 5
			1 2 3 4 5
			1 2 3 4 5
			1 2 3 4 5

INTENSITY: 1 – not vigorous at all (very light)

2 – somewhat vigorous (light)

3 – moderately vigorous (medium)

4 – vigorous (heavy)

5 – extremely vigorous (very heavy)

9. What is your occupation?

_____

Describe the activity level of your job (i.e., Sitting; Stair Climbing; Lifting)

_____

10. Is there any other information that you feel is important to this study?

_____

_____

_____

_____

Appendix C

Nutritional Information of Cliff Bar, Hormone Composition of Pharmaceutical Contraceptives taken by Participants and Specific Monoclonal Antibodies for Each Well

Table C.1. CLIF R Nutritional Information.

Mass (grams)	68 .0
Carbohydrates (grams)	43.0
Proteins (grams)	11.0
Fat (grams)	6.0
Energy (calories)	250.00

Table C.2. Composition of Pharmaceutical Contraceptives Taken by Participants.

Brand Name		Ethinyl Estradiol (mg)	Synthetic Progesterone (mg)	
Triphasil	Tri-phasic OCP	0.03/0.04/0.03	0.05/0.075/0.125	(LNG)
Ortho Tricyclen	Tri-phasic OCP	0.035	0.18/0.215/0.25	(NG)
Allesse	Mono-phasic OCP	0.02	0.1	(LNG)
Orthocyclen	Mono-phasic OCP	0.035	0.25	(NG)
Loestrin	Mono-phasic OCP	0.03	1.5	(NE)
Diane 35	Mono-phasic OCP	0.035	2.0	(CP)
Marvelon	Mono-phasic OCP	0.03	0.15	(DG)
Ortho-Eva	Trans-dermal Patch	0.2	0.15	(NLG)

OCP= oral contraceptive pill; LNG= levonorgestrel; NG=norgestimate; NE=norethindrone; CP= cyproterone; DG= desogestrel; NLG= norelgestromin

Table C.3. Specific mAB Added to Each Well.

Well	1	2	3	4	5	6	7
	Cells unlabeled	CD25 *F*, CD4 *PE*, CD3 *QR*, CD62L *APC*	CD25 *F*, CD8 *PE*, CD3 *QR*, CD62L *APC*	$CRTH_2$ *F*, CD25 *PE* CD8 *APC*,	$CRTH_2$ *F*, CD25 *PE*, CD4 *APC*,	CD3 *F*, CD16 *PE*, CD56 *B*	CD20 *F*, CD19 *PE*

F= fluorescein isothiocyanate, PE= phycoerythrin, APC= allophycocyanin, B= Biotin

Appendix D

Individual PHA stimulated IL-6 Cytokine Changes

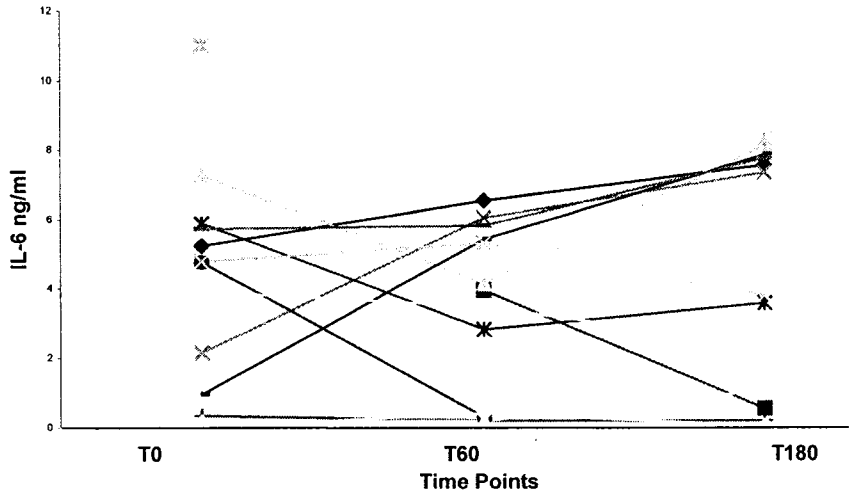


Figure D.1. Individual changes of L-6 in the untrained group following 60 minutes of endurance exercise.

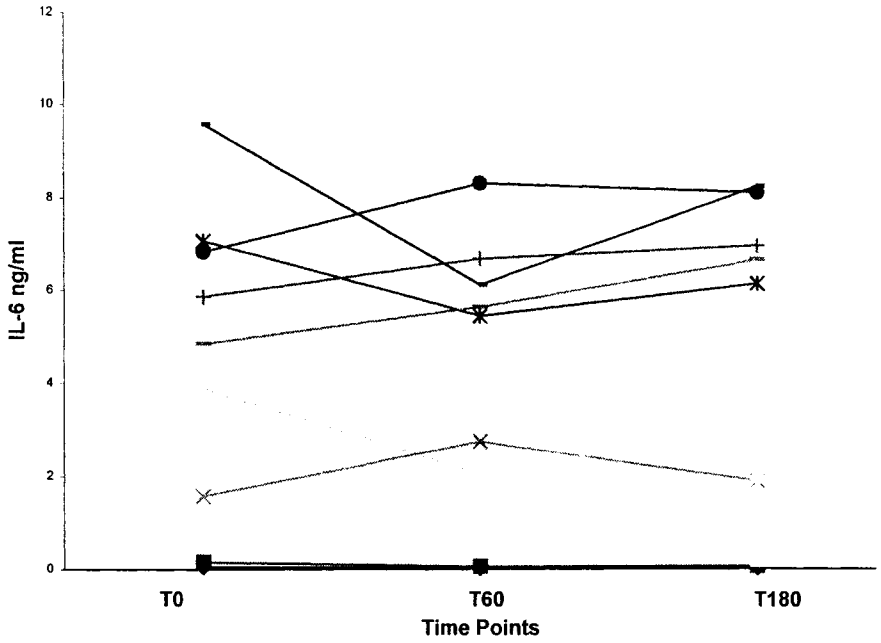


Figure D.2. Individual changes of IL-6 in the trained group following 60 minutes of endurance exercise.

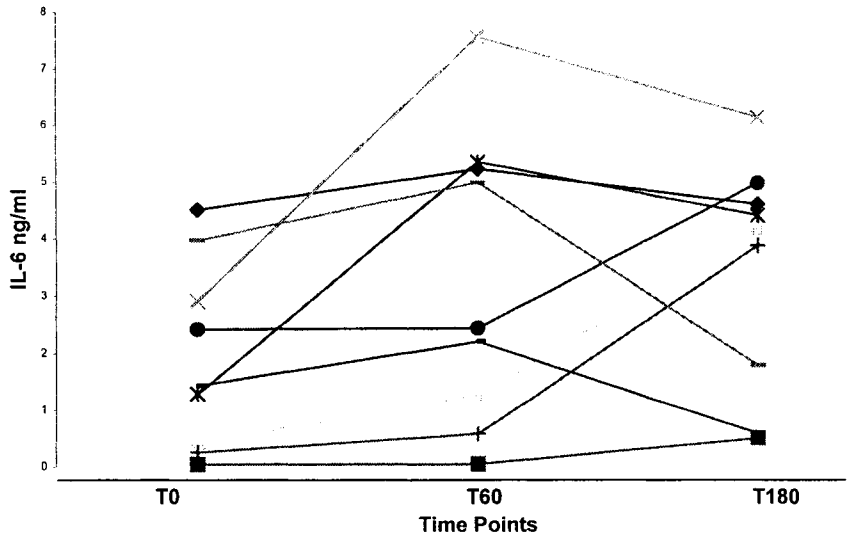


Figure D.3. Individual changes of IL-6 in the trained group following 60 minutes of endurance exercise.