









**Faculty of Applied Health Sciences  
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Masters of Science Degree**

**Relationship Between Physical Activity and  
Resting Secretory Immunity in Children**

**A Masters of Science Thesis**

**By**

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## **Abstract**

This study examined relationships among physical activity, body fat and salivary immunoglobulin A (sIgA) levels in adolescent children of Southern Ontario. Gender differences on these factors were also assessed. Sixty-one grade-five students (10-11yrs), males (n=29) and females (n=31), who had not received a flu vaccination in the past 12 months, participated in the study. They were assessed for: aerobic power (20-m shuttle run), relative body fat (bioelectrical impedance analysis), sIgA, sIgA/albumin ratio, and salivary cortisol. Each subject completed the Habitual Activity Estimation Scale and the Participation Questionnaire. Students wore a pedometer for 48h to estimate their average total distance traveled per day. The results show 40% of the children were over 25% body fat and 50% of them spend less than five hours per day in any physical activities. Salivary IgA was not related to salivary cortisol, physical activity, fitness level or body fat in this age group. There were no gender differences in sIgA and cortisol levels. Boys had a significantly higher aerobic power and daily distance traveled, but reported similar organized and free time activity participation levels as the girls. The test-retest reproducibility for salivary cortisol was 0.663 ( $p<0.01$ ), while long term sIgA and sIgA/albumin ratio reproducibility was non-significant for repeated measurements taken after six weeks. It was found that salivary IgA has not been shown to be a stable measure in children, in contrast to the results found in the literature that tested adults and the relationship with physical activity, fitness level and body fat.





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## List of Abbreviations

ACTH	– Adrenocorticotrophic Hormone
AIDS	– Acquired Immune Deficiency Syndrome
ALS	– Amyotropic Lateral Sclerosis
BIA	– Bioelectrical Impedance Analysis
BMI	– Body Mass Index
CH	– Constant heavy chain of the immunoglobulin structure
HAES	– Habitual Activity Estimation Scale
HGH	– Human Growth Hormone
HPA	– Hypothalamic-Pituitary Adrenocortical axis
Ig	– Immunoglobulin
MET	– Metabolic Equivalent Task value
MG	– Myastemia Gravis
MS	– Multiple Sclerosis
MTPAB	– Measures of Typical Physical Activity Behavior
NK cells	– Natural Killer cells
NKCA	– Natural Killer cell Cytotoxic Activity
PAQ-C	– Physical Activity Questionnaire for Children
PDPAR	– Previous Day Physical Activity Recall



SAM	– Sympathoadrenomedullary system
SAS	– Space Adaptation Syndrome
sIgA	– Salivary Immunoglobulin A
sC	– Salivary Cortisol
SP	– Sport Participation questionnaire
URTI	– Upper Respiratory Tract Infection
$\dot{V}O_2$ Max	– Maximal oxygen uptake
V region	– Variable region of the immunoglobulin protein



# Chapter 1

## INTRODUCTION

This research study was undertaken with the goal of searching for relationships between humoral immunity, physical fitness levels, stress levels, and percent body fat in children. The paper will provide an overview of the immune system of the human body. Further, recent literature will be reviewed bringing attention to the effects of physical activity on humoral immunity in adults. The focus will narrow further with an examination of the relevant few studies on children. The last section of the review leads to the purpose of the study: To examine the effects of physical fitness, stress, and relative body fat on humoral immunity in children. When stress is not a factor, the hypothesis is that lower levels of physical fitness combined with elevated levels of relative body fat will compromise humoral immunity in children.





## 1.2 Rationale

Much research has been done to determine the strengths and weaknesses of the defense mechanism that protects the human body from microbial invasion. While many advances in research on the human immune system have been made, many more questions remain unanswered. One area of inquiry is the possible relationship between humoral immunity and physical activity. For some time, exercise scientists have been trying to determine if a relationship exists between exercise and the ability of the human host to defend itself from pathogens. There is some evidence that exercise influences natural immunity, T- and B-cell functions, and cytokine responses through hemodynamic changes and hormonal secretion in response to physical stress in adults (Nieman et al., 1990).

The magnitude of the effect on the immune system depends on the intensity, duration, and chronicity of exercise. The response of immune function to exercise in adults appears to exhibit an “inverted J-shaped” curve, with benefits accruing from moderate exercise, and negative effects resulting from excessive, heavy exercise (Nieman & Pederson, 1999). As a result, it has been suggested that the stress of intense athletic competition and training may depress cellular immunity, predisposing athletes to an increased risk of infection (Heath et al., 1991; Baj et al., 1994). In contrast, a recent study demonstrated that moderate physical activity reduces the incidence of upper respiratory tract infections (URTI) by as much as 30% in physically active, as compared to inactive adults (Matthews et al., 2002).

Recent evidence indicates that moderate exercise can enhance cell-mediated immunity (CMI) and increase immunoglobulin A concentration leading to improved



immunity against infection (Mackinnon, 1996). In contrast, exercise-induced decreases in salivary IgA (sIgA) have been observed in a variety of athletes (Tharp & Barnes, 1989; Mackinnon & Jenkins, 1994; Mackinnon, 1996). It has also been noted that daily intensive training and psychological stress before major competitions often result in low resting salivary IgA levels, suggesting chronic immune suppression as antibodies of the IgA class are the primary mediators of the humoral immune response of mucosal surfaces.

A particularly common cause of absenteeism from school and work is upper respiratory tract infections (URTI). Susceptibility to URTI is substantially increased with a depressed immune system. At present, there are scant data comparing the response of sIgA levels and the incidence of URTI in moderately active children to those of sedentary children. Therefore, a potentially protective mechanism against URTI in children is poorly understood. More importantly, there are no reported data confirming the “inverted J-shaped” nature of immune function response and incidence of URTI in children and adolescents and so the effect of intense exercise on the immune system of children is also unknown. Although moderate exercise appears to decrease the risk of URTI in adults (Klentrou et al., 2002), there is a complete lack of information concerning the response of children and adolescents. The concern is that if children’s immunity is negatively affected by a lack of regular physical activity, the effects on the individual’s developing immunity may be cumulative.



## Objectives

There have been very few studies conducted on the effects of moderate exercise on sIgA levels in children (Nieman et al., 2002). Recent research has provided evidence that regular physical activity benefits secretory immunity in adults (Klentrou et al., 2002), but to date there is little research on the effects of regular physical activity in children and adolescents. Immunoglobulin A is present in all secretory areas of the body (Austyn & Wood, 1993; Underdown & Schiff, 1986) and is regarded as the primary humoral defense against antigens that may violate the organism whether by environment or diet. Immunoglobulin A (IgA) is of particular interest because of minimal amount of research connecting IgA to exercise research and because of its strong correlation with other humoral immunity markers. Salivary IgA will be used as one of the measures of immune function in the study.

More specifically, this study examined relationships among physical activity, stress-related and secretory immunity variables, such as habitual activity, organized activity, aerobic power, body fat, salivary cortisol (sC) and salivary immunoglobulin A (sIgA) levels in pre – teen children of Southern Ontario. Differences in the above mentioned variables between males and females were also assessed.





## CHAPTER 2

### REVIEW OF LITERATURE

#### 2.1 Humoral Immunity

##### 2.1.1 Introduction

Humoral immunity refers to the ability of the host body to defend itself from invaders/parasites that would negatively affect the host. These invaders/parasites would draw upon the resources of the host to reproduce and thrive. This is not a symbiotic relationship and the human host has developed a systematic defense system that includes: a) prevention or resistance, and b) aggressive defense. The second is activated only if the invader is able to gain access to the host. The key to all humoral defenses is the antibody. This section is a review of some of the relevant host defenses.





## 2.1.2 The Defense Mechanisms

*Adaptive Immunity:* The immune system features three general components to its approach to humoral immunity: adaptation, specificity and memory. These components are all part of the host's adaptive immunity. The immune system's most important trait is its ability to adapt to invasions by many different viruses, bacteria and parasites. This adaptability is sometimes referred to as *inductibility*. Inductibility is regulated by the systems' ability to deal with each case it is presented (Golub & Green, 1991). While action against multiple antigens can be referred to as inductibility, each individual action against an antigen is referred to as *specificity*. Specificity is the ability to pursue and disable an antibody by using a physiological 'exact fit' with an antibody using receptor sites as locations for binding and occupation of the cell. The advantage of specificity is the effect that the defenses have on the antibody is very effective for that specific antibody. However, specificity is specific for that particular antigen. If a different antigen or variation of the original antigen enters the host, that antibody will not be able to neutralize or destroy the subsequent antigen, or at least will be much less effective.

The last important component is the system's ability to deal with the same antigen on a repeat basis without going through protein differentiation each and every time. The system maintains the ability to utilize *memory* (Golub & Green, 1991). Memory ability enables the immune system to deal with a repeat invader specifically and more quickly than the first encounter (Golub & Green, 1991). The response to a second attack by the same antigen results in a quicker response and an accelerated production of antibody to neutralize the antigen.



*Natural Immunity:* In addition to the body's adaptive immunity, the human host has a second system that is not so discretionary. This system is termed *natural immunity* (Golub & Green, 1991). This system is much more basic in that it does not have the memory ability that the adaptive immunity utilizes, and it does not exhibit specificity (Golub & Green, 1991). It utilizes proteins such as macrophages to defend the host from invaders. It is inducible and displays non-self discrimination. Similar to natural immunity, the adaptive system is able to distinguish between invaders to the host and cells created by the host. An elicited response towards an antigen can be classified into two separate categories. The first classification is a *humoral response*. A humoral response begins with the host producing an antibody to respond to the antigen (Hood et al., 1978). The response can be measured according to the level of response. The response is generally a product of the lymphocytes. The second classification can be referred to as the *cell mediated response* (Hood et al., 1978). Essentially, the cells in the surrounding environment carry out the response.

Other aids to host defenses include maintenance of epithelial integrity. This ensures that no unprotected entry points exist in the body. In addition, the host must maintain equilibrium of proper pH levels throughout the body (Hood et al., 1978). This is especially effective in areas where contact with the surrounding environment is possible (stomach, vaginal areas etc). Lastly, mechanical expulsion of microorganisms by the host (sneezing) and a properly functioning phagocytic system (that stimulates B and T-cells) are all vital aids in maintaining host defense.

A further support mechanism for host defense is effective and timely secretion of lysozymes. Lysozymes work together with antibodies to strengthen host defenses.





Lysozymes are unique in their support in the manner in which they attack an invader. Through a method referred to as *opsonization*, the lysozymes continue to coat the antigen until it can no longer continue to thrive in the body (Hood et al., 1978). This facilitates digestion by phagocytes.

*The Antibody:* The antibody takes many different forms in the human body (and most higher level multi-cellular organisms), but is generally referred to as the protein structure that is best suited to attach and aid in the dismantling and/or neutralization of a specific invader. Antibodies do not have a specific purpose upon conception in the body. The selection process that antibodies go through after creation can be termed *clonal selection* (Golub & Green, 1991). The offspring or cells that are produced can be labeled as virgin/immature cells. These cells leave the Bone Marrow (BM) as base cells (Lawrence & Landy, 1969). In most cases, these cells are transported to various lymphoid sites and have a genetic code imprinted on them there. Clonal selection happens at extremely high rates, producing elevated numbers of cells. When left unchallenged, a normally functioning system could replenish the whole host's needs in approximately four to five days (Nossal & Ada, 1971). In most cases, antibodies originate from hosts' bone marrow, and are the starting point for the lymphoid system. The offspring proteins are not antigen reactive. In order for them to be so, they must differentiate first (Nossal & Ada, 1971).

The *thymus* is the site for the protein differentiation. The thymus' primary function/role seems to be to differentiate lymphoid (Hood et al., 1978). The spleen and lymph nodes would be classified as secondary sites for differentiation. The process takes approximately 2-3 weeks, with the cell going through complete maturation and cell



division prior to completion (Nossal & Ada, 1971). It is hypothesized that not all antibodies complete the process. Some are naturally selected out while others die before completing the process. Upon completion of this selective process, the cell that emerges from the differentiation site is now referred to as an Immunoglobulin.

### **2.1.3 Immunoglobulins**

The immunoglobulin is the eventual expression of the immature cells released by the bone marrow. There are currently five recognized classes of immunoglobulins (Ig). They are IgG, IgM, IgA, IgD, and IgE. IgG accounts for the majority of the protein content in serum (Hood et al., 1978; Austyn & Wood, 1993; Mackinnon, 1996). It is estimated that IgM accounts for approximately 10% of all normal human serum. IgA (serum) accounts for approximately 20% of total serum (Golub & Green, 1991). IgA, however, is the dominant protein in mucosal secretions such as saliva, tracheobronchial secretions, colostrums, milk and genito-urinary secretions. The body maintains a small IgD component of Ig content in the body. It is also most sensitive to proteolysis and heat, which cause the protein to fragment (Hood et al, 1978). IgE is involved in hypersensitivity and allergy. IgE has the lowest concentration in serum.

The IgA protein is of main interest to this study/paper. The effects of exercise on this specific protein will be covered more completely in the next section. However, it is worth mentioning here that the IgA antibody is the dominant Ig in seromucous secretions (Hood et al., 1978). Epithelial cells synthesize the secretory component, which is used as a transfer molecule through the epithelia (Underdown & Schiff, 1986). B lymphocytes at





the mucosae are primarily dedicated to IgA synthesis (Underdown & Schiff, 1986). The secretion sites are separated from the rest of the body by a basement membrane. It is believed that all sites are individually controlled (Underdown & Schiff, 1986). As a result of the individual control for each site, it is very difficult to immunize any epithelia with an injection or single site immunization.

The specificity between antibodies is determined by the differences of chains in the heavy and light chains in one antibody in comparison to the same chains in another (Golub & Green, 1991; Hood et al., 1978). In each specific immunoglobulin, there are subclasses of the same Immunoglobulin. The CH region of each Ig is designated a Roman letter to help distinguish the different subclasses. For example, in the IgG CH region, it is classified as  $\gamma$  chain, while the CH region of the IgM is a  $\mu$  chain (Ig A has an  $\alpha$  chain designation) (Golub & Green, 1991; Hood et al., 1978). While each Ig class can be composed of different polymers of the base Ig molecule, and thus have diverse molecular weights and properties, none of these characteristics determines the Ig class. Each class of Ig inherits the basic ability to combine with antigen, the primary function of antibody molecules (Small & Waldman, 1970). However, each Ig reacts differently to each stimulation.

It has been shown that Ig antibodies do not always remain the same class of Ig they began as. *Class switching* describes the process of changing the class or isotype of an antibody. For example, changing from an IgM to one of the other Ig antibodies. This is mediated by DNA rearrangement. The two key components for any combination/recombination of DNA strands for Ig antibodies are identified as promoters (responsible for accurate and efficient transcription) and enhancers (increase the rate of



transcription from promoters). The immediate environment can also influence transcription of DNA coding. Idiotypic determinants (meaning individual differences) are encoded into the V region of the Ig. This is evident when in response to antigen presence. Several idiotypes, as a result, are presented in response.

Class switching because of the environment relates to *clonal selection*. Clonal selection refers to the mediated response of the antibody to the antigen present (Brunet 1969; Golub & Green, 1991). However, this is unique in that the antigen does not specify what antibody to generate. It does activate the reproductive response from cells that can react with the antigen (Brunet, 1969; Golub & Green, 1991). Antigens act as a stimulus to the cells that have receptors for these antigens (Brunet, 1969; Golub & Green, 1991).

With all these adaptations, the Ig molecule would seem to be ably adapted to assume the role of defender of the host body single handedly. However, this is not the case. Ig molecules cannot be expressed without the presence of a B-lymphocyte cell. The final section explores the relationship and immune expression of Ig molecules in marriage with the B-lymphocyte.

#### **2.1.4 B-Lymphocyte and the Immunoglobulin Molecule**

The lung and intestine serve as major portals for invasion from external attack (antigen). Upon contact with an epithelial surface in the lower lung, mononuclear phagocytes and T lymphocytes attract (either destroy immediately or neutralize and transport) the antigen to the lymph nodes where the antigens are then further disassembled (Acha-Orbea et al., 1989). However, this is not the initial stage of defense in areas of potential contact with





the environment to the host. Previously mentioned lysozymes and Ig are present in nasal and stomach fluid and mucosae, which act as a superficial barrier against such contact. There are two types of receptor molecules that work together to defend the host from invasion. The two types are the T-cell receptor and the Ig. The T-cell receptor is expressed on the surface of the T-lymphocytes. The second is the previously mentioned Ig, which can only be expressed on the surface as a receptor of the B-lymphocytes (Acha-Orbea et al., 1989; Nossal & Ada, 1971). While both interact with antigen in similar but separate situations, they are connected by their reaction to each other. Specifically, the B-lymphocyte requires the T-lymphocyte for help to activate (Acha-Orbea et al., 1989). The specific interaction between the two lymphocytes is beyond the scope of this review.

The important components of an antibody distribution system include antibody formation, tolerance/recognition of self, immunological memory and feedback and control of response (Nossal & Ada, 1971). With one end of the Ig firmly attached to the B-lymphocyte, the two together form a complement that is fully capable of recognizing a wide variety of antigens (Acha-Orbea et al., 1989). As mentioned above, the Ig on the surface of B-cells act as a receptor. All five types of Ig are expressed on the surface of the B-cells (Hood et al., 1978). Since these Ig are the first to contact any antigen, they are the first antibodies present upon antibody stimulation as well (Hood et al., 1978).

At the point of contact between the antigen and antibody, a neutralization of the antigen occurs. This serves two primary purposes. Neutralization of the antigen prevents the release of toxins into the host and surrounding cells, and provides protection of the host from the virus (Hood et al., 1978; Mazanec et al., 1993). The second primary function is the blocking of the biological function of the receptor by binding of the



receptor (Hood et al., 1978; Mazanec et al., 1993). As a result, that specific receptor on the antigen could have been used to attach to other indefensible cells and causing destruction in the host. As a result of the blocking, that receptor is occupied by an antibody, and can no longer function in that way. Thus, when an individual is immunized with detoxified molecules, the individual develops antibody that reacts with the toxin in a future-invading virus (Mazanec et al., 1993).

Lymphocytes travel using the blood stream and lymphatic system. Using specific passage points, called lymph nodes, B and T lymphocytes have specialized receptors to locate and pass through these specialized venules (Hood et al., 1978). The ability to travel is necessary for humoral adaptations when defending the host. However, this travel is in contrast to the specific and local adaptations of epithelial surfaces (Small & Waldman, 1970). When attempting to immunize locally a surface/epithelial section, the adaptations remain local. This is in contrast to the humoral adaptations mentioned above. The secretory antibody is synthesized by epithelial surfaces directly in contact with the antigen (Small & Waldman, 1970). It seems to be able to adapt to protect the host against pathogens when stimulated appropriately. Liew et al. (1984) studied the local effects of epithelial immunization on rats. They found that mucosal adaptations to the immunization were the direct cause of successful defense of the host from the Influenza A virus. Liew et al. (1984) also determined that the primary response to the immunization was most likely a result of the specificity of secretory IgA.

According to Melchus & Anderson (1986), the antibody has the ability to select from a repertoire of resting B cells. B cells have been shown to have the ability to divide every 20 hours at 37 degrees Celsius (in vitro, for 5-15 divisions) (Melchus & Anderson,





1986). Upon maturation, B cells increase their rate of Ig synthesis and begin to actively secrete Ig (Melchus & Anderson, 1986). In addition to expanding the memory library of the B Lymphocytes, there is growing evidence that the process of expanding the memory library reverses itself in aging (Effros, 2000). In some cases, the repetitive exposure to some antigen (the hallmark of the normally behaving immunity) can be detrimental to the function of the immune system over time. This repeated overload causes senescence, thus potentially overloading the system (Effros, 2000).

There is a 2-dimensional interface on the surface membrane of the cell, between the cell and its immediate extra cellular or cellular environment (Cuatrecasas & Greaves, 1976). This interface provides the ideal venue and physical corridor for interactions and signal reception (Cuatrecasas & Greaves, 1976). The surface membrane can serve as a sensory device capable of detecting environmental signals. Upon detection of an external signal, the cell begins translating this signal into an internal reaction that induces, alters, or regulates cellular activity (Cuatrecasas & Greaves, 1976).

*Immune Tolerance.* Although the immune system is sensitive and reactive, there are some flaws that are exploited in naturally occurring instances. These flaws can prove to be quite problematic, regardless of how well pre-immunized or properly operating the system is. This potential problem is *immune tolerance* (Nossal & Ada, 1971).

Immune tolerance is defined as the body's inability to react to an antigen by selecting the antigen as tolerable and thus not reacting to eliminate it (Nossal & Ada, 1971). Immune tolerance can occur especially in younger individuals with weaker trapping and retention systems. This may explain why children are more prone to immune tolerance and, thus, more prone to higher rates of infection. Immune tolerance



can be characterized as either high dose or low dose tolerance. High dose tolerance is achieved when there is a very high exposure, and only takes 24 hours to reach (Nossal & Ada, 1971). Low dose tolerance is the opposite. It requires very small amounts of exposure to the antigen, and it must be quite regular and spread out over time (Nossal & Ada, 1971). High dose tolerance tends to be more complete and longer lasting. Low Dose is short-term and transient. While immunization creates a cell division reaction (amplification of response), tolerance cannot be transferred from cell to cell (Nossal & Ada, 1971). This does protect the host from complete tolerance in some cases. There is no fundamental difference found between tolerance induction between adults and neonatally induced tolerance (Nossal & Ada, 1971). However, it is still easier to induce tolerance in the neonatal (Nossal & Ada, 1971).





## 2.2 Effects of Exercise on Humoral Immunity

For some time, exercise physiologists have been searching for a relationship between exercise and the strength and quality of the immune system. Some general relationships have been established. Intense training in elite athletes has been linked to a weakened immune system, and increased risk of infection at the mucosal levels (Mackinnon et. al., 1993<sup>a,b</sup>). However, studies examining the effects of moderate training or decreasing levels of physical activity have had mixed results. It has been hypothesized that there would be positive effects on the immune system for a sedentary individual who undertook a regular moderate exercise routine (Klentrou et. al., 2002). Until recently, the literature has been dominated by cross sectional research on animals (e.g.: Liew et. al., 1984), high performance athletes (e.g.: Nieman, 1993), and the elderly (e.g.: Karper & Boschen, 1993). There are only a few longitudinal studies that have used average adult humans (Klentrou et. al., 2002) and children (Boas et. al., 1996) as subjects.

As most studies suggest, moderate exercise has a positive effect on the immune system while intense exercise evokes a negative response (Nieman, 1990<sup>a</sup>, 1993, 1998; Peters & Bateman, 1985; Mackinnon et al., 1993<sup>a,b</sup>). However, to accurately identify the effects, a further distinction must be made between chronic effects and acute effects of exercise on immunity. In most cases, effects of cross-sectional testing have revealed similar results. However, the duration of physical training is also a key influential factor in determining the effects on the immune system. In the next section, chronic effects of exercise on humoral immunity will be examined. Chronic, here, is defined as effects that exercise has on the resting levels of immune parameters. In contrast, acute effects are





defined as effects that exercise has on immune parameters immediately following a bout of physical activity. Acute effects are examined in section 2.2.3. Section 2.2.4 examines the effects of cortisol on the body when stressed and its relationship with exercise.

### **2.2.1 Effects of Intense Training in Athletes**

*Effects of Intense Training in Athletes.* It was hypothesized that elite athletes are at increased risk of contracting URTI in comparison to sedentary and non-elite trained individuals (Nieman, 1993). Elite level training (along with competitive/psychological stress) has been shown to increase the incidence of the contraction of URTI in these individuals (Nieman, 1993; Peters & Bateman, 1985). Gleeson et al. (1995, 1999) examined elite swimmers and found that the athletes were at increased risk of contracting URTI when compared to non-athletic controls. Gleeson et al. (1995, 1999) also found that the risk of contracting URTI increased as the athletes elevated training intensity and duration. Most interestingly, Gleeson et al. found a direct association between IgA levels and infection rates. They reported increases of infection rates as IgA decreased. The control group showed decreasing IgA levels over the course of the study at rates similar to the exercise subjects (Gleeson et al., 1999). Unfortunately, Gleeson et al. (1999) did not report the effect that the training from the whole month had on the corresponding IgA levels. This would seem to be important since post-training IgA levels seemed to be affected by the type of training of that month, when compared to pre-training levels.

Peters & Bateman (1983) randomly selected marathon runners to compare with non-runner controls. The participants who had the faster times and ran further during



weekly training, reported a higher incidence of URTI than the non-runner controls (Heath et. al., 1991; Peters & Bateman, 1983; Nieman et. al., 1990<sup>a</sup>). A number of other studies have confirmed these findings in similar studies with comparable controls (Nieman et. al., 1990<sup>a</sup>; Nieman et. al., 1998). Susceptibility of athletes to URTI and corresponding levels of IgA were examined by Mackinnon et. al. (1993<sup>a,b</sup>). Interestingly, Mackinnon et. al. (1993<sup>a,b</sup>) found that an inverse relationship did exist between decreasing levels of IgA and incidences of URTI. Drops in IgA levels were significantly related to subsequent increased recording of incidences of URTI. Depressed levels of IgA was also believed to be a contributing factor in the subjects' increase in URTI. This would indicate a similar effect on the individual's IgA effectiveness. Intensely active individuals would increase their risk of contracting URTI and, as a result, decrease the effectiveness of IgA to defend the host (Nieman, 1993).

There is much debate concerning the effects of the type of training an individual undertakes and the corresponding effect on the immune function. Baj et. al. (1994) could not find conclusive evidence that supported or disproved the hypothesis that there would be negative effects of intensive training on humoral immunity. Mackinnon & Hooper (1994) conducted tests that examined three aspects of intense training in elite athletes. The three aspects investigated were: the effects of single intense bouts, consecutive training days, and long-term over-training/staleness (Mackinnon & Hooper, 1994). Results of an acute nature are examined in the next section. Noticeable decreases were recorded in IgA levels in the athletes after consecutive days of training and in over-trained athletes (Mackinnon & Hooper, 1994). The decreases were attributed to the exercise intensity (Mackinnon & Hooper, 1994). In contrast, other researchers have





found that IgA decreased regardless of the intensity of training of athletes during the training season, with the largest decrease following moderate training (Tharp & Barnes, 1989; Mackinnon et. al., 1993<sup>a,b</sup>). Interestingly, results were conflicting for findings that illustrate negative effects on individuals in high intensity training programs. Kumae et. al. (1999) used elite runners as subjects and found that intense training did not negatively affect humoral defenses.

This increase in risk is now attributed to the body's lack of natural response to infection whilst participating in a strenuous exercise program. Nieman et. al. (1995<sup>a,b</sup>) assessed other indicators of humoral immunity such as: lymphocyte subsets, concavalin A/lymphocyte proliferation, NK cells and NK cytotoxic activity (NKCA) and did not find any significant differences between athletic and non-athletic control groups. Other research on marathon runners found that neutrophil levels were decreased and NKCA was found to increase compared to non-athlete controls (Nieman et. al., 1995<sup>a,b</sup>). Nieman et al. (1989<sup>a,b</sup>) found that proteins called complement C3 and C4 showed decreased levels post exercise in comparison to their non-exercise controls. In addition, Mackinnon & Hooper (1996) examined the levels of glutamine with respect to over-training and URTI incidence and found that no relationship existed between glutamine levels and the manifestation of URTI in athletes.

Both Schouten et. al. 1988 and Heath et. al., 1991 found that male and female changes in salivary IgA levels differ in reaction to exercise. Levels of sIgA were found to decrease in females, in contrast to the male subjects who were found to exhibit increasing levels of sIgA. In addition, Schouten et. al. (1988) reported that only women displayed a positive relationship between long-term physical activity and increasing



salivary IgA levels. Also, Heath et. al. (1991) found other sex differences. Heath et. al. (1991) found that males who consumed alcohol and trained in long distance running were more susceptible to exhibiting URTI than their female counterparts.

Nehlsen-Cannarella et. al. (2000) found that salivary IgA concentration was elevated in elite women rowers in contrast to non-exercising controls. Nieman et. al. (1989<sup>a,b</sup>) found that subjects who trained less (at moderate levels) were more susceptible to contracting URTI than marathon runners. One final study that deserves special mention is the work of Lindberg & Berglund (1996). Lindberg & Berglund (1996) treated their subjects with IgA /IgG nasal drops in an effort to immunize the epithelial cells, to monitor the effects on the elite athletes. Intense exercise did not illicit major differences in IgA levels between the elite exercisers and their comparative controls. Nor did Lindberg & Berglund find any difference in the URTI symptoms experienced by subjects treated by the nasal IgA drops and those who were not, nor was any significant difference found between groups. These results do not support the hypothesis that IgA levels (when increased) would increase the body's ability to defend against URTI. In contrast, Hemmingsson & Hammarstrom (1993) found that inoculation of adult elite cross-country skiers was effective in reducing URTI, thus improving humoral immunity.

### **2.2.2 Effects of Exercise Training on Immunity in Sedentary Individuals**

Studies have examined the effect of moderate exercise on immunity in sedentary subjects. The results are mixed, similar to research examining intense exercise. Nehlsen-Cannarella et. al. (1991<sup>a</sup>) reported that subjects in the exercise groups did not express any





significant differences in levels of IgA or other serum immunoglobulins. Karper & Goldfarb (1995) stated that they did not find conclusive evidence that moderate levels of physical activity in the elderly (which included light weight training and walking) positively affects humoral immunity. However, the subjects in the study acted as their own controls and were relying on their memory of the year prior to compare their URTI results. Both Nehlsen-Cannarella (1991<sup>a</sup>) and Karper & Goldfarb' (1995) studies examined chronic effects of exercise. In this paper, chronic refers to long term effects of physical activity in contrast to acute effects, which refers to effects after a single bout of physical activity.

In contrast, other researchers have found that moderate exercise is positively associated with long-term improvement in humoral immunity (Nieman et. al., 1990<sup>b</sup>; Nehlsen-Cannarella et. al., 1991<sup>b</sup>; Klentrou et. al., 2002). Nieman et. al. (1990<sup>b</sup>) and Klentrou et. al. (2002) reported that subjects in the exercise group reported significantly less URTI symptoms than their non-exercise counterparts. Klentrou et. al. (2002) found that sedentary individuals reported fewer incidents and decreased intensity of URTI symptoms after engaging in moderate exercise. This was in contrast to the sedentary controls. In addition, the exercising individuals increased their IgA concentration in contrast to a seasonal decrease in the controls (Klentrou et. al., 2002). Nehlsen-Cannarella et. al. (1991<sup>b</sup>) reported that serum immunoglobulin levels consistently rose after 45 minute bouts of moderate walking, over a 15-week period. Intriguingly, Nieman et. al. (1990<sup>b</sup>) reported that subjects in the control group recorded similar results in their percent NK cell activity to the exercise group, after 15-weeks. Nieman et. al. (1990<sup>b</sup>) speculated that the change might be attributed to seasonal change. However, none of the



other categories/variables examined exhibited similar effects, thus the results found in Nieman' study could be subject to reproducibility problems.

Karper & Boschen (1993) support the hypothesis that exercise is positively associated with humoral immunity in their research on older subjects. They also reported lower incidences of URTI in their subjects who took part in moderate exercise training over the course of one year. This is supported by Weidner et. al. (1998) hypothesis that subjects' condition, who were inoculated with a rhinovirus, did not deteriorate as a result of taking part in a moderate exercise program. The study compared results between an exercise and non-exercise group. The exercise group trained every other day to maintain a moderate level of exercise and symptoms were recorded throughout the testing period.

Mackinnon et. al. (1993<sup>b</sup>) conducted an eight-week intervention study using intense exercise. Mackinnon et. al. (1993<sup>b</sup>) took samples of sIgA from the subjects immediately following repeated intense exercise bouts on a cycle ergometer in an effort to determine if effects immediately post exercise change over time. The results showed a decreased overall IgA level (in comparison to samples taken prior to bout of exercise). The intense exercise decreased IgA levels further following the training.

### **2.2.3 Acute Effects of Exercise on Immune Function**

There are conflicting reports about the acute effects of exercise on immune function. Research on acute effects has examined the effects of low, moderate or intense exercise levels. Hanson & Flaherty (1981) found no change in IgA levels in research examining effects immediately following an intense bout of exercise in athletes and non-athlete





controls. Similar results were found by Mackinnon & Hooper (1994) and Nieman et. al. (1989<sup>a,b</sup>; Walsh et. al., 1999).

In contrast, Tharp & Barnes (1989) found that IgA decreased regardless of the intensity of the acute bout of exercise of athletes during training season, with the largest decrease following moderate training. Blannin et. al. (1998) also tested subjects at intense and exhaustion levels of exercise in an effort to determine the effects on IgA levels and the duration of these effects, if any. Results showed that overall, IgA levels in subjects who trained at intense levels, returned to pre-exercise levels by the first hour post-exercise. Mackinnon et. al. (1993<sup>a</sup>) found that levels of IgA decreased significantly immediately following intense training, and the decrease lasted at least 30 minutes. This decrease was supported by recent research conducted by Fahlman et.. al. (2001). Recently, Walsh et. al. (1999) suggested that perhaps simply the psychological anticipation of strenuous physical activity affects  $\alpha$  amylase (aids in inhibition of attachment of bacteria to oral surfaces). Walsh et. al. (1999) found that  $\alpha$  amylase levels were significantly depressed prior to the maximal intensity exercise in their study.

Fahlman et. al. (2001) tested females and found that only 8 minutes elapsed post intense levels of exercise before a significant decrease was recorded in salivary IgA (sIgA). Subjects recorded their symptoms of URTI for 3 weeks post testing and no significant relationship between the acute bout of exercise and any symptoms was found. In contrast, Gabriel et. al. (1992) found that plasma borne indicators of humoral defense (granulocytes, lymphocytes, leucocytes, NK cells,  $\beta$ -endorphins) were positively affected by intense exercise. As exercise intensity increased, so did concentration of the indicators. Similarly, the results from Reid et. al. (2001) supported a significant increase





in sIgA immediately post – exercise. Reid et. al. (2001) found increases in sIgA immediately following low-moderate exercise and short-intense exercise bouts. They also reported no significant difference in salivary IgA levels 30 minutes post training bout, in comparison to resting levels prior to training. McDowell et. al. (1992) supported these results. However, McDowell (1992) found that sIgA levels returned to pre-exercise levels one hour following the training bout.

Interestingly, when Fahlman et al. (2001) examined the findings of Schouten et. al. (1988), they suggested studying the effects of exercise on sIgA in subjects examining results from each gender separately. Unfortunately, Fahlman et. al. (2001) study only used female test subjects and could not draw comparisons from their results. Consequently, Fahlman et. al. were not able to support this approach by comparing their results to Schouten et al.' (1988) research.



## **2.3 Effects of Stress and Body Composition on Humoral Immunity**

The effects of stress span both physical and psychological components. Stress measures have been identified in both physiological mechanisms and psychological effects such as any vigorous activity, adverse environment or psychological pressure placed on the body (Brenner et. al., 1998). Stress can be exhibited either internally or externally (i.e. - illness or temperature). In addition, reactions can vary and simultaneously be multiple. The signs of stress can range from hypertension to perspiration. Assessing cortisol levels in the body has been reliably used to monitor effects of stress and yet expression varies between individuals. Cortisol has been most recently referred to as a central cellular predictor of stress.

### **2.3.1 Cortisol Response Cascade**

Responses to stress can vary, as do the type of stressors placed on the body. Types of stress can be vigorous activity, adverse environment or psychological pressure. Cortisol has been linked to the stimulation of the breakdown of protein to amino acids in all cells of the body (except the liver). Amino acids are then transported to the liver to be synthesized into glucose. Cortisol supports the gluconeogenesis of other proteins/hormones such as glycogen and human growth hormone (HGH). Cortisol also acts as an insulin antagonist – inhibiting glucose uptake and oxidation (al'Absi & Arnett, 2000). Prolonged, high serum concentrations of cortisol can lead to excessive protein breakdown, tissue wasting and negative nitrogen balance. Cortisol has shown the ability



to cause an acceleration of lipid mobilization for energy (al'Absi & Arnett, 2000). An increase in cortisol output is believed to cause the liver to split mobilized lipid into its simple ketoacid components (al'Absi & Arnett, 2000). An individual whose diet consists of a combined low carbohydrate - low caloric intake would most likely suffer from ketosis.

Any bodily response to stress, either physiological or psychological, is a multi-stage response where a number of components must be triggered in sequence for the body to adequately react. Increased levels of dopamine or norepinephrine trigger the release of corticotrophin releasing hormone (Brenner et. al., 1998). The release of this hormone stimulates the release of adrenocorticotrophic hormone (ACTH) and beta-endorphin from the corticotroph cells of the anterior pituitary. ACTH then induces production of cortisol through the adrenal cortex (Brenner et al., 1998). Simultaneously, activation of the sympathetic nervous system stimulates a release of catecholamines from the adrenal medulla and sympathetic nerve terminals. As a result, side effects of having overactive cortisol secretion include an enlarged adrenal gland, enlarged pituitary glands and a muting of neurotransmitters (Stokes, 1995). The pituitary generally decreases in size as we age.

Cortisol systematically affects many mechanisms in the body. Levels of cortisol in the plasma largely affect blood pressure. Effects of normal levels of cortisol directly affect the vascular tone of the cardiac system (al'Absi & Arnett, 2000). Presence of cortisol enhances fluid transport and volume by causing fluid to shift from an intracellular to an extra-cellular direction (al'Absi & Arnett, 2000). By increasing extra-cellular fluid volume, there is a corresponding increase of venous return. This cascade





results in increased cardiac output. However, to complicate the reaction, cortisol also causes vasoconstriction. As a result of the increased fluid volume in combination with vasoconstriction, there is an increase in hypertension (al'Absi & Arnett, 2000). However, research has failed to show that these vasoconstrictive reactions are a result of an abnormal production of cortisol (al'Absi & Arnett, 2000). Thus, many individuals experience this phenomenon regularly and the combination results in compounding a condition of hypertension or high blood pressure.

### **2.3.2 Relationship of Psychological Effects and Cortisol Levels**

The expression of stress in the human body is not uniform. For example, a positive psychological perception can produce effects similar in physiological response to negative effects (Brenner et. al., 1998). It is believed that a small increase in cortisol secretion is generally associated with passive coping and chronic stress response. However, a 'perceived' negative stress is not the only type of stress that leads to elevated cortisol levels. Thus, cortisol level monitoring is not always an effective measure/indicator of negative stress.

There are two primary systems that are hypothesized to be affected by a psychological stress placed on the body. The two systems are the hypothalamic-pituitary adrenocortical axis (HPA) and the sympathoadrenomedullary system (SAM) (al'Absi & Arnett, 2000). These two systems work together when engaging a response to a psychological stressor. The reaction begins chemically when the release of corticotropin-releasing factor from neuronal cell bodies of the paraventricular nucleus





initiates an HPA axis cascade (al'Absi & Arnett, 2000). A number of events happen subsequently after this release, which culminate in ACTH reaching the adrenal cortex. ACTH contacting the adrenal cortex causes the release of corticosteroids or cortisol (al'Absi & Arnett, 2000; Nejtek, 2002). The liver is the main site of cortisol synthesis. Upon release of cortisol, its main purpose is to enable peak energy release to respond to the stress (al'Absi & Arnett, 2000). Cortisol also enhances receptors in the sympathetic nervous system, which can have an effect on the ability of the individual to react to sudden stressors (al'Absi & Arnett, 2000).

Each stress placed on the body is unique. The uniqueness of each stress is gauged by the situation and/or environment. It is special because the perception of the stress is said to differ according to the aforementioned situation/environment. The major determining factor of the perception of a stress by each individual is their perception of their ability to deal with the stress (Nejtek, 2002). Nejtek (2002) also found evidence that may suggest a perceived emotional stress may influence the rate of cortisol release based upon the emotional significance the individual associates with the stressor. The importance of these findings relates to the aforementioned effects of chronic exposure to elevated levels of stress. Chronic exposure to stress of this nature could lead to clinical depression and has been shown to affect memory effectiveness (Brown et. al., 1999 in Nejtek, 2002).

There are two stages an individual proceeds through while appraising stress. These stages are referred to as the primary and secondary stages of appraisal (al'Absi & Arnett, 2000). The primary appraisal stage encompasses assessment of each of the following: potential threat, their ability to control the situation/threat, situational



ambiguity (a plus minus equation between items thought to be within the individual's realm of control versus items not within the individual's realm of control), and personal beliefs (al'Absi & Arnett, 2000). The secondary appraisal begins immediately after the primary, and most likely triggers the physical response (fight or flight syndrome). The secondary appraisal includes an individual's evaluation of personal resources, ability to deal with the stressor and likely effectiveness in coping with the stress (al'Absi & Arnett, 2000). Factors that affect this appraisal are task difficulty, lack of control of situation, social support, the amount and type of harassment while attempting the challenge, gender of the individual and the individual's age. These factors all help create inconsistencies in cortisol measurement upon assessment of the level and type of response to a stressor placed on the body. In fact, as Pruessner et. al. (1997) explains, these differences are exacerbated by the contrasting methods of measure in relation to the focus of the measure.

### **2.3.3 Cortisol and Disease**

Chronically elevated cortisol levels are believed to be a contributing factor in many degenerative and fatal diseases (Sapse, 1997). Originally, it was believed that cortisol was a result or a symptom of the prevailing disease. However, this is not the case anymore. For example, cancer was thought to produce elevated levels of cortisol. However, it has been shown that high concentrations of cortisol precede cancer identification, both in experimentally observed animals and humans (Sapse, 1997). Walker et. al., (1997, in Sapse, 1997) illustrated the effects of cortisol treatment by





injecting mice with variations of cortisol, and found that a significantly higher number of animals developed sarcoma when injected with large amounts of cortisol.

Cortisol acts on brain function by direct action on brain cells and/or by reducing availability of neurotransmitters (Stokes, 1995). These effects can alter mood and behavior. High amounts of cortisol are associated with Cushing's Disease. Normal cortisol secretion can be termed to have a "circadian rhythm" of release (Stokes, 1995, Adam & Gunnar, 2001). Pulses peak in the morning and then gradually decrease throughout the day to their lowest levels late in the evening (Stokes, 1995). These levels begin to rise again during sleep and continue to their peak in the morning, to complete the 'rhythm' (Stokes, 1995). However, stress levels or clinical depression can be initiated or altered by elevated secretion levels (Stokes, 1995). Rather than a normal peak, subjects demonstrated elevated levels of cortisol throughout the day. These levels were similar to those with documented levels of Cushing's Disease (Douyon & Schteingart, 2002).

While in most cases, levels return to normal post depression, there is evidence that lasting effects are present (Stokes, 1995). These effects included random elevated levels, especially in the early waking hours of the day (Stokes, 1995). It was hypothesized that the negative feedback loop in the HPA axis was affected (Stokes, 1995). In fact, it was believed that receptors seemed to be buffered against registering elevated levels of cortisol. Thus, elevated levels could be present (and the effects felt in the body) yet no reaction at the cellular level was commenced to counteract these elevated levels. These effects would be compounded by the continuation/resumption of the normal circadian pulses (Stokes, 1995).





Limited reaction to the elevated levels would predispose the subject to reoccurring depression (Greden, 1980, in Stokes, 1995). In fact, it was found that anorexia caused a significant increase in levels of serum cortisol, which would be similar in expression to those who are experiencing clinical depression (Douyon & Schteingart, 2002). Levine et. al. (1978, in Stokes, 1995) speculated that there could be a programming effect, especially in infants or children. When exposed to a stressful event simultaneously (temporary separation), the mother's cortisol levels returned to normal levels shortly after the stress was removed. However, the infant was not as efficient (Levine et. al., 1978 in Stokes, 1995).

Other diseases that may be correlated with high levels of cortisol include amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), myasthenia gravis (MG) (Sapsee, 1997) and even obesity (Douyon & Schteingart, 2002). Behaviors listed as affected by cortisol include the aforementioned depression, with associated conditions such as alcoholism (and other substance abuse), anorexia and smoking. Conditions linked to abnormal cortisol levels include: all types of cancers, ulcers, myocardial infarction, diabetes, arthritis, Parkinson's, psoriasis, acne and eczema (Sapsee, 1997). Most recently, other conditions added to the list include post-traumatic stress disease, Alzheimer's, acquired immune deficiency syndrome (AIDS), and even space adaptation syndrome (SAS) (Sapsee, 1997).



### 2.3.4 Cortisol and Exercise

Cortisol levels tend to vary according to exercise levels, both duration and intensity (Gabriel et. al., 1992; McDowell et. al., 1992; Nieman et. al., 1994), when measured using plasma samples. Increased intensity generally increases cortisol levels (Gabriel et. al., 1992; McDowell et. al., 1992; Nieman et. al., 1994). At maximal intensity exercises, cortisol levels have been found to increase (Gabriel et. al., 1992). Nieman et. al. (1994) indicated that plasma cortisol levels significantly increased following maximal intensity exercise compared to moderately exercised controls. Nieman et. al. (1994) also found that plasma cortisol levels returned to normal and/or sub-baseline levels 3.5 hours post single exercise bout for both levels of training. Gabriel et. al. (1992) found that in exercise levels below maximal output, increases were not significant. Gabriel et. al. (1992) speculated that elevated cortisol levels may affect granulocytosis and lymphocytopenia based on the results from their experimentation. Increased cortisol levels would lead to decreased immune function.

Interestingly, there were no changes in plasma cortisol levels during or after a psychological stressor (Moyna et. al., 1999). Moyna et. al. (1999) examined plasma cortisol levels in subjects who were placed under psychological (public speaking) and physical (stationary bike) stresses. Subjects were separated into three groups based on current physical activity levels (low, moderate, high) and did not find any significant relationship between group, time, or group time interaction. Moyna et al. (1999) suggested that immune indicators and cortisol levels are independent of aerobic fitness level, even though their study was cross-sectional in design. Dukor & Dietrich (1968)





have indicated that cortisol behaves as an immunosuppressant, thus supporting the theory that elevated levels of cortisol as a reaction to intense exercise is an adaptation of the body to the stress.

Similar increases in levels of cortisol were also found in long, steady state exercise, such as marathons (Brenner et. al., 1998; Chicarro et. al., 1998; Nieman, 2000). Elevated cortisol levels can last as long as 2 hours post exercise. Clearance of cortisol can be affected by low caloric diet or cases of clinical anorexia. Cortisol works in combination with other stress hormones to produce many reactions (catecholamines, HGH). The SAM system seems more sensitive to stressors than the HPA axis (Brenner et. al., 1998). The sympathetic response occurs within seconds of contact with the stressor while secretion of glucocorticoids may take 20 – 30 minutes (Brenner et. al., 1998). In a practical sense, a short bout/duration of exercise may have little affect on a glucocorticoid response.

Direct effects on resting secretory immunity are yet to be seen. Limited research has been done using direct measurement of secretory cortisol levels. Tsai et. al. (1991) found that females tended to have higher serum cortisol levels than their male counterparts when assessed in the off-season. However, the authors indicated that these differences were only prevalent in trained individuals, and not in untrained or sedentary controls.

When using cortisol levels to grade the response to a stressor, there are many methods to use, however, not all are very reliable. One possible method of cortisol measure (and possibly the most reliable) is to calculate according to binding capacity of corticosteroid-binding globulin (Chicharro et. al., 1998). It is not until the binding sites





of the carrier corticosteroid-binding globulin are exceeded, that the effects on the surrounding internal environment would be seen (Brenner et. al., 1998). Pre-exercise levels of cortisol have been known to have a significant influence upon the course of glycolytic metabolism during exercise. In addition, the intensity and duration of exercise also has direct effects.

Most notable are the effects when the exercise demand exceeds 60 percent of maximal oxygen intake (Brenner et. al., 1998). When comparing levels of cortisol in saliva versus plasma, this relationship could be defined as linear. However, it has been found that this relationship is only linear to a certain point. At the individual's anaerobic threshold, salivary cortisol (sC) levels drop below levels found in serum, indicating a less accurate indication of cortisol levels in the body (Chicharro et. al., 1998). This is important when conducting research that monitors effects of stress on the body using salivary cortisol as an indicator. When the effects of a combined of temperature and exercise stress are placed on the body, there are direct effects on cortisol levels (Brenner et. al., 1998). In addition, various leukocytes are known to carry cortisol receptors. There is an increase in the number of this type of receptor if cortisol reserves are depleted. These leukocytes can also be used to indicate depleted levels of cortisol when examining effects of physical activity on cortisol levels.

### **2.3.5 Cortisol and the Immune Response**

Cortisol typically stimulates migration of cells from the bone marrow into circulation. Elevated levels of cortisol correspond with increases in circulatory neutrophil counts, but decreases in lymphocytes, monocytes and eosinophils (Brenner et. al., 1998), and IgA



(Jemmott et. al., 1983). Initially, small increases of cortisol seem to have an enhancing effect on the immune response (Brenner et. al., 1998). However, as the level of cortisol increases, the immune response seems to exhibit a suppressive effect (Brenner et. al., 1998; Graham et. al., 1986). It is thought that glucocorticoids alter either density or characteristics of binding molecules (configuration) on the surface of the lymphocytes, thus, modifying the cell's binding reticulin fibers and endothelium (Brenner et. al., 1998).

High levels of cortisol diminish the effectiveness of macrophages, T-cells, and B cells. Cortisol limits the adhesion of the effectors to the target cells, and as a result, limits NK cell activity (Brenner et. al., 1998). Amino acid reserves are needed for proliferation of B cells and synthesis of Immunoglobulins. Cortisol has been shown to incite the reduction of these available amino acids (Brenner et. al., 1998). This reaction limits the ability of the body to respond to an invasion by antibody. However, physical stress does not act alone in compromising immune ability of the body. Psychological stresses throughout the daily routine of the subject generate a sustained physiological adaptation, thus predisposing a person to elevated levels of cortisol (al'Absi & Arnett). In fact, constant elevated levels could lead to predisposition to disease (al'Absi & Arnett). Research by Graham et. al. (1986) illustrated a strong correlation between subjects who experienced high stress levels and elevated episodes of URTI when compared to subjects who had low to moderate levels of stress. The findings were collected over a six-month period, and were typified by the significantly elevated numbers of separate episodes of URTI and total symptom days.





### 2.3.6 Effects of Body Composition on Immunity

Nieman (1990, 1995) did not find any relationship between URTI and Body Mass Index (BMI), using highly trained adult marathon runners as subjects. Nieman et. al. (1998) found a relationship between elevated levels of obesity and elevated levels of leukocytes and lymphocytes. Nieman et. al. (1998) also found that obesity was related to suppressed levels of monocyte and mitogen stimulated lymphocyte proliferation as well as other immune markers, supporting the concept that obesity is associated with alterations in and even suppression of immunity.

Nieman et. al. (1998) were trying to determine if there were any effects of weight loss and decreasing levels of body fat on immune parameters. The results from the study did not support their hypothesis that weight loss would be detrimental to immunity. However, the duration of the study was 12 weeks, and the effects of body composition on immunity may take longer to appear in the body.





## **2.3 Effects of Exercise on Humoral Immunity in Children and Adolescents**

Research into the effects of exercise on humoral immunity in children is quite limited. Many questions remain regarding the effects of exercise on a child's immune system and responses to increases or decreases of physical activity. Some aspects of the effects of physical activity on immunity have been examined in adults but this relationship has not been demonstrated in children and adolescents.

The younger the individual the less effective the immune defense and the following literature demonstrates the timing of maturity of the immune system. However, the question of a relationship between exercise and immunity remains unanswered. Questions regarding the role physical activity may play in the maturation of the immune system are unanswered. More interestingly, and the purpose of this chapter, is to determine if a significant relationship exists in children between levels of physical activity, cortisol, body composition, and mucosal immunity.

### **2.4.1 Immunity Markers in Children and Adolescents**

As mentioned above, minimal research has been conducted with children on the effects of exercise and immunity. Neither a baseline nor standard have been determined for a normal range of immune markers in young children and adolescents. This section will examine the immune markers in children, demonstrating how those levels differ from adults.



Gleeson et. al. (1982) examined salivary IgA, albumin and IgG levels in healthy infants from birth to their first year of life. The goal of the study was to assess the effectiveness of immunity transfer from mother to infant. A longitudinal design was used to track fluctuations in salivary IgA (sIgA) levels in children. A later publication by Gleeson et. al. (1987) found significant fluctuation in sIgA samples taken from the subjects. Salivary IgA was found to peak at the age of five years, and decreased slightly till the age of seven, where the levels remained relatively stable onward to the age of 9 (Gleeson et. al., 1987). However, Gleeson et. al. (1987) did not distinguish within groups of same aged children to characterize if any other factors such as diet, climate or amount of exposure/contact to densely populated areas could have significantly affected these outcomes.

Other research has shown that levels of Ig are comparable to adult levels by the conclusion of the infant's first year (Mellander et. al. 1984; Smith et. al., 1989). Surprisingly, the literature has record of expression of adult levels of sIgA in children at an even younger chronological age. In fact, Haworth & Dilling (1966) found adult levels of salivary IgA by the end of the infant's third week. These are just some of the examples of the inconsistencies regarding the age at which children express immunity similar to adults. However, much of the variability from earlier work could be explained by older (possibly less reliable) and dissimilar techniques used for collection and testing.

Burgio et. al. (1980) found that sIgA levels did not reach adult levels until approximately 6-8 years of age when unstimulated. In fact, Burgio et. al. (1980) were able to distinguish between stimulated and unstimulated secretory immunoglobulins. When stimulated, levels similar to adult values were then realized at the earlier





chronological age of between 2-4 years. Nuesslein et. al. (1995) tested children in the age range of 6.7 to 11.4 years and found that children did not achieve adult levels of immunoregulation until they were in the oldest age category tested. IgA steadily and significantly increased in their subjects over the age categories, peaking in the highest age group that was tested. These results were very similar to those reported by Hoelzer et. al. (2002). In fact, Hoelzer et. al. (2002) found that mean levels of IgA increased in all age groups up to the 12-15 year olds. Interestingly, there were subjects with and without respiratory disease or allergy.

Gleeson et. al. (1991) examined daily variation between Ig and how those levels correlated to URTI. During the 20-day sample period, IgA levels increased when an infectious episode was recorded. Raul et. al. (1996) examined infection rates of rotavirus in infants and children and found that after at least one previous infection (1-3 infectious cases, infection and diarrhea), the subject was at less risk and the symptoms less severe. These results provide evidence to support the theory that memory cells begin collecting at a substantial rate before the age of two years.

Measuring immune markers may not be sufficient to accurately predict immune strength in children. Gurwith et. al. (1981) found that most infants and children were incident free until approximately their second year. Then, over 60% of the subjects contracted an illness at least once (Gurwith et. al., 1981). This is contrary to the belief that a neonate would be most susceptible to contraction of illness, and as time went on, the child would become increasingly immune. There is some research to support the idea that children having immunoregulatory abilities. Olness et. al. (1989) found a significant relationship between children who received instruction on immunoregulation in contrast





to their non-instruction peers. Olness et. al. (1989) found that children who received instruction on immunoregulation were found to have elevated levels of IgA when compared to their non-trained peers. In fact, of the two groups that received instruction on increasing their Ig concentrations only one of the groups was subsequently asked to increase their Ig concentration. This group illustrated a significant increase in their concentration of IgA in comparison to both the other instruction group and the non-instructed group (Olness et. al., 1989).

One study was found in which the subjects were inoculated with an endonasal spray. In Giraudi et. al. (1997), the subjects chosen for the inoculation were patients who were statistically more frequently affected by URTI. The preparation consisted of high concentrations of serum IgA, IgG and IgM. Results showed a significantly reduced reporting of URTI by the subjects inoculated when compared to the placebo group. These results are somewhat in contrast to the results reported earlier by Weidner et. al. (1998) who infected their subjects and found that no change in immuno-efficiency was found. The differences in reported results support a difference between children and adults in immuno-efficiency.

#### **2.4.2 Exercise and Immunity in Children and Adolescents**

Research using children as subjects has not definitively found a significant relationship between heightened levels of exercise training and effects on humoral immunity. This is most likely because so little research has been conducted using children as subjects. However, some of the research supports that children's immune function reacts similar to



adults. Tharp (1991) found that sIgA levels increased over time in teenagers who trained and played basketball. An important aspect of this study was the time of year it was conducted. The research took place during the winter months in Nebraska (U.S.A.), the season that is hypothesized to potentially depress levels of sIgA naturally. Tharp (1991) tested the boys at the mid point of a basketball season, during heightened stress levels and training periods. The ages of the subjects ranged from 10 – 18 years old.

Eliakim et. al. (1997) conducted a study with elite child gymnasts and found no change in Ig levels occurred immediately after or 24 hours post exercise testing. Subjects were tested after a single bout of exercise. Subject's age ranged from 10 – 12 years of age. Chronic effects cannot be extrapolated from these results, nor can these results be compared to Tharp's study. Gleeson et. al. (1991) showed baseline variability of Ig levels in children according to age and reported increased levels of IgG and IgM as well as the concentration of IgA with age. The Ig variability could provide insight into why Tharp's (1991) study reported elevated levels of IgA in children who were highly active and under competitive stress. Recorded variable levels of Ig could be indicative of the children's immune system being in a state of flux while in development.

Boas et. al. (1996) conducted a study with similar objectives to the research study this paper was written. The study was designed to examine immunological reaction to physical activity. Sixteen subjects who participated in their own regular personal training and 17 untrained male subjects were examined during the study. Researchers assessed leukocyte and lymphocyte levels, NK cells and NK cell activity. The subjects were categorized according to the Tanner Developmental Stage criteria, and ages ranged from 9 – 17 years. Boas et. al. (1996) did not find any significant changes to those immune





markers examined in the study nor between the trained and untrained subjects. Nieman et al. (2002) conducted research with similar aims in children with mean age of  $9.9 \pm 1.7$  yrs, and found that there was no relationship between peak  $\dot{V}O_2$  and immune function. Nieman et al. (2002) also found a positive correlation between body mass and sIgA concentration, along with other immune markers such as serum subset leukocyte counts, monocytes, and granulocyte phagocytosis.

The limited number of studies illustrate that more research needs to be conducted in order to better understand if there is a relationship between the immune function and physical activity and body mass index in children. Some significant relationships have been identified in adult subjects. If a positive significant relationship is identified in children between physical activity and their immune function, the results may suggest a lack of physical activity could impair development of immune function in adults.

### **2.4.3 Measures of Physical Activity in Children**

As with assessment of any population, using tools that are reliable and accurate to assess physical activity levels in children is essential. Many different tools have been developed to assess different aspects of physical activity and fitness. However, inconsistencies of outcome measures plague many of these. Age differences and gender have been shown to significantly affect the outcome of measured immunity within groups of subjects (Washington et al., 1988). McMurray et al. (2002) examined the effects of age, gender and ethnicity on predicted maximal aerobic power in individuals aged 8 – 16. They found that the girls had lower absolute and relative (scaled to account subjects' mass)





$\dot{V}O_2$  max than the boys (Washington et al., 1988). Gender and age should be considered when choosing the type of measure and nature of subject groups (gender diverse or single gender groups) to examine physical activity.

One such method used to examine physical activity is the use of questionnaires to record overall time spent in physical activity, habitual physical activity and intensity of activity. Data collection can be done by asking subjects to record information from memory about physical activity from relatively recent or more distant past. One such example is the Previous Day Physical Activity Recall (PDPAR). Trost et al. (1999) evaluated the validity of using the PDPAR as an assessment instrument for recording physical activity behavior in children. They compared the data collected from the questionnaire to readings from an accelerometer from the previous day, using grade five students as subjects. PDPAR results were used in calculations to determine a metabolic equivalent task value (MET) level of physical activity. Trost et al. (1999) found the PDPAR to be only moderately accurate as an instrument to evaluate physical activity and to be inaccurate in prediction of physical activity.

Another example of questionnaire used to assess physical activity is the Measures of Typical Physical Activity Behavior (MTPAB). This questionnaire uses a seven day physical activity recall. The MTPAB is based upon the premise that the past week of physical activity is representative of the typical week for the subject. In addition, there is no conversion of data to a scale or score, which makes assessment difficult (Welk & Wood, 2001). A third example designed specifically for children is the Physical Activity Questionnaire for Children (PAQ-C). The PAQ-C uses a scale for assessment, and the questions assess activity at different times of the day. Scores can be used to target typical



activity or the average activity for the past seven days. Assessment is most useful in determining periods spent in activity and inactivity. However, when more detailed assessment is attempted, accuracy declines because no meaningful score is provided nor is there any distinction in the quality of the physical activity (Welk & Wood, 2001). In addition, when the PAQ-C was assessed for validity, it was found  $r = 0.39$  (Sirard & Pate, 2001). Thus, subjectivity becomes an issue in using self-recall.

The questionnaire selected for assessment of physical activity in the present study was the Habitual Activity Estimation Scale (HAES). The HAES was initially developed to monitor physical activity in children with acute lymphoblastic leukemia (Hay, 1997). The HAES has demonstrated test-retest reliability greater than 0.80 when assessing at least two days (Hay, 1997). The questionnaire divides an individual day into 4 periods. In addition, activities are then ranked according to intensity. In the testing of the HAES, parents completed the scale for the children and the results were compared to clinical and anthropometric measures, producing the high reliability (Hay, 1997).

Some of the other methods of assessment include heart rate monitors, direct observation, indirect calorimetry, doubly labeled water and motion sensors (pedometers and/or accelerometers) (Sirard & Pate, 2001; Welk & Wood, 2001). Cost, accessibility, subject characteristics and availability (time) must all be considered regarding choice of method of assessment. In addition, children's activity patterns differ from adults in that their activity tends to be random and intermittent (Nilsson et al., 2002). The median duration of activity at moderate levels tends to last approximately 10 seconds, while intense levels of activity only 3 seconds (Nilsson et al., 2002). Nilsson et al (2002) examined the effectiveness of using an accelerometer (The Computer Science and





Applications Inc. Uniaxial Activity Monitor) to monitor different levels of physical activity in children, aged 7 years old. The accelerometer proved to be reliable when appropriate time sampling intervals were used for detecting specific activity levels (Nilsson et al., 2002).

The Digi-Walker® pedometer was chosen because of stability and reliability in addition to cost effectiveness and ease of operation of the device. Welk et al. (2000) examined the Digi-Walker® pedometer to determine its effectiveness as a tool for assessing physical activity patterns. An analysis was done in two stages. The first was a controlled comparison of steps on an outdoor track to steps in a treadmill test. In the second test, the pedometer was compared in field conditions. The pedometer was worn for one week for all waking hours, and the second week the pedometer was worn for all but highly active periods. Differences in stride length between genders were explained to be due to height differences, rather than gender differences (Welk et al., 2000). In addition, in the field portion, the pedometers tended to under-predict high intensity activities simply because fewer steps were needed to complete the activity. As such, distinguishing between differing levels of physical activity may not be possible with the pedometer (Welk et al., 2000). However, Welk et al. (2000) do support use of the pedometer as a useful indicator of daily activity.



## Chapter 3

### Methodology

#### 3.1 Subjects

Brock University Human Ethics Review Board as well as the Hamilton Catholic District School Board approved the research project and all protocols. Table 1 illustrates subject characteristics. Subjects were recruited from three schools in Southwestern Ontario. Randomization of subjects was achieved by including full classes of students from randomly selected schools that agreed to participate. All grade-5 students enrolled in the selected schools were provided with a project package containing a study description and a parental consent form. Permission was obtained from school officials while the purpose and potential risks of the study were explained carefully to parents before obtaining consent.

Of the initially recruited cohort, 80% returned a signed parental consent. The only major exclusion criteria were the presence of chronic medical conditions such as asthma or heart conditions (or any other condition that would put the subject at risk when performing the activities during testing) and/or if subjects had received a flu vaccination in the previous 12 months. All testing was performed in the schools during the months of May and June. Any medication taken for treatment of illness was recorded.



**Table 1: Subjects Characteristics**

	<b>Males</b>	<b>Females</b>	<b>Overall</b>
<b>N (number)</b>	29	32	61
<b>Age (years)</b>	10.5	10.4	10.4
<b>Mean Height (cm)</b>	142.6 $\pm$ 1.4	142.2 $\pm$ 1.5	142.4 $\pm$ 1.0
<b>Mean Weight (kg)</b>	87.6 $\pm$ 3.9	87.7 $\pm$ 3.5	87.6 $\pm$ 2.5

### 3.2 Experimental Protocol

At the onset of testing, all subjects were split into separate groups according to gender in order to maintain a manageable size of test group. Each group was then separately given instructions for the 20m-shuttle run of Léger & Lambert (1982), and given opportunities for practice and to ask questions about the activity. While one group participated in the shuttle run, the data on the second group's mass (kg), height (cm), and stride length were collected.

Stride length was determined by using a measuring tape secured to the floor. Subjects were instructed to walk on/alongside the tape. Landing toe position of both feet for one stride were marked and distance was calculated. The toe was used because it was the last part of the foot to leave the floor thus providing a more accurate reading when measuring the subject's stride length. Subjects were given 10m to walk, prior to measurement, in order for them to assume normal walking stride. Once completed, the groups then rotated to the opposite station. In order to not collect stimulated saliva samples too soon after the maximal physical activity, both groups were then instructed to complete the questionnaires (HAES and Sport Participation (SP)) to provide adequate 'cool-down' time.





After all had completed the questionnaires, each subject submitted to assessment of body fat using Bioelectrical Impedance Analysis (BIA). Following collection of body fat data, all submitted for collection of saliva samples using cylinder-shape swabs. Two samples were collected, one for IgA and the second for cortisol. The swabs were placed in the subjects' mouth and they were instructed to moisten/chew lightly on the swab for a specified duration. Elapsed collection time for each sample was one minute, with a two-minute interval between samples.

Following collection of the first saliva sample, temperature of the subject was taken using the auditory thermometer (FIRSTTEMP GENIUS Model 3000A Tympanic Thermometer, Mansfield, MA). The temperature was taken to ensure cortisol contained in the saliva samples was not affected by acute stress. The experimental procedure is presented in Table 2. One randomly selected group ( $n = 15$ ) was assessed a second time, six weeks after initial testing. The second testing was conducted to test the stability of results from initial testing. Following all on-site testing, Digi-walker© pedometers were provided for each subject. All subjects were required to wear the pedometer for a period of 48 hours and to keep a log of activity (during sleep or bathing they were instructed to remove the device).



**Table 2: Timetable of Experimental Measurements for Research**

Variables	Week 1 n= 61	Week 2	Week 3	Week 4	Week 5	Week 6 * n= 15 only
$\dot{V}O_{2\max}$ (Leger Shuttle Run)	✓					✓
sIgA (Saliva/Cortisol Testing)	✓✓					✓✓
Body Fat (BIA)	✓					✓
Habitual Physical Activity (HAES Questionnaire)	✓					✓
SportParticipation Questionnaire	✓					✓
Body Temperature	✓					✓
Pedometer (for 48hr period with Activity Log)	✓					✓

\* denotes experimental retest group

### 3.3 Measurements

Maximal aerobic power was estimated using the 20m shuttle run of Léger & Lambert (1982). Two lines were marked 20m apart on the gym floor. Subjects lined up on one line, and ran back and forth between the marked lines to a cadence provided by an instructional tape. The initial cadence corresponded to a speed of  $8.5 \text{ km} \cdot \text{h}^{-1}$ , with the speed increasing by  $0.5 \text{ km} \cdot \text{h}^{-1}$  every minute. Subjects continued through increasing levels of intensity until they could no longer keep pace with the cadence of the tape. Once a subject “fell off the pace”, the last completed stage was recorded.

An estimate of each individual's  $\dot{V}O_{2\max}$  ( $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was determined by multiplying the MET value associated with the final completed level of activity by  $4.6 \text{ ml}$





$\text{kg}^{-1} \cdot \text{min}^{-1}$  for 1 MET as recently suggested by Allor & Pivarnik (2001). This test has been validated against a direct laboratory protocol ( $r=0.91$ ,  $\text{SEE}=4.16$ ), and the reproducibility has been reported ( $r=0.975$ ) for measurements taken on the same subject within a one-week period (Léger & Lambert, 1982). In addition, this measurement has been shown to be a valid test in a school setting for children 6 to 17 years of age (Leger et. al., 1984).

BIA was used to estimate the relative percent body fat (%BF) using the input variables of physical activity level, body frame size, height, mass, and sex in combination with the body fat measurement equipment (Quantum II, RJL Systems, U.S.A.). Recently, the validity and reliability of this method has been demonstrated successfully in children and adolescents (Okasora et. al., 1999). Whole body resistance was measured with four surface electrodes: two on the right hand at the third metacarpal and the wrist between the styloid processes of the radius and the ulna, and two on the right foot at the third metatarsal and the ankle between the medial and lateral malleoli (Nash, 1985). The skin was cleaned with 70% alcohol prior to application of the electrodes. An electrical current of 50 kHz and 0.8 mA was produced by a generator (Quantum II, RJL Systems, U.S.A.) and conducted to the skin through the adhesive electrodes with the subject in the supine position. Short and long term reproducibility was recently reported as  $r = 0.999$  for measurements taken in the same subject within one week, and 0.977 for repeat measurements up to one month apart for a 2.5% coefficient of variance (Kyle et. al., 2001). BIA measurements were made over one hour after the shuttle run to avoid problems associated with dehydration and changes in skin temperature, electrolyte concentration and glycogen stores (Kyle et. al., 2001).



All subjects provided two saliva samples. For each of these samples, one milliliter of unstimulated whole mixed saliva was collected using cylinder-shape swabs placed in the mouth for one minute. After sampling, the swabs were placed directly into plastic tubes. The samples were then stored using standard procedures at  $-20^{\circ}\text{C}$  (Gleeson et. al. 1999) until the samples were assayed. The subjects were asked not to consume any food or drink for at least one hour prior to saliva collection, and the mouth was not rinsed with water prior to sampling to avoid altering resting sIgA levels. The sIgA concentration was measured by radial immunodiffusion using a BINARID<sup>TM</sup> kit (Binding Site Limited UK).

Cortisol levels were assessed in saliva samples using a DPC coat-a-count Cortisol Kit. Total plasma concentrations of cortisol were measured in duplicate by commercial solid-phase  $^{125}\text{I}$  radio-immunoassay kits.  $^{125}\text{I}$ -labeled cortisol competes for antibody sites for cortisol within the unknown sample. The antibody is immobilized to the wall of the polypropylene tube, so when the supernatant is decanted the antibody bound fraction of the radiolabeled cortisol is still present. Counting the tube in a gamma counter yields the measurement of cortisol present in the unknown sample. The reference ranges are from 3.5 - 27.0 nmol/L at 8am and  $< 6.0$  nmol/L at 10pm for both sexes, all ages (including children) and women on contraceptives.

Total distance traveled per day (TD) was measured using a pedometer (Digi-walker©). The device recorded the child's physical activity in steps using a step counter. Each individual's stride was measured to the nearest centimeter. The steps counted by the pedometer were then multiplied by the individual's step length to determine total distance traveled in meters. All subjects were required to utilize the Digi-walker© Pedometer for two consecutive days. Each monitor was calibrated to record the proper movements of





the subject. A 2-day activity log accompanied the pedometer. The subjects recorded daily physical activities other than general locomotion. Utilization of the log ensured that the data recorded on the pedometer were valid and accurately and reliably recorded.

### 3.4 Questionnaires

Past reports on the physical activity patterns of adolescents show considerable variability, which has been attributed, in part, to the difficulty of measuring physical activity in youth using self-report methods. To address this issue, two distinctly different questionnaires were used in the present study. The HAES (Hay, 1997) was used to measure the intensity and duration of habitual activity. It allows measurement of the duration and intensity of daily activity of children past infancy through adolescence. The validity of the HAES has been evaluated by Hay (1997), and the reliability was found to be  $>0.80$ , using generalizability theory (Hay, 1997; Hay et. al., 1995). Intensity and duration of activity was measured for both a typical school weekday and a typical weekend day during the previous month using the HAES, in order to calculate the total weekly habitual physical activity (HPA). The Participation Questionnaire (PQ) was used to estimate both the amount of participation in physical activity and nature of the participation under three categories: free time activity (FTA), organized activity time (OAT), and total time spent in activities (SAT) (Hay, 1992). Participation scores are referred to as "activity units". Each unit refers to participation in one activity on a regular or seasonal basis. An activity unit in the Organized Sport section refers to participation on a single sport team (either school or community), playing on a single intramural team, or participating in a single





series of lessons during the past year etc. An activity unit in the Free Choice section refers to the identification of any active leisure pursued as a preferred choice after school, or on weekends, with family and friends (Hay, 1992).

### **3.5 Statistical Analysis**

One-way ANOVA were used to compare males and females on physical activity, %BF, aerobic power, sIgA and sC. Pearson correlation analysis was used to detect possible relationships among all the variables, as well as stability of measures (pre and six weeks post). All data analyses were conducted using SPSS 11 for Windows. A value of  $p \leq 0.05$  was accepted to indicate a statistically significant result.



## Chapter 4

### Results

#### 4.1 Descriptive and correlation data

There were no statistically significant differences found in subject characteristics. However, the results of the BIA analysis showed a 40% obesity level (>25% B.F.). In addition, 50% of the subjects spent <5 h d<sup>-1</sup> in any physical activity, and 41% were physically mobile less than 110 meters per day (just over one tenth of a kilometer). As a group, approximately 69% of the subjects had a salivary cortisol level at or below the recommended reference range beginning at 3.5 nmol L<sup>-1</sup> while body temperature recorded using an auditory thermometer had no significant effect on either of the saliva samples acquired from the subjects. Physical activity levels, cortisol, sIgA, body fat and aerobic power are presented in Table 3.

As shown in Table 4, organized activity and free time activity were significantly related to  $\dot{V}O_2$  max ( $p < 0.05$ ). The total activity score (TA) was significantly correlated with  $\dot{V}O_2$  max ( $p < 0.01$ ), distance traveled per day ( $p < 0.01$ ) as well as resting salivary cortisol levels ( $p < 0.05$ ). Distance traveled per day (m d<sup>-1</sup>) was also significantly correlated with  $\dot{V}O_2$  max ( $p < 0.01$ ) as well as with time spent in organized sport activities ( $p < 0.01$ ). Salivary cortisol was significantly correlated with body fat ( $p < 0.05$ ) and time





spent in organized sport ( $p < 0.05$ ). Salivary IgA and sIgA/Albumin ratio did not demonstrate a significant relationship with any of the tested measures (Table 4). Refer to Table 5 (Males) and Table 6 (Females) for specific relationships by gender.

**Table 3.** Salivary IgA, sIgA/Albumin ratio, cortisol, body fat, aerobic power and physical activity levels in male and female children

Variables	Males (n=29) (mean $\pm$ SE)	Females (n=32) (mean $\pm$ SE)
sIgA ( $\text{ml} \cdot \text{L}^{-1}$ )	$133.4 \pm 17.4$	$134.8 \pm 20.7$
sIgA/albumin ratio	$2.4 \pm 0.1$	$2.4 \pm 0.1$
Salivary Cortisol ( $\text{nmol} \cdot \text{L}^{-1}$ )	$3.0 \pm 0.5$	$3.0 \pm 0.3$
$\dot{\text{V}}\text{O}_2$ max ( $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ )	$48.2 \pm 0.9^{**}$	$45.3 \pm 0.7^{**}$
Body Fat (%)	$25.3 \pm 1.3$	$21.5 \pm 1.8$
HPA ( $\text{h} \cdot \text{wk}^{-1}$ )	$4.9 \pm 0.4$	$5.4 \pm 0.9$
Free Time Activity (score)	$13.0 \pm 0.7$	$13.2 \pm 0.6$
Organized Activity (score)	$10.0 \pm 1.1$	$9.6 \pm 0.9$
Total Activity (score)	$23.0 \pm 1.4$	$22.8 \pm 1.2$
Locomotion ( $\text{m} \cdot \text{day}^{-1}$ )	$120.5 \pm 13.7^{**}$	$95.4 \pm 6.5^{**}$

**\*\***  $p < 0.01$  between genders

Stability of measurement (pre and 6 weeks post) for the scores of organized sport activities, free time activities and total activity was measured as  $r = 0.517$  ( $p < 0.05$ ),  $r = 0.777$  ( $p < 0.01$ ) and  $r = 0.651$  ( $p < 0.01$ ) respectively for measurements taken from the same subjects after six weeks. The test and 6 week post-test for stability for sC was found to be  $0.663$  ( $p < 0.01$ ). Salivary IgA and sIgA/Albumin ratio stability over time was non-



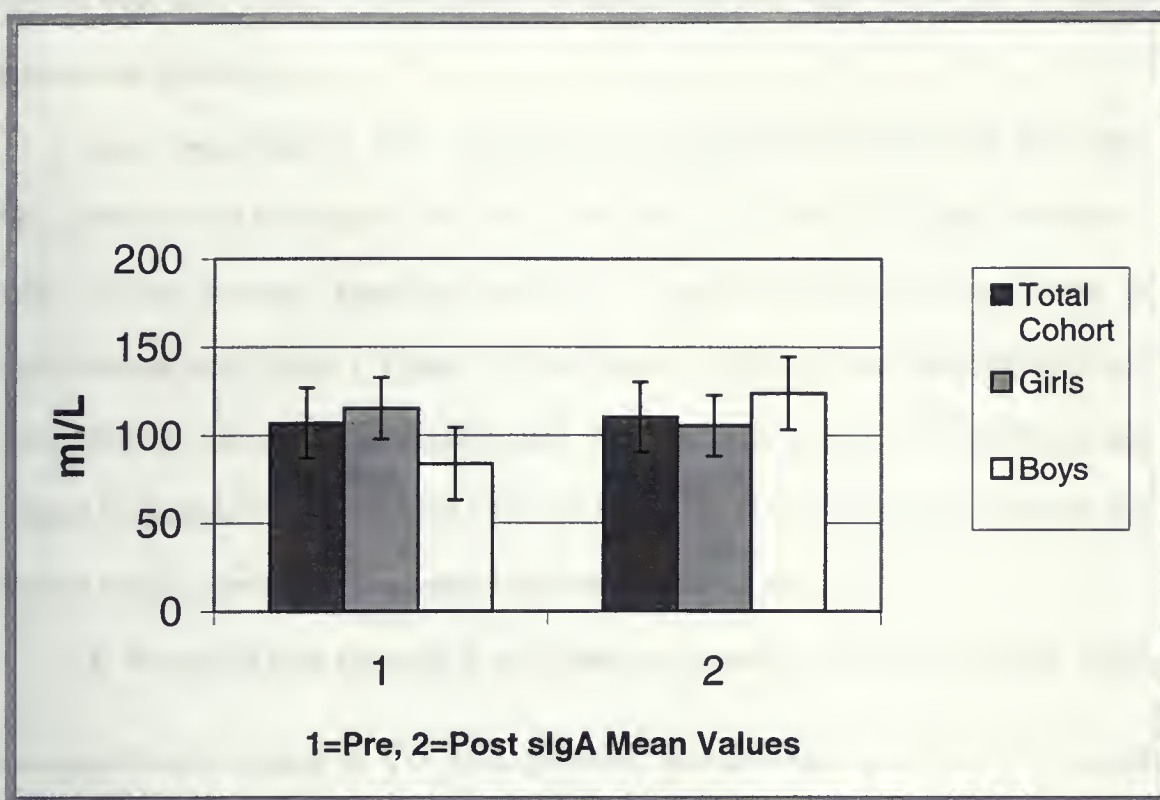
significant. Initial and 6-week post measurements of sIgA and sIgA/Albumin were not significantly different ( $p < 0.05$ ) when compared for both males and females (Figure 1).

## 4.2 Gender Differences

A significant difference ( $p < 0.05$ ) in  $\dot{V}O_2$  max was revealed between genders (Table 3).

A second significant difference was revealed between genders in distance traveled by each participant, on a daily basis ( $p < 0.01$ ). No significant difference was evident in organized sport or free time activity, nor was there a significant difference between genders in daily and weekly habitual physical activity. Only 10% of girls had a  $\dot{V}O_2$  max of greater than  $50 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , while 31% of boys attained that level or higher. When examining physical activity, 100m traveled per day was used to classify levels of activity. Subjects who traveled less than  $100 \text{ m} \cdot \text{day}^{-1}$  were considered sedentary, and those who recorded values above  $100 \text{ m} \cdot \text{day}^{-1}$  were considered active. Using this criterion, 44% of the boys did not attain at least 100m per day of locomotion, while almost 60% of girls did not attain this level of locomotion, as recorded by the pedometer. Scores from the HAES questionnaire that indicated that the children were active less than  $3 \text{ h} \cdot \text{day}^{-1}$  were considered inactive, while those who recorded activity levels above  $3 \text{ h} \cdot \text{day}^{-1}$  were considered active. More than one fifth of the boys (22%) averaged less than  $3 \text{ h} \cdot \text{day}^{-1}$  of physical activity, while almost 32% of girls did not achieve this level. This was further broken down into time spent in free-play and organized sport. Scores from the HAES questionnaire that indicated less than  $10 \text{ h} \cdot \text{wk}^{-1}$  in free-play were considered inactive,





**Figure 1.** The mean sIgA values for select subgroup of total cohort, then broken down by gender for males and females. Pre scores refer to values obtained from initial testing ( $n=15$ ). Post scores illustrate sIgA values obtained from the same sample six weeks after initial testing.

while those above  $10 \text{ h} \cdot \text{wk}^{-1}$  in free-play were considered active. About 62% of boys spend less than  $10 \text{ h} \cdot \text{wk}^{-1}$  in free-play. Of those same boys, only 52% spent at least  $10 \text{ h} \cdot \text{wk}^{-1}$  in an organized sporting activity. Meanwhile, 62% of girls spent less than  $10 \text{ h} \cdot \text{wk}^{-1}$  in free-play, and only 53% spent at least  $10 \text{ h} \cdot \text{wk}^{-1}$  in an organized sporting activity. When using the criteria for total time spent in activity, greater than  $20 \text{ h} \cdot \text{wk}^{-1}$  was considered active while less than  $20 \text{ h} \cdot \text{wk}^{-1}$  was considered inactive. Based on this value,





47% of boys were found to have scores considered inactive, while 44% of girls did not achieve this minimum level.

Boys were found to have slightly but not significantly higher %BF than girls. About 50% of boys had percent body fat above 25%, while only 42% of girls were above 25%. There was no significant difference between genders in either sIgA or sIgA/Albumin ratio (Table 1, Figure 1). Sixty percent of the girls had sIgA  $>88.0 \text{ ml} \cdot \text{L}^{-1}$ , while 59% of boys had that concentration or higher. In addition, 72% of boys had cortisol levels below recommended reference levels, while less than 69% of females had cortisol levels below the recommended reference level ( $3.5 \text{ nmol} \cdot \text{L}^{-1}$ ).

In the results from the boys, it was found that distance traveled per day ( $\text{m} \cdot \text{day}^{-1}$ ) was significantly related to  $\dot{V}\text{O}_2 \text{ max}$  ( $p<0.05$ ), average hours per week of physical activity ( $p<0.05$ ), total activity ( $p<0.01$ ) and organized activities ( $p<0.01$ ). Cortisol was significantly correlated with organized activities ( $p<0.05$ ) and  $\dot{V}\text{O}_2 \text{ max}$  was significantly correlated with free time activities ( $p<0.05$ ) (Table 5). None of the above, or any other significant relationships were found in the female cohort (Table 6).



**Table 4 Correlation Coefficients among Time Spent in Sport Activities (OSA), Free Time Activity (FTA), Total Activity (TA), Salivary IgA (sIgA), Body Fat (%BF), Aerobic Fitness (VO2max), Salivary Cortisol (sC), Distance traveled per day (m d<sup>-1</sup>), sIgA/Albumin Ration (sIgA/Alb) and weekly habitual activity (HPW).**

	TA (score)	OSA (score)	FTA (score)	sIgA (ml·L <sup>-1</sup> )	%BF	VO2max (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	sC (nmol·L <sup>-1</sup> )	m·d <sup>-1</sup>	sIgA/Alb (ratio)	HPW (d·wk <sup>-1</sup> )
TA (score)	-	0.865**	0.667**	-0.003	0.146	0.340**	0.290*	0.475**	0.009	-0.010
OSA (score)		-	0.200	0.002	0.102	0.270*	0.300*	0.450**	0.040	0.140
FTA (score)			-	-0.003	0.134	0.260*	0.122	0.280	-0.040	-0.220
sIgA (ml·L <sup>-1</sup> )				-	0.080	-0.006	0.003	0.002	0.880***	-0.120
%BF					-	0.114	0.060	0.180	0.140	-0.230
VO2max (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )						-	0.010	0.550***	0.120	0.080
sC (mmol·L <sup>-1</sup> )							-	-0.030	0.030	0.003
m·d <sup>-1</sup>								-	0.270	-0.020
sIgA/Alb (ratio)									-	-0.110
HPW (d·wk <sup>-1</sup> )										-

\* p<0.05    \*\* p<0.01    \*\*\* p<0.001





**Table 5 Correlation Coefficients for Males among Time Spent in Sport Activities (OSA), Free Time Activity (FTA), Total Activity (TA), Salivary IgA (sIgA), Body Fat (%BF), Aerobic Fitness (VO2max), Salivary Cortisol (sC), Distance traveled per day ( $\text{m d}^{-1}$ ), sIgA/Albumin Ratio (sIgA/Alb) and weekly habitual activity (HPW).**

	TA (score)	OSA (score)	FTA (score)	sIgA ( $\text{ml L}^{-1}$ )	%BF	VO2max ( $\text{ml kg}^{-1} \text{min}^{-1}$ )	sC ( $\text{nmol L}^{-1}$ )	$\text{m d}^{-1}$	sIgA/Alb (ratio)	HPW ( $\text{d} \cdot \text{wk}^{-1}$ )
TA (score)	-	0.877**	0.636**	0/165	0.385*	0.412*	0.319	0.621*	0.113	0.195
OSA (score)		-	0.187	0.080	0.267	0.293	0.428*	0.652**	0.079	0.353
FTA (score)			-	0.208	0.367	0.374*	-0.035	0.265	0.207	-0.154
sIgA ( $\text{ml L}^{-1}$ )				-	0.154	0.145	0.042	0.453	1.000***	-0.167
%BF					-	0.165	0.149	0.233	0.154	0.117
VO2max ( $\text{ml kg}^{-1} \text{min}^{-1}$ )						-	0.073	0.593**	0.143	0.246
sC ( $\text{mmol L}^{-1}$ )							-	0.039	0.041	0.130
$\text{m d}^{-1}$								-	0.453	0.534*
sIgA/Alb (ratio)									-	-0.169
HPW ( $\text{d} \cdot \text{wk}^{-1}$ )										-

\*  $p < 0.05$     \*\*  $p < 0.01$     \*\*\*  $p < 0.001$



**Table 6 Correlation Coefficients for Females among Time Spent in Sport Activities (OSA), Free Time Activity (FTA), Total Activity (TA), Salivary IgA (sIgA), Body Fat (%BF), Aerobic Fitness (VO2max), Salivary Cortisol (sC), Distance traveled per day (m d<sup>-1</sup>), sIgA/Albumin Ratio (sIgA/Alb) and weekly habitual activity (HPW).**

	TA (score)	OSA (score)	FTA (score)	sIgA (ml·L <sup>-1</sup> )	%BF	VO2max (ml·kg <sup>-1</sup> min <sup>-1</sup> )	sC (nmol·L <sup>-1</sup> )	m·d <sup>-1</sup>	sIgA/Alb (ratio)	HPW (d·wk <sup>-1</sup> )
TA (score)	-	0.852**	0.703**	-0.142	-0.039	0.262	0.252	0.383	-0.102	-0.092
OSA (score)		-	0.227	-0.074	-0.047	0.233	0.097	0.322	-0.073	0.082
FTA (score)			-	-0.163	-0.008	0.172	0.338	0.294	-0.162	-0.277
sIgA (ml·L <sup>-1</sup> )				-	0.055	-0.164	-0.036	-0.151	1.000***	-0.115
%BF					-	-0.030	-0.020	0.130	0.056	-0.326
VO2max (ml·kg <sup>-1</sup> min <sup>-1</sup> )						-	-0.095	0.327	-0.162	0.064
sC (mmol·L <sup>-1</sup> )							-	0.000	-0.036	-0.058
m·d <sup>-1</sup>								-	-0.148	-0.157
sIgA/Alb (ratio)									-	-0.115
HPW (d·wk <sup>-1</sup> )										-

\* p<0.05      \*\* p<0.01      \*\*\* p<0.001



## **Chapter 5**

### **Discussion**

#### **5.1 Physical Activity and sIgA**

Salivary IgA is believed to be the first line of defense for the human body against pathogenic invasion. What is of importance to this study is that recent studies have suggested a direct association between sIgA levels and exercise in adults (Gleeson et. al., 1995, 1999; Klentrou et. al., 2002). The most important finding of the present study is the lack of any relationship between sIgA and all the physical activity variables. This is quite surprising since the literature states that sedentary adults have a weakened immune system when compared to active adults (Nieman et. al., 1990; Nehlsen-Cannarella et. al., 1991; Klentrou et al., 2002). This is surprising because research on the functioning immune system has shown that children achieve adult levels of immune markers at a very early age and thus it could be assumed that they would achieve adult levels of efficiency in protection of the host shortly after. However, recent studies in children (Boas et. al., 1996; Nieman et. al., 2002) also showed no significant relationship between sIgA and physical activity markers. Thus, presence of sIgA in high concentrations may not be a sufficient measure in children for immune function. Boas et. al. (1996) tested male subjects 9 to 17 years old and found the same lack of relationship between sIgA and physical activity. The findings from this study support findings by Boas et al. (1996) and Nieman et al. (2002).





One possible reason for the lack of relationship between sIgA and physical activity may be due to sIgA weekly fluctuations in this age group. It was found in this study that 60% of the subjects had sIgA levels above normal adult levels reported in previous studies (Gleeson et al, 1982). According to the literature, adult levels of sIgA were found to be developed from one to seven years of age (Mellander et. al., 1984; Smith et. al., 1989; Gleeson et. al., 1987) but there are no published reports on short or long-term reliability or stability of these findings. The fact that these researchers found adult levels in their young subjects may suggest that the children's humoral immunity has the potential to reach adult levels but this does not mean that there are consistent concentration of adult sIgA present in children. In the present study, sIgA was reassessed 6 weeks later and it was found that sIgA and sIgA/Albumin ratio measurements were not significantly stable ( $r = 0.258$ ) but non significantly different either. These findings suggest that fluctuations in sIgA may be a regular occurrence. In fact, the sIgA levels measured in this study were higher than what is considered as the normal range for adults. One might even surmise that the levels children reach may be the peak level in humoral development, and that healthy adults should be measured against these levels as an indicator of a normally functioning immune system.

Another possible theory to explain why there was no significant relationship between sIgA and physical activity, as suggested by Gleeson et. al. (1987), is that the quality of the sIgA protein may not be as high in children as in adults who have had many more years of exposure to bacteria and viruses leading to improved protection from re-infection by the same or similar infection. This could not be supported or contraindicated by the results in this study since concentration was examined in the sIgA samples, not



quality/diversity of the sIgA protein. However, it could be inferred that this may not be the case since children have a high rate of exposure to situations where they could develop their immune system and this would account for an improved immune system, i.e., attending school (Gleeson et. al., 1987). The children in this study have been exposed to a school setting for approximately four years. This leads us to believe that more exposure time may be needed for their immune system to exhibit adult sIgA consistency and quality.

An important distinction was made in the analysis of the results by separating data collected from the boys and girls. It was found that the boys' results had significant relationships between locomotion recorded by the pedometer and  $\dot{V}O_2$  max, average hours of activity per week, and time spent in organized sport (Table 5). None of these relationships were found in the girls' results (Table 6). The difference between genders may be a result of the types of activities that females engage in would be different than their male peers. In addition it is likely that the boys' physical activity is directly related with the time spent in activities that increased their  $\dot{V}O_2$  max, while for the girls their activities may not positively affect their  $\dot{V}O_2$  max.

## **5.2 Cortisol, Body Fat and Salivary IgA**

Equally surprising were the results for cortisol, body fat and sIgA. This research found that over 40% of the total cohort could be defined as obese, when using 25% body fat as a determinant. Though the difference was not significant, the girls had lower mean





percent body fat than the boys. There was no significant relationship between sIgA and body fat. This was in contrast to the results of Nieman et. al. (2002), who found that there was a correlation between body fat levels and sIgA. Salivary IgA levels and sIgA/albumin ratio were not significantly related to cortisol levels. Cortisol has been shown to be an indicator of stress (Brenner et. al., 1998). It is, therefore, an important finding that stress may not have any more of an effect on secretory immunity in children than physical activity does.

Since there is evidence that acute maximal exercise produces a delayed onset of cortisol secretion (Brenner et. al., 1998). Careful consideration was taken to ensure that the saliva samples were taken with sufficient time after the aerobic power test to avoid these possible effects in the saliva samples as a result of the physical activity the children engaged. Other studies (Moyna et. al., 1999; Nieman et. al., 2000) demonstrated that immune indicators and cortisol levels are independent of aerobic fitness level. The results from this study seem to support this.

Although there was no difference in the mean levels of resting salivary cortisol between genders, cortisol was significantly correlated with time spent in organized sport ( $p < 0.05$ ) for boys, but not for the girls. Tsai et. al. (1991) found that females tended to have higher serum cortisol levels than their male counterparts. However, Tsai et al. (1991) indicated that these differences were only prevalent in trained individuals, and not in untrained or sedentary controls (Tsai et. al., 1991). The results in the present study would seem to confirm this. In fact, as a result of the significant relationship between sC and time spent in organized sport in only the boys, the opposite may occur in untrained





individuals. This may support the notion that males and females should be tested separately and development in children may be different by gender.

No gender differences were found in sIgA and sIgA/albumin ratio in either the initial or the 6-week post measurements (Figure 1). Schouten et al. (1988) stated that there were gender different sIgA levels in adults. However, the adults in Schouten et al.'s (1988) study were in job situations where they were not necessarily exposed to increased infection levels, and not all the subjects were exposed to the same environment. The children's elevated levels of sIgA may be explained by their exposure in the school environment. It is very possible that outside this environment, or if the subjects were not all in the same environment, their sIgA levels might be significantly different, as was found by Schouten et al. (1988).

### **5.3 Stability of Measures over Time**

Research on adults has used sIgA as an assessment tool in gauging the state and ability of the immune system to fight infection (Mackinnon et. al., 1993<sup>a,b</sup>; Gleeson et. al., 1995; 1999). One of the objectives of the present study was to determine if measuring sIgA was an appropriate assessment tool for testing immune function in younger individuals. Gleeson et. al. (1987) found a fluctuation in the children's saliva samples from the age of one to five, but concluded that since there seemed to be a plateau from the age of five to seven years of age, sIgA would remain relatively stable from that point on. If Gleeson et al.'s (1987) hypothesis were true, sIgA should be a stable measure in 10-11 year olds.



The present research study does not support the hypothesis by Gleeson et. al. (1987) since the results from the present study are somewhat conflicting. Salivary IgA and sIgA/Albumin ratio reproducibility over time was non-significant for repeated measurements taken after six weeks. In contrast, pre and 6-week post measurements of sIgA and sIgA/Albumin were not significantly different ( $p < 0.05$ ) when compared for both males and females (Figure 1). In conclusion, sIgA seems to continue fluctuating throughout the child's developing years. Since sIgA did not have any significant relationship with any of the other variables, it is reasonable to believe that sIgA as a tool for assessing state of immunity is not necessarily stable. It would be premature to conclude that it is not a useful tool in measuring immunity in children without further study. Thus, weekly and monthly variability of sIgA should be considered when testing young individuals.

Gleeson et al. (1982) stated the normal adult range is between  $2 - 88 \text{ mmol} \cdot \text{L}^{-1}$ . Sixty percent of the sample in the present study had sIgA levels above this stated norm. This may be explained by class switching. It has been found that if there is a deficiency of IgA production in secretory areas, IgM has been found to have elevated levels and the body increases production of IgM to compensate (Nossal & Ada, 1971). This may be what is occurring here, but in reverse. While the research in literature has not addressed this issue, this may provide support for further research of the possibility of this occurring. The children may not have sufficient levels of IgM present in their secretory areas, thus their immune system stimulates increased production of IgA to compensate. In addition, since sIgA takes on the primary responsibility of host defense, IgM may



undergo class switch to aid in host defense and as a result, increase levels of IgA in secretory areas.

A second possibility when considering the elevated levels of sIgA may be that the children have spent the past 6 months in a school setting. The subjects were surrounded by large numbers of children, in concentrated areas, and not all individuals maintained a clean environment (i.e. – washing hands). The subjects' exposure to antigens may often be at a high level. The present study did not examine the subject for the presence of infection (bacterial or viral), nor was a health log used to record symptoms prior to the testing date. Thus, it can only be speculated that the children with elevated levels may have had recent exposure to infection and the elevated levels of sIgA were a result of this exposure. In addition, being present in the school and the increased opportunity to exposure of infection may induce chronic type elevations in sIgA. This may be a form of low zone immune tolerance, as described earlier (Nossal & Ada, 1971). These levels may decrease over the course of the summer (natural seasonal decrease and not spending the majority of their days in the classroom). However, during the months that children attend school, the constant exposure to infection may produce a chronically elevated level of sIgA. This elevated level induces the low zone tolerance, and the proteins become less effective.

The first HAES reporting was not significantly related to the reporting six weeks later. This could be deemed cohort specific since this would be the first time the HAES had been completed by the children themselves. The HAES had previously proven to be a reliable method (Hay, 1997), but under different circumstances, when the children's parents or guardian were filling out the questionnaire. This could result in an over or







under estimation of habitual activity levels by the children. In a study by Boucher et. al. (1997), activity level reported by Cystic Fibrosis patients 12 years and older was on average 24.1% higher ( $p<0.05$ ) than that reported by their parents, but the two reportings were related ( $r=0.758$ ;  $p<0.01$ ). Since our subjects were younger than 12 years of age, this will be an interesting point to be examined in future research. In contrast, the test-retest results for the Participation Questionnaire were significantly related ( $p<0.05$ ), ensuring stability of this measure, which was significantly ( $p<0.05$ ) correlated with locomotion and  $\dot{V}O_2$  max.



## Chapter 6

### Conclusions and Recommendations

#### 6.1 Conclusions

As a result of the data collected and the resulting analysis, it is this researcher's conclusion that salivary IgA is not significantly correlated with salivary cortisol levels, physical activity, body fat and cardiorespiratory fitness for this age group. What is evident from the results of this study is that children are becoming more obese as a group. The time spent by children in physical activity is very minimal and the effects this is having on their immune system are yet to be determined.

A second conclusion based on the results of the present study is that sIgA seems to fluctuate in this age group. Salivary IgA has not been shown to be a stable measure in children, in contrast to the research in adults. It is also possible that the time of the month, or year or the type of environmental exposure (i.e. – school) could be affecting the fluctuations. Salivary IgA levels fluctuate on a yearly circadian rhythm in adults. The circadian rhythm in children may be multiple (one within another). Circadian rhythm in this case refers to a predictable fluctuation of sIgA and serum IgA that is determined by seasonal fluctuations. Winter or colder temperatures cause an increase in concentration of sIgA and serum IgA, while warmer temperatures or the summer are linked to a decrease in sIgA and serum IgA.



## 6.2 Limitations of the Study and Recommendations

Some limitations of the study include access to laboratory analysis equipment/funding. Increased funding/increased access to more diverse laboratory equipment would have provided the opportunity to test for other immune markers in saliva (i.e. - lymphocytes, leukocytes) and even test for the range of sIgM or serum IgA. As stated earlier, suppressed levels of IgA in some of the subjects could be further examined to see if the subjects were IgM deficient. IgM has been known to increase in concentration to accommodate suppression or absence of sIgA (Nossal & Ada, 1971). In addition, comparing the values found for sIgA against serum IgA might have provided more insight into the differences found between adult subjects and the subjects in the present study. These tests could provide more definitive answers about the levels of sIgA from this subject pool.

Another limitation of the study is the time of the year that the data were collected from the subjects. Testing was initiated at the end of March, and proceeded until mid June. While a cool spring was experienced (temperature was well below average for that time of the year) in the Southern Ontario area, the effects of weather may not have been enough of an influence on the change in immunological defense. It has been reported in other research that in areas where there is seasonal change, there is a variation of sIgA levels throughout the year (Nehlsen-Cannarella et al., 1997), with peak levels present in the colder months, and lower levels present in the warmer months. While the testing times could not be circumvented in this case, a follow-up study with similar protocols in





late fall or the middle of winter would be ideal in order to compare results to this study, and others that were conducted during those seasons.

While testing for sIgA proved to be a reliable measure in adult subjects, it may not be so for children in this age group or younger, since there was no correlation between sIgA and any of the other testing variables in the study. Future studies could test stability and reliability of other methods of examining sIgA, to help decide which would provide the most reliable results for detecting sIgA levels, as well as other immune markers, and could be used to test reliability of this and other studies that used similar methods.



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## Appendices



# THE HAES (HABITUAL ACTIVITY ESTIMATION SCALE)



This questionnaire will ask you questions about your daily activities. Please read all of the instructions carefully and answer each question as truthfully as you can.

Name: \_\_\_\_\_

Date: \_\_\_\_\_

## INSTRUCTIONS (please read!)

Please recall the activities of *one typical weekday* (choose from Tuesday, Wednesday or Thursday) and *one typical Saturday within the past 2 weeks*. For each given time period, please estimate the percentage of time that you spent in each of 4 different activity levels. For each of the time periods, the total time spent in all activity levels must add up to 100%.

The different activity levels are described below:

### ACTIVITY LEVEL DESCRIPTIONS

These descriptions give you examples of activities that are typical of each activity level. You should refer back to these descriptions as often as you need when completing your estimates.

- a) inactive – *lying down*, sleeping, resting, napping
- b) somewhat inactive – *sitting*, reading, watching television, playing video games, time in front of the computer, playing games or activities which are mostly done sitting down
- c) somewhat active – *walking*, shopping, light household chores
- d) very active – *running*, jumping, skipping, bicycling, skating, swimming, games that require lots of movement and make you breathe/sweat hard

Following is a sample of a completed time period:

### SAMPLE

From when you finished breakfast until when you started lunch, please estimate the percentage of time that you spent in each of the following activity levels:

a) inactive	5% (i.e., having a nap)
b) somewhat inactive	60% (i.e., watching TV)
c) somewhat active	25% (i.e., shopping)
d) <u>very active</u>	10% (i.e., riding a bicycle)
TOTAL	100%



## WEEKDAY ACTIVITY

For one typical weekday in the past 2 weeks, (choose from one of Tuesday, Wednesday or Thursday), please estimate the percentage of time that you spent in each activity level.

1. After getting out of bed until starting breakfast:

- a) inactive \_\_\_\_\_ %  
b) somewhat inactive \_\_\_\_\_ %  
c) somewhat active \_\_\_\_\_ %  
d) very active \_\_\_\_\_ %

TOTAL	100%
-------	------



INACTIVE

2. After finishing breakfast until starting lunch:

- a) inactive \_\_\_\_\_ %  
b) somewhat inactive \_\_\_\_\_ %  
c) somewhat active \_\_\_\_\_ %  
d) very active \_\_\_\_\_ %

TOTAL	100%
-------	------



SOMEWHAT INACTIVE

3. After finishing lunch until starting supper:

- a) inactive \_\_\_\_\_ %  
b) somewhat inactive \_\_\_\_\_ %  
c) somewhat active \_\_\_\_\_ %  
d) very active \_\_\_\_\_ %

TOTAL	100%
-------	------



SOMEWHAT ACTIVE

4. After finishing supper until bedtime:

- a) inactive \_\_\_\_\_ %  
b) somewhat inactive \_\_\_\_\_ %  
c) somewhat active \_\_\_\_\_ %  
d) very active \_\_\_\_\_ %

TOTAL	100%
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VERY ACTIVE





For the *typical weekday* that you are referring to, please answer the following questions as accurately as possible in the spaces provided.

5. At what time did you get out of bed in the morning? \_\_\_\_\_
6. At what time did you start eating breakfast? \_\_\_\_\_
7. How long did you spend eating breakfast? \_\_\_\_\_ minutes
8. At what time did you start eating lunch? \_\_\_\_\_
9. How long did you spend eating lunch? \_\_\_\_\_ minutes
10. At what time did you start eating supper? \_\_\_\_\_
11. How long did you spend eating supper? \_\_\_\_\_ minutes
12. At what time did you go to bed that evening? \_\_\_\_\_

13. For the *typical weekday* that this questionnaire has asked you about, please rate your overall level of activity (please circle one response only):

- a) very inactive
- b) inactive
- c) somewhat inactive
- d) somewhat active
- e) active
- f) very active

14. Is this "typical" Tuesday, Wednesday or Thursday that you described in this questionnaire (please circle one response only):

- a) a lot like most weekdays
- b) a little bit like most weekdays
- c) a little bit different from most weekdays
- d) a lot different from most weekdays



## SATURDAY ACTIVITY

For one typical Saturday in the past 2 weeks, please estimate the percentage of time that you spent in each activity level.

15. After getting out of bed until starting breakfast:

- a) inactive \_\_\_\_\_ %  
 b) somewhat inactive \_\_\_\_\_ %  
 c) somewhat active \_\_\_\_\_ %  
 d) very active \_\_\_\_\_ %

TOTAL	100%
-------	------



INACTIVE

16. After finishing breakfast until starting lunch:

- a) inactive \_\_\_\_\_ %  
 b) somewhat inactive \_\_\_\_\_ %  
 c) somewhat active \_\_\_\_\_ %  
 d) very active \_\_\_\_\_ %

TOTAL	100%
-------	------



SOMEWHAT INACTIVE

17. After finishing lunch until starting supper:

- a) inactive \_\_\_\_\_ %  
 b) somewhat inactive \_\_\_\_\_ %  
 c) somewhat active \_\_\_\_\_ %  
 d) very active \_\_\_\_\_ %

TOTAL	100%
-------	------



SOMEWHAT ACTIVE

18. After finishing supper until bedtime:

- a) inactive \_\_\_\_\_ %  
 b) somewhat inactive \_\_\_\_\_ %  
 c) somewhat active \_\_\_\_\_ %  
 d) very active \_\_\_\_\_ %

TOTAL	100%
-------	------



VERY ACTIVE



For the *typical Saturday* that you are referring to, please answer the following questions as accurately as possible in the spaces provided.

19. At what time did you get out of bed in the morning? \_\_\_\_\_
20. At what time did you start eating breakfast? \_\_\_\_\_
21. How long did you spend eating breakfast? \_\_\_\_\_ minutes
22. At what time did you start eating lunch? \_\_\_\_\_
23. How long did you spend eating lunch? \_\_\_\_\_ minutes
24. At what time did you start eating supper? \_\_\_\_\_
25. How long did you spend eating supper? \_\_\_\_\_ minutes
26. At what time did you go to bed that evening? \_\_\_\_\_

27. For the *typical Saturday* that this questionnaire has asked you about, please rate your overall level of activity (please circle one response only):

- a) very inactive
- b) inactive
- c) somewhat inactive
- d) somewhat active
- e) active
- f) very active

28. Is the "typical" Saturday that you described in this questionnaire (please circle one response only):

- a) a lot like most Saturdays
- b) a little bit like most Saturdays
- c) a little bit different from most Saturdays
- d) a lot different from most Saturdays

29. If you have any comments about your activity patterns that you think are important, please mention them on the back of this page. Thank-you.





## PARTICIPATION QUESTIONNAIRE

NAME ..... BIRTHDATE (month)..... (day).... (year).....

GRADE ..... AGE ..... Do you take physical education? yes / no

**INSTRUCTIONS:** In this survey you will be asked about the activities that you do at school and in your spare time. There are no right or wrong answers because this is not a test! Just answer each question as best as you can remember. Please read each question carefully before you answer it. TO ANSWER A QUESTION JUST CIRCLE YOUR ANSWER OR PRINT YOUR ANSWER IN THE SPACE PROVIDED. Only circle one answer for each question.

Here is an example question to practice on.

How often do you eat an apple?

1. never    2. once a month    3. once a week    4. once a day

|||||  
|||||

### SECTION 1

**FREE TIME ACTIVITIES:** This section asks questions about what you do during your free time. Some of the questions will be about recess, some about what you like to do after school and others will be about what you do on weekends and holidays. Active games mean things like tag or skipping or playing catch.

1. During recess (or spares) do you spend most of your time:

1. Talking with friends    2. Doing schoolwork    3. Playing active games

2. After school and before you eat supper, most of the time do you:

1. Watch T.V.    2. Talk with friends    3. Play active games    4. Play video games  
5. Do other things (.....)

3. After supper and before you go to bed, do you spend most of your time:

1. Watching t.v.    2. Talking with friends    3. Reading books  
4. Playing active games    5. Doing other things (.....)

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12. When you have finished swimming do you usually feel:

1. very tired      2. tired      3. a little tired      4. not tired at all

PART D

13. During the winter how often do you go cross-country skiing?

1. never (If you answered never go directly to (PART E))  
2. once a month      3. once a week      4. once a day      5. all the time

14. When you finish skiing are you usually:

1. very tired      2. tired      3. a little tired      4. not tired at all

PART E

15. If there are other activities that you do once a week or more please list them below:

1. ....      2. ....      3. ....

16. How often do you watch T.V.?

1. every day      2. almost every day      3. hardly ever      4. never

17. How many hours a day do you usually watch t.v.?

- 0-1      1-2      2-3      3-4      4-5      5 or more

18. How often do you read a book in your free time?

1. every day      2. almost every day      3. hardly ever      4. never

19. How many hours a day do you usually read books?

- 0-1      1-2      2-3      3-4      4-5      5 or more

20. How often do you play video games in your spare time?

1. every day      2. almost every day      3. hardly ever      4. never

21. How often do you play active games with your friends after school?

1. every day      2. almost every day      3. hardly ever      4. never

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32. During your intramural games how often did you have to work hard (breathing heavily, sweating, heart beating quickly)?
1. very often    2. often    3. sometimes    4. hardly ever    5. never
33. After playing games in intramurals are you usually:
1. very tired    2. tired    3. a little tired    4. not tired at all
34. How many times a week, on the average, do you play intramural games?
- 0    1    2    3    4    5 or more
35. How many hours each week do you think that you spend playing intramural games at school?
- 0    1    2    3    4    5 or more
36. How many of your friends play intramural games?
1. most of them    2. a few of them    3. none of them

### SECTION 3

#### SCHOOL SPORTS TEAMS

These questions are about school teams that play sports against teams from other schools. If you don't play for any of your school's sports teams check here [ ] and go directly to section 4.

37. This school year how many school sports teams have you belonged to?
- 0    1    2    3    4
- (If you answered 0 go directly to SECTION 4)

38. After a game or practice are you usually:
1. very tired    2. tired    3. a little tired    4. not tired at all
39. During games or practices did you have to work hard (breathing heavily, sweating, heart beating quickly):
1. very often    2. often    3. sometimes    4. hardly ever    5. never

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## SECTION 5

### SPORTS AND DANCE CLUBS

These are clubs like gymnastics, martial arts (karate, judo, etc.), tennis, golf, swimming, horseback riding, and dance (jazz, ballet, tap). It doesn't include groups like Cubs or Guides or 4H. If you didn't belong to any sports or dance clubs in the last year check here [ ] and go directly to section 6.

49. In the last year how many DANCE clubs have you belonged to?  
0    1    2    3    4    5 or more
50. In the last year how many SPORTS clubs did you belong to?  
0    1    2    3    4    5 or more
51. How many times a week, on average, do you go to a sport or dance club competition or practice?  
0    1    2    3    4    5 or more
52. How many hours a week, on average, do you think that you spend at sport or dance club activities?  
0    1    2    3    4    5 or more
53. During practices or competitions how often did you have to work hard (breathing heavily, sweating, heart beating quickly):  
1. very often    2. often    3. sometimes    4. hardly ever    5. never
54. How tired do you feel after a sport club practice or competition?  
1. very tired    2. tired    3. a little tired    4. not tired at all
55. How many of your friends belong to sports clubs?  
1. most of them    2. a few of them    3. none of them

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