IMMUNE SYSTEM ADAPTATIONS DURING COMPETITION PERIOD IN FEMALE CROSS-COUNTRY SKIERS

Johanna Stenholm

Master's Thesis in Exercise Physiology
Spring 2011
Department of Biology of
Physical Activity
University of Jyväskylä
Supervisors: Antti Mero, Heikki
Kyröläinen

Abstract

Stenholm, Johanna. **Immune system adaptations during competition period in female cross-country skiers.** Master's Thesis in Exercise Physiology, Department of Biology of Physical Activity. University of Jyväskylä. 95pp.

Purpose. This study was undertaken to characterize the extent of immune and endocrine changes in competition period and related to two competition weekends in well trained athletes in different parts of the competition period. An additional purpose was to evaluate if the changes in immunological variables would have an effect on the incidence of upper-respiratory-tract infections. The effect of the nutrition, nutrient and training status was also studied.
Methods. Ten (10) national level female skiers were investigated as they followed their normal race preparation routines. Blood samples were taken for basal and recovery measurements in the mornings approximately an hour after the athletes had woken up. Saliva samples were taken in the basal measurement morning, in the first normal distance competition morning, immediately after finishing the race and in the recovery measurement morning. The ski race distances were between 4-12 kilometres and the race finishing time were between 12 and 45 minutes. The first competition was in early December and the second took place in Finnish championships in late January. The second competition of the present athletes took place in extremely cold weather (approximately -20 Celsius degrees). If the athletes were not able to participate to the scheduled competition, they were measured during the next possible weekend. The athletes filled their daily training diaries, food diaries both 3 days before the competition and during the competition weekend, and they reported upper-respiratory tract (URTI)-symptoms using WURSS-21 questionnaire.
Results. URTI-symptoms were the most common reason for the athletes to miss training. There was a significant difference ($p<0.05$) in URTI-symptoms between the control group and the athlete group in the main competition period measurements. Blood leukocytes of the athletes were at a clinically normal level but were slightly higher in athletes in the competition period than in the controls but lower in the transition phase. The athletes had significantly ($p<0.05$) lower salivary IgA levels in the competition period than in the transition phase. In the basal measurements there were significant correlations between average serum IgA ($R=0.820$; $p<0.05$) and serum IgG ($R=-0.857$, $p<0.05$) levels to the number of days that athletes were unable to participate in their training sessions. Iron intake correlated negatively with testosterone/cortisol ratio ($R= -0.750$; $p<0.05$) and with the total amount of blood leukocytes ($R= -0.778$; $p<0.01$) in the main competition period. The amount of low and moderate training had a significant negative correlation with salivary IgA levels ($R= -0.750$; $p<0.05$) and with testosterone/cortisol -ratio($R= -0.714$; $p<0.05$).
Discussion and conclusion. The significant increase in the upper-respiratory tract infection was seen to be related to competitions when compared to the transition phase. Previously, it has been shown that the international level cross-country skiers adapt well to an acute exhaustive exercise test of short duration in a laboratory settings, even during the periods of heavy training and multiple competitions. But the effect on the athlete's health still remains unclear. In conclusion, substantial chances were observed in several immune-endocrine, substrate and metabolic measurements related to competition period and competition weekend. The immunoglobulin levels of A and G and testosterone/cortisol-ratio might be good indicators of the immunological state of the athletes. When designing training, competition and nutrition of the athletes, the sufficient recovery time has to be taken into account to avoid catabolic state and immune suppression. It is also recommended to take care of the adequate intake of nutrients but avoid excess doses, especially iron, as they might have suppressant effect on immunity of the athletes.

Key words: endurance training, immunology, upper-respiratory tract symptoms, serum IgA, serum IgG, testosterone/cortisol ratio

CONTENTS

ABSTRACT 2

1 INTRODUCTION 5

2 CROSS-COUNTRY SKIING 8

2.1 Physiology of cross-country skiing 8
2.1.1 Training and racing in cross-country skiing 10
2.2 Environmental factors in cross-country skiing 13

3 IMMUNE SYSTEM 15

3.1 Anatomy of the Immune System 16
3.2 Innate immune system 18
3.3 Acquired immune system 19

4 EXERCISE AND IMMUNE FUNCTIONS 22

4.1 Acute effects of exercise on immune functions 22
4.1.1 Exercise and leukocyte subpopulations 23
4.1.2 Soluble components 26
4.2 Chronic effects of training on immune system 27
4.2.1 Leukocyte count 28
4.2.2 Soluble components 29
4.3 Mechanism of Action 31
4.3.1 Catecholamines 31
4.3.2 Growth hormone and IGF-1 32
4.3.3 Cortisol 33
4.3.4 Sex hormones 34
4.4 Exercise and upper-respiratory tract infections 35
4.5 Overtraining and immune functions 39
4.6 Environmental factors 39

5 NUTRITION AND IMMUNE FUNCTIONS 41

5.1 Carbohydrates and immune response 41
5.2 Lipids and immune response 42
5.3 Protein and immune response 43
5.4 Vitamins, Minerals and Immune response 44

6 PURPOSE OF THE STUDY AND HYPOTHESES 46

7 MATERIALS AND METHODS 50

7.1 Study design 50
7.2 Subjects 50
7.3 Data collection and analysis 52

7.3.1 Blood samples 52
7.3.2 Saliva samples 53
7.3.3 Questionnaires 53
7.3.4 Anthropometry 54
7.4 Statistical analyses 55

8 RESULTS 56

8.1 Basal measurements 56
8.1.1 Incidence of upper-respiratory tract symptoms 56
8.1.2 Blood leukocytes 58
8.1.3 Salivary and Serum Immunoglobulins 60
8.1.4 Hormones 63
8.1.5 Other blood parameters 65
8.2 Effect of the competition weekend 65
8.2.1 Leukocytes 65
8.2.2 Salivary and Serum Immunoglobulins 67
8.2.3 Hormones 68
8.3 Relationships between measured parameters 69
8.3.1 Nutrition 69
8.3.2 Training 72
8.3.3 Body composition 73

9 DISCUSSION 74

9.1 Upper-respiratory tract symptoms and seasonal variation in immune parameters 75
9.2 Effect of competition weekend on immune parameters 77
9.3 Effect of nutrition, training and body composition on immune parameters 78
9.4 Critical evaluation 80
9.5 Conclusion 80

10. REFERENCES 81

APPENDIX 1. 94

APPENDIX 2. 95

APPENDIX 3. 96

1 INTRODUCTION

The mechanisms governing the body's response to physical exercise have been investigated from various perspectives including metabolism, nutrition, age and gender. Increased attention to the immune system during recent decades is reflected by a rapidly growing number of publications in the field. (Malm 2004.) Sport immunology is a relatively new field that examines the interaction of physical, psychological, and environmental stress on immune functions. Recently clinicians and scientists have begun to understand the complex interaction between exercise and immune functions. (Brolinson & Elliott 2007.) The biggest reason why knowledge on immune system modulations has increased is that it is relevant from public health as well as from elite athlete's point of view. (Malm 2004)

The immune system protects against, recognizes, attacks and destroys elements that are foreign to the body and the most important function of the immune system is to protect body against the infectious diseases. Immune system is influenced by genetics as well as environmental factors and thus there is some degree of variability in resistance to infections within the normal population. Since physical inactivity is now acknowledged as a major risk factor for several life-style associated diseases (e.g. cardiovascular and metabolic diseases), there is a major interest in whether a lifetime of regular exercise helps to prevent infectious diseases and cancer (Mackinnon 2001). Exercise may have both positive and negative effects on immune function and susceptibility to minor illnesses. The relationship between exercise and susceptibility to infections has been modeled in the form of a "J"-shaped curve. (Nieman 1994.) The model presented by Nieman (1994) suggests that while engaging in moderate activity may enhance immune function above sedentary levels, excessive amounts of prolonged, high intensity exercise may impair immune function.

In the field of sports, one of the most common reasons for poor performance at a major sport event is an acute respiratory infection. (Malm 2004) The upper respiratory tract infections are an international problem. (Brolinson & Elliott 2007.) One of the biggest

questions in sports is why endurance athletes are susceptible to upper respiratory tract infections during intense training and competition and how can they reduce the risk of getting sick. By studying athletes it is also possible to find, for example, clinical applications and identify the optimal amount of physical activity that protects against cancer or form guidelines of exercise for individuals in immune compromised state (e.g. aging and HIV infection). Athletes experience high levels of stress related to quantity and quality of training and competing. Managing large training and competition loads requires a fine balance between total stress and daily training (Rosen 2003.)

There is a close relation between the neurological, endocrinal and immune systems. It implies that activation of the neuroendocrinal system has potential to modulate immune function (e.g. the release of stress hormones during exercise). (Mackinnon 2002.) Exercise alters immune functions in many ways; stimulating some immune parameters while suppressing others. In general, there is a dose-response relationship between exercise amount and specific immune responses. The significance of these changes on long-term health is still unclear. Immune-endocrine responses are related to intensity and duration of the acute exercise stress (Fry et. al. 1992, Nieman et. al. 1994). It has been also demonstrated that certain parameters of the immune and endocrine systems are affected by chronic exercise. (Pyne & Gleeson, 1998) Cortisol is a key mediator of systemic and psychological stress responses. It has been proposed that exercise might stimulate the hypothalamic-pituitary-adrenal (HPA) axis leading to secretion of cortisol which is an immunosuppressive hormone that might increase the susceptibility to infections (Smith 1997). There is strong evidence that overtraining has an effect on immunity. (Mackinnon 2000) A decrease in the ratio between testosterone or free testosterone and cortisol has been suggested as a marker of 'anabolic-catabolic balance' and as a tool in the diagnosis of overtraining (Adlercreutz et al.1986.)

Low levels of immunoglobulin A have been observed in athletes suffering of overtraining syndrome, and it has been suggested that serum immunoglobulin A levels would be a potential marker of URTI risk (Gleeson et al. 1995, 1999; Mackinnon et al. 1993; Pyne and Gleeson 1998). It has been found that saliva immunoglobin A (saIgA) concentrations may be lower in elite cross-country skiers at rest than in age-matched controls (Tomasi et al. 1982). It has also been suggested that serum IgA may decrease

over prolonged periods of intensive training in elite athletes (Gleeson et al. 1995; Pyne and Gleeson 1998) due to neuroendocrinal factors related to physical and psychological stress resulting from intensive daily exercise (Mackinnon 2009, 144.)

The purpose of this study was to determine cross-country skiers' immune and hormone modulations during a competition period and to further analyze what is the contribution of training volume, nutrition, hormones, body composition changes and exercise to these changes.

2 CROSS-COUNTRY SKIING

Cross-country skiing is one of the most demanding endurance sports. It imposes extensive physiological challenges. Cross-country skiing challenges the athletes in the areas of physiological, biomechanical, psychological and environmental conditions. (Lindinger & Holmberg 2010.)

2.1 Physiology of cross-country skiing

High level cross-country skiers have traditionally been characterized by high aerobic power and endurance (Rusko 1987). The single most important physiological determinant of cross country skiing is maximum oxygen uptake, VO_2max, which integrates the ability of the lungs to transfer oxygen from air to blood, the blood and red blood cell to bind to oxygen, the heart to pump the blood, the circulation to distribute blood to muscles, and the muscles to use oxygen (Hoffman et al. 1994, Rusko 2003, 1.) Anaerobic power does not limit the skiing endurance performance, but anaerobic capacity is very important for sprint skiing and during uphill skiing in long distances. (Rusko 2003, 109.)

In untrained subjects maximal oxygen uptake per kg of body mass appears to plateau after 8-10 years of age. (Rowland 1990.) Longitudinal studies on runners and skiers have indicated that VO_{2max} increases with the age and training through 15-20 years of age. The annual increase of maximal oxygen uptake in cross-country skiers, with age and training, amounts to 1-3 ml/kg/min between the years 15-20 years in age. (Rusko 1992.) Ingjer (1991) studied data of test results from elite Norwegian cross-country skiers. His conclusion was that the best cross-country ski athletes have the greatest yearly variations in maximal oxygen uptake (VO_{2max}) and anaerobic threshold oxygen uptake ($VO_{2threshold}$) with the highest values being reached in the competitive season and the lowest values being reached in the spring. (Ingjer 1991.)

VO_{2max} is directly proportional to maximal cardiac output (Rusko 2003, 1). Maximal cardiac outputs in excess of 40 l/min and maximal oxygen uptake values over 6 l/min have been measured and stroke volumes can increase up to 200 ml in elite cross-country skiers. (Ekblom & Hermansen 1968.) The maximal heart rate does not change very much with training; elite skiers have almost the same maximal heart rate as untrained persons. The heart size and stroke volume are much higher in skiers when compared to untrained. Those two factors are responsible for the increase in maximum cardiac output and VO_{2max} with training. (Rusko 2003, 1-2.) Peripheral factors do not seem to be very important for $VO_{2max.}$ During prolonged exercise, such as 30 and 50 km ski races, capillarity, number of mitochondria and oxidative capacity of muscles might be important. (Rusko 2003, 3-8.)

Total mass of red blood cells (RCM), hemoglobin mass (HbM) and total blood volume are the most important blood variables for elite cross-country skiers. Hemoglobin (Hb) and hematocrit (Hct) usually adjust to the optimal individual level for each skier. If RCM, HbM and Hb are increased by endurance training, VO_{2max} also increases. The Hb concentration of elite skier should not significantly differ from that of normal individuals, because plasma volume usually increases concomitantly with the increase in HbM and RCM. (Rusko 2003, 3.) In cross-country skiing, the advantage of having a high haemoglobin value was illustrated by the fact that 50% of the medal winners at the World Cross-country Championships (WCCs) in Lahti in 2001 had a highly abnormal [Hb], whereas only 3% of the skiers placing between the 41st and 51st place had an abnormal [Hb] (Stray-Gundersen et al. 2003).

In addition to endurance capacity, upper body power is also known to play a major role in the performance of cross-country skiing. Rundell & Bacharach (1995) showed a positive correlation ($r > 0.80$) between upper body power and competitive results in U.S. Biathlon Team members as well as a strong relationship ($r = -0.79$) between a 1-km uphill double-pole test and ski racing time. (Rundell & Bacharach 1995.) Since the introduction of sprint event, higher speeds and new technical modifications have emerged, which requires an increased emphasis on strength and power. Stöggl et al. (2007) studied relationships between general strength tests, kinetics and V_{max} at high speeds. Upper body strength has been shown to be a major predictor of cross-country

skiing performance. The results of the study of Stöggl et al. (2007) revealed that general strength is not directly coupled to V_{max} but some level of general strength is needed to perform all the needed techniques. (Stöggl et. al 2007.)

2.1.1 Training and racing in cross-country skiing

There are a few basic principles behind training for cross-country skiing. First of all, the training of athletes is based on the stress theory which includes the stages of alarm reaction and acute adaptation to the demand of exercise, resistance development and fatigue-exhaustion. Repeated exercise stress leads to training-induced adaptations resulting in an improved resistance to disturbances of homeostasis. Normal overload training means that a skier has one demanding training session after which he or she recovers and performance is improved. Overreaching means that skiers intentionally increase the total training load for a short period of time to attain further training adaptations. If the training stress is too high, the body enters the stage of acute short-term fatigue and/or chronic long-term fatigue and overtraining syndrome may develop. (Rusko 2003, 62-64.)

Cross-country skiers normal training includes building a base with high volumes of training during the off-season, adding speed during the pre-competition period, and engaging in higher intensity intervals and training in the final preparation for competition with a final taper into the most important events. (Gaskill 1998; Bompa 1983, 211; Sharkey 1986.) Figure 1 demonstrates monthly training volumes of example athlete.

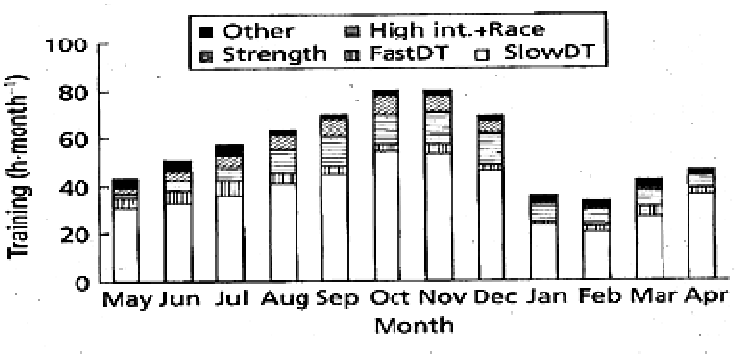

FIGURE 1. Monthly volume of training from the age of 15-16 years performed by another world champion skier to demonstrate periodization. FastDT =fast distance training SlowDT=slow distance training (Modified by Rusko 2003 from Ingjer 1992.)

Training for cross-country skiing becomes more serious and regular after puberty. In Finland training volume increases gradually, from about 50 km and six times a week at the age of 15 to 140-150 kilometers and 8 times a week at the age of 25. The best range for optimal annual improvements is accomplished by 5-10% increase in volume of training (Rusko 1992). According to Rusko (1992), young female cross-country skiers (age 17-21) seem to train as much and as frequently as young male athletes. The proportions of roller-skiing (13-19%), running or Nordic walking (37-43%) and skiing (40-48%) are similar in different junior skiers although there is a tendency to increase the proportion of skiing on snow in older skiers. The quantities of the training modes vary between the different countries. (Rusko 1992, 5-7)

Cross-country skiing requires a huge amount of energy to be produced for energy intake (carbohydrates, fats and proteins). The energy demand in cross-country skiing is saturated with aerobic and anaerobic energy production. The average proportion of aerobic and anaerobic energy yield depends on the distance, but generally anaerobic energy production is quite low. During ski-racing, the blood lactate concentration increases quickly during the first 5-10 minutes and thereafter slowly increases up to the end of the race. Blood lactate concentrations after 10 km (women) and 15 km (men) are presented in figure 2. (Rusko 2003, 5-7.)

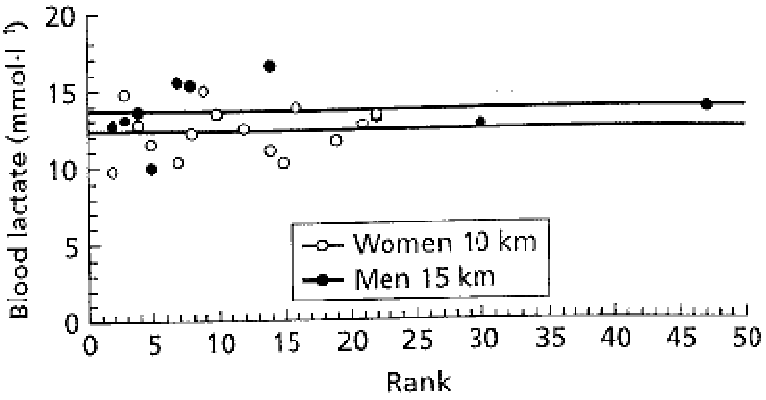

FIGURE 2. Blood lactate concentration after a classic style race in Finnish championships in 2000. (Rusko 2003, 6)

Rosen et al. (2004) studied the magnitude of change in immune, hormonal substrate and metabolic variance during a long distance ski race among 10 male and 6 female international level cross-country skiers. They found increased concentrations of granulocytes, natural killer (NK) cells, epinephrine (EPI), norepinephrine (NE), growth hormone (GH), cortisol, glucose, free fatty acid, creatine kinase, uric acid, non-organic phosphate, and a decrease in insulin concentration when they compared the values measured before and immediately after the race. (Rosen 2003)

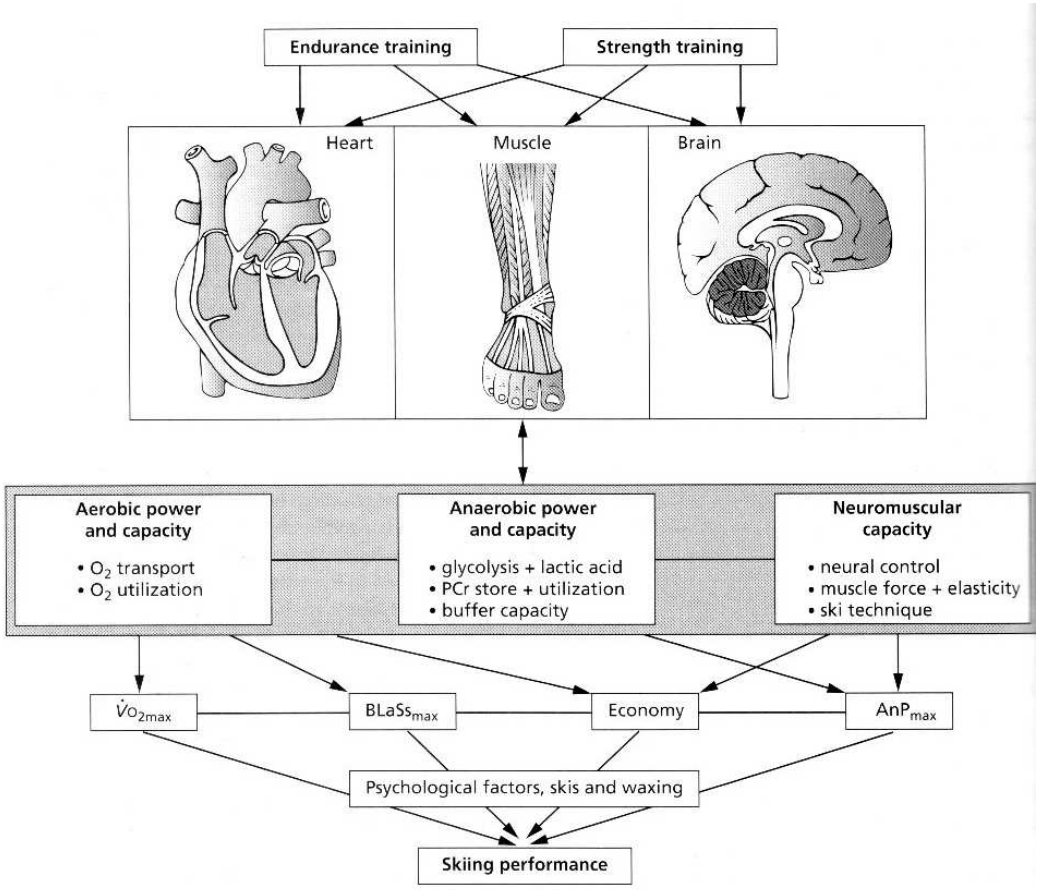

FIGURE 3. Factors affecting cross-country skiing performance. (Modified by Rusko 2003 from Noakes et al. 2001 and Paavolainen et. al. 1999.

2.2 Environmental factors in cross-country skiing

Physical training is only one of the stressors that have effect on body's stress responses. The non-training stressors or stimuli that induce stress are physical environment (temperature, humidity, oxygen content, time zone shift); psychological environment (personal and situational factors); primary and secondary needs (rest, sleep, nutrition, sexual instinct). The body integrates the stress factors and responses to total amount of stress. (Rusko 2003, 62.)

Weather conditions vary a lot when training and competing in cross-country skiing. Races are held at different altitudes, from sea level to 1800m, and training camps are held at 2500-3000m altitude. Weather conditions may vary from -20 to + 10 during

training and racing. (Rusko 2003, 64.) Cross-country skiing differs from most other sports, in that the athletic effort is conducted at low temperature. Trainings and competitions are performed in temperatures below - 15°C, implying ventilation with large amounts of cold air for several hours a week, for several months of the year. (Gleeson et al. 2000.)

Heir et al. (1993) studied exercise induced asthma symptoms in cross-country skiers by a postal survey. The prevalence percent of asthma and exercise-induced respiratory symptoms in 153 high-level Norwegian cross-country skiers and 306 control subjects were 100% for the skiers and 79% for the controls. (Heir & Oseid 1993.)

3 IMMUNE SYSTEM

The immune system is composed of many interdependent cell types that collectively protect the body from bacterial, parasitic, fungal, viral infections and from the growth of tumor cells. Many of these cell types have specialized functions. The cells of the immune system can engulf bacteria, kill parasites or tumor cells, or kill viral-infected cells. Often, these cells depend on the T helper subset for activation signals in the form of secretions formally known as cytokines, lymphokines, or more specifically interleukins. (Silverthorne 2007, 780-781.) The immune system is very complex system. (Brolinson & Elliot 2007.) Human immune system has developed as a means of self-identification and homeostasis. Self identification means distinguishing the body's own cells from those originating outside of the body. (Mackinnon 1999, 27-29.) Dysfunction in immune system may lead to a wide variety of diseases. The immune system comprises two basic components: the innate immune system and the adaptive immune system. (Simon 1987.) Both components of immune system involve various blood-borne factors and cells (Table 1). All cells of the immune system are originated from bone marrow. They are found circulating in the bloodstream, organized into lymphoid organs such as the thymus, spleen, lymph nodes, and gut-associated lymphoid tissue, or dispersed in other locations around the body. (Calder 2007.)

TABLE 1. Components of the innate and acquired immune system. (Modified from Shephard 1997, 4.)

	Innate	Acquired
Psychochemical barriers	Skin Mucosal membranes Lysozymes Stomach acid	Cutaneos and mucosal immune system Antibodies in mucosal secretion
Circulating molecules	Complements	Antibodies
Cells	Granulocytes Monocytes / Magrophages Natural Killer Cells	B-lymphocytes T-lymphocytes
Soluble mediators	Macrophage-derived cytokines	Lymphocyte-derived cytokines

The innate immune system contains exterior defenses (such as skin and mucosal membranes), non specific phagocytic leukocytes, and serum proteins. Pathogens that escape this outer barrier then come in contact with adaptive system, which is made up of T and B cells. These two elements provide a formidable obstacle to the establishment and long-term survival of infectious agents. (Guyton & Hall 2000, 402). In this study the main focus is on immune functions against infections and the purpose of this chapter is to provide a simple picture what is known about the anatomy and the functions of the immune system.

3.1 Anatomy of the Immune System

Immune system is very complex and multidimensional system which integrates into the tissues of other organs, such as gastrointestinal tract. The immune system is composed of two components: lymphoid tissues and the cells responsible for the immune system. Hematopoietic stem cell–derived cell lineage is presented in figure 4.

Lymphoid tissue. The body has two primary lymphoid tissues; the thymus gland and the bone marrow, both are the sites where cells involved in the immune response form and

mature. Secondary lymphoid tissues are sites where mature immune cells interact with pathogens and initiate a response. Secondary tissues are divided into two groups; the encapsulated lymphoid tissue is found from the spleen and the lymphoid nodes. (Silverthorn 2007, 780-781.)

Leukocytes. The primary cells of the immune system are the white blood cells and leukocytes. Leukocytes are divided into six subgroups: eosinophils, basophiles, neutrophils, monocytes (macrophages), lymphocytes and dendritic cells. Neutrophils, macrophages, monocytes and eosinophils form a functional group, phagocytes. This is a group of white blood cells, which are capable of engulfing and ingesting their targets by phagocytosis. A second functional group is the cytotoxic cells; those cells are capable of killing other cells. This group contains eosinophils and some types of lymphocytes. The third group is made up of antigen-presenting cells (APCs), which display fragments of foreign proteins on their cell surface. This group includes certain lymphocytes, dendritic cells, macrophages and monocytes. (Silverthorn 2007, 780-782.)

Eosinophils are associated with allergic reactions and parasitic diseases. Normally 1-3% of the white blood cells are eosinophils. Most of the eosinophils are found in the digestive tract and lungs, urinary and genital epithelia. Basophiles are very rare in the circulation. Basophiles release mediators that contribute to inflammation. The granules of basophiles contain histamine, heparin, cytokines and other chemicals involved in allergic reactions and immune responses. Neutrophils are the most abundant leukocytes; 50-70% of the white blood cells are neutrophiles. Neutrophils are phagocytic cells that typically ingest and kill 5-20 bacteria during their life span (1-2 days). Neutrophils also release variety of cytokines. Monocytes are precursor cells of tissue macrophages. Only 1-6 % of the all white blood cells are monocytes. Macrophages are antigen-presenting cells and that is why they do play a very important role in development of acquired immunity. About 20-35 % of the leucocytes are lymphocytes and they are the key cells that mediate the acquired immune responses of the body. (Silverthorn 2007, 780-784.)

Soluble elements. Soluble elements of the immune system include immunoglobulins, cytokines and other soluble factors such as complement and acute phase proteins. Soluble factors are found in the blood and other body fluids and act as a mediator of

immune function by activating cells, by mediating communication between different types of cells, by mediating movement of the cell throughout body, by directly killing certain pathogens or by providing nutrients or substrates to immune cells. (Mackinnon 2000, 12-13; Shephard 1997, 16-18.)

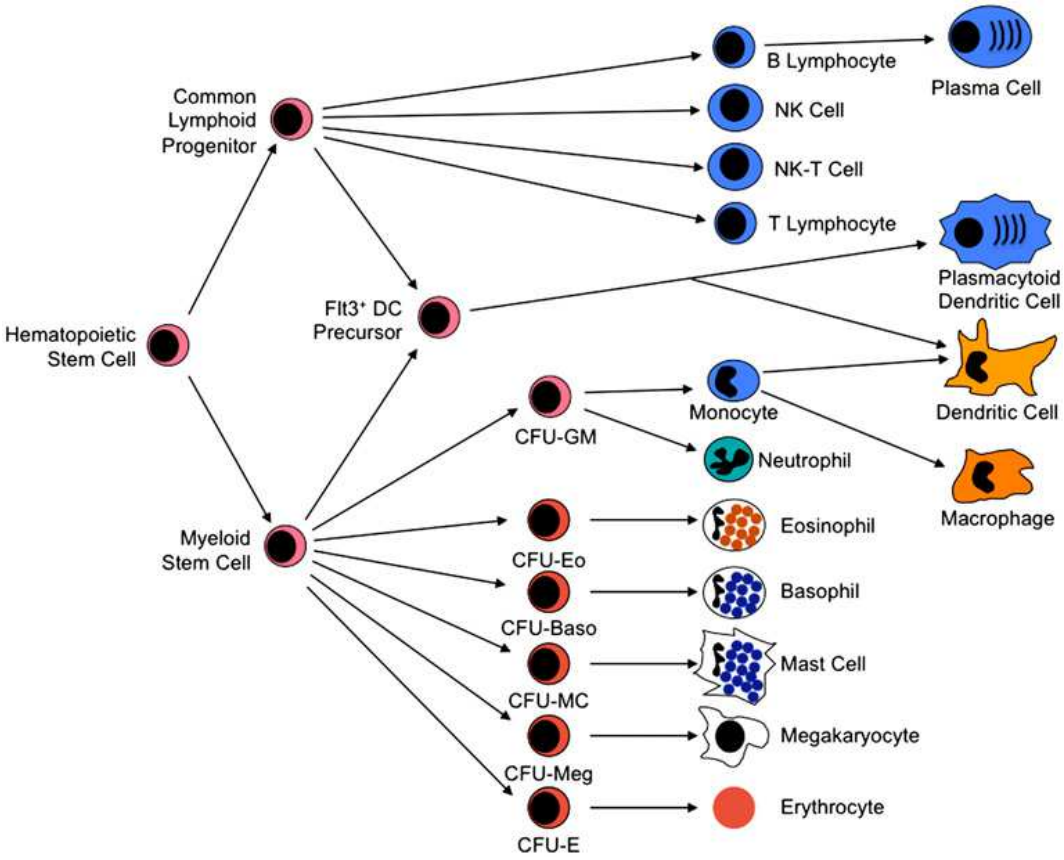

FIGURE 4. Hematopoietic stem cell–derived cell lineages. (Chaplin 2010)

3.2 Innate immune system

Innate cellular defenses are particularly important in combating viruses, since these micro-organisms have only limited susceptibility to the physical and chemical defense mechanisms. The simplest way to avoid infections is to prevent the micro-organisms from gaining access to the body; these are called external barriers against infections. The major line of defense is skin, which is impermeable to most infectious agents. Mucus secreted by the membranes lining the inner surfaces of the body, act also as a protective barrier to block the adherence of bacteria to epithelial cells. Microbial and

other foreign particles are removed from the mucosal membranes by mechanical stress, like coughing. One of the most important mechanical factors that protect mucosal membranes is washing action of tears, saliva and urine. A totally different mechanism is the microbial antagonism associated with the normal bacterial flora of the body that suppresses the growth of many potential pathogenic bacteria and fungi. (Roitt 2001, 1-4.) If the micro-organisms do penetrate in to the body, there are two main defensive operations, the destructive effect of soluble chemical factors and the mechanism of phagocytosis. The main phagocytic cells are polymorphonuclear neutrophils and macrophages. Phagocytic cells have evolved a system of receptors capable to recognizing molecular patterns expressed on the surface of the pathogens (PAMPs). Engagement of the pattern recognition receptor generates a signal which alerts the cell to danger and initiates the phagocytic process. After adherence of microbe to the surface of the neutrophil or macrophage, the resulting signal initiates the ingestion phase. There is an array of killing mechanisms. These include: killing by reactive oxygen intermediates, nitrogen intermediates and preformed antimicrobials. (Roitt 2001, 2-6.)

Humoral mechanism provides a second defense strategy. Acute phase proteins increase in response to infection. They show a dramatic increase in concentrations in response to early ‘alarm’ mediators such as IL-1 released as a result of infection or tissue injury. These include C-reactive protein (CRP), mannose-binding protein (MBP) and serum amyloid P complement. Recovery from viral infections can be affected by the interferons which block viral replication. (Roitt 2001, 16.) Virally infected cells can be killed by large granular lymphocytes with NK activity through a proforin/granzyme and separate Fas-mediate pathway, leading to programmed cell death (apoptosis) mediated by activation of the caspase protease cascade which fragments the nuclear DNA. This is called extracellular killing. (Roitt 2001, 19.)

3.3 Acquired immune system

Failure of the innate system and the resulting infection activates the acquired system, which aids recovery from infection. (Roitt 2001, 30) Acquired immunity is caused by a special immune system that forms antibodies and/or activates lymphocytes which attack

and destroy the specific invading organism or toxin. The adaptive components of the immune system comprise of characteristic cell types (T- and B lymphocytes), antibodies in mucosal secretion, and the plasma (immunoglobulins) and lymphocyte derived cytokines. The adaptive components are specific to a given foreign macromolecule. (Guyton & Hall 2006, 409-410; Shephard 1997, 4; Roitt 2001, 22.)

Monocytes or macrophages first ingest and process and then present foreign material (antigens) to lymphocytes. This is followed by clonal proliferation of T- and B lymphocytes that possess receptors that recognize the antigen, engendering specificity and 'memory' that enable the immune system to mount an augmented cell-mediated and humoral response when the host is re-infected by the same pathogen. Antibodies differentiate between antigens because recognition is based on molecular shape complementarity. That is why memory induced by one antigen will not extend to another unrelated antigen. The immune system differentiates self-components from foreign antigens by making immature self-reacting lymphocytes unresponsive through contact with host molecules. (Guyton & Hall 2006, 402-403; Shephard 1997, 37; Roitt 2001, 24.)

Lymphocytes are the basis of acquired immunity. The lymphocytes are located mostly in the lymphoid node, but they are also found from the special lymphoid tissues, such as the spleen and the bone marrow. There are two main subgroups in lymphocytes. "Cell-mediated immunity" is provided by T lymphocytes and "humoral immunity" by B lymphocytes. When a specific antigen comes to contact with the T and B lymphocytes, certain T lymphocytes become activate to form activated T cells and certain of the B lymphocytes become active to activate the formation of the antibodies. The antibodies are gammaglobulins called immunoglobulins. There are five general classes of antibodies, respectively named IgM, IgG, IgA, IgD and IgE. Immunoglobulin A is the principal immunoglobulin found on mucosal surfaces and plays a significant role in mucosal defense. IgG plasma cells are in low numbers at mucosal sites but serum-derived and locally produced IgG antibodies are important in protection of the respiratory tracts (Persson et al. 1998, Brandtzaeg et al. 1997) Antibodies act mainly in two ways to protect the body against invading agents: (1) by direct attack on the invader and (2) by activating of the complement system which then has multiple means of its

own for destroying the invader. (Guyton & Hall 2006, 402-403; Shephard 1997, 328; Roitt 2001, 24.)

4 EXERCISE AND IMMUNE FUNCTIONS

There is a general perception among athletes, coaches and sports physicians that athletes are susceptible to infectious illness, such as upper respiratory tract infection (URTI) during intensive training and major competitions. Experimental findings suggest that regular physical training may induce proper immune responses. It is generally believed that a program of moderate intensity exercise may have positive effect on immune functions (Kwak 2006). Low intensity exercise might be beneficial for immune system as well (Fitzgerald 1991); whereas heavy prolonged exercise can have negative effects (Kim & Kwak 2004). The negative effects of heavy exercise are best documented as an increased vulnerability to viral infections, allergic reactions and URTI symptoms (Mackinnon and Hooper 1994). J-curve model has been proposed to describe the relationship between exercise volume (duration/intensity) and probability of URTI symptoms, in which risk of URTI would be reduced by moderate exercise but elevated because of more intense training. While there is data of URTI that supports this model, the data is still limited to prove that moderate exercise lowers the risk of URTI. (Mackinnon 2000.) The following chapter summarizes cellular and soluble component responses to acute exercise and the chronic effect of training on the immune system.

4.1 Acute effects of exercise on immune functions

Acute bout of prolonged strenuous exercise has a temporary depressive effect on immune system. The changes during recovery have been suggested to provide an ‘open window’ for infections. The effects are attributed to rises in circulating stress hormones such as adrenaline, cortisol, growth hormone and prolactin. (Gleeson 2009.) Responses of blood leukocyte subpopulations to an acute exercise are quite stereotypical. However there are individual differences between individuals and the exercise stimulus varies between the subpopulations of the leukocytes. (Pedersen 2005, 321-324) There are a lot of variables affecting the acute exercise induced immune functions. These variables include, for example, blood volume, autonomic activity, secretion of hormones

(especially cortisol), exercise related modifications in diet, nutrient deficiencies, type, intensity and duration of exercise, associated stress (psychological) and recovery from prior activity. (Shephard, 1997, 51-54.) This chapter will focus on the changes in the amounts of leukocyte subpopulations and responses of soluble components of the immune system in the context of exercise.

4.1.1 Exercise and leukocyte subpopulations

The sudden, temporary changes in the immune system caused by one bout of exercise are called acute responses to exercise, and disappear shortly (usually in 6 h) after the exercise is finished. There are quite a few limitations in access to study the human immune responses via leukocytes obtained from peripheral blood; the amount of leukocytes may present only 1-2% of all the immune cells in the body at a given time. Sampling immune cells from human blood is still said to be a “window” with which to view immune events occurring throughout the body. The underlying assumption is that the activity of the cells in the blood reflects the activity of the cells in the entire body. (Mackinnon 2000.) It is a well known fact that the overall leukocyte count in peripheral venous blood shows substantial increases during and following a bout of physical activity. This reflects the combined influence of granulocytosis, lymphocytosis, and monocytosis. Granulocytosis and monocytosis usually persist into the recovery phase so the leukocyte count remains elevated for as long as 24 hours. In contrast, lymphocyte count decreases rapidly and the values may drop under resting values. (Shephard 1997, 51.) To illustrate the effect of exercise on leukocytes the total leukocyte and neutrophil responses in two types of 60 minute treadmill running exercise are presented in Figure 5.

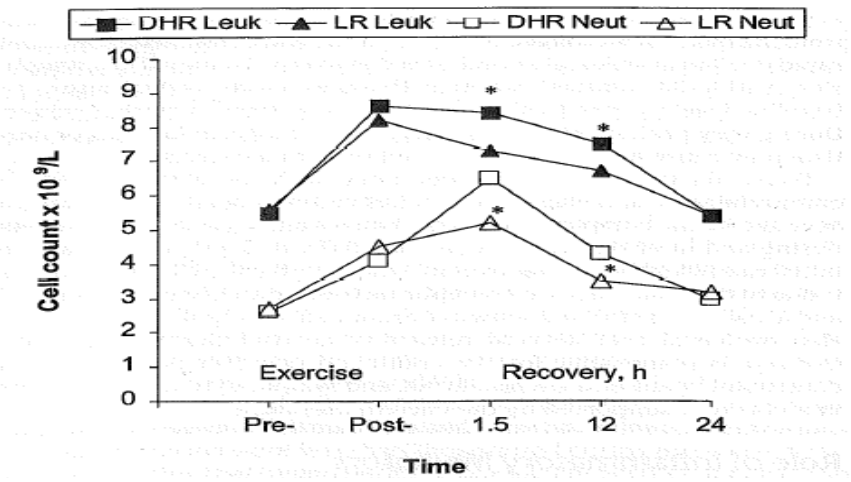

FIGURE 5. The total leukocyte and neutrophil responses after exercise (Adapted by Mackinnon 1999 from Pizza et al. 1995)

Neutrophils. Neurophil count largely increases after a period of physical activity (Eliakim et al.1999, Gabriel and Kindermann 1997, Robson et. al. 1999) but returns close to baseline values usually within 30 minutes. Cell counts often show a delayed rise from one to several hours following exercise, as mature cells are mobilized from the bone marrow in response to such signals as increase in plasma concentration of cortisol and various cytokines. (Pyne 1994, Shephard 1997, 56.) Nieman et al. (1994) studied the effect of 45 minutes of high- (80% $\dot{V}O_2$max) versus moderate- (50% $\dot{V}O_2$max) intensity treadmill exercise on circulating leukocytes. There was no significant increase in neutrophil amount in low-intensity training but the high-intensity training increased neutrophil count 48 % during exercise and 208 % two hours after the exercise. (Nieman et. al. 1994.)

Eosinophils and basophils. Experimental data suggest that sustained moderate exercise leads to decreased eosinophil count. Aerobic exercise does not have any great effect on basophil counts. (Shephard 1997, 75.)

Monocytes and macrophages. Most reports suggest that an acute bout of physical activity increases the monocyte activity and augments the macrophage activity. (Shephard 1997, 75.) Nieman et al. (1994) found a 27 % increase in monocyte count after moderate intensity (50% $\dot{V}O_2$max) treadmill exercise. Bailey et al. (1991) tested highly active individuals' responses to 30 seconds maximal cycle-ergometer test. They

found 44% increase in monocyte count after exercise. The reason for the exercise-induced increase in circulating monocytes is not entirely clears (Shephard 1997, 81). Macrophage activity is important for the ingestion of bacteria; destroy tumor cells and secretion of IL-1 and other cytokines. Woods and Davis (1994) concluded that the results in macrophage activity are complex and dependent on the exercise dose, as well as the functional state of the macrophages at the time of the exercise. They, at least partially, supported the hypothesis that moderate exercise might have a positive and exhaustive exercise a negative effect on macrophage antitumor function. (Wood & Davis 1994.)

Lymphocytes. Lymphocytes contribute to the acute leukocytosis, but unlike the neutrophils the lymphocyte numbers may decline below basal values in the hours after exercise. This lymphocytopenia, together with the decrease in lymphocyte cell function, has been suggested to be one of the reasons for an "open window" period, during which the athletes might be more susceptible to infections (Pedersen and Ullum, 1994). The number of natural killer (NK) cells increase sharply during moderate to high- intensity activity. (Del Giacco et. al. 2004.) For example Kendal et al. (1990) found 275 percent increase in natural killer cell count after 120 minutes cycle ergometer exercise at 65 % of $VO2_{max}$.The exercise data has reported responses of either total T cell count or T cell subsets to sustained bouts of moderate or vigorous aerobic activity. There is usually a large increase in overall T cell count (up to 150 percent) during and immediately after bouts of vigorous or maximal exercise. (Shephard 1997, 90-91.) Both CD4 (helper/inflammatory) and CD8 (cytotoxic/suppressor) T cell counts increase, the ration of CD4 to CD8 is declined due to larger increase in CD8 cell number. There is relative little change in B-cell number. (Mackinnon 2001, 143.)

TABLE 2. Effect of strenuous exercise on the immune system. (Modified from Pedersen & Hoffman-Goetz 2000.)

	During exercise	After exercise
Neutrophil count	↑	↑↑
Monocyte count		↑
Lymphocyte count	↑	↓
CD4 + T cell count	↑	↓
CD8 + T cell count	↑	↓
CD19 + B cell count	↑	↓
CD16 + 56 + NK cell count	↑	↓
Lymphocyte apoptosis	↑	↑
Proliferative response to mitogens	↓	↓
Antibody response in vitro	↓	↓
Saliva IgA	↓	↓
Delayed type hypersensitivity response (skin test)		↓
NK cell activity	↑	↓
Lymphokine activated killer cell activity	↑	↑
C-reactive protein		↓
Neopterin		↑
Plasma concentration of TNF-a	↑	↑
Plasma concentration of IL-1	↑	↑
Plasma concentration of IL-6	↑↑	↑
Plasma concentration of IL-1ra	↑↑	↑
Plasma concentration of IL-10	↑	↑
Plasma concentration of TNF-R	↑	↑
Plasma concentration of MIP-1b,IL-8		↑

4.1.2 Soluble components

During and following intense exercise there is evidence of overall inflammatory response with substantial changes in the blood levels of cytokines, immunoglobulins and other soluble components of immune defenses. (Shephard 1997, 137.)

Cytokines. Increases of cytokine levels in blood (Interleukin-1, Interleukin-6, and Tumor Necrosis factor- α) have been shown immediately after strenuous exercise (Northoff and Berg 1991; Northoff et al. 1994). In the study of Weinstock et al. (1997), one hour of an exhaustive exercise markedly decreased the levels of TNF-α, IL-2, and

Interferon-g. The levels of cytokines return to pre-exercise levels 20 hours after exercise (Weinstocket al. 1997.)

Immunoglobulins. Moderate physical activity has no significant influence on salivary immunoglobulin A production. Intense exercise seems to lead to a substantial and sustained reduction in salivary IgA output. (Shephard 1997, 158.) For example Mackinnon et al. (1989) found 65 percent reduction in salivary IgA levels after 120 minute cycle ergometer test at 70-80 % of $VO_{2max.}$ A 24.4 percent decrease in salivary IgA level concentration was reported to occur after exhaustive treadmill exercise, which remained depressed (16.9 %) one hour later (McDowell et al., 1992). Schouten et al. (1988) found the opposite to be true in young, healthy habitual exercisers.

There have been some changes in serum immunoglobulin levels during and after physical activity, but the results have been inconsistent. (Shephard 1997, 140.) Of the group of cytokines, it appears that only IL-2 is adversely affected by exercise. (Mackinnon 1992, 148.)

4.2 Chronic effects of training on immune system

The acute changes in immune function are well-documented. Less is known about the chronic effects of training and competing on immune functions. Exercise –induced changes in circulating leukocyte and leukocyte subset numbers are transitory, and cell counts usually return to normal levels by 12-24 hours after exercise. There appears to be few chronic effects of training on immune-cell number. Thus, clinically normal levels of immune cells are observed in most athletes. Possible exception is during prolonged periods of very intense training. Researchers have tried to find differences in resting immune function between athletes and non-athletes. Thus so far they have failed to prove any evidence that athletic endeavour is linked to clinically important changes in immunity. (Nieman 2001, Mackinnon 2000.)

Mackinnon (1999) has presented a modified version (Figure 6) of “open window” hypothesis concerning the chronic effect of training on immune system. (Mackinnon

1999, 314.) According to Bruunsgard (1997), the immune system is only suppressed following exercise and lasts an hour or longer. While this seems to be an oversimplification, because exercise intensity is not taken into account, it follows that prolonged; frequent, high-intensity bouts of activity potentiate the immunosuppressive effect. (Gleeson & Bishop 2000.) The effect of training on leukocyte count and soluble factors is discussed next.

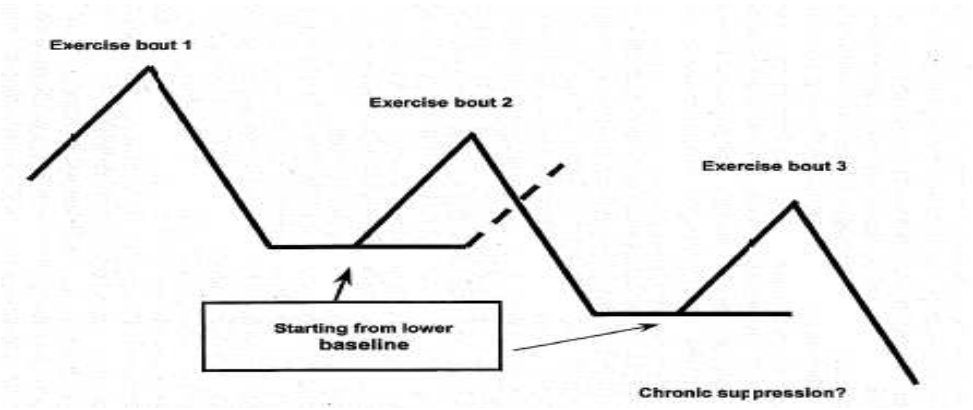

FIGURE 6. Mackinnon's modification of "open window" theory hypothesises immunosuppressant effect of repeated bouts of exercise. (Mackinnon 1999, 314.)

4.2.1 Leukocyte count

Lymphocytes. The relative proportions of lymphocyte proportions (T, B, and NK cells) obtained in rest are not differing between athletes and non-athletes. The data of Gleeson et al. (1995), Mujika et al. (1996) and Gray et al. (1993) have suggested that exercise training has no long-term effect on lymphocyte subset distribution. (Mackinnon 1999, 75-79.) Of all the possible immune measures, only NK cell activity has emerged as a consistent indicator differentiating the immune systems of athletes and non-athletes. (Mackinnon, 2000.)

Neutrophils. Neutrophils are important components of the innate immune system, aiding in the phagocytosis of many bacterial and viral pathogens, and the release of immune modulator cytokines. Neutrophil function has been reported to be suppressed in athletes, but this has not been a consistent finding, and may depend on the severity of training (Mackinnon 1999, 122). Although neutrophil number appears to be within the

clinically normal range in athletes, several recent studies have observed lower neutrophil functional capacity in athletes compared to control subjects. (Mackinnon 1999, 123.) Whereas moderate training may have a slight beneficial influence on neutrophil counts, intensive training seems to have a suppressant effect. (Shephard 1997, 169).

Monocytes. There is inconsistent data on the monocyte response to periods of training in athletes. Baum et al. (1994) observed a significance increase in the number of circulating monocytes between early season endurance training phase and latter competition phase in male track athletes (Baum et al. 1994.). In contrast, Mackinnon et al. (1997) found a significance 45% decrease in monocyte number within the first two weeks of intensified training. The data suggest that moderate normal training does not affect the monocyte number but very intense longer periods might have suppressive effect. (Mackinnon 1999, 106.)

4.2.2 Soluble components

Immunoglobulins. The first research into the effects of exercise on mucosal immune parameters was published by Tomasi et al. in 1982. They reported that salivary IgA levels were lower in elite cross-country skiers compared to recreational athletes and that the levels were further lowered after a competitive race. Tomasi et al. (1982) speculated that the 'temporary antibody deficiency on the mucosal surface might lead to a susceptibility to acquiring viral and bacterial infections' particularly after strenuous exercise. Mackinnon & Hopper (1994) have reported that serum IgA concentrations were lower in stale compared to well-trained swimmers during 6-months follow-up. The impact of long-term training on systemic and mucosal immunity was assessed prospectively in a cohort of elite Australian swimmers over a 7-month training season in preparation for national championships (Gleeson et al. 1995, Gleeson 2005.) The results indicated significant suppression of resting serum IgA, IgG and IgM concentration in athletes, associated with long-term training at an intensive level. Resting serum IgA concentrations at the start of the training period showed significant correlation with infection rates, and the number of infections observed in the swimmers was predicted

by the preseason and mean pre-training serum IgA levels. (Gleeson 2005.) This kind of chronic suppression of salivary IgA has previously been reported in swimmers undertaking a 3-month training program (Tharp et al. 1990). Gleeson (1999) studied elite kayak competitors' salivary IgA levels during intensive 2 week training period. In this case study, pre-exercise salivary IgA concentrations failed to recover to the initial IgA concentration before the first training session of the day prior to subsequent sessions. (Figure 7). Concentrations of serum IgA measured in swimmers before training sessions have shown correlations with URTI rates, and it has been found that the number of infections observed in the swimmers could be predicated by the pre-season and the mean pre-training sIgA concentrations (Gleeson et al. 1999). It has been suggested that sIgA may decrease over prolonged periods of intensive training in elite athletes (Gleeson et al. 1995; Pyne and Gleeson 1998) due to neurohormonal factors related to physical and psychological stress resulting from intensive daily exercise (Mackinnon 1996).

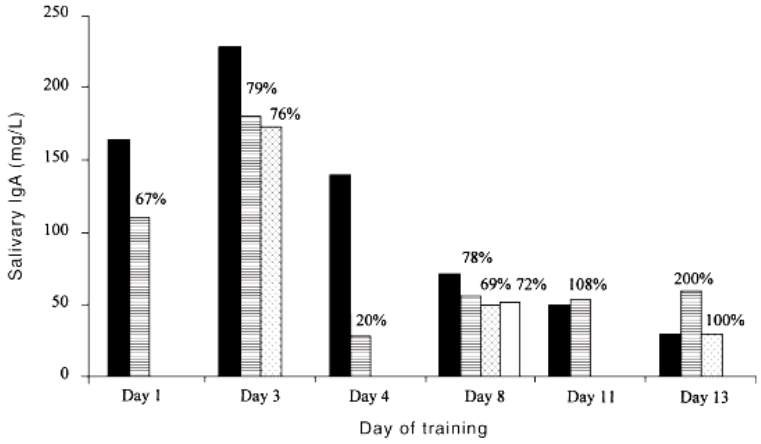

FIGURE 7. Pre-exercise salivary IgA concentrations for each training session over a 2 week period for an elite kayaker and the percentage of change from the initial concentration of the day. (Gleeson 1999.)

These data suggest the possibility of clinically relevant immune suppression mediated by salivary IgA suppression in well-trained athletes when training load is high. Psychological stress associated with training and competition at the elite level may be an additive factor to the effects of intensive exercise on immune function (Selby et al.1990).

4.3 Mechanism of Action

Acute, intense exercise increases the concentration of numerous hormones in the blood. These hormones include adrenalin, noradrenalin, growth hormone, beta-endorphins, and cortisol, whereas the concentration of insulin slightly decreases. The acute and chronic exercise-induced changes in hormone- immune system interactions are discussed next.

4.3.1 Catecholamines

During exercise, adrenaline is released from the adrenal medulla, and noradrenalin is released from the sympathetic nerve terminals. Arterial plasma concentrations of adrenalin and noradrenalin increase almost linearly with the duration of dynamic exercise and exponentially with intensity, when expressed relative to the individual's maximal performance (VO_{2max}). (Kjaer & Dela 1996.) The expression of beta-adrenoceptors on T-, B-, and NK-cells, macrophages, and neutrophils provides the molecular basis for these cells to be targets for catecholamine signaling. (Madden & Felten 1995.) β-receptors on lymphocytes are intracellularly linked to the adenyl cyclase system for generation of cAMP as a second messenger and the β-receptor density appears to change in conjunction with lymphocyte activation and differentiation. (Ackerman et al. 1991.) The number of adrenergic receptors on the individual lymphocyte subpopulation may determine the degree to which the cells are mobilized in response to catecholamines. NK cells have the highest number of adrenergic receptors, with CD4+ having the lowest number. Dynamic exercise upregulates the β-adenergic density but only on NK-cells. Interestingly, NK-cells are most responsive to exercise and other stressors than any other subpopulations. Thus there seems to be a correlation between number of adrenergic receptors on lymphocyte subpopulations and their responsiveness to exercise. (Pedersen & Hoffman-Goetz 2000.)

4.3.2 Growth hormone and IGF-1

Growth hormone (GH) is released from the anterior pituitary in a pulsatile fashion, and irregular time courses in plasma GH levels have been found. Plasma levels of pituitary hormones increase in response to exercise both with duration and with intensity. Growth hormone responses are more related to the peak exercise intensity than to duration of exercise or total work output. (Kjaer & Dela 1996.) Growth hormone does not have a major role in the exercise-induced requirement of lymphocytes to circulation. However, epinephrine and growth hormone in combination are probably responsible for the recruitment of neutrophils to the blood during physical stress. (Pedersen & Hoffman-Goetz 2000.) Blalock (1994) found that GH is an activator of neutrophil phagocytosis and may influence the proliferation of T lymphocytes and their differentiation into effectors cells.

The main regulators of the Insulin-like growth factor 1 (IGF-1) levels in blood are nutrition and growth hormone status (Clark 1997). IGF-1 levels are higher in fitter subjects which are correlated with lean body mass. That is why one might expect that exercise would increase IGF-1 levels, but there are still mixed results about exercise and IGF-1 levels. These seemingly contradictory findings might be explained, in part, by the substantial role that overall energy balance plays in the regulation of circulating IGF-1. Negative energy balance, whether caused by increasing exercise energy expenditure with an exercise training program or by reducing energy intake without increasing exercise, causes a reduction in circulating IGF-1 within several days (Nemet et al. 2004)

There is evidence that IGF-1 plays significant role in regulating hematopoesis, especially lymphopoesis and immune functions. (Clark 1997.) IGF-1 is synthesized and secreted by various immune competent cells. In addition IGF-1 receptors are expressed on immune cells. IGF-1 stimulate the proliferation of immune competent cells and modulate humoral and cellular immune functions, i.e. immunoglobulin secretion of B cells, thymulin secretion of thymic epithelial cells, natural killer cell activity, phagocytosis, oxidative burst and killing capacity of neutrophils and macrophages. (Auernhammer & Strasburger 1995)

Several immunological parameters and functions are altered in GH- deficient patients when compared to normal controls. Auernhammer & Strasburger (1995) reported that the data available indicates that endocrine and pleiotropic para- and autocrine mechanisms of action are involved in a neuropeptide immune network, including GH PRL and IGF-I as modulators of immune function. (Auernhammer & Strasburger 1995)

4.3.3 Cortisol

Exercise is a potential stimulus for cortisol secretion. The plasma cortisol levels increase only in relation to exercise of long duration, thus short-term exercise has no effect on plasma cortisol levels (Galbo 1984.) The cortisol response is dependent on the relative exercise work load, but there are also numerous other factors modulating these hormonal responses, including mode and duration of exercise, the relationship between anaerobic and aerobic exercise, prior meal, pulsate and circadian manor of secretion of cortisol and fitness of the subject. (Brandenberger & Follenius 1975, Brandenberger et. al 1982, Few 1974, Van Cauter et al. 1996) Circulating cortisol concentrations are maximal in the early morning hours just before awakening as a result of increased cortisol secretary pulse amplitude and frequency. The amplitude of cortisol secretary pulses progressively decreases throughout the day until cortisol concentrations are quite low in the evening. (Veldhuis et al.1989.)

It is well documented that corticosteroids given intravenously to humans cause lymphocytopenia, monocytopenia, eosinocytopenia, and neutrophilia, which reach their maximum in about 4 hours after the administration. (Rabin et al. 1996.) The increase in cortisol during and after exercise is mediated by IL-6 (Bruungaard et al. 1997). The link between exercise-induced lymphocyte changes and the effect of IL-6 on cortisol production is further supported by several studies demonstrating that carbohydrate loading during exercise attenuates both exercise-induced increase in circulating IL-6 and the exercise effect on lymphocyte number and function. (Nieman et al. 1997.)

The relative level of physical activity varies greatly in endurance athletes between training and competition periods. It has been suggested that female athletes are more vulnerable to physical stress than male athletes (Highet 1989; Keizer et al. 1987). Thus,

it is possible that an increased intensity of physical activity could increase the concentrations of cortisol more in females than in males. Tegelman et al. (1989) demonstrated that female athletes had higher off-season cortisol values and lower androgen values compared to sedentary controls.

4.3.4 Sex hormones

Klarlund et al. (2000) reported that testosterone influences both cellular and humoral components of the immune system. Acute, short-time exercise of high intensity and moderate exercise have been reported to increase serum testosterone (Volek et al., 1997, Zmunda et. al. 1997) where as prolonged physical activity reduces serum testosterone concentration, possibly by suppressing gonagotropin-releasing hormone secretion. The levels of testosterone might stay suppressed several hours or even days. (Lac & Berthon 2000.) Daly (2004) found the testosterone levels of the endurance athletes were suppressed still 24 hours after the maximal endurance load. This is presented in figure 8. (Daly 2004.)

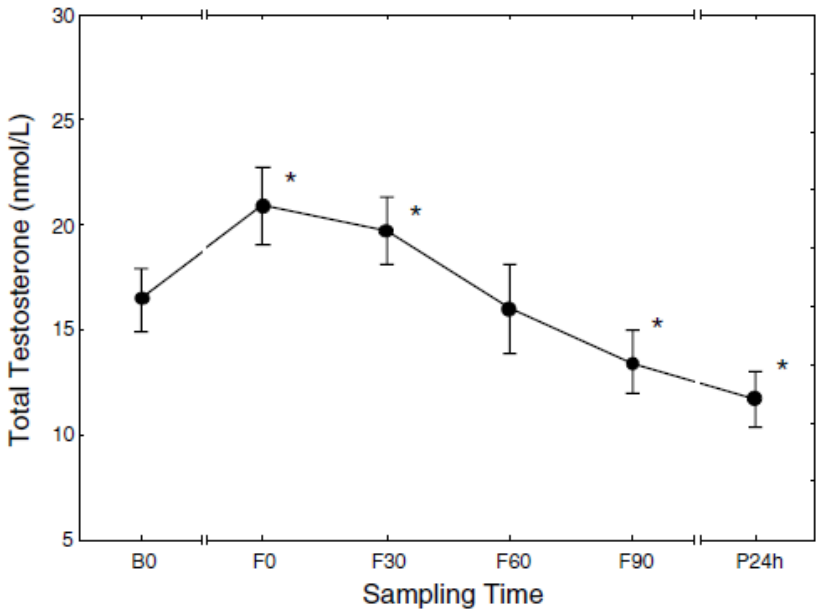

FIGURE 8. Testosterone levels before and immediately, 30 minutes, 60 minutes, 90 minutes and 24 hours after maximal endurance exercise. (Daly et al. 2004).

The role of testosterone on the immune system has been investigated in various models with conflicting results. (Smith & Myburgh 2006.) Testosterone is shown to have a stimulatory effect on both white and red blood cell counts (Ellegala et al. 2003; Palacios et al. 1983) but one the other hand, another group reported an immunosuppressive role for testosterone (Yesilova et al. 2000).

4.4 Exercise and upper-respiratory tract infections

Without doubt exercise and training influence the concentrations of immune competent cells in the circulating pool, the proportional distribution of lymphocyte subpopulations, and the function of these cells. An important question is, to what degree are these cellular changes of clinical significance, especially with respect to resistance to infectious diseases. (Pedersen & Hoffman-Goetz 2000.) Immune system is influenced by a variety of stressors that can be broadly categorized as physical (e.g. physical activity, exercise and athletic training), environmental (e.g. heat and humidity, cold conditions, altitude, air pollution), psychological (personal and psychosocial factors), and other lifestyle factors (e.g. transmission of infectious agents, cohabitation, dietary practices). (Hoffman-Goetz & Pedersen 1997.) Figure 9 summarizes the effect of stress on immunological factors which affect susceptibility to infections in athletes.

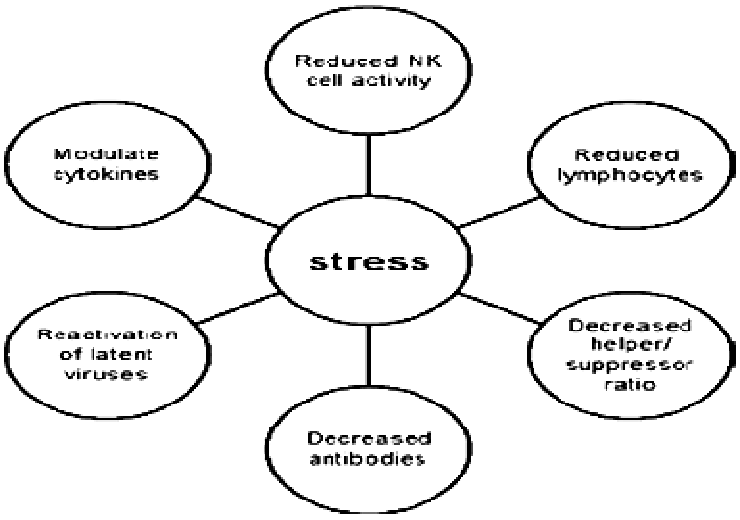

FIGURE 9. Stress has multiple effects on immune system. (Webster Marketon & Glaser 2007).

Athletes are subject to the same infections suffered by the rest of the community. However, there are also some special circumstances that may increase the susceptibility of athlete to infections. Infections of the upper and lower respiratory tracts are very common, especially during winter months. (Brukner & Khan 1993, 611-616.) One of the most common reasons for poor performance at a major sporting event is an acute respiratory tract infection (Brolinson & Elliot 2007). Viral respiratory tract infections involved are adenovirus, influenza virus, echovirus, cytomegalovirus and rhinovirus. Treatment for viral infections is usually aimed at controlling the accompanying fever and a reduction of symptoms. (Brukner & Khan 1993, 611-616.) Figure 10 collects together the factors affecting susceptibility to infection in athletes.

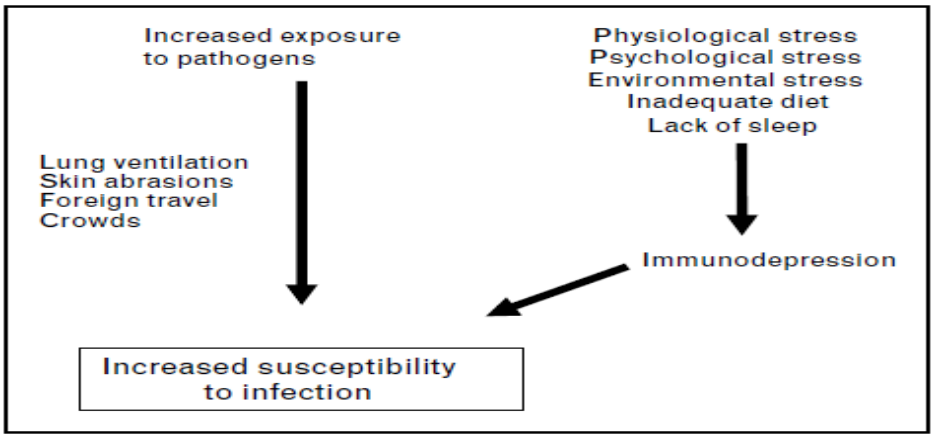

FIGURE 10. Factors affecting susceptibility to infection in athletes (Gleeson 2005).

A common perception among athletes and coaches is that heavy exercise may lower immune functions and lead to upper-respiratory tract infection. (Nieman 1997.) The relationship between physical activity and upper respiratory tract infections (URTI) has been described with “J-shaped” curve. This model suggests that the lowest risk of URTI is found among moderately active individuals, and that risk of URTI is increased for both physically inactive and highly active individuals. The J-shaped curve is presented in the figure 11. Epidemiologic data supports the contention that moderately active nonathletic individuals are at lowest risk for URTI. To date, available evidence suggests that moderate levels of activity are associated with a reduced duration of URTI episodes. Research data has shown a 29% reduction in risk of picking up upper respiratory tract infection (URTI) compared with the risk of infection associated with a sedentary lifestyle (Matthews, 2002, Nieman et al. 1993, Nieman et. al. 1998)

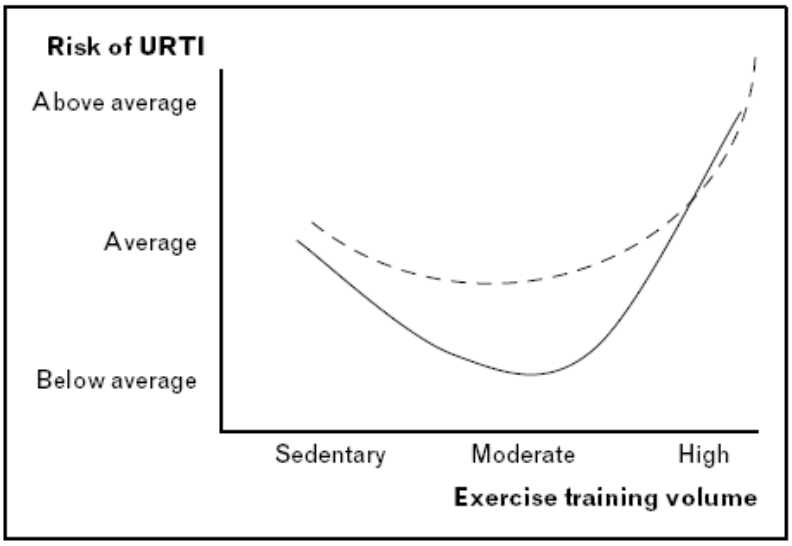

FIGURE 11. The relationship of the risk of URTI and amount of exercise (Gleeson 2007).

A common perception among athletes and coaches is that heavy exercise may lower immune functions and lead to upper-respiratory tract infection (Nieman 1997). The study of Pyne (1999) supported the link between training volume and URTI episodes during a spring-summer training and competition season. Elite swimmers had more URTI symptoms during the highest volume of training and after competitions. (Pyne 1999.) Figure 12 illustrates the results of Pyne (1999). Over the past decade, there has been considerable interest in the link between URTI and immune suppression in elite athletes. Although it has been reported that highly conditioned athletes have a higher incidence of URTI than control groups during and after events or training (Brenner 1994, Douglas & Hanson 1978; Petters & Bateman 1983.), the link with immune suppression has not been clearly established (Nieman 1994, Peters-Futre 1997).

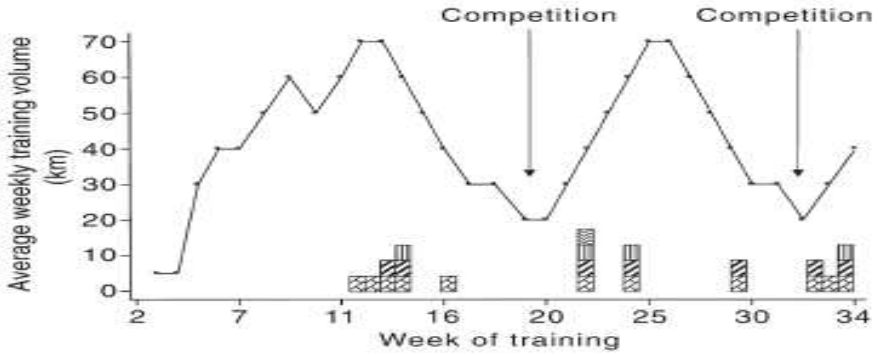

FIGURE 12. Training volume and URTI episodes during a spring-summer training and competition season for elite swimmers (*n* = 22). Each shaded block indicates an episode of URTI in a swimmer. (Pyne 1999.)

Moreira et al. (2009) reviewed the research about URTI so far. They retrieved 30 studies, comprising 4 descriptive, 18 observational and 8 randomized or controlled reports, which included a total of 8595 athletes and 1798 non-athletes. Only one study used objective URTI measurement, other used medical records or self-reported symptoms by questionnaires. Severity assessments were not performed. Other factors, like nutrition, influencing the immune system were rarely reported. The results suggested that moderate activity may enhance immune function, whereas prolonged; high-intensity exercise temporarily impairs the immune competence. Athletes suffer from higher incidence of URTI compared to the less active individuals.

It has been documented that URTIs are the illnesses to which athletes are most susceptible (Mackinnon 2000). They are the most frequently reported disability among athletes and cause more lost training days than all the other infectious diseases combined (Beck 2000; Weidner 2001). Intense exercise affects the immune system and may increase susceptibility to infection. (Nieman et. al. 1990, Fitzgerald 1991, Berglund & Henningson 1990.) Moreover, training during the incubation period of infectious diseases can aggravate the clinical course of the developing disease (Gautmanitan et al. 1970, Russell 1949). Elite endurance athletes do not have the option of training moderately on a regular basis. Instead they must go through various phases of intense training and competition to achieve their personal goals. Nonetheless several precautions can help athletes reduce their risk of URTI. Pyne et al. (2000) reviewed the factors that are influencing immune competence and provide a comprehensive set of

practical guidelines and strategies for the management of illness and infection in athletes.

4.5 Overtraining and immune functions

Many athletes incorporate high training volumes and limited recovery periods. Overreaching is defined as an accumulation of the training and/or non-training stress resulting in a short-term decrement in performance capacity. It is generally accepted that longer imbalance between training and recovery results for overtraining in which restoration of performance capacity may take several weeks or months to recover. (Kreider et. al. 1998.) Given the high volume of training and limited recovery periods often associated with overreaching and overtraining, it has been suggested that immunosuppression may occur in over trained athletes. To diagnose overtraining of the athletes, for example, testosterone/cortisol –ratio has been used. Depending on the intensity and duration of a preceding physical load, hormones with anabolic or catabolic properties, such as testosterone and cortisol respectively, show quantitative changes signalling a catabolic state (Busso et al. 1992). The ratio of the hormones is considered to reflect the states of anabolism when it is high and catabolism when it falls by 30 % or more (Maso et al. 2004). Athletes and coaches associate overtraining with frequent illness, especially URTI. One unanswered question is whether the immune suppression of the endurance athletes is result from overtraining syndrome or rather from the long-term stress of intense daily training. (Mackinnon 2000.)

4.6 Environmental factors

Although endurance athletes may be at increased infection risk during heavy training or competitive cycles, they must exercise intensively to compete successfully. Athletes appear less interested in reducing training workloads, and more receptive to ingesting drugs or nutrient supplements that have the potential to counter exercise-induced inflammation and immune alterations. There is some preliminary data that various immunomodulator drugs may afford athletes some protection against inflammation, negative immune changes, and infection during competitive cycles, but much more

research is needed before any of these can be recommended (Pizza et al., 1999; Pedersen et. al. 2003). Researchers have suggested that cold exposure may increase an organism's susceptibility to infection (Chen et al. 1993, Ben-Nathan et al. 1996.)

5 NUTRITION AND IMMUNE FUNCTIONS

Nutrition influences the development of the immune system. Nutrients are also necessary for the immune response to pathogens so that cells can divide and produce antibodies and cytokines. Many enzymes in immune cells require the presence of micronutrients, and critical roles have been defined for zinc, iron, copper, selenium, vitamins A, B6, C, and E in the maintenance of optimum immune function (Nieman and Pedersen, 2000). The diet of the athlete appears to be a further potential cause for immune suppression. Many athletes try to control their body weight by restricting the dietary intake in order to gain competitive edge. Kono (1988) demonstrated that a loss of 2 kilograms over a 2 week period had an adverse effect on the defense mechanism of even healthy athletes. According to Kono (1988) the further energy restriction might lead to extensive dysfunction of immune system. The influence of many nutritional supplements on the immune and infection response to intense and prolonged exercise has been assessed (Nieman and Pedersen, 2000). Supplements studied thus far include zinc, dietary fat, plant sterols, antioxidants (e.g., vitamins C and E, (B-carotene, N-acetylcysteine, and butylated hydroxyanisole), glutamine, and carbohydrate. (Nieman 2001.) As well as the deficiencies, also the excessive intakes of individual micronutrients (e.g. n-3 unsaturated fatty acids, iron, zinc, vitamin A and E) can increase the risk of infection (Chandra 1997).

5.1 Carbohydrates and immune response

Athletes in endurance events attempt to boost their muscle and liver glycogen stores by providing 60 % or more of the energy in the form of carbohydrates. (Shephard 1997.) Research has established that a reduction in blood glucose levels is linked to HPA-axis activation and this way to increased release of adrenocorticotrophic hormone and cortisol and variable effect on blood epinephrine levels (Murray et al. 1991). It has been proposed that elevated carbohydrate supplementation increases plasma glucose, decreases stress hormone release and this way reduces immune stress (Nieman 1997).

5.2 Lipids and immune response

Relatively little is known about the potential contribution of dietary fatty acids to the regulation of exercise induced modification of immune function (Gleeson et al. 2003). Lipids are powerful mediators of the immune system, and they are known to exert their effect on cytokines, hormones, etc. It is important that athletes involved in intense training receive nutritionally correct and well balanced diet. As intensive training can be very demanding all athletes need sufficient number of calories to meet their energy requirements. Low dietary fat has been related to hormone irregularities, such as amenorrhea. It is clear that diets under 20% of fat intake are insufficient to maintain normal immune functions. (Venkatraman et al. 2000.)

Two groups of polyunsaturated fatty acids (PUFA) are essential to the body as they cannot be synthesized in the body and therefore must be derived from the diet: the omega-6 (n-6) series, derived from linoleic acid, and the omega-3 (n-3) series, derived from alfalinolenic acid. Calder (1996) reported that diets rich in either of these polyunsaturated fatty acids improve the conditions of patients suffering from diseases characterized by an over-active immune system, such as rheumatoid arthritis. That might be because n-6 and n-3 have anti-inflammatory effects (Calder, 1996; 2001). Polyunsaturated fats are necessary for T-cell functions, and a deficiency of essential fatty acids leads to deterioration of immune responses. (De Wille et. al. 1979.) Excessive intake of omega-3 fatty acids may also suppress immune functions, both neutrophil and monocyte functions with decreased secretion of IL-2, IL-1 and TNF-alpha (Johnston 1988, Maki et. al. 1992).

The sources of the energy (fat, carbohydrate, and protein) should meet the energy expenditure. Failure to accomplish this results in depletion of intramuscular stores of glycogen and fats and reduces athlete’s performance. It is clear that diets containing less than 20% of fat are insufficient to maintain intramuscular stores. Fat intake might be particularly important in female athletes for whom low-caloric and low-fat intake diets are associated with amenorrhea. (Venkatraman 2000.) Figure 13 summarizes how the lipids might modulate the exercise-induced effects on the immune system.

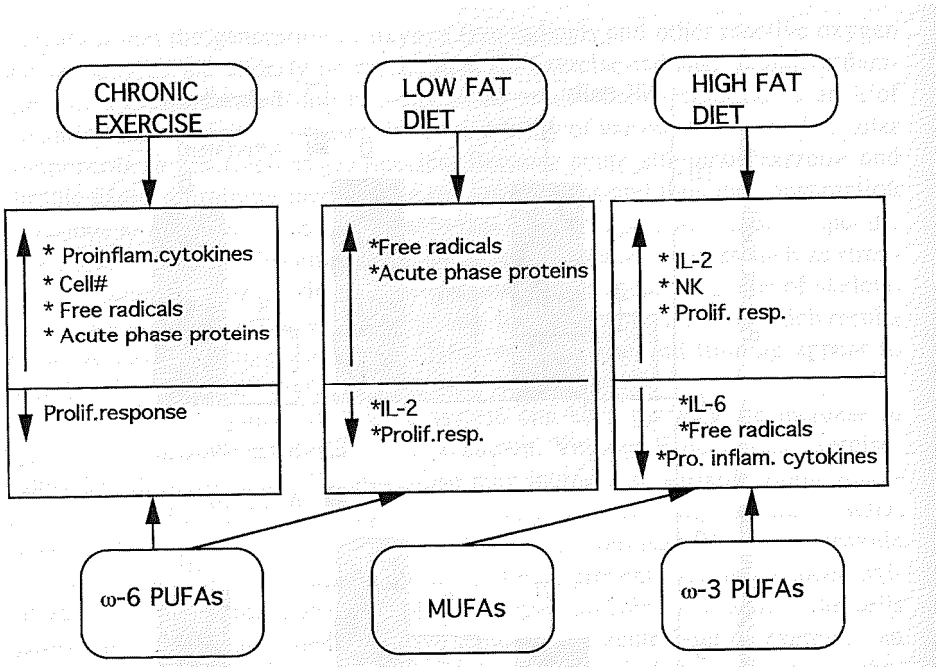

FIGURE 13. Summary of how dietary lipids may modulate the exercise–induced effects on the immune system (modified from Venkatraman 2000). MUFA=monounsaturated fatty acid, PUFA=Polyunsaturated fatty acid.

5.3 Protein and immune response

Protein deficiency is observed to interfere with resistance to infections because most of the immune mechanism is dependent on cell replication or production of active protein compounds. The available literature suggests that the lack of protein has no effect on immune system until decrease in serum albumin concentration occurs. (Rowbottom 2000.) The recommendations for protein intake differ between sedentary people and athletes. WHO recommendation for ordinary people is 0.8 g protein/body weight/day. There is catabolism, even in sedentary individuals. The physical activity increases the protein catabolism and increases the protein requirement, which is why endurance athletes require 1.2-1.6 g protein/bodyweight and athletes undertaking strength training 1.6-2.0 g protein/kg body (Ziegenfuss et al. 2010, Lemon 1998, Lemon et. al. 1992). Infection also affects requirements of protein intake. The average loss of protein during infection is 0,6g/bodyweigth (Rowbottom 2000). The optimal protein intake can reduce catabolic effects of training, which can cause depressed immune function by decreasing the number of immunological proteins in blood (Taimasoz et al. 2003, Olejnika &

Gununoi 2008). Many studies have used serum concentrations of albumin as a quantitative indicator of the degree of malnutrition (Rowbottom 2000).

Glutamine is a neutral glycogenic, non-essential and most abundant amino acid in the human body. Cells utilize glutamine at high rate and only small amounts enter the circulation. (Felig 1975.) Glutamine is an important source of energy for lymphocytes and macrophages (Ardawi & Newsholm 1983; Antonio & Street 1999). Exercise affects plasma glutamine concentrations depending on the type, duration and intensity of exercise (Rohde et. al. 2000). It has been hypothesized that during physical exercise, the demand of muscle or other organs for glutamine is so high that the immune system may suffer from lack of glutamine (Rohde et. al. 2000). Infections decrease plasma glutamine concentration (Rowbottom et. all 1996). It remains to be shown if glutamine supplementation has an effect on immune system (Rohde et. al. 2000).

5.4 Vitamins, Minerals and Immune response

Exercise can produce an imbalance between the exercise induced increase in the amount of free radicals and reactive oxygen species (ROS) and the nutrition and exercise-induced immune depression of antioxidant defense system. The antioxidant defense mechanism prevents the generation of ROS or intercepts any that is formed. The dose–response between vitamin intake and immune stimulatory effect is not linear. In fact, excess doses of vitamins may even impair immune function in athletes. (Prasad, 1980.)

Vitamins and minerals are essential nutrients for optimal performance. Deficiencies impair general health and human functions. In modern society vitamins deficiencies are quite rare. Cross-country skiers have quite a high energy intake. The amount of nutrient intake mainly follows energy intake. So when the athlete consumes ‘normal’ food there is a general agreement that the risk of an inadequate nutrient is low. (Ekblom & Bry 2000.)

Several vitamins are essential for normal immune function. Deficiencies of fat-soluble vitamins A and E and water-soluble vitamins folic acid, B6, B12 and C impair immune function and decrease the body’s resistance to infection (Calder and Jackson, 2000;

Calder et al., 2002). Specific vitamin supplements can be effective in restoring immune functions to normal (Calder and Jackson, 2000). Consuming mega doses of individual vitamins, which appears to be a common practice in athletes, can impair immune function and have other toxic effects (Calder et al., 2002) Peters et al. (1993) studied the affect of vitamin C supplementation (600 mg) to upper-respiratory tract symptoms after participation in a competitive ultra marathon race. Runners in the placebo group reported significantly more URTI symptoms than C-supplemented runners. This study provided evidence that vitamin C supplementation might enhance the resistance to post race upper-respiratory tract infections.

Several minerals are known to affect immune function. These minerals include zinc, iron, magnesium, manganese, selenium and copper, yet with the exception of zinc and iron, isolated deficiencies are rare. Exercise has a pronounced effect on both zinc and iron metabolism (Gleeson & Bishop, 2000). Mineral requirements are higher in athletes than sedentary individuals because of the increased loss in sweat and urine. However, excesses of some minerals (particularly iron and zinc) can impair immune function and increase susceptibility to infection. Hence, supplements should be taken only as required and regular monitoring of iron status (serum ferritin and blood hemoglobin) and zinc status (erythrocyte zinc) is probably a good idea (Gleeson & Bishop, 2000).

6 PURPOSE OF THE STUDY AND HYPOTHESES

The stress related to the training and competing has been proposed to have immunosuppressant effect which lead to the upper-respiratory tract infections. As one of the most common reasons for poor performance at a major sport event is an acute respiratory infection. (Malm 2004.) It is important to recognize the effect of nutrition and training for the endocrine-immunological system of the athletes. The purpose of this study was to determine the total load of competition period on female cross-country skiers' immunological variables and on the body's endocrinal regulation mechanisms. Both chronic effect followed by training and the recovery of acute effect followed by competition were investigated. The results of the study may be used when programming the training and nutrition of the athletes in the competition period to avoid immunological problems and upper-respiratory tract infections. The study questions and hypotheses have been presented as follows:

1. **Are the athletes more susceptible to upper-respiratory tract infections than the controls?**

H1: Athletes are more susceptible to infections compared to the control group

Research data has shown a reduction of 29-40% the risk for upper respiratory tract infection compared with infection risk associated with a sedentary lifestyle (Matthews et al., 2002, Nieman et al. 2001)

2. **Is there a difference in immunological and endocrinal state between the athletes and the controls? Is there a difference in immunological state of the athletes between pre-competition phase, main competition phase and transition phases?**

H2: White blood cell levels are lower in the athletes than in the controls during the competition period because of the suppressant effect of training and competing.

Gleeson et al. (1995), Mujika et al. (1996) and Gray et al. (1993) have found no significant differences between the athletes and the controls in lymphocyte counts. Although neutrophil number appears to be within the clinically normal range in the athletes, several studies have observed lower neutrophil count and functional capacity in the athletes compared to the control subjects. (Robson et al.1996, Smith 1990.)

H3: The athletes have lower basal level of immunoglobulins A and G than the controls and the levels are reduced after the competition.

As early as 1982, Tomasi et al. reported that salivary IgA levels were lower in elite cross-country skiers, compared with recreational athletes. Gleeson et al. (1995, 2005) found significant suppression of resting serum IgA, IgG and IgM concentration in athletes, associated with long-term training at an intensive level. Resting s-IgA concentrations in the beginning of the training period showed a significant negative correlation coefficient with infection rates, and the number of infections observed in the swimmers was predicted by the preseason and mean pre-training s-IgA levels.

H4: Salivary cortisol is affected by cross-country skiing competition and the basal level of cortisol is higher in the athletes than in the controls.

Cortisol levels are affected by long duration exercise stimulus (Galbo 1983). Tegelman et al. (1989) demonstrated that the female athletes had higher off-seasonal cortisol values than the sedentary controls.

H5: The testosterone levels and the testosterone-cortisol ratio are lower in the athletes than in the controls and the testosterone-cortisol levels are related to white blood cell levels.

Endurance training has been shown to reduce the level of testosterone. (Volek et. al 1997.) Testosterone is shown to have a stimulatory effect on both the white and red blood cell counts (Ellegala et al. 2003, Palacios et al 1983.)

3. **What is the immunological recovery state of athletes 20-40 hours after the competition?**

H6: The total white blood cell count, neutrophil, mixed size cells and lymphocytes are returned to basal level in the 40 hours group (the athletes that competed on Sunday) but not in the 20 hours group (the athletes that competed on Saturday). Neutrophil count is reduced in the 40 hours' group.

Leukocyte number may increase related to physical exertion, but returns to the basal level within hours. Monocyte and neutrophil counts are returned to basal level in six hours. Malm (2004) has found that leukocyte count was reduced 24 hours after loading when compared to basal levels. Mars et al. (1998) found that the level of lymphocytes was also reduced after loading. Peake et al. (2005) found that the neutrophil count increased to basal level 24 hours after loading. Malm (2004) has also found that neutrophil count was reduced 24 hours after loading compared to the basal level.

H7: The immunoglobulin levels are reduced after the competition and stay suppressed up to 40 hours.

Tomasi et al. (1982) found that immunoglobulin levels decrease after skiing competition. Many researchers have found that saliva immunoglobulin levels decreased significantly after physical activity (Nieman et al. 2002, Ronsen et al. 2004). The ratio between immunoglobulin A and T-protein have also found to decrease (Moreira et al. 2009).

4. **Do nutrition, body composition and training play a role in immunological variation?**

H8: The total caloric intake, fat intake, Vitamin C, zinc, iron and seleen have effects on immunological variables and URTI-symptoms.

Several vitamins are essential for normal immune function. Deficiencies of fat-soluble vitamins A and E and water-soluble vitamins folic acid, B6, B12 and C impair immune

function and decrease the body's resistance to infection (Calder and Jackson, 2000; Calder et al., 2002).

H9: The volume of total training and the volume of maximal intensity training correlate with immunological variables and with URTI-symptoms.

The infections of the athletes are very common, especially in winter months (Brukner & Khan 1993). The J-shaped curve between training and URTI-symptoms have been proposed (Nieman 1997). It has been shown that the athletes are more vulnerable to URTI symptoms and the stress related to training and competing has been proposed as the reason. (Brenner 1994, Douglas & Hanson 1978, Petters 1983.)

7 MATERIALS AND METHODS

7.1 Study design

This study consists of two measurements during the competitive phase and basal level measurements after transition phase. Measurements included basal level morning's blood and saliva samples in a fasting state, body composition measurements and counter movement jump (CMJ). On weekend the athletes took part in competitions and recovery measurements took place on Monday morning in a laboratory of the Department of Biology of Physical Activity. Saliva samples were also taken in the competition morning and right after finishing the race. The first set of measurements was in the early competition phase in December, second in the end of January related to athletes' main competition (Finnish Championships) and third basal level measurements in May. Each set of measurements included also health questionnaire, self reported URTI symptoms and food diaries. The training load was estimated using volume and intensity of training. Body composition was measured in January and in May.

7.2 Subjects

Ten healthy national level female cross-country skiers served as the subjects for the study. Group of ten non-athlete moderately active females served as their controls. Each subject was informed of the potential risks and discomforts associated with the measurements, and all the subjects gave their written informed consent to participate. The Ethics Committee of the University of Jyväskylä approved the study. Anthropometric details of the subjects are presented in Table 3.

TABLE 3. Descriptive (mead and SD) characteristics of the anthropometric details of the subjects and their training load. (*= training of the control group was reported only for 25 weeks, on the average the control group exercised 3 hours per week (n=9), SMM=skeletal muscle mass)

	n	Age (years)	Body mass (kg)	Height (cm)	Fat-%	SMM (kg)	Hb (g/l)	Yearly training (h)	Finnish rank.
Athletes	10	23.6 (2.8)	60.4 (6.2)	167 (5.6)	19.9 (4.0)	28.0 (3.0)	136 (7.2)	663 (101)	44 (25.8)
Controls	10	24.8 (3.2)	58.5 (6.2)	165 (5.0)	27.2 (5.4)	24.7 (2.7)	135± (9.2)	*	no

Figure 14 presents the annual training volume of the group of the athletes. In all the three measurement sets, the athletes were measured on Friday between 7am and 9:30 am. All the athletes were not able to participate to the same competition. In the first measurements, seven of the athletes had only one national level competition during weekend and they were measured 40 hours after finishing the race. Three of the athletes had two competitions and were measured 20 hours after finishing the second race. In the second competition measurements which took place at the end of January in Finnish championships, seven of the athletes were able to participate in two races. After the second competition weekend all the seven athletes were measured 20 hours after finishing the race, while three athletes were measured 40 hours after finishing the race. When the differences between the basal level and the recovery level (on Monday) measurements are discussed, the athletes who had the same immunological recovery time (20 or 40 hours) are considered as two separate groups.

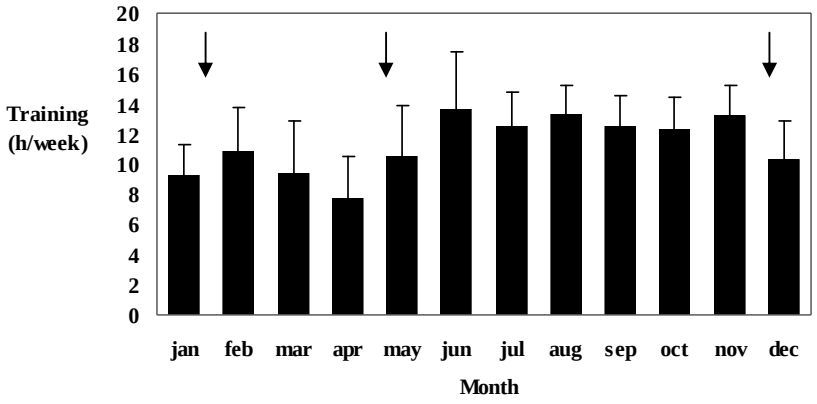

FIGURE 14. Training of the athletes and measurement points.↓=measurements.

7.3 Data collection and analysis

7.3.1 Blood samples

The blood samples were drawn into K2-EDTA-tubes (Terumo Medical Co., Leuven, Belgium) from the antecubital vein using 21-gauge disposable needles. In all the measurements the samples were taken in standardized fasting state (i.e. 12 hours overnight fast) in a sitting position. The cycle phase of the menstrual cycle was not controlled. The samples were centrifuged 3500 rpm for 10 minutes. The serum was divided into three Eppendorf-tubes and kept frozen at –80°C until assayed.

Whole blood samples were analyzed within 30 min. Total and differential white blood cells (WBC), platelets and hemoglobin and hematocrit were determined with Sysmex KX-21N (TOA Medical Electronics Co., Ltd., Kobe, Japan). Of WBC, neutrophils, lymphocytes and mixed cells (monocytes, eosinophils, basophils and immature precursor cells) were analyzed. The coefficient of variation (CV %) for leukocytes was 2.2 % and for platelets, hemoglobin and hematocrit 4.0 %, 1.5 % and 2.0 %, respectively.

Serum cortisol, testosterone, sex-hormone-binding globulin (SHBG) and insulin concentrations were analyzed by an immunometric chemiluminence method with an Immunite R 1000 (DPC, Los Angeles, USA) The sensitivity of the assay for cortisol is 5,5 nmol/L and CV 7,4%, for testosterone 0,5 nmol/l and CV of 5,7%, for SHBG 0,2 nmol/l and 2,4 %, and for insulin 2mIU/L and 3,4% respectively. Free testosterone was calculated from total testosterone and SHBG concentration (Vermeulen 1999). The data presented is not corrected for plasma volume changes. Serum IGF-I was analyzed in duplicate with an OCTEIA IGF-I kit, which is a two-site immunoenzymometric assay for the quantitative determination of IGF-I in human serum. The method incorporates a sample pretreatment to avoid interference from binding proteins. The absolute sensitivity of the kit, defined as the concentration corresponding to the mean + 2 SDs of 20 replicates of the zero calibrator is 0.25 nmol/l. The functional sensitivity, defined as the concentration at which the coefficient of variation falls <10% and is 1.2 nmol/l. Serum IgA and IgG and saliva IgA were analyzed with clinical chemistry analyzer

KONE Specific Supra. The methods are based on measurement of immunoprecipitation enhanced by polyethylene glycol at 340 nm. Specific antiserum is added in excess to buffered samples. The increase in absorbance caused by immunoprecipitation is recorded when the reaction has reached its end point. The change in absorbance is proportional to the amount of antigen in the solution.Serum Albumin was analyzed by Konelab 20 XTi (Thermo Fisher Scientific, Finland). Precision for T-protein was between day saliva 410 mg/l and coefficient of variation was 3.6%.

7.3.2 Saliva samples

The subjects gave fasting saliva samples during the Friday and Monday measurements in the laboratory. They collected saliva samples independently in the competition morning and after the competition. Saliva samples were taken with a cotton-chew salivette which was chewed for 1–2 minutes. The time points for sampling were as follows:

- on Friday in the laboratory (approximately 30 minutes after the wake-up)
- 30 minutes after the wake-up in the competition morning before the first normal distance competition of the weekend
- one minute after finishing the competition
- on Monday morning in the laboratory

Saliva cortisol level analyses were analyzed with Immulite 1000. Normal cortisol values for adults measured in saliva are in the morning 13.8–48.9 nmol/l and eight hours after the peak it decreases to 1.4–8.6 nmol/l. The sensitivity of the method was 0.15 ng/ml, intra-assay precision was 4.6% of coefficient of variation and between days the CV precision was 15.3 %. The saliva samples for IgA-levels were analyzed with Konelab 20 XTi (Thermo Fisher Scientific, Vantaa Finland). The coefficient of variation precision for Sa-IgA was 5.0%.

7.3.3 Questionnaires

Health questionnaires. Information on living habits and health status such as injuries, known diseases, medication, diet, menstrual cycle and the consumption of cigarettes or alcohol was obtained with detailed questionnaires.

Volume of training. Subjects were training and preparing for competitions normally, according to their own training programs. They filled in their own training diaries normally. The yearly training volumes were calculated from the training diary. The quality and quantity of training during measurement week was reported more in detail with a questionnaire.

Upper-respiratory tract infections symptoms. The amount and severity of URTI-symptoms was evaluated by using the short version of The Wisconsin Upper Respiratory Symptom Survey (WURSS-21). WURSS-21 is an evaluative illness-specific quality of life instrument, designed to assess the negative impact of the common cold. (Appendix 1)

Dietary intake. The dietary intake of all subjects was registered with dietary diaries for five days, 3 days before the Friday measurements and during the competition weekend. The diaries were analyzed using the Micro Nutrica nutrient-analysis version 3.0 (The Social Insurance Institution of Finland).

7.3.4 Anthropometry

After an overnight fasting, body mass (kg), fat percentage and lean mass were measured by DEXA device (Prodigy, USA). Norcross & Van Loan (2004) examined the validity of DEXA device (Prodigy) against two and three compartment models in normal weighed men and women. In this study, analysis of variance (ANOVA) indicated no significant differences in % body fat (%BF) between the three methods. However, Bland-Altman plots showed a trend for DEXA measurements to overestimate %BF compared with the reference methods. Thus, the %BF values measured by DEXA were 0.68-1.45 % higher than body fat-% values measured by the reference methods. (Norcross & Van Loan 2004.) Williams et al. (2006) showed that DEXA (Lunar

Prodigy, USA) compared with four component model produced absolute biases ranging from -1.97 kg (obese women) to 0.56 (non-obese boys). Aasen et al. (2006) have showed that the reproducibility of lean mass measurement is good, since the coefficient of variation was 0.7% for lean mass.

7.4 Statistical analyses

Results are presented as average ± SD. Statistical analyses were performed with PAWS Statistics 18.0 for Windows (SPSS, Inc., Chicago, IL). All the measured parameters were not normally distributed, and sample sizes were relatively small. Therefore statistical analyses were performed with the non-parametric tests. Differences between study groups were tested with the Mann Whitney U -test. Control measurements at rest were compared with the Wilcoxon signed ranks test. Correlation coefficients were calculated with the Spearman's correlation coefficients. Level of significance was set at $p<0.05$. In the figures and tables, star symbols are used to express statistical significance as follows: $p \leq 0.05$ = *, $p \leq 0.01$ = **, and $p \leq 0.001$ ***.

8 RESULTS

8.1 Basal measurements

8.1.1 Incidence of upper-respiratory tract symptoms

The upper-respiratory tract infection (URTI)-symptoms were the most common reason for athletes to miss training. The athletes missed 19 (±12) training days during the training and competition season 2009-2010. The individual URTI symptom map and the number of missed training days are presented in figure 15. Upper-respiratory tract symptoms before and after the competition in both measurements and in total are presented in table 4. There were no significant differences in URTI symptoms before and after the competition in the first, the second or the third measurements. Five of the ten athletes had upper-respiratory tract symptoms related to the competition weekend when only one of the athletes had URTI symptoms in transition phase measurement. All the symptoms during three sets of measurements (6 weeks) had a significant positive correlation ($R=0.82$; $p<0.05$) with the days the athletes were unable to participate to training during the whole year because of upper-respiratory tract symptoms.

TABLE 4. Upper-respiratory tract symptoms before (pre), after (post) the competitions (early competition period, main competition period), related to the transition phase measurements and in total and the percent of the athletes who had some upper-respiratory tract symptoms in different measurement points (mean ± SD).

	Early comp. (n=10)	**Symptoms (%) (n=10)**	**Main comp. (n=10)**	**Symptoms (%) (n=10)**	**Transition phase (n=10)**	**Symptoms (%) (n=10)**
pre URTI	21 ±27	50	17 ±23	50	5 ±14.9	10
post URTI	16 ±37	50	20 ±47	30	2 ±6.0	10
total URTI	37 ±60	50	37 ±55	60	7 ±23.1	10

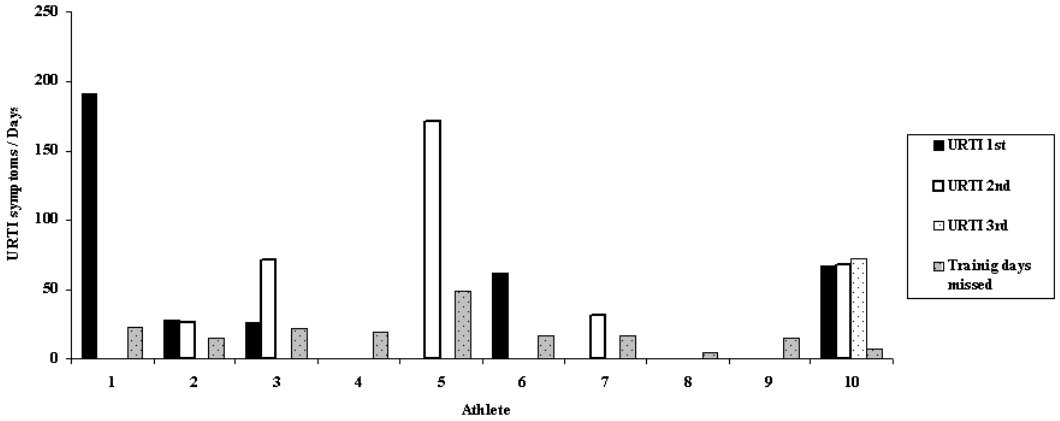

FIGURE 15. Individual upper-respiratory tract symptoms (14 days) in different measurements and the total amount of days which the athletes were unable to train because of the upper-respiratory tract symptoms.

Figure 16 illustrates the total number of days that athletes and controls had URTI symtoms. There was a significant difference ($p<0.05$) between the control group and athletes group during the main competition period measurements in the amount of URTI-symptoms related to the competition weekend. This is presented in the figure 17. No significant differences were found related to the first and the third measurement or in the total amount of URTI symptoms. There were no significant changes in the URTI symptoms between the first and the second ($p=0.730$), between the first and the third ($p=0.080$) and between the second and the third ($p=0.080$) measurements.

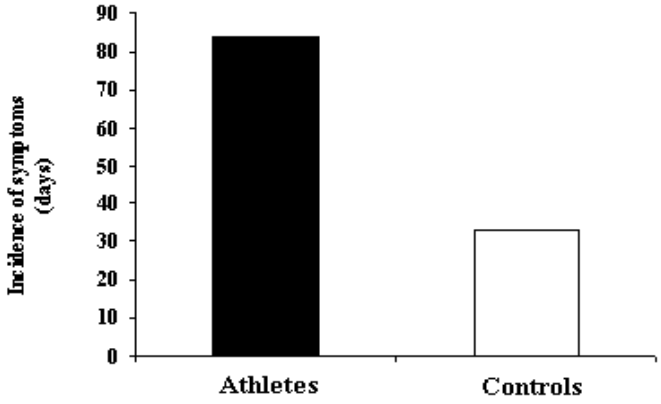

FIGURE 16. The total number of days that athletes (n=10) and controls (n=10) as a group had URTI symptoms.

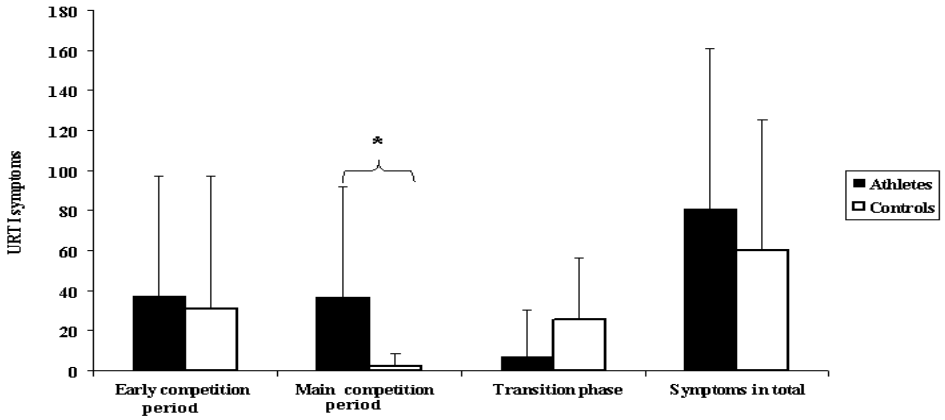

FIGURE 17. URTI-symptoms (mean ± SD) reported in total seven days before and seven days after the Friday measurements ($p<0.05$) and the sum of all the symptoms related to three measurements.

8.1.2 Blood leukocytes

There was a significant difference between the Athletes group and the Control group in neutrophil concentration in the third measurement ($p<0.05$) so that Control group had higher neutrophil concentrations between the measurements in blood leukocytes and blood leukocyte subgroups. Other significant changes were not found, either in the athletes group or in the control group. The trend was that the athletes had higher white blood cell content in the competition period but the control group had higher white blood cell content in the transition phase. The blood leukocyte count in early and main competition period and in transition phase is presented in figure 18.

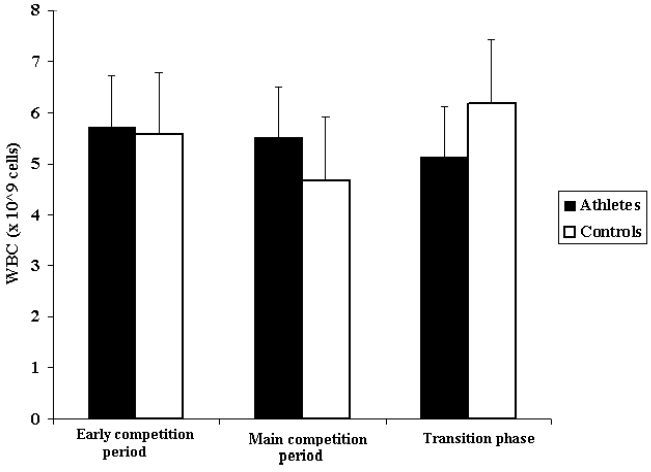

FIGURE 18. Leukocyte counts in athletes and control group in the early- and main competition period and transition phase. (Difference between athletes and controls: 1st measurement p=.806, 2nd measurement p=0.14, 3rd measurement p=0.06).

There were significant differences between the groups considering the changes between the main competition period measurements and transition phase measurements. The significant differences in absolute cell count changes where found in white blood cells ($p<0.01$) and neutrophils ($p<0.01$). The changes in blood cell counts are presented in figure 19. The complete results considering blood leukocytes and subgroups in basal levels are presented in Appendix 2.

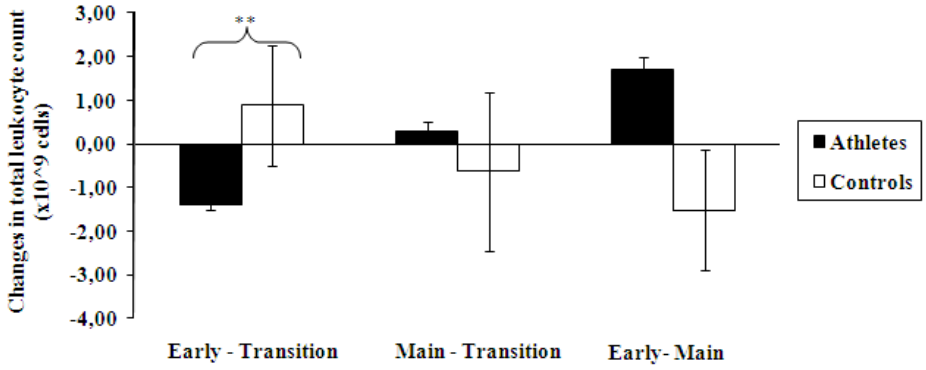

FIGURE 19. Changes (mean ± SD) in total number of leukocytes between a) the first and the third ($p<0.01$) b) the second and third and c) the first and second measurement.

There was a significant correlation between total number of blood leukocytes and testosterone-cortisol ratio in the early competition period ($R=0.810$; $p<0.01$) and in the main competition period ($R=0.879$, $p<0.05$) but not in the transition phase measurements ($p=0.402$). These results are presented in figure 20. Blood leukocyte concentration also correlated significantly with testosterone in the competition period (first measurement ($R=0.750$; $p<0.05$) and the second measurement ($R=0.681$; $p<0.05$), but not in the transition phase ($p=0.347$)

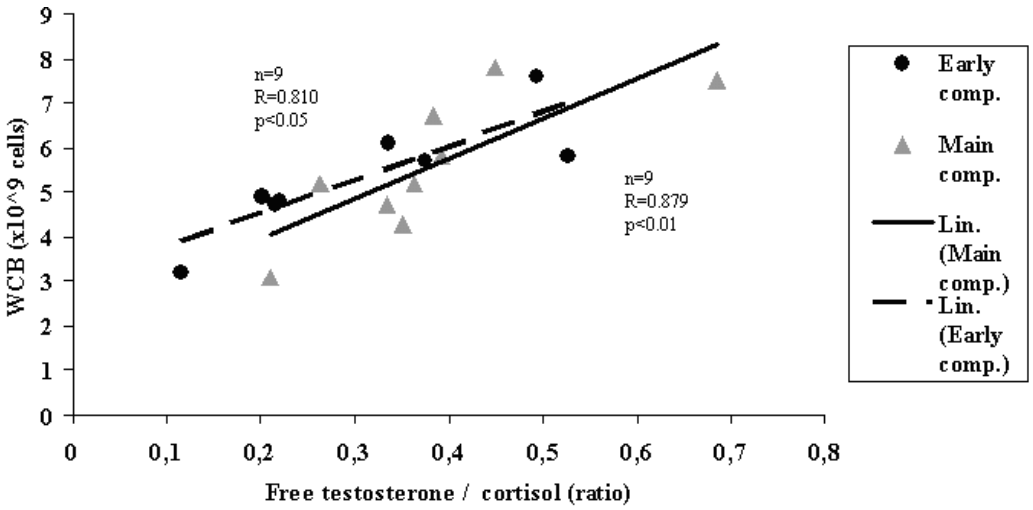

FIGURE 20. Correlation coefficient between white blood cells and testosterone-cortisol ratio was significant in the first (R=0.810; p<0.05) and the second (R=0.879; p<0.01) measurements in athletes. No significant correlation was found in control group.

When considering the leukocyte subpopulations (lymphocytes, mixed size cells and neutrophiles), there were significant correlation coefficients between testosterone-cortisol ratio and lymphocytes in the first (R=0.812; p<0.01), mixed size cells in the first (R=0.797; p<0.01) and second (R=0.763; p<0.05) and neutrophiles in the second (R=0.800; p<0.01) measurement. No significant differences were found between testosterone-cortisol ratio and blood leukocyte count or any blood leukocyte subgroups in the transition phase measurement.

Creatine kinase levels in blood correlated highly negatively with the total number of white blood cells (R=-0.809; p<0.01) and with neutrophil count (R=-0.770; p<0.01) in athletes in the transition phase. This was not found in the competition period. The total cholesterol level correlated also with the total amount of white blood cells in the early competition (R=-0,718, p<0.01) and in the main competition measurements (R=-0.802; p<0.05) in athletes.

8.1.3 Salivary and Serum Immunoglobulins

Immunoglobulin levels during different training phases are presented in table 5. There was significant difference between the athletes (n=10) and control group (n=10) in serum albumin level so that it was higher in control group compared to the athletes

group in the transition phase measurement ($p<0.01$). Also the salivary IgA/Tprotein -ratio was significantly lower in athletes than in controls in the main competition period ($p<0.05$).

In athletes group serum IgA levels were significantly lower in competition period measurements than in transition phase. Immunoglobulin levels in athletes and controls and differences between groups are presented in table 5.

TABLE 5. Mean (+SD) serum and saliva immunoglobulin and serum albumin levels in both groups at different measurement points.

	Early competition period			**Main competition period**			**Transition phase**		
	Athletes (n=10)	**Controls (n=10)**	**p**	**Athletes (n=10)**	**Controls (n=10)**	**p**	**Athletes (n=10)**	**Controls (n=10)**	**p**
S-IgA (g/l)	3.00 (2.58)	2.40 (1.03)	0.71	3.02 (2.65)	2.41 (1.10)	0.71	3.05 (2.70)	2.32 (1.00)	0.76
Sa-IgA (mg/l)	84.8 (30.7)	80.7 (45.4)	0.32	74.1 (33.7)	96.7 (55.0)	0.13	106 (60.6)	124 (108)	0.92
Sa-Tprot (mg/l)	425 (137)	452 (362)	0.41	407 (225)	365 (188)	0.71	461 (157)	586 (337)	0.71
Sa-IgA/ Tprot (ratio)	0.21 (0.05)	0.21 (0.11)	0.82	0.21 (0.08)	0.29 (0.13)	0.04*	0.23 (0.11)	0.22 (0.11)	0.57
S-IgG (g/l)	10.5 (3.27)	12.2 (2.10)	0.35	10.4 (3.26)	11.6 (2.40)	0.71	10.8 (3.49)	12.1 (1.90)	0.51
S-Albumin (mg/l)	39.8 (3.45)	42.4 (3.43)	0.06	39.1 (2.17)	39.8 (3.05)	0.85	39.4 (3.00)	43.6 (3.70)	0.01 **

The salivary Immunoglobulin A in different measurements and in both groups is presented in the figure 21. The athletes had significantly lower salivary IgA-levels in the competition period than in transition phase ($p<0.05$). No significant differences were found either in the control group or between the groups. When considering the basal IgA/T-protein-ratio there was a significant difference ($p<0.05$) between the athletes and the controls in main competition phase but not in early competition phase or transition phase. This is presented in the figure 22.

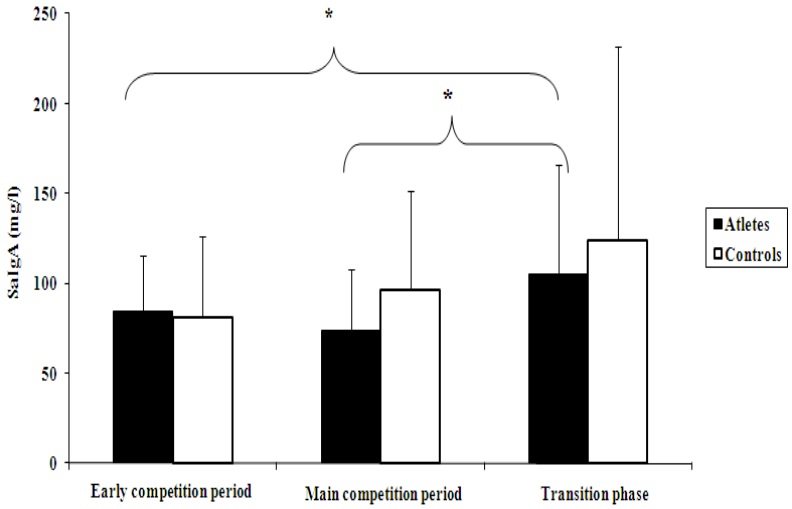

FIGURE 21. Differences (mean ±SD) in salivary IgA-levels in early (1) and main (2) competition period and transition phase (3) (*= $p<0.05$).

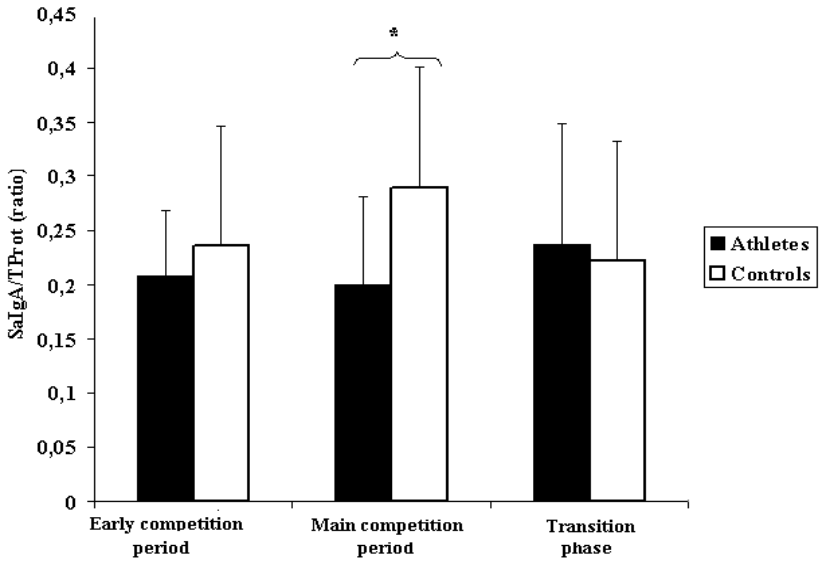

FIGURE 22. Controls had significantly ($p<0.05$) higher serum IgA levels in main competition period (n=8) but not in early competition period or transition phase.

In serum Immunoglobulin G levels there were no significant differences either between groups or in of the groups between the different measurements but there was less variation in the athletes group and that they had lower values in every measurement. Immunoglobulin G levels are presented in figure 23.

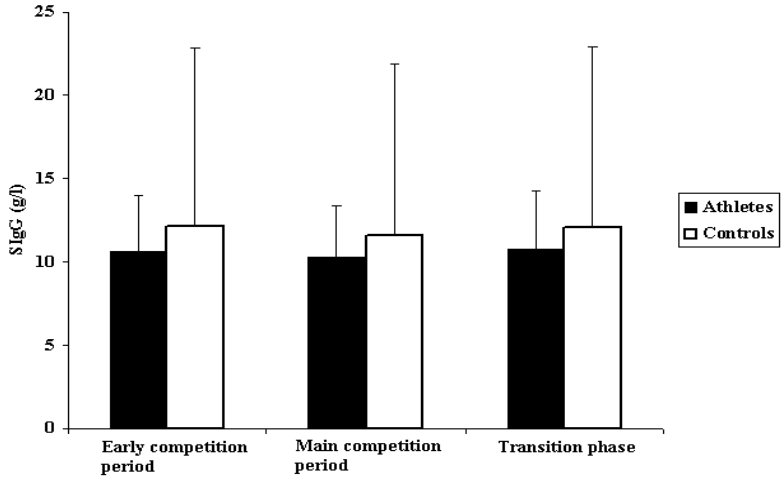

FIGURE 23. Serum immunoglobulin G levels in early (1), main (2) competition period and transition phase (3).

There were significant correlations between the basal measurements average serum IgA($R=-0.820$, $p<0.05$) and serum IgG ($R=-0.857$; $p<0.01$) levels to the days that athletes were unable to participate to the trainings. The relation of serum IgG level and the missed training days is presented in figure 24. The average serum IgA and IgG-levels also correlated significantly with the URTI-symptoms in the main competition measurements.

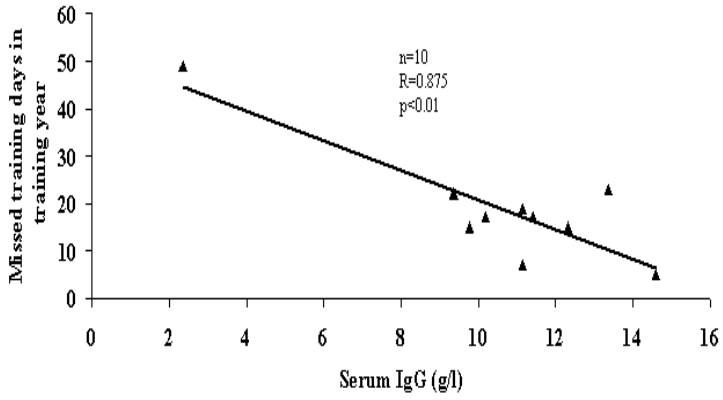

FIGURE 24. Correlation coefficient ($R=0.875$, $p<0.01$) between the average IgG-levels and the missed training days in athletes.

8.1.4 Hormones

Figure 25 presents the differences in testosterone/cortisol-ratio in the different measurements and between the athletes (n=9) and control group (n=10). No significant changes were found between the measurements or between the groups.

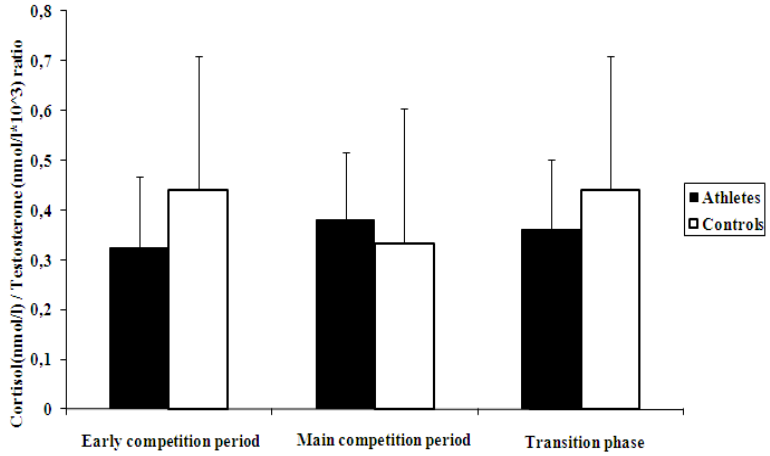

FIGURE 25. No significant seasonal variation of testosterone/cortisol –ratios (mean ± SD) in the athletes and in the controls.

There was a significant positive correlation between the upper-respiratory tract symptoms and salivary cortisol levels both in Friday measurements ($p<0.01$) and Monday measurements ($p<0.05$) when the athletes without symptoms were excluded. There was a significant difference between the control group and the athletes in IGF-1 levels in the early competition period ($p<0.05$) and the main competition phase ($p<0.05$) but not in the transition phase. This is presented in Figure 26. There was also a significant difference in the athletes group between the first and the third ($p<0.05$) and the second and the third ($p<0.05$) measurements.

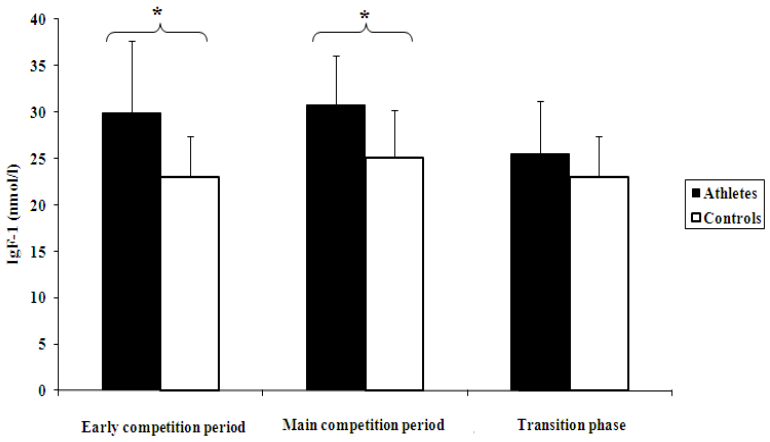

FIGURE 26. Seasonal variation in IgF-1 levels in athletes and in controls in early ($p<0.05$), main ($p<0.05$) competition period and transition phase.

No significant differences where found between the immunological parameter (blood leukocytes, Immunoglobulin A and G) and IgF-1 levels.

8.1.5 Other blood parameters

There was a significant difference in the average red cell mass between the athletes and the controls ($p<0.05$) in the main competition period. No other significant differences were found in red blood cell size, count or in hemoglobin concentrations between the athletes and the controls. No significant differences between the groups were found in blood lipids.

8.2 Effect of the competition weekend

8.2.1 Leukocytes

There was a significant difference in the change of total amount of blood leukocytes ($p<0.05$) and in the change of the amount of neutrophiles ($p<0.05$) between the athletes in the 20 hours and in the 40 hours group in the early competition phase. The same was found in leukocyte count but not in neutrophils in the main competition period. The effects of competition weekend on blood leukocytes are presented in figure 27. In the 20 hour group the total amount of white blood cells was decreased in both competition weekends and in the 40 hours group the amount was increased. In the early competition period the difference between Friday and Monday measurement was significantly different between 20 and 40 hours group ($p<0.05$). In the main competition period the change was not significantly different between 20 and 40 hours group.

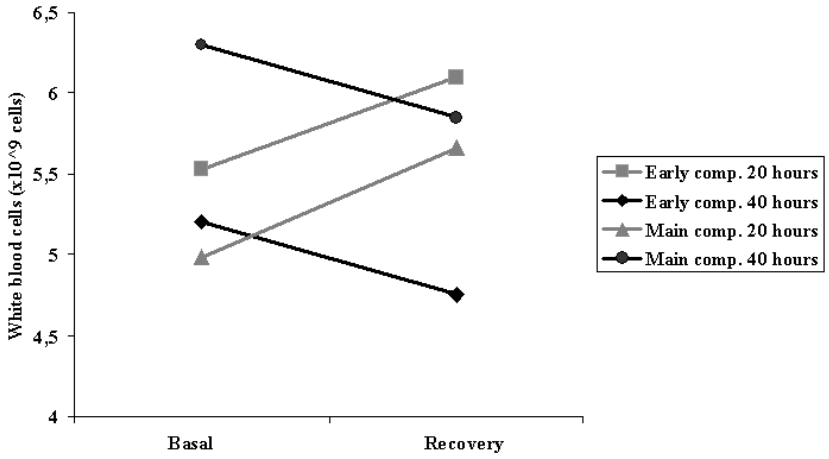

FIGURE 27. White blood cell count before and after the competition weekend. 1) The basal level on Friday, 2) After competition weekend on Monday.

When considering the leukocyte subgroups the lymphocyte and neutrophil concentrations are presented in figures 28 and 29. The change in Leukocyte count correlated ($p<0.05$) with the change in neutrophil concentrations. There was a significant change in neutrophil count between the 20 and 40 hours group. The neutrophil count increased in 40 hours group in the early competition and the main competition period competitions. In the early competition period the neutrophil count decreased significantly in 20 hours group but increased in the main competition period. The change in neutrophil count between 20 and 40 hour groups was significant only in the early competition period ($p<0.05$).

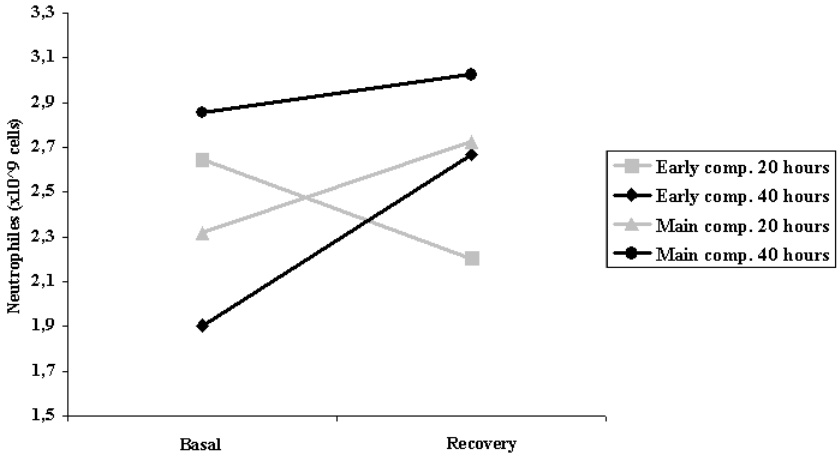

FIGURE 28. Neutrophil count related to competition weekend in the early and main competition phase 1) Basal level on Friday, 2) After competition weekend on Monday.

The response of the lymphocytes to the competition weekend was also different between groups. In the early competition period the 40 hour group's lymphocyte counts decreased significantly related to the competition weekend. In the early competition period 20 hours group and in both groups in the main competition the lymphocyte count increased related to the competition weekend. The differences between the Friday and the Monday measurements were not significant.

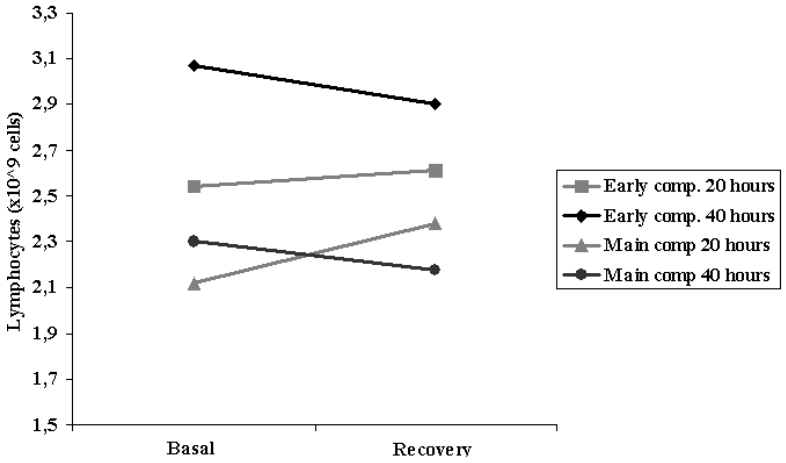

FIGURE 29. Lymphocyte count in athletes related to competition weekend in the early and main competition phase 1) Basal level on Friday, 2) After competition weekend on Monday.

8.2.2 Salivary and Serum Immunoglobulins

There were no significant changes in salivary immunoglobulin A levels, salivary immunoglobulinA/T-protein ratio nor serum immunoglobulin A levels on Monday measurements between the 20 and the 40 hours group. The changes in immunoglobulin A related to competition period are presented in figure 30. There was no significant difference between the Friday and the competition morning salivary immunoglobulinA/Tprotein ratio. The decrease immediately after the competition was significantly high (early competition period $p<0.01$, main competition period $p<0.01$). The difference between the Friday morning measurements and the Monday morning measurements were not significant (early competition period $p=0.102$, main competition period $p=0.093$). There were no significant changes in serum immunoglobulin G levels related to competitions.

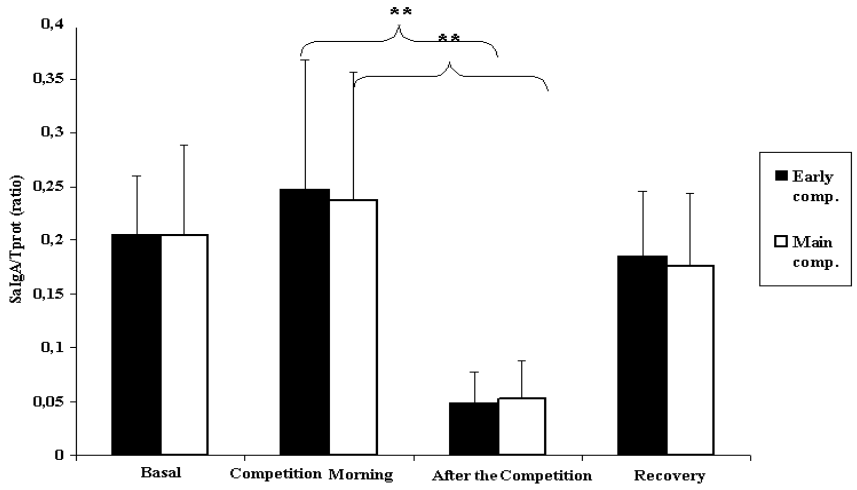

FIGURE 30. Mean ± SD salivary IgA/Tprot levels related to competitions. The basal level and the level on competition morning in fasting state and the level immediately after the competition and on the recovery measurements.

8.2.3 Hormones

Cortisol levels related to the main competition are presented in figure 31. There was a significant increase in the cortisol level related to the competition both in the early ($p<0.05$) and the main competition period ($p<0.05$). No significant differences were found between cortisol level on Friday and Monday measurements.

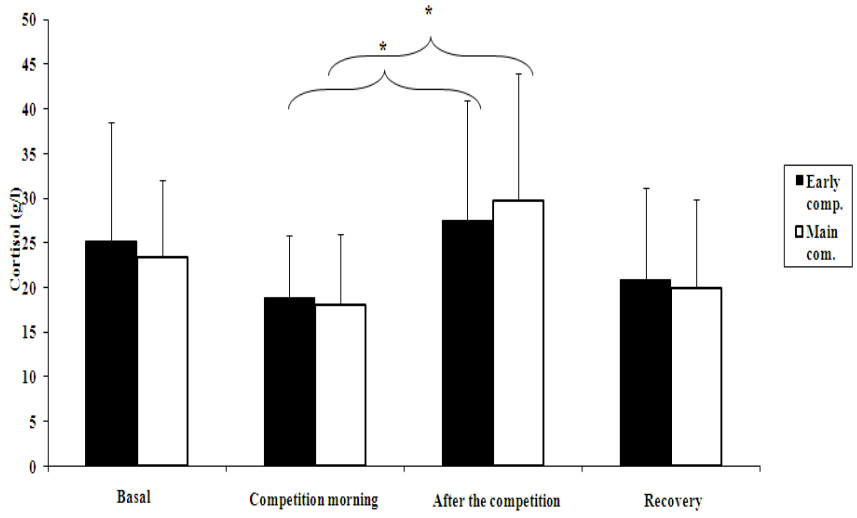

FIGURE 31. Cortisol levels related to competitions in basal level, on a competition morning, immediately after the competition and on the recovery measurements.

Cortisol/Testosterone ratios related to competitions in different competition period are presented in figure 32. No significant changes were found between the measurements.

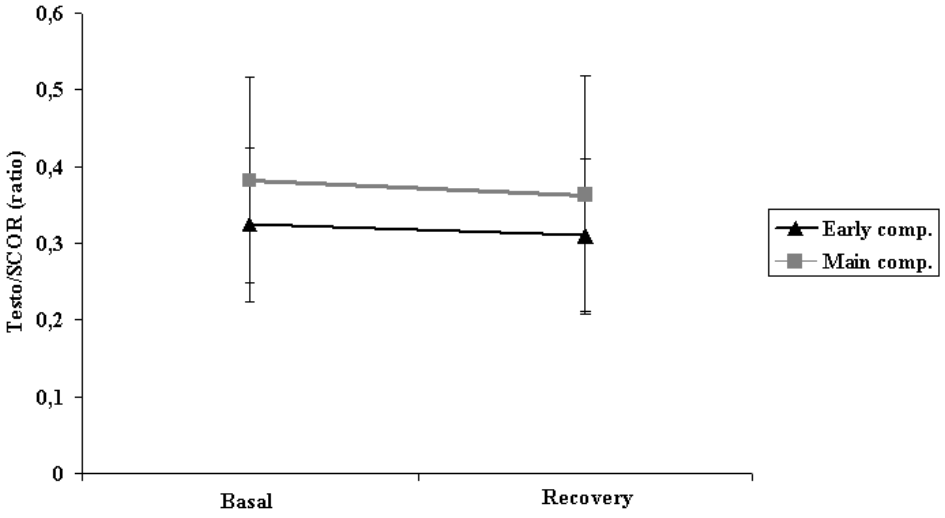

FIGURE 32. Free testosterone/cortisol rations (mean ± SD) related to competitions.

8.3 Relationships between measured parameters

8.3.1 Nutrition

The differences in nutrition intake between the athletes and control groups in different competition periods and transition phase are presented in table 6. The presented values are the averages of the three (3) days before the basal level measurements (weekday) and two (2) days after (weekend day, competition days.

TABLE 6. Differences in nutrition and nutrient intake (mean ± SD) of the athletes and the controls.

Nutrient	1. Athletes n=10	Controls n=10	p	2. Athletes n=10	Controls n=10	p	3. Athletes n=10	Controls n=9	p
Total caloric intake (kcal)	9227 (2080)	7500 (960)	0.06	9970 (1513)	8365 (1220)	0.04*	8570 (1930)	8014 (1240)	0.41
Water (ml)	2290 (865)	1990 (480)	0.65	2511 (791)	2800 (1120)	0.65	2250 (670)	2180 (491)	0.51
Total dietary Protein (g)	103 (22.7)	79.0 (16.1)	0.04 *	106 (20.1)	88.5 (12.2)	0.02*	103 (32.0)	84.0 (13.3)	0.09
Protein of TCI (%)	19 (3.1)	17.9 (3.70)	0.45	19.0 (3.9)	18.0 (1.96)	0.76	21.0 (5.20)	37.6 (61.7)	0.33
Total dietary Fat (g)	70.5 (25.0)	62.1 (12.8)	0.29	67.4 (18.7)	66.0 (15.0)	0.88	71.5 (11.6)	60.5 (8.62)	0.10

Fat of TCI (%)	28.0 (7.1)	30.6 (4.70)	0.36	25.2 (4.3)	28.0 (5.60)	0.20	31.6 (4.7)	28.1 (2.70)	0.06
Saturated FA (g)	23.1 (9.8)	20.5 (4.16)	0.41	20.3 (5.20)	22.4 (4.60)	0.60	24.0 (5.90)	21.5 (4.33)	0.23
Carbohydrates (g)	285 (80)	216 (39.0)	0.03*	322 (55.0)	246 (40.0)	0.01**	245 (87.0)	238 (42.4)	0.77
Carbohydrates of TCI (%)	52.6 (7.6)	48.9 (5.10)	0.16	56.0 (5.90)	50.3 (5.00)	0.06	48.0 (8.20)	50.1 (3.48)	0.43
Alcohol (g)	1.31 (2.61)	6.60 (7.70)	0.07	0.06 (0.19)	8.30 (7.70)	0.004**	0.78 (1.50)	10.6 (7.88)	0.003**
Vitamin A (µg)	1780 (1320)	1880 (203)	0.71	1440 (856)	1540 (990)	0.82	1750 (1190)	1720 (1230)	0.87
Vitamin D (µg)	5.33 (2.90)	5.50 (5.20)	0.41	7.6 (4.20)	6.40 (5.00)	0.45	6.10 (5.70)	7.03 (3.85)	0.462
Vitamin E (mg)	12.7(4.20)	11.50 (7.60)	0.364	23.1 (22.0)	13.5 (7.20)	0.65	21.30 (24.0)	10.9 (3.90)	0.744
Vitamin B1 (mg)	7.71 (11.6)	1.43 (0.90)	0.01*	2.70 (1.45)	1.54 (0.81)	0.023*	2.0 (1.50)	1.51 (0.51)	0.568
Vitamin B2 (mg)	3.38 (1.61)	2.40 (1.35)	0.151	3.30 (1.82)	2.70 (1.25)	0.406	2.70 (1.93	2.70 (0.93)	0.514
Niacin (mg)	45.7 (12.6)	30 (12.4)	0.004**	40.2 (12.0)	35.1 (9.80)	0.226	34.0 (11.0)	32.2 (7.25)	0.744
Vitamin B12	7.4 (4.70)	9.60 (9.10)	1	104 (308)	7.50 (3.40)	0.734	7.80 (5.30)	7.30 (4.53)	0.744
Vitamin C (mg)	190 (77.5)	133 (86.0)	0.041*	216 (120)	162 (76.0)	0.326	158 (128)	122 (45.0)	0.935
Natrium (mg)	3180 (712)	2190 (480)	0.007**	2880 (620)	2450 (444)	0.151	2910 (645)	2340 (440)	0.051
Kalium (mg)	3640 (750)	4740 (4890)	0.45	3820 (670)	3600 (464)	0.406	3570 (760)	3520 (719)	1
Calcium (mg)	1170 (305)	1270 (400)	0.496	1350 (330)	1430 (325)	0.65	1270 (429)	1320 (330)	0.744
Ferritin (mg)	18.4 (7.20)	9.90 (2.20)	0.001**	26.2 (24.7)	11.1 (1.80)	0.07	19.8 (24.50)	10.5 (2.30)	0.935
Zinc (mg)	17.2 (6.40)	12.60 (4.20)	0.059	16.15 (5.2)	13.4 (2.92)	0.151	13.70 (5.90)	12.4 (4.31)	0.87
Selene (µg)	96.0 (29.2)	73.4 (22.0)	0.082	101 (29.1)	85.0 (15.5)	0.151	91.0 (32.1)	75.7 (24.3)	0.327

No significant differences were found between the nutrition intake and immune parameters when the athletes and controls were considered as one group. No significant differences were either found from vitamin or mineral intakes and immune parameters. When athletes were considered as one group, there was a significant positive correlation between the C-vitamin intake and neutrophil cell count. That is presented in figure 33. Vitamin C intake also had negative correlations with testosterone/cortisol –ratio ($R=-0.733$; $p<0.05$) and mixed size cell ($R=-0.692$; $p<0.05$) but positive correlation with Friday measurements serum IgA-levels ($R=709$; $p<0.05$) in the main competition period. No significant correlations were found in the early competition period.

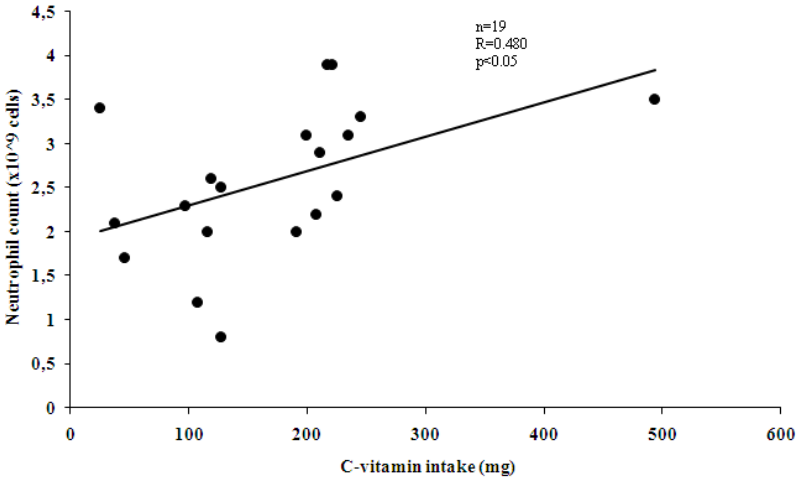

FIGURE 33. Correlation coefficient between vitamin C intake during competition weekend and Monday neutrophil count (R=0.480; p<0.05).

Iron intake correlated negatively with testosterone/cortisol ratio (R=-0.750; p<0.05) and total amount of blood leukocytes (R=-0.778; p<0.01) in the main competition period. This is illustrated in figure 34. No significant correlations were found in the early competition period. Total caloric intake correlated significantly with IGF-1 levels in the early competition period in athletes (p<0.05) but not in the main competition period or transition phase.

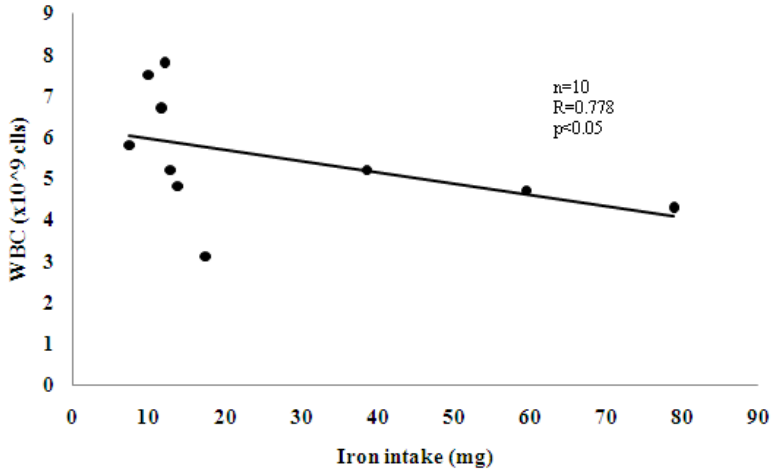

FIGURE 34. Correlation coefficient between iron intake and total number of leukocytes (n=10; R= -0.778; p<0.01)

8.3.2 Training

The volume and intensity of training of the athletes' week before are presented in table 7. The amounts of average training hours in month were presented in the method part in figure 12. There was a significant difference between the maximal intensity training between the early competition period and transition phase ($p<0.05$) and between the main competition period and transition phase ($p<0.05$) measurement.

TABLE 7. Training loads and intensities in the early competition period, the main competition period and the transition phase.

	Low intensity (%)	Moderate intensity (%)	High intensity (%)	Maximal intensity (%)	Training in total (min)
Early competition period	34.7	59.0	3.7	3.4	634
Main competition period	35.4	53.8	4.0	7.2	551
Transition phase	17.4	70.6	3.6	0.4	617

A significant negative correlation was found between the sum of low and moderate intensity training and salivary immunoglobulin A levels in the early competition period ($R=-0.750$; $p<0.05$) Friday measurement. This is presented in figure 35. In the main competition period the percent of low intensity training correlated with salivary IgA level ($R=-0.683$; $p<0.05$) but the absolute low and moderate intensity training amount did not correlate significantly. No significant correlations were found between the immune parameters and the volume of high or maximal training.

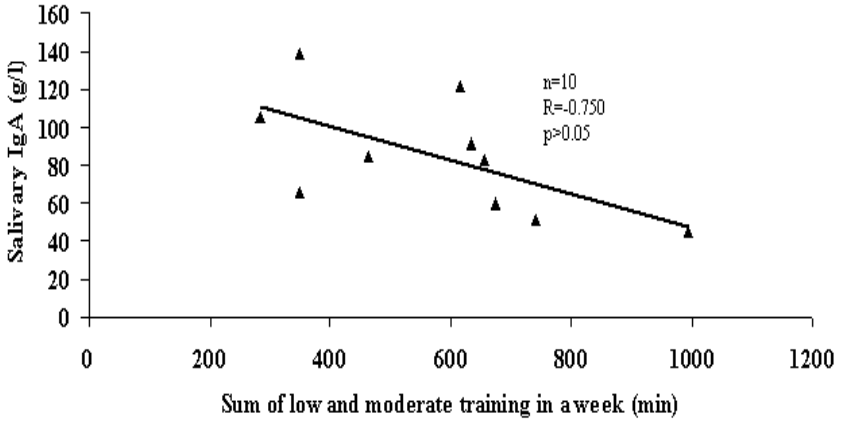

FIGURE 35. Correlation coefficient between salivary IgA levels in basal measurements and the volume of low and moderate training ($n=10$, $R= -0.750$; $p<0.05$) in early competition phase.

Significant negative correlations were found between the sum of low and moderate training and testosterone/cortisol-ratio in the early competition period ($R=-0.714$; $p<0.05$) and in transition phase ($R=-0.850$; $p<0.05$) but not in the main competition phase in athletes. The sum of low and moderate training also correlated negatively with the athletes blood leukocyte count in the early competition period ($R=-756$; $p<0.05$) recovery measurements. The same was seen in neutrophil count ($R=-0.753$; $p<0.05$). In the main competition period no significant correlations were found between the low intensity exercise and total number of white blood cells but there was a significant negative correlation ($R=-0.757$; $p<0.05$) between the percentage of high intensity training of total volume of training and total number of white blood cells. No significant correlations were found in the transition phase.

8.3.3 Body composition

There was a significant negative correlation between the serum cortisol levels in Friday ($R=0.806$; $p<0.01$) and Monday ($R=0.770$; $p<0.01$) measurements and lean mass of the athletes in main competition phase. The same was seen in saliva cortisol in competition morning ($R=-0.758$; $p<0.01$). The fat mass correlated positively with the saliva cortisol immediately after the competition ($R=0.697$; $p<0.05$). Unfortunately there was no DXA measurement in the early competition period. There was no significant correlation between the immunoglobulin levels or blood leukocytes and body composition. No significant correlations where found between lean mass, fat mass or fat percent of the athletes and immune parameter or hormone concentrations in transition phase in DXA measurements.

9 DISCUSSION

The present study focused on endocrinal and immunological state of the female cross-country skiers in different periods of training and after the competition weekend and on the relationship of nutrition, training, body composition and the immunological state of the athletes. The main findings of the present study were:

1) Upper-respiratory tract infections were the most common reason for athletes to miss training. The athletes had more severe upper-respiratory tract infection symptoms related to main competitions than the controls at the same period of time ($p<0.05$).
2) Baseline salivary immunoglobulin A - levels were significantly lower in the competition phase than in the transition phase in the athletes ($p<0.05$). The athletes, who had the lowest serum IgA and serum IgG levels, were those who were most absent from the training because of URTI-symptoms ($p<0.05$). Salivary IgA / T-protein -ratios were significantly lower in athletes than in controls in main competition phase ($p<0.05$).
3) Blood leukocytes of the athletes were at a clinically normal level but were slightly higher (no significant differences) in the athletes in the competition period and lower in the transition phase than in the controls.
4) Testosterone-cortisol ratio correlated significantly with blood leukocyte count in the early competition phase ($R=0.810$; $p<0.05$) and in the main competition phase ($R=0.879$; $p<0.01$) but not in the transition phase.
5) IGF-1 was significantly higher in the athletes than in the controls in the competition period measurements ($p<0.05$) but not in the transition phase measurements.
6) No significant changes were found in blood leukocyte count related to the competition weekend but in the 40 hours group a slight decrease and in the 20 hours group a slight increase was seen in the total amount of blood leukocytes.
7) The cross-country skiing competition had a significant decreasing effect on immunoglobulin A levels ($p<0.01$) but no significant differences were found 20-40 hours after the competition.

8) Vitamin C intake had a significant positive correlation with immunoglobulin A levels in saliva ($R=0.709$; $p<0.05$) but a significantly negative correlation with mixed size cell count ($R= -0.692$; $p<0.05$) and with testosterone-cortisol ratio ($R= -0.733$; $p<0.05$).
9) A significant negative correlation was found between the total number of blood leukocytes and iron intake ($R= -0.778$; $p<0.01$) and testosterone-cortisol ratio ($r= -0.750$; $p<0.05$).
10) The volume of low and moderate training had a significant negative correlation with salivary IgA-levels ($R= -0.750$; $p<0.05$) in the early competition phase. Also the sum had a negative significant correlation with testosterone/cortisol-ratio in the early competition period ($R=-0.714$; $p<0.05$) and in the transition phase ($R= -0.850$; $p<0.05$) but not in the main competition phase in the Ag.

9.1 Upper-respiratory tract symptoms and seasonal variation in immune parameters

As discussed before there is a general perception that the athletes are more susceptible to infection during intensive training and major competitions than in the transition phase. In this study the upper-respiratory tract symptoms were the most common reason for the athletes to miss training. Other reasons from absence in training were rare.

The severity of URTI symptoms related to the athlete's main competition was significantly higher in the athletes than in the control group but there was no significant differences in the early competition period. Matthews (2002), Nieman et al. (1993) and Nieman et al. (1998) have proposed that there might be a J-shaped curve between the intensity and amount of training and the upper-respiratory tract symptoms. The amount of symptoms related to the early competition period and the main competition period were similar. This could be explained with the higher training load before the first competition and the higher intensity of training related to the second competition.

It could be assumed that the same athletes that where sick related to the measured competition weekends were more frequently absent in training because of the URTI-

symptoms as the URTI symptoms correlated with missed training days. When considering the individual URTI symptoms and the missed training days, different kind of “sick-profiles” are found. For example, the athletes number 2 and number 10 had symptoms related to both competitions and number 10 also in the transition phase measurement, but the symptoms were not very severe and at the same time they missed only a couple of training days during the year. The athletes number 4, 8 and 9 were not sick at all related to the measured competition weekends and they had very few missed training days during the year. Two of the athletes, numbers 1 and 5, had very severe symptoms related to one of the competition and also had the highest number of the missed training days in the last year. The athlete number 3 could also part of that group. The athlete number 7 had symptoms only related to the first competition and the amount of missed training day was at the average level in this group. It might be well advised to collect data for a large number of athletes to find out if this kind of profiles can be found and what are the physiological changes behind this phenomenon. When we look at the figure 18, the athlete number 5 in the individual map is distinguished from the other athletes with remarkable low serum IgG levels.

The only significant seasonal variation in the athletes’ leukocyte counts were found in the change between the first and the third measurement. There was a significant difference in the change of leukocytes and neutrophils between the first and the third measurement so that the leukocyte and neutrophil concentration of the athletes in Friday measurement decreased whereas the leukocyte and neutrophil counts in the controls increased when comparing the early competition and transition phase measurements. The same trend was observed between the main competition and the transition phase but this was not statistically significant. This could be due to the alert state caused by the training of the athletes in the competition periods. This might be the reason, why the immunological suppression in the athletes, was not seen in Friday measurements. As the athletes had the lowest values in the transition phase, it might be due to the effect of the acute training responses keep the leukocytes at a higher level and the suppression comes when the athletes are recovered (see figure 6). This might be one reason why the athletes get sick before the main competitions as training is lighter and more time for recovery is given.

Tomasi et al. (1982) reported that the salivary IgA levels were lower in elite cross-country skiers compared with recreational athletes. There was a significant difference between the athletes and the control group in basal level salivary IgA / T-protein ratio in main competition period but not in early competition period or transition phase but the trend also in early competition phase was that the athletes had lower IgA levels than the controls. This might be one of the reasons why athletes had more URTI-symptoms in main competition phase than controls did. When considering the seasonal variation of the immunoglobulin A levels of the athletes, there were significant differences between the competition and transition phase measurements. The immunoglobulin A levels were significantly lower in the competition period when compared to the transition phase. There was also a significant correlation between the IgA levels and the severity of the URTI-symptoms. In the immune function tests, which have shown some changes with the athletic performance, only sIgA has been found to be a potential marker of URTI risk (Gleeson et al. 1995, 1999; Mackinnon et al. 1993; Pyne and Gleeson 1998). In this study the serum IgG levels correlated with the sick days and the levels of serum IgA and serum IgG might be good tools for monitoring the immunological state of the athlete. The saliva samples would be easier to collect, but in this study the serum samples were found to be more valid.

Testosterone-cortisol ratio was found to be a good tool in evaluation of metabolic and immunological state of the athletes as it correlated highly with the white blood cell count in the athletes. Significant changes were not found in the variation of testosterone/cortisol-ratio in the athletes.

9.2 Effect of competition weekend on immune parameters

There were no significant differences between the athletes Friday and Monday measurements blood leukocyte levels. But there was a trend that those athletes who competed on Sunday (20 hours from the competition) had higher blood leukocyte counts on Monday compared to those who were competing only on Saturday (40 hours recovery) having decreased level of blood white cell count. As expected the acute effect of leukocytosis might last as long as 24 hours.

The Salivary IgA levels were significantly dropped immediately after the competition. This might be one of the factors for “open a window” for bacterium and viruses, especially, in cross-country skiing where the athletes ventilate large amounts of air and the air might be extremely cold. But there was no significant correlation between saliva IgA level immediately after the competition and the URTI symptoms. The salivary IgA level decreased in Monday measurements in both 20 and 40 hours groups but the change was not significant. Gleeson (2000) studied IgA levels of the elite kayak competitors during an intensive 2 week training period. In this case-study pre-exercise salivary IgA concentrations failed to recover to the initial IgA concentration for the first training session of the day prior to subsequent sessions. The competition weekend had no effects on the IgG level. No significant changes were seen in testosterone-cortisol ratio in the athletes.

No significant changes after the competition weekend were found in immune parameters. As there was a trend that immunoglobulin A levels were lower, even if not significantly, on Monday morning it would be recommended to take that into account in training to avoid the MacKinnon’s modification of “open window” immunosuppression. (Figure 7).

9.3 Effect of nutrition, training and body composition on immune parameters

The total amount of energy intake or the intake of most of the nutrients didn’t have relation to the immunological state of the athletes. The vitamin intake of the athletes was more than double to the recommended vitamin intake. Ekblom & Bay (2000) found that the cross-country skiers have quite a high energy intake. Since the amount of nutrient intake mainly follows energy intake when, the athlete consumes ‘normal’ food there is a general agreement that the risk of an inadequate nutrient is low in the athletes with high total energy intakes. As all the athletes ate normal and well-balanced diet no significant alterations in immunological state related to nutrition of the athletes was seen.

Consuming excessive doses of individual vitamins, which appears to be a common practice in athletes, can impair immune function and have other toxic effects (e.g. Calder et al., 2002). Probably the same effect was seen in this study related to the main competition period. The megadoses of vitamin C and iron supplements, taken to be on the safe side, might have suppressant effect on immune system as there was a negative correlation with testosterone/cortisol –ratio ($R=-0.733$; $p<0.05$) and mixed size cell ($R=-0.692$; $p<0.05$) but positive correlation with Friday measurements serum IgA-levels ($R=709$; $p<0.05$) with vitamin C intake in the main competition period. No significant correlations were found in the early competition period. Iron intake correlated significantly and negatively with the total number of blood leukocytes related to the main competition basal measurements. It might be possible that consuming excessive doses of vitamin C related to competition might increase the effect of oxidative stress. The athletes and coaches should keep in mind that when the doses of iron or vitamin C are thousand percent of recommended it might have a suppressant effect on athletes' immune system. This relation was not seen in the early competition phase when the supplement intakes were more reasonable. At the same time, vitamin C intake had positive correlation with salivary IgA-level in the main competition period.

When considering the training volume of the athletes, there was a significant correlation between the sum of low and moderate training and the total number of leukocytes in the early competition period and there was a significant correlation between the percent of the sum of the low and moderate training of the total amount of training in the main competition phase. No significant correlations were found in transition phase. No significant correlations were either seen when the maximal and high intensity training were considered. The training of the athletes was quite homogenous and that might be the reason why there were no significant correlations. The training also affected testosterone-cortsol-ratio significantly and that bound training, testosterone-cortisol-ratio and total amount of blood leukocytes together. Testosterone-cortisol-ratio has been used to evaluate the overtraining and overreaching of the athletes. (Busso et al. 1992) This study confirms the use of the testosterone/cortisol –ratio as a tool to diagnose the total stress of the athletes, including the immunological stress.

9.4 Critical evaluation

Some weaknesses of the present study are recognized. The major one of these was that there was no blood sampling for acute responses to the competition. The changes in blood parameters such as in neutrophiles do not necessary mean changes in their activation (Peake et. al. 2005). The lymphocyte subpopulations and for example interleukin-6 could have brought some added value to this research.

The number of athletes that participated in this research was only 10 and all the athletes were not able to participate to the same competitions. In the future studies it would also bring added value to increase the amount of subjects. It was planned that all the athletes would participate to the same number of competitions but this was not possible. The weather during both competition weekends was cold (13-23˚C) so it might have had effects on the training of the athletes before the competitions.

9.5 Conclusion

Total stress related to training and racing in cross-country skiing has an effect on athletes' immunoendocrinal state. To avoid URTI-symptoms related to competition weekend the training and the nutrition of the athletes must be in balance. For monitoring the immunoendocrinal state of the athletes the testosterone-cortisol ratio and immunoglobulin A and G levels might have extra value to blood leukocyte count. The results followed mostly hypothesizes but most of the changes were not statistically significant.

10. REFERENCES

Aasen, G., Fagertun, H. & Halse, J. 2006. Body composition analysis by dual X-ray absorptiometry: in vivo and in vitro comparison of three different fan-beam instruments. Scandinavian Journal of Clinical and Laboratory Investigation. 66:8, 659-666.

Ackerman, K. D, Bellinger, D. L., Felten S. Y. & Felten D. L. 1991. Ontogeny and senescence of noradrenergic intervention of the rodent thymus and spleen. Psychoneuroimmunology, edited by R. Ader, D. L., Felten, & N. Cohen. San Diego, CA: Academic, 71–125.

Adlercreutz, H., Härkönen, M. & Kuoppasalmi, K. 1986. Effect of training on plasma anabolic and catabolic steroid hormones and their response during physical exercise. International Journal of Sports Medicine. 1, 27-28.

Antonio, J. & Street, C. 1999. Glutamine: A potentially useful supplement for athletes. Canadian Journal of Applied. Physiology. 2:1, 1-14.

Ardawi, M. S. & Newsholme, E. A. 1984. Glutaminen metabolism in lymphocytes of the rat. Journal of biochemistry, 212, 835.

Auernhammer, C. J. & Strasburger C. J.1995. Effects of growth hormone and insulin-like growth factor I on the immune system. European Journal Endocrinology 133, 635–45.

Bailey, F., Maresh C. M., Hoffmann J. F., Gabaree, C. L., Hannon, D., Deschenes, M. R., Abraham , A., Kraemer, W. J. and Armstrong L. E. 1991. Immune factor responses to Wingate anaerobic power test. Medicine Science in Sport and Exercise. 23-62.

Baum, M., Liesen, H. and Enneper, J. 1994. Leucocytes, lymphocytes, activation parameters and cell adhesion molecules in middle-distance runners under different training conditions. International Jounal of Sport Medicine. 15, 122-126.

Beck, C.K. 2000. Infectious diseases in sports. Medicine and Science in Sports and Exercise 32, 431–438.

Ben-Nathan, D. S., Lustig & Kobiler, D. 1996. Cold stress induced neuro invasiveness of attenuated arboviruses is not solely mediated by corticosterone. Achieved Virology. 141, 1221–1229.

Berglund, B. & Hemmingson, B. 1990. Infectious diseases in cross-country skiers: one year incidence study. Clinical Sports Medicine. 2, 19-23.

Blalock, J. E. 1994. The syntax of in immunoendocrine communication. Immunology today 15, 504-511.

Bompa, T. O. 1983. Theory and Methodology of Training: The Key to Athletic Performance. Dubuque, IA: Kendall/Hunt Publishing, 63, 76, 132-140, 188-193.

Brandenberger, G. & Follenius, M. 1975. Influence of timing and intensity of muscular exercise on temporal patterns of plasma cortisol levels. Journal of Clinical Endocrinology and Metabolism. 40, 845– 849.

Brandenberger, G., Follenius, M. & Hietter B. 1982 Feedback from meal-related peaks determines diurnal changes in cortisol response to exercise. Journal of Clinical Endocrinology and Metabolism 54, 592–596.

Brandtzaeg, P., Jahnsen, F. L. & Farstad, I. N.1996. Immune functions and immunopathology of the mucosa of the upper respiratory pathways. Acta Otolaryngol. 116: 149–59.

Brandtzaeg, P. 1997. Mucosal immunity in the female genital tract. Journal Reproductive Immunogy. 36: 23–50.

Brenner, I. ,K., M., Shek, P., N. & Shephard, R.,J. 1994. Acute infections and exercise. Sports Medicine 17:86-107.

Brolinson, G. & Elliott, D. 2007. Exercise and the Immune System. Clinics in Sports MedicineVolume 26: 3, 311-319

Brukner, P. & Khan, K. 1993. Clinical Sports Medicine. McGraw-Hill Book Company. Australia.

Bruunsgaard, H., Galbo, H., Halkjaer-Kristensen, J., Johansen, T.L., Maclean, D.A. & Pedersen B.K. 1997. Exercise-induced increase in interleukin-6 is related to muscle damage. Journal of Physiology. 499, 833-841.

Busso, T., Häkkinen, K. &Pakarinen, A. 1992. Hormonal adaptations modelled responses in elite weightlifters during 6 weeks of training. European Journal of Applied Physiology 64, 381–6.

Calder, P.C. 1996. Fatty acids, dietary lipids and lymphocyte functions. Biochemical Society Transactions. 2, 302–309.

Calder, P.C.2001. Polyunsaturated fatty acids, inflammation, and immunity. Lipids. Volume 34:1.

Calder, P.C. 2007. Immunological Parameters: What Do They Mean? The Journal of Nutrition 137, 773 – 780.

Calder, P. C. & Jackson, A. A. 2000. Undernutrition, infection and immune function. Nutrition Research Reviews. 13, 3–29.

Calder, P.C., Field, C.J. & Gill, H.S. 2002. Nutrition and Immune Function. Oxford: CABI Publishing.

Cashmore, G., C., Davies, C., T, & Few, J.D. 1977. Relationship between increase in plasma cortisol concentration and rate of cortisol secretion during exercise in man, Journal of Endocrinology, 72, 109.

Chandra, R. K. 1989. Immune response in under nutrition and over nutrition: Basic considerations and applied significance. Nutrition 5, 297-302.

Chandra, R. K. 1997. Nutrition and the immune system: an introduction. American Journal of Clinical Nutrition, 66: 460–463.

Chaplin, D.D. 2010. Overview of the immune response. Journal of Allergy and Clinical Immunology.

Chicharro, J.L, Lucia, A., Perez, M., Vaquero, A.F. & Urena, R. 1998. Saliva composition and exercise. Sports Medicine 26, 17-27.

Chen, X., Ou Y.X. & Chen, W. J. 1993. Sequential observation on the influence of artificial wind-cold environment to immunologic function of mononuclear phagocytic system in mice. Chung Kuo Chung Hsi I Chieh Ho Tsa Chih 13: 739–740.

Clark, R. 1997. The somatogenic hormones and insulin-like growth factor-1: Stimulators of lymphopoiesis and immune function. Endocrinial. Review. 18, 157-179.

Daly, W. 2004. Relationship between stress hormones and testosterone with prolonged endurance exercise. European Journal of Applied Physiology 93, 375-380.

Del Giacco, S.R. , Tocco, F., Melis, F. , Crisafulli, A. , Gessa, M. , Santoboni, U. , Caria, M. , Tavera, C., Del Giacco, S. G. &Concu, A. 2004. Responsiveness of human natural killer cells during acute, incremental exercise up to exhaustion Sport Science and Health 1:36–40

De Ville, J.W. Fraker, P.J. and Romsos, D.R. 1979. Effects of essential fatty acid deficiency and various levels of dietary polyunsaturated fatty acids on humoral immunity in mice. Journal of nutrition. 109, 1018-1027.

Douglas, D. J., &Hanson, P. G. 1978. Upper respiratory infections in the conditioned athlete. Medicine Science Sports Exercise 10, 55-58.

Ekblom, B. & Bergh, U. 2000. Cross-country skiing. Nutrition in sport. Blackwell Science. Australia.

Ekblom, H. & Hermansen, E.1968. Cardiac output in athletes. Journal of Applied Physiology.

Eliakim, A, Wolach, B. and Kodesh, E. 1997. Cellular and humoral immune response to exercise among gymnasts and untrained girls. International Journal Sports Medicine 18:3, 208-12

Ellegala, D., Alden, T., Couture, D., Vance, M., Nicholas, F., Maartens, F. and Edward, L.R..2003. Anemia, testosterone, and pituitary adenoma in men. Journal of neurosurgery. 98: 5.

Felig, P. 1975. Amino acid metabolism in man. Annual Review Biochemistry 44, 933-955.

Few, J.D. 1974 Effect of exercise on the secretion and metabolism of cortisol in man. Journal of Endocrinology 62, 341–353.

Fitzgerald, L. 1991. Overtraining increases the susceptibility to infections. International Journal of Sports Medicine. 12: 5-8.

Fry, R., Morton, A. R., Crawford, G. P. M. & Keast D. 1992. Cell numbers and in vitro responses of leucocytes and lymphocyte subpopulations following maximal exercise and interval training sessions of different intensities. European Journal of Applied Physiology and Occupational Physiology 64:3, 218-227.

Gabriel, H., Kindermann W. 1997. The acute immune response to exercise: what does it mean? International Journal Sports Medicine 18:981, 28-45

Galbo, H. 1984. Hormonal and metabolic adaptations to exercise. Journal of Applied Physiology October 57,1288-1295.

Gaskill, S. E.1998. Fitness Cross-Country Skiing. Champaign, IL: Human Kinetics Publishers, 53-165.

Gautmanitan, B.G., Ghason, J.L. and Lerner, A.M. 1970. Augmentation of the virulence of murine Coxsackie virus B3 myocardiopathy by exercise. Journal Explorative Medicine, 1121–5.

Gleeson, M. 1995. The effect on immunity of long term intensive training in elite swimmers. Clinical and experimental Immunology. 102:1, 210.

Gleeson, M. editor.2005. Immune function in sport and exercise. Advances in sport and exercise science series. Edinburgh, UK: Elsevier; 2005.

Gleeson, M. 2007. Immune function in sport and exercise. Journal of Applied Physiology 106, 693–699.

Gleeson, M. 2009. Immune System Adaptation in Elite athletes. Current Opinion in Clinical Nutrition and Metabolic Care 9, 659–665

Gleeson, M. & Bishop, N.C. 2000. Elite Athlete Immunology: Importance of nutrition. International Journal of Sports Medicine 21:1, 44-50

Gleeson, M., McDonald, W. and Cripps, A. 1995. The effect of intensive training on systemic and mucosal immunity in elite swimmers. Clinical Experimental Immunology 102, 210-216.

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

Gleeson, M., Nieman, D.& Pederssen, B.K., 2004. Exercise, nutrition and immune function Journal of Sports Sciences. 22, 115–125.

Gray, A.B., Telford, R.D., Collins M., Baker, M.S. &Weidemann M.J. 1993. Granylocyte activation induced by intensive interval running. Journal of leukocyte Biology 53, 591-597.

Guyton, A. C. & Hall J. E. 2000. Textbook of medical physiology. 10th ed. Philadelphia: W. B. Saunders Company.

Haymes, E. M., Puhl J.L., & Temples T. E. 1986. Training for cross-country skiing and iron status. Medicine, Sports and Exercise 18: 2, 162-167.

Heir, T. & Oseid, S. 1993. Self-reported asthma and exercise-induced asthma symptoms in high-level competitive cross-country skiers. Scandinavian Journal of sport and Medicine in Sport. 4:2, 128 – 133.

Highet, R. 1989. Athletic amenorrhea. An update on aetiology, complications and management. Sports Medicine 7, 82-108

Hoffman-Goetz, L. & Pedersen B.K. 1994. Exercise and the immune system: a model of the stress response? Immunology Today 15, 382–387

Hoffman, Martin D., Clifford, Philip S. Watts, Phillip B.; Drobish and Kip, M.1994. Physiological comparison of uphill roller skiing: diagonal stride versus double pole. Medicine & Science in Sports & Exercise 26:10.

Ingjer, F. 1991. Maximal oxygen uptake as a predictor of performance ability in women and men elite cross-country skiers. Scandinavian Journal Medicine Science in Sports 1, 25-30.

Johnston , P.V. 1988. Lipid modulation and immune responses. In nutrition and Immunology, Contemporary Issues in clinical Nutrition. Vol. 2. Edited by Liss A. Inc. New York, 37, 231-235.

Keizer, H.A. 1987 Hormonal responses in women as a function of physical exercise and training. General Discussion. International Journal Sports Medicine 8, 137-138 and 168-174

Kendall, A., Hoffman-Goestz L., Houston, M., MacNeil, B & Arumagam, Y. 1990.Exercise and blood lymphocyte subset resposnses: intensity duration and subject fitness effects. Journal of applied Physiology 69, 251-261.

Klarlund, Pedersen B. & Hoffman-Goetz L. 2000. Exercise and Immune system: regulation, Integration and Adaptation. Physiological reviews. 80:3.

Kim, C.H & Kwak Y.S. 2004. Swim training increases ovalbumin induced active systemic anaphylaxis in mice. Immunological Investigation 33, 469-480.

Kjaer, M. & Dela F. 1996. Endocrine responses to exercise. In Exercise and Immune function, edited by Hoeffman-Goetz. Boca Raton. FL: CRC, 1996, p. 1-20.

Kono 1988. Review of insulin-sensitive glucose transport. Vitamins and Hormones, 44,103-110.

Kreider, R., Fry, A. C. & M. O'Toole. 1998. Overtraining in sport: terms, definitions, and prevalence. In: Overtraining in Sport, R. Kreider, A. C. Fry, and M. O'Toole (Eds.). Champaign, IL: Human Kinetics.

Kwak Y.S. 2006. Effects of training onspleen and peritoneal exudate reactive oxygen specises and lymphocyte proliferation by splenocytes at rest and after an acute bout of exercise. Journal Sports Science 224, 973-978.

Lac, G. & Berthon, P. 2000. Changes in cortisol and testosterone levels and T/C ratio during an endurance competition and recovery. Journal of Sports Medicine and Physical Fitness 40:2, 139-144.

Larsson, K., Ohlsen, P., Larsson, L., Malmberg, Rydstrom, P. & Ulriksen H. 1993. High prevalence of asthma in cross country skiers. BMJ 307, 1326-9.

Lemon, P.W.R. 1998. Effect of exercise on dietary protein requirements. International Journal of Sports Nutrition. 8, 426-433.

Lemon, P.W.R. Tarnopolsky, M.A., MacDougall, J.D. & Atkinson, S.A. 1992. Protein requirements and muscle mass/strength changes during intensive training in novice bodybuilders. Journal of Applied Physiology. 73, 767-769.

Lindinger, S. J. & Holmberg H. 2010. How do elite cross-country skiers adapt to different double poling frequencies at low to high speeds? European Journal of Applied Physiology 38:10, 1853-1860.

Mackinnon, L.T. 1997. Immunity in Athletes. International Journal of Sports Medicine 18: S62-S68.

Mackinnon, L.T. 1999. Advances in Exercise Immunology. Champaign, IL: Human Kinetics.

Mackinnon, L. T. 2000. Overtraining effects on immunity and performance in athletes. Immunology and cell Biology. 78, 502-509.

Mackinnon, L.T. 2001. Exercise Immunology: Current issues. In Nutrition and exercise immunology.

Mackinnon L.T. &Hooper S.1994. Mucosal (secretory) immune system responses to exercise of varying intensity and during overtraining. International Journal of Sports Medicine 15, 179-183.

Madden, K. & Felten, D.L., 1995. Experimental basis for neural-immune interactions. Physiological reviews. 75:1, 77-106.

Mahood, N. V., Kenefick R. W.. Kertzer R. & Quinn T. J. 2001. Physiological determinants of cross-country ski racing performance. Medicine and Science in Sports and Exercise. 33:8, 1379–1384

Maki, P.A. 1992. Dietary lipids and immune function The Journal of nutrition: 122:3, 610.

Malm, C. 2004. Exercise Immunology. The Current State of Man and Mouse. Sports Medicine; 34 :2, 555-566.

Mars, M., Govender, S., Weston, A., Naicker, V. & Chuturgoon A. 1998. High Intensity Exercise: A Cause of Lymphocyte Apoptosis? Biochemical and Biophysical Research Communications. 249:2, 366.

Maso, F., Lac, G., Filaire, E., Michaux, O. and Robert, A. 2004. Salivary testosterone and cortisol in rugby players: correlation with psychological overtraining items. British Journal of Sports Medicine 38, 260–263.

Matthews, C.E, Ockene, I.S. and Freedson, P.S. 2002. Moderate to vigorous physical activity and the risk of upper-respiratory tract infection. Medicine Science Sports Exercise 34, 1242–1248.

McDowell, S.L., Hughes, R.A., Hughes, D.J., Housh, D.J. & Johnson G.O. 1992. The effect of exercise training on salivary immunoglobulin A and cortisol responses to maximal exercise. Intenational Journal of Sports Medicine. 13, 577-580.

Moreira, A. , Delgado L., Moreira P. & Haahtela T. .2009. Does exercise increase the risk of upper respiratory tract infections? British Medical Bulletin 31.

Mujika, I. Chartad C.J. &Geyssant A. 1996. Effects of training and taper on blood leukocyte populations in competitive swimmers: Relationship with cortisol and performance. Internetiopnal Journal of Sports Medicine 17, 213-217.

Murray, R., Seinfelt P. and Eddy D.E. 1991. Responses to varying rates of carbonhydrate ingestion during exercise. Medicine and Science in Sport and Exercise. 23, 713-718.

Nemet, D., Connolly P., Pontello-Pescatello A., Rose-Gottron C., Larson, J.K, Galassetti P. & Cooper M.2004. Negative energy balance plays a major role in the IGF-I response to exercise training. Journal of Applied Physiology 96, 276–282.

Nieman, D.C. 1997. Immune response to heavy exertion. Journal of Applied Physiology 82, 1385-1394.

Nieman, D.C. 2001. Exercise immunology: Nutritional countermeasures. Canadian Journal of Applied Physiology 26, 45-55.

Nieman, D.C., Miller A. R. , Henson D. A. , Warren B. J. , Gusewitch G. , Johnson R. L. , Davis J. M. , Butterworth D. E. , Herring J. L. & Nehlsen-Cannarella S. L.1994. Effect of High- Versus Moderate-Intensity Exercise on Lymphocyte Subpopulations and Proliferative Response International Journal Sports Medicine 15,199-206.

Nieman, D.C, Fagoaga O.A., Butterworth D.E, Warren, B.C.,Utler B.J., Davis M.J., Henderson D.A. & Nelhlsen-Cannarella S.L.. 1997. Carbohydrate supplementation affects blood granulocyte and monocyte trafficking but not function following 2.5 hours running. American Journal of clinical nutrition. 66, 153-159.

Nieman, D.C.,& Pedersen, B.K. 2000. Nutrition and Exercise Immunology. Boca Raton, FL: CRC Press.

Nieman, D.C., Henson D. A., McAnulty S. R., McAnulty L.M., Swick N. S., Utter, A. C., Vinci D. M. Opiela S., J. & Morrow, J.D. 2002. Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. Journal of applied physiology. 92:5.

Noakes, T. 2001. Lore of Running, 4th ed. Champaign, IL: Human Kinetics, 282–284.

Norcross, J. & Van Loan, M. D. 2004. Validation of fan beam dual energy x ray absorptiometry for body composition assessment in adults aged 18-45 years. British Journal of Sports Medicine. 38, 472-476.

Northoff , H. & Berg A.1991 Immunologic mediators as parameters of the reaction to strenuous exercise. International Journal of Sports Medicine 12: 9-15.

Northoff, H., Weinstock C. &Berg A. 1994. The cytokine response to strenuous exercise. International Journal of Sports Medicine 15, 167-171.

Olejnika, S.A. & Gununoi, L.M. 2008. Sportivnaja farmakologija i dietologia, Moscow Russian.

Paavolainen, L., Häkkinen, K., Hämäläinen, L., Nummela, A. & Rusko, H. 1999. Explosive-strength training improves 5-km running time by improving running economy and muscle power. Journal of Applied Physiology. 86:5, 1527-1533.

Palacios, A., Campfield, L.A., McClure, R.D., Steiner, B. &Swerdloff R.S. 1983. Effect of testosterone enanthate on hematopoiesis in normal men. Fertilization and Steril. 40, 100-104.

Paulsen, G., Benestad H., Stöm-Gundersem I., Morkrid L., Lappegård K., Knut T. & Raastad T.2005. Delayed Leukocytosis and Cytokine Response to High-Force Eccentric Exercise. Medicine & Science in Sports & Exercise. November Volume 37: 11, 1877-1883.

Peake, J.M., Suzuki K., Wilson G., Hordern M., Nosaka K., MacKinnon L. & Coombes J.S. 2005. Exercise-induced muscle damage, plasma cytokines, and markers of neutrophil activation. Medicine & science in sports & exercise. 37: 737-745.

Pedersen, B.K. & Ullum H. 1994. NK cell response to physical activity: Possible mechanisms of action. Medicine and Science in Sports and Exercise 26:140-146.

Pedersen, B.K. 2005 Leukocytes. Molecular and cellular exercise physiology. Human Kinetics. United States of America.

Pedersen, B.K. & Hoffman-Goetz L. 2000. Exercise and the Immune System: Regulation, Integration, and Adaptation. Physiological Reviews 80: 1055–1081.

Persson, C.G., Erjefält, J.S., Greiff L. 1998. Contribution of plasma-derived molecules to mucosal immune defence, disease and repair in the airways. Scandinavian Journal of Immunology 47: 302–13.

Petters, E.M. & Bateman, E.D. 1983. Ultra marathon running and URTI: an epidemiological survey. S A Medicine Journal 64, 582–584.

Peters, E., Goetzshe J., Grobbelaar B & Noakes T. 1993. Vitamin C supplementation reduces the incidence of post race symptoms of upper-respiratory-tract infection in ultra-marathon runners. American Journal of Clinical Nutrition 57:170-174

Peterson, J. M., Trappe, T. A., Mylone, E. White, F. Lambert, C. P. Evans, W. J., & Pizza, F. X. 2003. Ibuprofen and Acetaminophen: Effect on Muscle Inflammation after Eccentric Exercise. Medicine and Science in Sports and Exercise, Vol. 35, No. 6, 892–896.

Pizza, F. X., Mitchell J. B., Davis B. H., Starling R. D., Holtz R. W., Bigelow N.1995. Exercise-induced muscle damage: effect on circulating leukocyte and lymphocyte subsets. Medicine and Science in Sports and Exercise 27, 363–37.

Pizza, D., Calende A., Stockard, H., Baylies, Beighle L. 1999. Anti-Inflammatory Doses of Ibuprofen: Effect on Neutrophils and Exercise-Induced Muscle Injury. International Journal Sports Medicine 1: 98-102.

Prasad, J.S .1980. Effect of vitamin E supplementation on leukocyte function. American Journal Clinical Nutrition 33, 606–608.

Prestes, J., Ferreira, C.K.O, Frollini, A.B., Dias, R., Donatto, F.F, & Guereschi M.G. 2007. Influence of acute physical exercise on leukocytes, lymphocytes and circulating cytokines levels. Fit Performance Journal 6, 32-37.

Pyne, D. 1994. Regulation of neutrophils during exercise. Sports Medicine. 17, 245-258.

Pyne. D. Balancing training to keep your athletes healthy. Sports Coaching: 20–22.

Pyne, D., & Gleeson M. 1998. Effects of Intensive Exercise Training on Immunity in Athletes. International Journal Sports Medicine 19, 183-194

Rabin, B.S., Moyna, M.N., Kusnecow, A., Zhou, D. & Shurin M.R. 1996. Neuroendocrine effects of immunity In: Exercise and immune function, edited by Hoefman-Goetz. 1: 21-38.

Robson, P.J, Blannin, A.K. & Walsh, N.P. 1999. Effects of exercise intensity, duration and recovery on in vitro neutrophil function in male athletes. International Journal of Sports Medicine 20: 128-135.

Rohde, T., Maclean D.A. & Pedersen, B.K. 1996. Glutamine, lymphocyte proliferation and cytokine production. Scandinavian journal of Immunology. 3:648-650.

Roitt, I. 2001. Roitt's essential immunology . Blackwell Science, 2001.

Rosen, O. 2003. Immune, endocrine and metabolic changes related to exhaustive and repeated exercise sessions. Series of dissertations submitted to the Faculty of Medicine, University of Oslo; No. 111. Oslo : Universitetet i Oslo.

Rosen, O., Børsheim E., Bahr, R., Klarlund-Pedersen, B., Haug, E., Kjeldsen-Kragh, J. and Høstmark, A.T. 2004. Immuno-endocrine and metabolic responses to long distance ski racing in world-class male and female cross-country skiers. Scandinavian Journal Medicine and Science in Sports: 14, 39–48.

Rotfeld, B. 1974. Plasma cortisol. In nuclear medicine in vitro 20.

Rowland, T.W. 1990. Developmental aspects of physiological function relating to aerobic exercise in children. Sports Medicine.10,225-266.

Rowbottom, D. 2000. Protein, exercise and immunity. Nutrition and Exercise Immunology. Boca Raton, FL: CRC Press.

Rowbottom, D., Keast, D., Morton, A. 1996. The emerging role of glutamine as an indicator of exercise stress and overtraining. Sports Medicine 21, 80-97.

Rundell, K. W., and Bacharach, D. W. 1995. Physiological characteristics and performance of top U.S. biathletes. Medicine and Science in Sports and Exercise. 27, 1302-1310.

Rusko, H. 1987. The effect of training on aerobic power characteristics of young cross-country skiers. Journal of Sport Sciences, 5, 273-286.

Rusko, H. K. 1992. Development of aerobic power in relation to age and training in cross-country skiers. Medicine and. Science in Sports and Exercise, 24:9, 1040-1047.

Rusko, H. 2003. Handbook of sports medicine and science: Cross-country skiing. Blackwell science, ltd. Malden.

Russel, R.W.1949. Paralytic poliomyelitis. The early symptoms and the effect of physical activity on the course of the disease. British Medicine Journal. 1:465-471.

Saxton, J. M., Claxton D., Winter E. &Pockley A. G. 2003. Peripheral blood leucocyte functional responses to acute eccentric exercise in humans are influenced by systemic stress, but not by exercise-induced muscle damage. Clinical Science 104, 69–77.

Schouten, W.J., Verschuur, R. &Kemper, H.C.G.1988. Physical activity and upper respiratory tract infections in a population of young men and women: the Amsterdam growth and health. International Journal of Sports Medicine. 9:451-455.

Selby, R, Weinstein, H.M. & Bird, T S. 1990. The health of university athletes: attitudes, behaviors, and stressors. Journal American Coll-Health. 39: 11-8

Sharkey, B. 1986. Training for Cross-country Ski Racing. Champaign, IL: Human Kinetics Publishers, 1986, pp. 61-68, 123-126.

Shephard, R. J. 1997. Physical Activity, Training and the Immune system. Cooper Publishing Group, United States of America.

Silverthorne, D. U. 2007. Human physiology: an integrated approach. 4th edition. Benjamin Cummings.

Simon, H.B. 1987. Exercise and infections. The physician and Sports medicine 15:135-141.

Smith, J.A. 1997. Exercise immunology and neutrophils. International Journal of Sports Medicine. 18: 46–55

Smith, C. & Myburg G. 2006. Are the relationships between early activation of lymphocytes and cortisol or testosterone influenced by intensified cycling training in men? Applied Physiology, Nutrition & Metabolism 31:3, 226.

Strauss,R.H, McFaddan ER, Ingram RH & Jaeger J. 1977 Enhancement of exercise induced asthma by cold air. New English Medicine 297:743-7.

Stöggl, T., Lindinger S. & Müller E. 2007. Analysis of a simulated sprint competition in classical cross country skiing. Scandinavian Journal of Medicine & Science in Sports 17:4, 362–372.

Stray-Gundersen, J., Videman T, Penttila I. & Lereim I. 2003. Abnormal hematologic profiles in elite cross-country skiers: blood doping or? Clinical Journal Sport Medicine 13: 132–137.

Tegelman, R., Johansson ,C., Hemmingsson P, Ekliof R, Carlström, K. & Pousette.1989. Endogenous anabolic and catabolic steroid hormones in male and female athletes during off season. International Journal Sports Medicine 11 : 103-106.

Venkatraman, J.T., Horvath, P., J. & Pendergast D., R. 2000. Lipids, Exercise, and immunology. in Nieman , C.D and Pedersen, B. K. (ed.) Nutrition and exercise immunology.

Taimazov, V.A., Tzygan, V.N. & Mokejeva, E.G. 2003. Sport i Immunitet, Sankt Peterburg. Russia.

Tharp, G. D., & Barne, M. W. Reduction of saliva immunoglobulin levels by swim training. European Journal of Applied Physiology 60, 61-64, 1990.

Tomasi, T. B., Trudeau F.B., Czerwinski D. and Erredge S. 1982. Immuneparameters in athletes before and after strenuous exercise. Journal of Clinical Immunology. 2, 173-178.

Van Cauter, E, Leproult R. & Kupfer DJ. 1996 Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. Journal of Clinical Endocrinology and Metabolism 81, 2468 –2476.

Veldhuis, J.D., Iranamesh, A., Lizarralde, G. & Johnson, M.L. 1989. Amplitude modulation of a burstlike mode of cortisol secretion subserves the circadian glucocorticoid rhythm. American Journal of Physiology. 257, 6 –14.

Volek, J.S., Kraemer, W, J., Bush, J.A., Incledon, T., & Boetes, M. 1997. Testosterone and cortisol in relations.ip to dietary nutrients and resistance exercise. Journal of Applied Physiology 82, 49–54.

Webster, Marketon, J. & Glaser, R. 2007. Stress hormones and immune function. Cellular Immunology 252, 16-26.

Weidner, T.G.2001.Preventing the common cold and associated secondary problems in athletes. Athletic Therapy Today 6, 44–45.

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

Williams, J, E., Wells, J. C. K., Wilson, C. M., Haroun, D., Lucas, A. & Fewtrell, M. S. 2006. Evaluation of Lunar Prodigy dual-energy X-ray absorptiometry for assessing body composition in healthy persons and patients by comparison with the criterion 4-component model. American Journal of Clinical Nutrition. 83, 1047-1054.

Woods, J. A. & Davis, J. M. 1994. Exercise, monocyte/macrophage function, and cancer. Medicine & Science in Sports & Exercise. 26 :2.

Yesilova, Z., Metin, O., Kocar, I. H., Turan, M., Pekel, A., Sengul, A. & Ozdemir, C. 2000. The Effects of Gonadotropin Treatment on the Immunological Features of Male Patients with Idiopathic Hypogonadotropic Hypogonadism The Journal of Clinical Endocrinology & Metabolism 85:1.

Ziegenfuss, T. Landis, J. A. & Lemieux R. A. 2010. Protein for Sports—New Data and New Recommendations. Sport and Conditioning Journal. 32: 1.

Zmuda, J.M., Thompson, P.D., & Winters S.J. 1996. Exercise increases serum testosterone and sex hormone-binding globulin levels in older men. Metabolism 45, 935–939.

APPENDIX 1. WURSS-21 questionnaire.

Wisconsin Upper Respiratory Symptom Survey – 21 --- *Daily Symptom Report*

Day:	*Date:*	*Time:*	*ID:*

Please fill in one circle for each of the following items:

	Not sick	Very mildly		Mildly		Moderately		Severely
	0	1	2	3	4	5	6	7
How sick do you feel **today**?	O	O	O	O	O	O	O	O

Please rate the **average severity of your cold symptoms over the last 24 hours** for each symptom:

	Do not have this symptom	Very mild		Mild		Moderate		Severe
	0	1	2	3	4	5	6	7
Runny nose	O	O	O	O	O	O	O	O
Plugged nose	O	O	O	O	O	O	O	O
Sneezing	O	O	O	O	O	O	O	O
Sore throat	O	O	O	O	O	O	O	O
Scratchy throat	O	O	O	O	O	O	O	O
Cough	O	O	O	O	O	O	O	O
Hoarseness	O	O	O	O	O	O	O	O
Head congestion	O	O	O	O	O	O	O	O
Chest congestion	O	O	O	O	O	O	O	O
Feeling tired	O	O	O	O	O	O	O	O

Over the last 24 hours, how much has your cold interfered with your ability to:

	Not at all	Very mildly		Mildly		Moderately		Severely
	0	1	2	3	4	5	6	7
Think clearly	O	O	O	O	O	O	O	O
Sleep well	O	O	O	O	O	O	O	O
Breathe easily	O	O	O	O	O	O	O	O
Walk, climb stairs, exercise	O	O	O	O	O	O	O	O
Accomplish daily activities	O	O	O	O	O	O	O	O
Work outside the home	O	O	O	O	O	O	O	O
Work inside the home	O	O	O	O	O	O	O	O
Interact with others	O	O	O	O	O	O	O	O
Live your personal life	O	O	O	O	O	O	O	O

Compared to yesterday, I feel that my cold is…

Very much better	Somewhat better	A little better	The same	A little worse	Somewhat worse	Very much worse
O	O	O	O	O	O	O

WURSS -21® (Wisconsin Upper Respiratory Symptom Survey) 2004
Created by Bruce Barrett MD PhD et al., UW Department of Family Medicine, 777 S. Mills St. Madison, WI 53715, USA

APPENDIX 2. Mean ±SD Blood leukocytes.

Leukocyte subset	Units	Early competition period Athletes (n=10)	Controls (n=10)	p	Main competition period Athletes (n=10)	Controls (n=10)	p	Transition phase Athletes (n=10)	Controls (n=10)	p
Total leukocyte count	x 10 ^9 cells	5.69 (1.71)	5.56 (1.51)	0.94	5.51 (1.46)	4.68 (1.01)	0.13	5.11 (1.00)	6.19 (1.23)	0.063
Lymphocyte no.	x 10 ^9 cells	2.62 (0.67)	2.24 (1.06)	0.12	2.19 (0.49)	1.98 (0.46)	0.60	2.16 (0.512)	2.22 (0.73)	0.10
Lymphocyte (%)	% of WBC	47.26 (8.8)	40.3 (10.2)	0.817	40.1 (4.7)	43.0 (10.3)	0.88	43.0 (8.28)	36.2 (10.60)	0.325
Mixed size cells no.	x 10 ^9 cells	0.62 (0.18)	0.6 (0.18)	0.449	0.59 (0.14)	0.48 (0.13)	0.705	0.74 (0.35)	0.65 (0.17)	0.28 *
Mixed size cells (%)	% of WBC	10.69 (2.48)	11.52 (4.60)	0.131	10.2 (2.43)	10.01 (2.43)	0.176	14.1 (5.80)	10.60 (2.87)	0.97
Neutrophile count	x 10 ^9 cells	2.45 (1.18)	2.72 (1.11)	0.76	2.73 (1.01)	2.22 (0.87)	0.25	2.21 (0.68)	3.32 (1.17)	0.91
Neutrophile (%)	% of WBC	42.05 (8.77)	48.15 (12.2)	0.364	48.2 (6.25)	47.0 (10.3)	0.256	42.96 (8.70)	53.16 (11.0)	.023*

Leukocyte subset	Units	Differences Early - Main Athletes (n=10)	Controls (n=10)	p	Difference Early – Transition Athletes (n=10)	Controls (n=10)	p	Differences Main - Transition Athletes (n=10)	Controls (n=10)	p
Total leukocyte count	x 10 ^9 cells	-1.4 (0.1)	0.88 (1.37)	0.211	0.30 (-0.2)	- 0.63 (1.82)	0.121	1.7 (-0.3)	- 1.51 (1.39)	.008 **
Lymphocyte no.	x 10 ^9 cells	0.60 (0.20)	0.26 (0.78)	0.112	1.2 (0)	0.02 (0.60)	0.272	0.6 (0.2)	- 0.24 (0.58)	0.271
Lymphocyte (%)	% of WBC	16.5 (6.8)	- 2.66 (9.62)	0.496	17.2 (3.3)	4.07 (9.89)	0.94	0.7 (3.5)	6.73 (10.7)	0.13
Mixed size cells no.	x 10 ^9 cells	-0.1(0)	0.12 (0.16)	0.326	- 0.1 (0.1)	- 0.05 (0.16)	0.877	0 (0.1)	- 0.17 (0.15)	0.512
Mixed size cells (%)	% of WBC	0.9 (0.2)	1.51 (3.12)	0.272	- 1.1 (3.4)	0.94 (3.01)	0.112	-2.0 (3.2)	- 0.57 (2.60)	0.038*
Neutrophile count	x 10 ^9 cells	-1.9 (0.1)	0.5 (1.066)	0.416	-0.8 (0.1)	- 0.6 (1.63)	0.186	1.1 (0)	- 1.1 (1.31)	0.04*
Neutrophile (%)	% of WBC	-17.4 (-6.6)	1.15 (10.1)	0.24	-16.1 (0.1)	- 5.01 (11.6)	0.406	1.3 (6.7)	- 6.16 (11.49)	0.021*

APPENDIX 3. Mean ± SD Testosterone, SHBG, Cortisol and Testosterone/Cortisol –ratio.

		Early comp.			Main comp.			Transition phase		
Hormones	**Units**	**Athletes (n=10)**	**Controls (n=10)**	**p**	**Athletes (n=10)**	**Controls (n=10)**	**p**	**Athletes (n=10)**	**Controls (n=10)**	**p**
Serum Cortisol	nmol/l	650 (132)	581 (230)	0.364	611 (161)	603 (253)	0.677	560 (86)	581 (230)	0.762
SHBG	nmol/l	121 (90)	150 (130)	0.705	122 (87)	144 (121)	0.821	135 (123)	150 (133)	0.762
Serum Testosterone	nmol/l	1.96 (1.08)	2.15 (0.95)	0.821	2.22 (1.18)	1.70 (0.82)	0.257	1.82 (0.84)	2.15 (0.95)	0.406
Testosterone/ Cortisol ratio	ratio	0.32 (0.14)	0.44 (0.26)	0.514	0.38 (0.13)	0.33 (0.19)	0.514	0.36 (0.14)	0.44 (0.26)	0.683