UNIVERSITY OF KWAZULU-NATAL

SALIVARY BIOMARKERS OF MUCOSAL IMMUNITY AND SYMPATHETIC ACTIVATION IN CHILDREN: EFFECTS OF BODY COMPOSITION, CARDIO-RESPIRATORY FITNESS AND EXERCISE

By
Ms. Kristen F. Konkol (BS Exercise Science, MA Kinesiology)
209540389

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Supervisor: Associate Professor Andrew McKune (DTech)

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DECLARATION

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LIST OF ABBREVIATIONS

1. ANS – Autonomic Nervous System
2. SNS – Sympathetic Nervous System
3. PNS – Parasympathetic Nervous System
4. HR – Heart Rate
5. RHR – Resting Heart Rate
6. sIgA – Salivary Immunoglobulin A
7. HPA axis – Hypothalamic Pituitary Adrenal Axis
8. sAA – Salivary Alpha Amylase
9. CHD – Coronary Heart Disease
10. BMI – Body Mass Index
11. CVD – Cardiovascular Disease
12. BP – Blood Pressure
13. IPAQ – International Physical Activity Questionnaire
14. SBP – Systolic Blood Pressure
15. DBP – Diastolic Blood Pressure
16. BF% – Body Fat Percentage
17. LMM – Lean Muscle Mass
18. URTI – Upper Respiratory Tract Infection
19. VO_2 max – Maximal Oxygen Consumption
20. CRP – C-Reactive Protein
21. HDL – High-Density Lipoprotein
22. IDF – International Diabetes Foundation
23. DHEA-S – Dehydroepiandrosterone Sulfate
24. CMIS – Common Mucosal Immune System
25. GALT – Gut-Associated Lymphoid Tissue
26. BALT – Bronchus-Associated Lymphoid Tissue
27. NALT – Nasal-Associated Lymphoid Tissue
28. CAs – Catecholamines
29. NE – Norepinephrine
30. TGF – Tumor Growth Factor
31. IL – Interleukin

32. T_H – T Helper
33. Ig – Immunoglobulin
34. OHDA – Hydroxydopamine
35. cAMP – Cyclic Adenosine Monophosphate
36. SC – Secretory Component
37. CRH – Corticotropin Releasing Hormone
38. ACTH – Adrenocorticotropic Hormone
39. ACh – Acetylcholine
40. NPY – Neuropeptide Y
41. VIP – Vasoactive Intestinal Peptide
42. sC – Salivary Cortisol
43. RMSSD – Root Mean Square of Successive Differences
44. ELISA – Enzyme-Linked Immunoassay
45. sAA con – Salivary Alpha Amylase Concentration
46. sAA SR – Salivary Alpha Amylase Secretion Rate
47. sIgA con – Salivary Immunoglobulin A Concentration
48. sIgA SR – Salivary Immunoglobulin A Secretion Rate
49. PA – Physical Activity

ABSTRACT

Introduction

Worldwide, overweight/obesity and associated chronic diseases such as type 2 diabetes, have reached epidemic proportions. Statistics show that overweight/obesity and chronic disease is prevalent amongst adults and children in South Africa. In addition to chronic disease/non-communicable diseases, overweight/obesity has been shown to alter immune and sympathetic activation. There is limited information on immune function (mucosal) and sympathetic activation on children both internationally and nationally and in particular investigating these parameters using non-invasive methods such as salivary biomarkers. The aim of this thesis was to investigate the levels of salivary biomarkers of immune function and sympathetic activation in children and determine the association with overweight/obesity, cardiorespiratory fitness (CRF) and increased physical activity (PA).

Methods

This thesis is divided into six chapters. These include an introductory chapter (Chapter One), a review of the literature (Chapter Two) and then three chapters that are written in article format and that have each been submitted to accredited journals for publication. Chapter Three is a review article that discusses salivary biomarkers in children as they relate to exercise, PA and obesity. Chapter Four is a study that examined salivary biomarkers of mucosal immunity and sympathetic activation as predicted by age, body composition and cardiorespiratory variables in one hundred and thirty-two black South African children (age 10.05 ± 1.68y, 74 females, 58 males). Chapter Five is a study that investigated salivary biomarkers of mucosal immunity and sympathetic activation in response to 12 weeks of soccer training in thirty-four black male South African children (11 – 13y) from a youth football training academy. Chapter Six includes a summary of the research findings, conclusions and well as recommendations for future research.

Results

Review Paper: A review of the literature revealed that participation in regular moderate intensity PA or exercise appears to enhance mucosal immunity (increases salivary IgA (sIgA)) in preadolescent children. In contrast, poor fitness and inactivity as well as strenuous training appear to compromise the mucosal immune system thereby increasing the risk of upper respiratory tract infections (URTIs). Children reporting higher levels of body fat and with a greater BMI appear to have lower sIgA levels and a greater incidence of infections. The limited research examining salivary C-reactive protein (sCRP) suggests a strong association between poor cardio-respiratory fitness (CRF) and/or overweight/obesity and inflammatory status in children based on elevated sCRP levels. Research surrounding salivary alpha-amylase (sAA) indicates that exercise can result in a marked increase in sAA as seen by an increase sympathetic activity via increased adrenergic activity in the salivary glands. The limited research suggests exercise may also pose a high stress on young athletes as seen with an increase in sAA. Additionally it appears that BMI may be a strong predictor of stress-induced sAA increases in children. Greater hypothalamic pituitary adrenal (HPA) axis response, as seen by increases in salivary cortisol, appear to be influenced greatly by increases in obesity. Higher salivary cortisol secretions have been observed in obese versus lean children in response to exercise.

School study: The outcomes of the one-way ANOVAs examining the differences by body mass index (BMI) categories showed there were significant differences in weight ($F = 83.64$, $df = 2, 129$, $P < 0.0001$), BMI ($F = 193.36$, $df = 2, 129$, $P < 0.0001$), waist-to-hip ratio ($F = 193.36$, $df = 2, 129$, $P < 0.0001$), body fat percentage ($F = 336.98$, $df = 2, 129$, $P = 0.0001$), SBP ($F = 5.72$, $df = 2, 129$, $P = 0.0042$), DBP ($F = 291.76$, $df = 2, 129$, $P < 0.0001$), VO_{2max} ($F = 521.00$, $df = 2, 129$, $P < 0.0001$), sAA concentration ($F = 17.05$, $df = 2, 129$, $P < 0.0001$), sAA secretion rate ($F = 15.15$, $df = 2, 129$, $P < 0.0001$), sIgA concentration ($F = 11.30$, $df = 2, 129$, $P < 0.0001$), and sIgA secretion rate ($F = 8.08$, $df = 2, 129$, $P = 0.0005$), between children of different BMI categories. According to the CDC-BMI-for-age standards, the participants were grouped into the following CDC-BMI-for-age categories: normal weight ($< 85^{th}$ percentile), overweight ($\geq 85^{th}$ percentile to $< 95^{th}$ percentile), and obese ($\geq 95^{th}$ percentile) (Ogden and Flegal, 2010). Tukey's post hoc analyses revealed that obese children had significantly ($P < 0.01$) higher weight, BMI, body fat percentage, DBP, SBP,

sAA concentration and secretion rate, compared to overweight and normal weight children, as well as a significantly lower aerobic capacity (VO_{2max}) than both normal ($P < 0.001$) weight and overweight ($P < 0.05$) children. In addition, sIgA concentration and secretion rate were significantly lower between normal weight and obese children ($P < 0.01$). Multiple linear regression revealed that BMI, DBP and VO_{2max} predicted sAA. BMI ($P = 0.04$) and DBP ($P = 0.04$) were found to be independent predictors of sAA concentration. Age and BMI category predicted sIgA secretion rate. BMI category ($P = 0.0006$) was found to be an independent predictor of sIgA secretion rate.

Soccer study: Significant differences after 12 weeks of soccer specific training were found to be significant between pre vs. post for BMI ($P =0.034$), waist-to-hip ratio ($P = 0.046$), age ($P < 0.0001$), height ($P < 0.0001$), body fat % ($P < 0.0001$) and LMM ($P < 0.0001$). Decreases in BMI, waist-to-hip ratio, body fat % and LMM were found while age and height increased throughout the 12 weeks. Significant differences were also found between sIgA secretion rate pre vs. post training ($P =0.025$) as increases in these values pre to post were observed.

Conclusions

The results from the studies on the school children and soccer players suggested that mucosal immune function and sympathetic activation appear to be affected by body composition, CRF and chronic exercise training. The main findings for the school study revealed that BMI, DBP and VO_2 predict sAA and that age and BMI category predict sIgA. This study also found that obesity (based on BMI) has a major role to play and that obese children have elevated sAA, lowered sIgA, and poor CRF. The finding of an increase in sIgA secretion rate in the soccer study suggested that a structured 12 week exercise programme can elevate mucosal immune function in youth soccer players. The underlying mechanism responsible may be an exercise-induced increase in the transport of sIgA across the mucosal epithelium and/or enhanced production of IgA in the mucosa via mediating cytokines. The literature review demonstrated that PA and overweight/obesity may have an impact on salivary biomarkers of mucosal immunity and sympathetic activation in children, however further

research with regards to optimal intensity, duration and modality need to be assessed in the pre-pubescent population.

Keywords: Physical activity, obesity, immunity, neuro-endocrine, children, salivary biomarkers, sympathetic activation

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CHAPTER 1: INTRODUCTION

Worldwide, overweight/obesity and associated chronic diseases such as type 2 diabetes, have reached epidemic proportions. Statistics show that overweight/obesity and chronic disease is prevalent amongst adults and children in South Africa. A study of obesity in South Africa showed the overall prevalence of adults who are overweight (Body Mass Index (BMI) > 25 kg/m^2) and obese (BMI >30 kg/m^2) to be high, with more than 29% of men and 56% of women being classified as overweight or obese (Puoane et al., 2002). Additionally, childhood obesity worldwide is estimated at 22 million children under aged five being classified as overweight (Goedecke et al., 2005). One study reported that 17.1% of South African children between the ages of 1-9 years living in urban areas are overweight (Steyn et al., 2005).

The role of physical activity (PA) in the prevention and management of overweight and obesity is linked, in part, to the impact of PA on energy expenditure, body composition, and substrate oxidation and metabolism (Donnelly et al., 2004). Furthermore, regular exercise is associated with increased adherence to dietary intervention for weight loss and weight control, improved self-efficacy and better long-term weight loss maintenance (Donnelly et al., 2004). As such, PA has the potential to be a powerful "agent of change" in the prevention and management of overweight and obesity. Moreover, associated cardiovascular disease risk factors can be attenuated by those who are physically active, even at the same level of obesity (Kruger et al., 2003). The protective effect of PA for obesity is not limited to adults, and, in fact, an inverse association between activity levels and fat mass, measured by television viewing time, has been reported in South African children (Kruger et al., 2003).

There is limited research regarding the association between salivary biomarkers of health status, body composition, cardiovascular variables and cardio-respiratory fitness (CRF), especially in children. However, obesity and lack of PA have been suggested to have negative affects on immune and neuro-endocrine function (Cieslak et al., 2003, Granger et al., 2007). In the last two decades, saliva has been advocated as a non-invasive alternative to blood as a diagnostic fluid. This is especially important for research with children as it is a more convenient tool for the analysis of biomarkers of health in this population (Christodoulides and Mohanty, 2005). In addition to being more straight forward and

economical to obtain than blood, saliva has the added advantage of being easier to handle for diagnostic purposes because it does not clot once it comes in to contact with ambient air (Gutie´rrez and Martı´nez-Subiela, 2009) and is an important and painless research tool for assessing health in adults and children alike (Christodoulides and Mohanty, 2005).

Research is required to examine mucosal immune function (salivary secretory immunoglobulin A (sIgA) is a biomarker) and sympathetic (salivary alpha-amylase (sAA) is a biomarker) activation in children. To the best of this author's knowledge, there is currently little research using saliva as a test medium in relation to the effect of body composition, cardiovascular variables and CRF on immune function and sympathetic activation in normal and overweight black South African children (Naidoo et al., 2012). Examining these parameters in South African children is important because the findings have implications for understanding their health status, particularly where the double edged sword of chronic diseases (cardiovascular disease, diabetes, obesity) as well as infectious diseases (HIV/AIDS) have reached epidemic proportions (Steyn, 2006, Puoane et al., 2002). Research with children in South Africa has focused primarily on the areas of obesity, body fat, nutrition, urbanization, gender, race, age and HIV/AIDS (Steyn, 2006, Puoane et al., 2002).

Although the research in South Africa is limited, a few international studies have examined the effect that PA on salivary biomarkers of immune function and sympathetic activation in normal and overweight children and a few of these studies are highlighted here. Dorrington et al., (2003) reported that sIgA levels were enhanced in children following moderate intensity exercise, but suppressed following high intensity exercise. Further, a study by Tharp (1991) on 27 prepubescent boys aged 10-12y, and 23 post-pubescent boys aged 16-18y examined sIgA before and after three games and three practice sessions during the basketball season. The results showed that sIgA levels were significantly elevated following basketball practice and games, suggesting that basketball exercise can increase sIgA levels and that chronic exercise over the basketball season may increase the resting levels of sIgA. Additionally, the limited research on children surrounding sAA suggests that exercise may impose a high stress on young athletes as seen with an increase in sAA (Capranica et al., 2012). Additionally, it appears that elevated BMI may be a strong predictor of stress-induced sympathetic activation in children as indicated by an increase in sAA in children aged 6-10y (Strahler et al., 2010).

The research studies conducted for this thesis will help to add to the body of knowledge as to effects that body composition, cardiovascular variables, CRF and exercise have on mucosal immune function and sympathetic activation in school children as well as in youth soccer players. The majority of current research on salivary biomarkers examines the effect of high intensity exercise or chronic training in the adult athletic population. Understanding the relationship between these variables will help in the development of safe and effective PA and exercise prescription guidelines for health and exercise professionals, coaches and teachers. Such guidelines will be particularly relevant for children whose immune function is compromised and/or sympathetic activation is dysfunctional.

Therefore, the aim of this thesis was to examine how salivary biomarkers of immune function (sIgA) and sympathetic activation (sAA) were associated with overweight/obesity, cardiorespiratory variables and CRF in a paediatric population. The thesis is divided into six chapters. Chapter Two is a comprehensive review of literature. Chapters three through five include three papers for scientific publication. Chapter Three is a review of literature for salivary biomarkers in children as they relate to exercise, PA and obesity. The second paper, Chapter Four, examined mucosal immunity and sympathetic activation salivary biomarkers in African children as predicted by age, body composition and cardiorespiratory variables. Chapter Five includes the final study that investigated salivary biomarkers of mucosal immunity and sympathetic activation in black male African children after 12 weeks of soccer training. Chapter Six includes a summary of the research findings, conclusions and well as recommendations for future research.

CHAPTER 2: REVIEW OF LITERATURE

2.1 Introduction

Worldwide, obesity and associated diseases such as type 2 diabetes, have reached epidemic proportions. Internationally, there is a considerable amount of research that is focused on identifying the aetiology, prevention and treatment of obesity, particularly in children, as it is felt that this is where interventions to prevent obesity should be targeted. Statistics show that obesity is prevalent amongst adults and children alike in South Africa (Goedecke et al., 2005). Traditional and cultural perceptions regarding body size, urbanization, poor diet, low socioeconomic status and lack of PA are a few of the suggested contributing factors (Steyn, 2006, Puoane et al., 2002).

The role of PA in the prevention and management of overweight and obesity is linked, in part, to the impact of PA on energy expenditure, body composition, and substrate oxidation and metabolism (Donnelly et al., 2004). Furthermore, regular exercise is associated with increased adherence to dietary intervention for weight loss and weight control, improved self-efficacy and better long-term weight loss maintenance (Donnelly et al., 2004). As such, PA has the potential to be a powerful "agent of change" in the prevention and management of overweight and obesity. Moreover, associated cardiovascular risk factors can be attenuated by those who are physically active, even at the same level of obesity (Kruger et al., 2003).

Obesity and/or a lack of PA /CRF have been suggested to have negative affects on immune and neuro-endocrine function (Cieslak et al., 2003, Granger et al., 2007). Currently there is limited research worldwide and in South Africa as to the effect these factors have on salivary biomarkers of immune and sympathetic activation in the paediatric population.

The following review of literature will look into the issues of obesity, poor CRF, physical inactivity, immune function, sympathetic activation, salivary biomarkers and exercise in the paediatric population.

2.2 Epidemiology of Obesity

Obesity has become a global epidemic. Worldwide obesity statistics have now soared to an estimated 1.3 billion people who are either overweight or obese (Goedecke et al., 2005). The prevalence of obesity, and subsequently obesity-attributable deaths, in developed countries is high. However, obesity is a problem for developing countries as well, including South Africa. A study of obesity in South Africa showed the overall prevalence of adults who are overweight (BMI > 25 kg/m^2) and obese (BMI >30 kg/m^2) to be high, with more than 29% of men and 56% of women being classified as overweight or obese (Puoane et al., 2002). Additionally, childhood obesity worldwide is estimated at 22 million children under aged five being classified as overweight (Goedecke et al., 2005). One study (Steyn et al., 2005) reported that 17.1% of South African children between the ages of 1-9y living in urban areas are overweight. The THUSA BANA study (Mukuddem-Petersen and Kruger, 2004) examined the role of ethnicity and gender as factors influencing body fat percentage and prevalence of childhood obesity in 10-15y old children from five different regions in the North West province in South Africa. The highest body fat percentages were amongst girls of all races (23%) compared to boys (15.2%) and higher in white (19.0% 20.8%) and Indian (17.5%, 20.2%) children compared to black (17.4%, 19.9%) and mixed origin (16.8%, 17.6%) children (Mukuddem-Petersen and Kruger, 2004). It has been suggested that many factors including gender, race, socioeconomic status and urbanization play a role in the prevalence of obesity in South African children.

2.3 Possible Causes of Obesity in South Africa

Unlike other parts of the African continent, South Africa has a pattern that is predominantly one of over-nutrition rather than under-nutrition (Puoane et al., 2002). There are many factors that could explain the high obesity rates in South Africans including changes in nutritional patterns over time, PA, the degree of urbanization and traditional and cultural perceptions concerning body size (Goedecke et al., 2005, Steyn, 2006). Being overweight has positive connotations in the African community as it may be seen as the ability of a husband to take care of his family or be perceived as an indicator of health in a society where there is a high prevalence of HIV/AIDS (Mvo et al., 1999). As many people move from more rural to urban

settings, the availability of low-cost, unhealthy, and fast food is greater (Puoane et al., 2002). In addition, less PA is being performed as a result of labour-saving mechanical devices and passive entertainment (television, computers, electronic games) (Goedecke et al., 2005). There is a higher prevalence of children experiencing excess calorie intake and decreased energy expenditure compared to previous generations (Steyn, 2006). Because habits are learnt at a young age, obesity in children must be targeted early to prevent the continuation to adult obesity as many studies have shown a strong correlation between overweight adults who were overweight as children (Puoane et al., 2002, Goedecke et al., 2005).

The protective effect of PA for obesity is not limited to adults, and, in fact, an inverse association between activity levels and fat mass, measured by television viewing time, has been reported in South African children (McVeigh et al., 2004, Kruger et al., 2003). In a regional, cross-sectional survey of children's health and fitness status of 12-18y old children in 14 schools in the Western Cape (boys n=2 026, girls n=2 792), current levels of obesity were associated with inactivity as measured by television time, lower fitness levels and a low reported daily intake of fruit and vegetables. Moreover, television viewing time was greater, and opportunities for school-based or after school sports and PA were fewer, in persons of lower socio-economic status (Lambert et al., 2000). The National Youth Risk Behaviour Survey looked at the self-reported data available for activity levels in children and youth (Reddy et al., 2003). These data (shown in Table I below) suggested that more than one-third of children surveyed participate in insufficient or no moderate-to-vigorous activity weekly. In the 2002 study, only 45% of adolescents participated in sufficient vigorous PA to be considered health-enhancing (Reddy et al., 2003). In 2008, this decreased to only 43% of those surveyed. Less than 1 in 3 of youth surveyed in 2008 participated in moderate activity, and nearly 42% did little or no PA weekly (Reddy et al., 2010). Additionally, more than 25% of the youth surveyed reported watching more than three hours of television per day in 2002 (Reddy et al., 2003) which increased to nearly 30% in the 2008 survey (Reddy et al., 2010). This emerging formative evidence necessitates public health focus and strategies, so that prevention can be implemented, particularly in children and youth.

Table I: Percentage of 13-19 year olds who participated in insufficient or no physical activity. Source: (Reddy et al., 2003)

	Males	Females	All
Black	34.4	42.4	37.5
Mixed Ancestry	36.8	56.8	45.6
White	28.2	37	29.4
Indian	40.8	36	33
RSA	34.4	43	37.5

Total n=10 100

2.4 Co-morbid Diseases of Obesity

Obesity is not commonly thought of as a disease, but it is in fact a serious medical problem. Although many people don't die from obesity, there are a number of obesity-attributable deaths due to some co-morbid disease. A study in the United States found that more than 80% of the estimated obesity-attributed deaths occurred among individuals with a BMI of more than 30 kg/m^2 (Allison et al., 1999). Obesity has been closely associated with many chronic conditions including Type 2 diabetes, coronary heart disease, hypertension, cancer, psychological implications and osteoarthritis, some of which can have life-threatening consequences (Goedecke et al., 2005). Childhood obesity can also result in the development of co-morbid conditions as one study showed approximately 60% of overweight 5-10y old children presenting with at least one associated cardiovascular risk factor and 25% presenting with two or more risk factors (Koplan and Dietz, 1999). These conditions begin in childhood due to obesity and persist into adulthood affecting morbidity and quality of life (Koplan and Dietz, 1999).

The metabolic syndrome in adults is defined as a cluster of cardiovascular and diabetes risk factors including abdominal obesity, dyslipidemia, glucose intolerance, and hypertension (Alberti et al., 2006). While the danger associated with clustering of components of the metabolic syndrome has been demonstrated in adults, where the presence of three or more components significantly increases the risk for coronary heart disease death/non-fatal myocardial infarction and the onset of new diabetes (Sattar et al., 2003), few, if any, outcome

data in children exist (Zimmet et al., 2007). While one definition, although with gender- and ethnicity-specific cut off points, is suitable for use in the at-risk adult population (Alberti et al., 2006), transposing a single definition to children and adolescents is problematic (Zimmet et al., 2007). Blood pressure, lipid levels, and anthropometric variables change with age and pubertal development (Zimmet et al., 2007). Puberty impacts on fat distribution (both gonoidal and androidal) and is known to cause a decrease both in insulin sensitivity, of approximately 30% with a complementary increase in insulin secretion (Bloch et al., 1987) and in adiponectin levels (Reinehr et al., 2004). Therefore, single cut off points cannot be used to define abnormalities in children (Zimmet et al., 2007). Instead, values above the 90th, 95th, or 97th percentile for gender and age are used (Zimmet et al., 2007). However, there has not been universal agreement as to which level to use for the criteria for the metabolic syndrome (Zimmet et al., 2007).

The importance of the early identification of children at risk of developing the metabolic syndrome and subsequently progressing to type 2 diabetes and cardiovascular disease in later life must not be forgotten. From birth and before, circumstances can predispose a child to conditions such as obesity or dysglycemia (Zimmet et al., 2007). The presence of maternal gestational diabetes (Pettitt et al., 1993), low birth weight (Wei et al., 2003), infant feeding practices (Pettitt et al., 1997), early adiposity rebound (Eriksson et al., 2003), and genetic factors may all contribute to a child's future level of risk. Being raised in an obesogenic' environment can also have a strong impact, as can the influence of socioeconomic factors (Abu Sayeed et al., 1997), with weight gain often being observed as a positive correlate to affluence in developing countries.

The International Diabetes Foundation (IDF) suggests that below the age of 10y the metabolic syndrome as an entity should not be diagnosed, although a strong message for weight reduction should be made for these children (Zimmet et al., 2007). At the age of 10y and more, a diagnosis of metabolic syndrome can be made. It requires the presence of abdominal obesity plus the presence of two or more of the other components (elevated triglycerides, low high-density lipoprotein (HDL)-cholesterol, high blood pressure, and elevated plasma glucose) (Zimmet et al., 2007). It is further suggested by the IDF that adult criteria (Alberti et al., 2006) can be used for adolescents aged greater than or equal to 16y while a modified version of these criteria should be applied to those aged 10 to < 16y (use

90th percentile cut off point for waist and < 40 mg/dL of HDL for both sexes) (Zimmet et al., 2007).

2.4.1 Consequences of Overweight in Children

Adverse health consequences related to overweight can begin in childhood or adolescence. Overweight children and adolescents are at increased risk for various chronic diseases in later life. In a study conducted by (Freedman et al., 1999), nearly 60 percent of overweight children had at least one cardiovascular risk factor compared to 10 percent of those with a BMI-for-age < 85th percentile while 25 percent of overweight children had two or more risk factors. Common medical consequences of overweight in children (Dietz, 1998) include hyperlipidemia, glucose intolerance, hepatic steatosis, cholelithiasis and early maturation. Overweight in children increases the risk for cardiovascular disease and premature death in adulthood (Power et al., 1997, Must et al., 1992). One study (Freedman et al., 1999) found 90 percent of the children with high levels of triglycerides were also overweight. Additionally, the incidence of Type 2 diabetes among adolescents is increasing and accompanying the national rise in overweight among teens (Pinhas-Hamiel et al., 1996). Acanthosis nigricans is associated with glucose intolerance in children and adolescents and is characterized by increased thickness and pigmentation of the skin between folds or juxtaposed surfaces (Richards et al., 1985). It has also been found that high concentrations of liver enzymes are associated with hepatic steatosis and have been found in overweight youth (Kinugasa et al., 1984). Although gallstones occur less frequently among children and adolescents who are overweight than in obese adults, nearly 50 percent of the cases of cholecystitis (i.e., inflammation of the gallbladder) in adolescents may be associated with overweight (Crichlow et al., 1972). Finally, early maturation, characterized by adolescents with a skeletal age > 3 months in advance of chronological age, is associated with increased fatness in adulthood (Van Lenthe et al., 1996).

There are some additional medical consequences that occur in overweight children, including hypertension and acute complications (Lauer et al., 1975). Hypertension, where persistently elevated blood pressure occurs, has been found to occur approximately nine times more frequently among children who are overweight compared with other children (Lauer et al.,

1975). In Lauer's study, almost 60 percent of children with persistently elevated blood pressure had relative weights >120 percent of the median for their sex, height and age. Childhood blood pressure and change in BMI were consistently the two most powerful predictors of adult blood pressure across all ages and both genders (Lauer et al., 1975). Freedman et al. (1999) found overweight children were 2.4 times as likely to have elevated diastolic blood pressure and 4.5 times as likely to have elevated systolic blood pressure. Acute complications are those that require immediate medical attention and should be referred to a paediatric obesity centre (Barlow and Dietz, 1998), including sleep apnoea, obesity hypoventilation syndrome and a variety of orthopaedic complications affecting the feet, legs and hips. It is estimated that sleep apnoea occurs in approximately seven percent of obese children (Mallory et al., 1989).

2.4.2 Assessment of Overweight Children

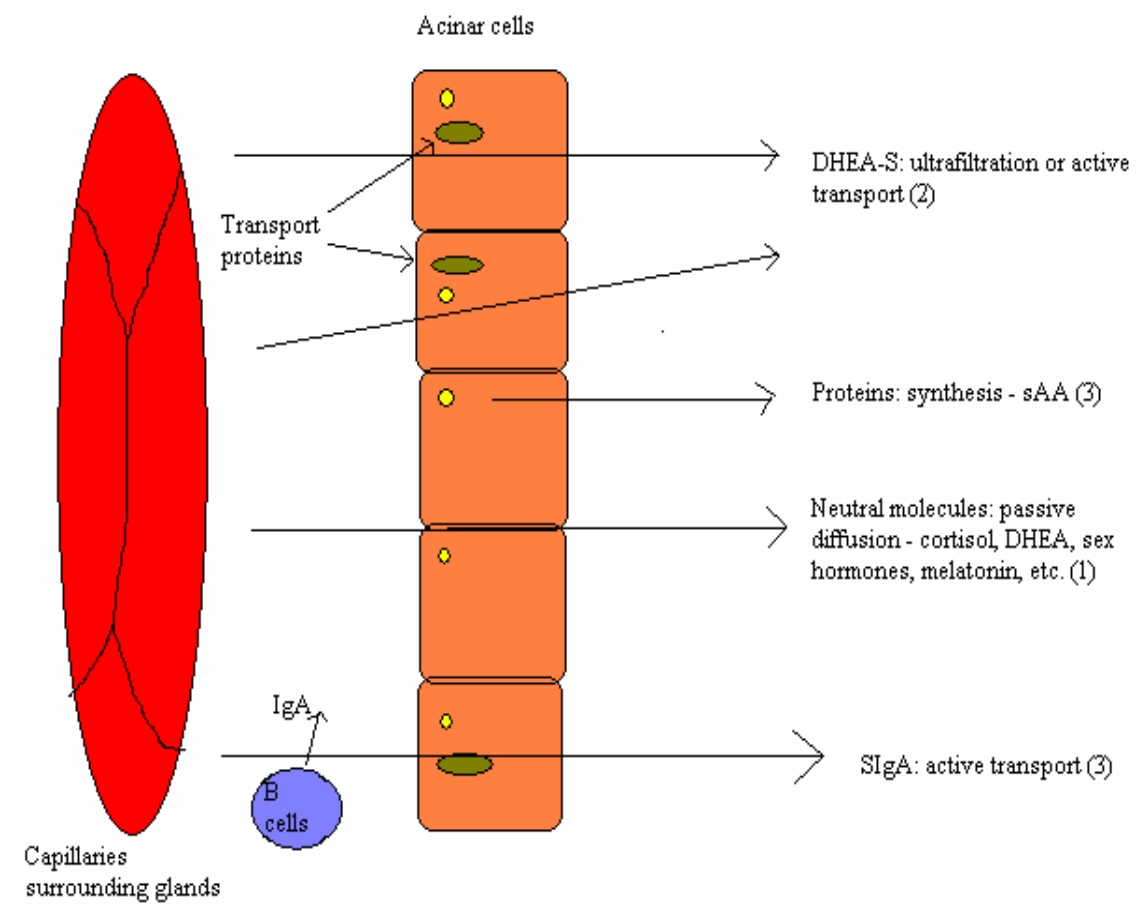
In-depth assessments are required to determine if children and adolescents with a BMI-for-age at > 95th percentile are overweight/obese and at increased risk for health complications related to increased weight. BMI-for-age is an assessment recommended to screen children aged 2 to 20y who are at risk of being overweight/obese (Barlow and Dietz, 1998). Children who are found to be overweight/obese may need further assessment and possible treatment. In-depth assessments allow for a diagnosis of the underlying causes of overweight/obesity and provide a basis for management plans. An assessment may include a medical history, family history, dietary assessment, PA assessment, physical examination, laboratory tests, and psychological evaluation (Dietz, 1998). Laboratory tests administered will help to determine the degree of overweight. These may include screening for cholesterol, blood pressure, and blood sugar and diabetes screening (Dietz, 1998). Although the aforementioned assessments are a positive step in the screening and intervention process in overweight children, it does not give us the full picture of the immune or neuro-endocrine function or sympathetic activation of the child. Additionally, invasive testing procedures for the purposes of acquiring blood diagnostics may warrant hesitancy amongst not only children, but parents and/or guardians.

2.5 Saliva as a Diagnostic Tool

The use of saliva assays to examine biomarkers of health has increased over the last two decades. Currently many analytes can be accurately and reliably measured in saliva. Some of the salivary analytes currently available for testing include alpha amylase, androstenedione, transferrin, cortisol, cotinine, C-reactive protein, DHEA, DHEA-S, estradiol, estriol, estrone, progesterone, 17 OH progesterone, sIgA and testosterone (Granger et al., 2012)

Saliva can be considered as gland-specific saliva and whole saliva. Gland-specific saliva can be collected directly from individual salivary glands: parotid, submandibular, sublingual and minor salivary glands. Secretions from both the submandibular and sublingual salivary glands enter the oral cavity through Wharton's duct, and thus the separate collection of saliva from each of these two glands is difficult (Christodoulides and Mohanty, 2005). The collection and evaluation of the secretions from the individual salivary glands are primarily useful for the detection of gland-specific pathology, such as, infection and obstruction (Christodoulides and Mohanty, 2005). However, whole saliva is most frequently studied when salivary analysis is used for the evaluation of systemic disorders. Whole saliva (mixed saliva) is a mixture of oral fluids and includes secretions from both the major and minor salivary glands (Kaufman and Lamster, 2002). Saliva has protective properties and contains a variety of antimicrobial constituents and growth factors (Kaufman and Lamster, 2002). In addition, saliva has lubricating functions and aids in the digestion of food (Kaufman and Lamster, 2002).

When saliva is used as a testing fluid, it is important to note that biomarkers enter saliva through a number of different pathways (Figure 1). Analytes that originate in the salivary glands or that enter by active transport will behave differently than those that enter by passive diffusion; consequently, some analytes may be sensitive to mouth location and/or saliva flow rates (Salimetrics, 2010). Different responses and recovery characteristics to stressors may also exist among analytes, and other factors such as diurnal rhythm must be considered as well. Some of these factors will be discussed later in this review as analytes are discussed in depth**.**



(1)Not affected by location or flow

(2)Affected by flow

(3)Affected by location and flow

Figure 1: Movement of analytes into saliva (adapted from Salimetrics, 2010)

Beltzer et al. (2010) explored the impact of saliva flow rate on sAA measurement by examining the influence of (1) the technique used to collect oral fluid-synthetic swab, cotton pledget, hydrocellulose microsponge, or passive drool; (2) collection point duration--the length of time the technique is employed (1-5min); and (3) oral fluid type--whole unstimulated saliva (not absorbed by any material) or oral fluid sampled from areas near the parotid, submandibular, or sublingual salivary glands. sAA activity (U/mL) was the highest in oral fluid collected from the parotid and submandibular gland areas. The volume (mL) of oral fluid collected increased, and the activity of sAA (U/mL) decreased, as collection point duration lengthened. The magnitude of these effects varied according to collection technique

and oral fluid type. Across all conditions, there were positive correlations (range .70-.88) between sAA activity (U/mL) and sAA output (U/min). The results suggested management of these potential sources of measurement error is essential to ensuring the success of future research on the correlates and concomitants of sAA activity, stress-related reactivity and recovery, and diurnal variation (Beltzer et al., 2010).

Saliva is an ideal testing fluid for studies in the field that involve athletes and exercise. However, the paths of entry into saliva for protein biomarkers such as sIgA, sAA, lysozyme, and lactoferrin are different from those of other analytes often measured in saliva, such as the steroid hormones (Bishop and Gleeson, 2009), as was previously mentioned. It has been suggested that inconsistency in various factors such as hydration of subjects, different methods of collecting saliva, and the presence or absence of salivary stimulation makes it difficult to compare findings among studies (Bishop and Gleeson, 2009). The need to standardize the method of reporting sIgA data and the merits of expressing assay results in relation to total protein, saliva flow rates, or saliva osmolality is of importance (Bishop and Gleeson, 2009). The concentrations of some biomarkers such as sIgA (Kugler et al., 1992) and sAA (Granger et al., 2012) can also be subtly influenced by salivary flow rate. Stimulating saliva flow may require that concentration per volume units need to be adjusted for flow rate (volume/min) (Kugler et al., 1992).

Saliva contains a certain level of previously circulating elements/molecules (i.e. from the blood) which are secreted consistently into the saliva and can be measured using biological assays (Lac, 2001). In the last two decades, saliva has been advocated as a non-invasive alternative to blood as a diagnostic fluid. In addition to being more straightforward and economical to obtain than blood, saliva has the added advantage of being easier to handle for diagnostic purposes because it does not clot once it comes in to contact with ambient air (Gutie´rrez and Martı´nez-Subiela, 2009). Therefore collection of saliva may be done using procedures that are considered to be non-invasive, painless and convenient and is an important research tool for assessing health in adults and children (Christodoulides and Mohanty, 2005).

2.5.1 Strategies for Collecting Salivary Analytes Children

Several factors need to be addressed before incorporating measurements of salivary analytes into research with children. Consistent collection processes are important that include controlling for factors that might interfere with accurate measurements of analytes in saliva (Hanrahan et al., 2006). Strategies for saliva collection include standardizing the time for sample collection, using consistent collection materials and methods, controlling for certain drinks and foods and establishing procedures for handling, storing and analysis.

2.5.1.1 Standardize the Time for Sample Collection

To control for the effects of circadian and diurnal rhythms, the time of day samples are collected should be standardized (Hanrahan et al., 2006). Ideally, samples are collected at a similar time of day for all study participants. However, this is not always possible in a research study involving children. The number of samples obtained must also be established. Having more samples provides more information on individual fluctuations. However, the number of samples obtained in a study may be limited by the availability of funds and the clinical situation. For intervention studies, the number of samples should include both measurements of baseline levels for the child and the child's response to the interventions (Hanrahan et al., 2006). The individual child acting as his or her own control in experimental situations may be the best reference value (Schmidt, 1997).

2.5.1.2 Use Consistent Collection Materials and Methods

The materials and techniques used to collect samples may influence the accuracy of testing (Hanrahan et al., 2006). One process for collecting saliva in children involves asking the child to spit (also referred to as passive drool) through a short straw into a plastic collection tube (Schwartz et al., 1998, Granger et al., 1999). Other options for saliva collection in younger children or infant populations include using a syringe or absorbent cotton swabs (Schwartz et al., 1998, Herrington et al., 2004, Joyce et al., 2001). More recently a collection device has also been developed by Salimetrics © called the Saliva Collection Aid ® which is similar to the straw system but fits perfectly with the collection tube. When saliva samples

are collected via unstimulated passive drool, the time period for collection is recorded. Whilst seated, children are asked to lean slightly forward and tilt their heads down to accumulate saliva in the floor of the mouth for a minute, which is subsequently swallowed. Following this there is a preset time of collection where the children dribble saliva through a 5 cm plastic straw into a pre-weighed polypropylene cryovial (5 ml capacity). Care must be taken to allow saliva to dribble into the collecting tubes with minimal orofacial movement. After collection the cryovial is weighed in order to determine the saliva flow rate. Saliva volume is estimated by weighing the cryovials to the nearest milligram with the saliva density assumed to be 1.0 g/ml (Cole et al., 1988). The saliva volume (calculated by post weight subtracted by the pre weight of the tube) is then used to calculate salivary flow rate. Saliva flow rate (ml/min) is determined by dividing the volume of saliva by the collection time.

2.5.1.3 Control for Certain Drinks and Foods

Certain foods, medications, and diagnoses can affect salivary analyte levels and should be documented, and exclusion criteria should be determined (Hanrahan et al., 2006). For example, in studies with young children, food or drinks that may be potentially problematic are milk products and those that contain caffeine or that alter salivary pH (Hanrahan et al., 2006). To control for alterations in salivary analytes, standardized saliva collection procedures should be followed (Salimetrics, 2010). The parents/guardians and children are requested to adhere as closely as possible to the following guidelines: The children should 1) not eat a major meal within 60 minutes of sample collection, 2) not brush their teeth (this may cause the gums to bleed causing blood contamination of the saliva), 3) avoid dairy products for 20 minutes before sample collection, 4) avoid foods with high sugar or acidity, or high caffeine content, immediately before sample collection (these have all been shown to impact on the saliva pH altering assay results), 5) rinse their mouths with water to remove food residue before sample collection, and swallow to increase hydration, and 6) wait at least 10 minutes after rinsing before collecting saliva to avoid sample dilution. Following these guidelines will help to control for factors that might interfere with accurate measurements of analytes in saliva.

2.5.1.4 Establish Procedures for Handling, Storing and Analysis

To ensure that samples are handled in a consistent manner, protocols for research team members should be developed. Samples should be refrigerated immediately in dry ice and kept frozen until reaching the laboratory (if in the field), upon which they should be stored at -70^{o}C until analysis. The reliability of the measurement of salivary analytes levels are determined by the assay selected. Researchers should first review the various types of assays available, identify the assay most appropriate for their needs, and then identify a salivary kit that is able to carry out the appropriate analysis (Hanrahan et al., 2006). Consultation with experts familiar with the assays and performance criteria helps in identifying an appropriate assay for researchers' needs (Hanrahan et al., 2006).

Saliva provides the researcher a tool to examine and test for a variety of analytes. The research studies carried out for this thesis collected and examined salivary analytes that provided valuable insights into salivary biomarkers of mucosal immunity and sympathetic activation and their association with body composition, cardiovascular variables and CRF in children. The next sections will provide an introduction to the immune system and then will discuss the mucosal immune system in general, with a specific emphasis on sIgA and its regulation.

2.6 Introduction to Immune System

The immune system is the collection of cells, tissues and molecules that protects the body from numerous pathogenic microbes and toxins in our environment (Crotty and Ahmed, 2004). This defence against microbes has been divided into two general types of reactions: reactions of innate immunity and reactions of adaptive immunity. Thus, innate and adaptive immunity can be thought of as two equally important aspects of the immune system.

The first arm to be deployed in the defence against pathogens is the innate immune system that is non-specific, but capable of rapid defence against infectious agents. This engagement is followed by the adaptive arm of the immune system with its humoral and cellular response,

capable of, responding to, and developing memory to pathogen encounters. This ensures that subsequent encounters are specific and up-regulated swiftly (Crotty and Ahmed, 2004).

Most of the pathogens that cause infectious disease multiply in the extra cellular spaces of the body, while intracellular pathogens spread by moving from cell to cell through the extra cellular fluids (Crotty and Ahmed, 2004). These extra cellular spaces are protected by the humoral immune (HI) response with antibodies at the front-line. The antibody response depends on the interaction between antigen, B-cell receptors /immunoglobulin (Ig), CD4+ T helper (TH) cells, and the differentiation of B-cells into antibody-secreting plasma cells (Crotty and Ahmed, 2004).

2.7 Mucosal Immunity

The Common Mucosal Immune System (CMIS) is a network of immune structures at mucosal surfaces throughout the body. The network incorporates the gut-associated lymphoid tissue (GALT) / Peyer's Patches, urogenital tracts, lachrymal glands, lactating mammary glands and, in the respiratory tract, the bronchus-associated lymphoid tissue (BALT), salivary glands and nasal-associated lymphoid tissue (NALT) (Gleeson, 2000).

These external surfaces provide a large surface area that can be colonized by pathogens or exposed to antigens and allergens (Mackinnon and Hooper, 1996). For example, the mucosal surface of the airways has a surface area of $500m^2$, which is continually exposed to a large number of antigens present in the 10,000 L of daily inhaled ambient air, and in the nasopharyngeal secretions that trickle into the lung during sleep (Salvi and Holgate, 1999).

Host defence against pathogens, antigens or allergens involves several mechanisms, including biochemical, physical and immunological barriers that provide a first line of defence (Salvi and Holgate, 1999, Mackinnon and Hooper, 1996, Gleeson, 2000). The secretory immune system is a major effector of host resistance to colonization of external surfaces of the eyes, nose, upper and lower respiratory tracts, gastrointestinal tract, and genitourinary tract by pathogens (Mackinnon and Hooper, 1996, Gleeson, 2000, Salvi and Holgate, 1999). Antibodies (e.g. sIgA) and other substances in mucosal secretions such as sAA, interact with a variety of pathogens, antigens or allergens deposited on the mucosal

surfaces of the body (Gleeson, 2000, Mackinnon and Hooper, 1996, Salvi and Holgate, 1999).

2.7.1 Primary and Secondary Antibody Responses

Immune responses to antigens may be categorised as primary or secondary responses. The primary immune response of the body to antigen occurs on the first occasion it is encountered. Depending on the nature of the antigen and the site of entry this response can take up to 14 days to resolve and leads to the generation of memory cells with a high specificity for the inducing antigen (Ademokun and Dunn-Walters, 2010, Janeway et al., 2001). The humoral response, mediated by B cells with the help of T cells, produces high-affinity and antigen-specific antibodies (Ademokun and Dunn-Walters, 2010). This is in contrast with the CD8 T-cell response which leads to the generation of large numbers of antigen-specific cells that are capable of directly killing infected cells (Ademokun and Dunn-Walters, 2010). Antigen-specific CD4 T cells, which provide help to B cells in the form of cytokines and other stimulatory factors, can also be expanded upon antigenic stimulation (Ademokun and Dunn-Walters, 2010).

The secondary response of both B- and T cells is observed following subsequent encounter with the same antigen and is more rapid leading to the activation of previously generated memory cells. This has some quantitative and qualitative differences from the primary response (Ademokun and Dunn-Walters, 2010, Janeway et al., 2001).

2.7.2 Regulation of Antibody Response: Linking the Sympathetic Nervous System and Hypothalamic Pituitary Adrenal Axis

It has been convincingly demonstrated that immune responses are not autonomous and that functional interactions exist between the central nervous system (CNS), endocrine system and the immune system (Elenkov et al., 2000, Silberman et al., 2003). The exact nature and type of neurohormonal-immune reactions that occur vary widely and depend on the ‘communication’ pathway that is involved. Specifically, two pathways link the brain and the immune system: the autonomic nervous system (ANS) via direct neural influences, and the

neuro-endocrine humoral outflow via the pituitary gland (Elenkov et al., 2000). Adrenal glucocorticoids, the end-products of the hypothalamic-pituitary-adrenal (HPA) axis, are generally immunosuppressive (Silberman et al., 2003). Similarly, the sympathetic nervous system (SNS), via its end-products, catecholamines (CA), is regarded as immunosuppressive (Elenkov et al., 2000). There has been accumulating evidence that both CAs and glucocorticoids, under physiologic, constitutive conditions or at levels that can be achieved during stress, are required for successful adaptive immune responses (Elenkov et al., 2000, Fleshner, 2000).

Although many organs of the body, such as the heart and the gastrointestinal tract, receive both sympathetic (noradrenergic) and parasympathetic (cholinergic) innervation, it is usual for one to predominate over the other. The primary and secondary lymphoid organs received predominantly sympathetic/noradrenergic innervation (Elenkov et al., 2000, Fleshner, 2000), and immune cells, such as T- and B-cells express β-adrenoreceptors. Through stimulation of lymphoid organs and β-adrenoreceptors, locally released norepinephrine (NE), or circulating catecholamines such as epinephrine, regulate lymphocyte trafficking, circulation, and proliferation (Elenkov et al., 2000).

The HPA via glucocorticoids, and SNS, via NE and epinephrine have also been shown to modulate cytokine production. Specifically, they inhibit the production of type 1/proinflammatory cytokines, such as IL-12, tumor necrosis factor-α, and interferon-γ by antigen-presenting cells and T_H1 cells (Elenkov et al., 2000), whereas they stimulate the production of the type 2/anti-inflammatory cytokines such as IL-10 and TGF- β. Through this mechanism, systemic, endogenous catecholamines may cause a selective suppression of T_H 1 responses and cellular immunity, and a T_H 2 shift toward the dominance of T_H2 humoral immunity B-cell proliferation and differentiation as well as antibody production (Elenkov et al., 2000).

Although lymphoid organs are predominantly innervated by the SNS and, that B-cells express β-adrenergic receptors it has been demonstrated that B-cells, including antibody-secreting plasma B-cells, express cholinergic receptors (Brink et al., 1994) and react to acetylcholine (Rinner et al., 1995) with enhanced synthesis of IgG antibody (Cameron et al., 1995, Brink et al., 1994). Therefore it has been suggested that the immune system, in

particular the adaptive response, is also highly integrated with the parasympathetic nervous system (Cameron et al., 1995, Kawashima and Fujiia, 2000, Kawashima and Fujiia, 2003b, Kawashima and Fujiia, 2003a).

It can be seen from the above information that there are numerous different components of the neuro-endocrine system that may regulate the antibody response (Fleshner, 2000). Figure 2 gives a schematic illustration of connections between the nervous and immune systems**.** Signalling between the immune system and the central nervous system (CNS) through systemic routes, the vagus nerve, the HPA axis, the SNS and the peripheral nervous system (PNS) are shown. The next sections will discuss the effects of the HPA axis and SNS on the antibody response to antigen.

2.7.2.1 Hypothalamic-Pituitary-Adrenal Axis and Antibody Responses

After an organism is challenged with an antigen, there is often a small rise in adrenal serum corticosterone that corresponds with a primary antibody response including the clonal expansion of B-cells. A more prolific B-cell response is associated with a high corticosterone response (Miller et al., 1997, Stenzel-Poore et al., 1993). Research has shown that an intact HPA is required for the generation of an optimal antibody response (Fleshner, 2000). Adrenalectomy or treatment with glucocorticoid receptor antagonists both reduced IgM and IgG antibody responses (Fleshner et al., 1995, Fleshner et al., 1997).

The exact role of the HPA response after antigenic challenge is still not clear. The stimulation of the HPA axis by antigen could be involved with providing immune feedback inhibition, given that glucocorticoids have well-known anti-inflammatory effects on the immune response. However, this role is questionable in the case of the in vivo antibody response. If glucocorticoids were providing immune inhibition, one would expect an increase, rather than the observed decrease, in the antibody response when corticosterone was removed however this has not been shown (Fleshner, 2000). Another possible role of the HPA interactions is that, 4-7 days after antigenic challenge (during the primary antibody response), the small rise in coticosterone provides a stimulatory signal for antigen-specific B-cell proliferation (Fleshner, 2000).

Research has provided evidence that basal levels of corticosterone may be important during the generation of an in vivo antibody response (Fleshner, 2000, Silberman et al., 2003). After exposure to physical or psychological stress, corticosterone is greatly increased and the antibody response is suppressed (Fleshner, 2000). However, it has been shown that although elevated glucocorticoids are necessary, they are not sufficient to suppress the antibody response to antigen (Fleshner, 2000).

2.7.2.2 Sympathetic Nervous System and Antibody Responses

Many primary and secondary lymphoid organs are densely innervated by efferent branches of the SNS (Felton et al., 1987). It has been reported that splenic neural activity increases during an immune response (MacNeil et al., 1996) and that surgical transection of sympathetic input into the spleen alters immune responses (Zalcman et al., 1994). In addition, chemical sympathectomy, by injection of the neurotoxin 6-hydroxydopamine (6-OHDA), reduces the formation of B-cells (Fleshner et al., 1997), and low-dose β-adrenergic receptor stimulation increases the formation of IgM secreting B-cells (Sanders and Powell-Oliver, 1992).

These in vitro studies may suggest that a low-moderate level of catecholamines facilitates B-cell proliferation. However, the precise role of catecholamines in the development of a normal in vivo antibody response, remains unclear. One possibility is that norepinephrine regulates lymphocyte migration into and out of the spleen during the generation of the antibody response. The antibody response requires the interaction of several different cells of the immune system (i.e. dendritic cells, macrophages, B-cells, T-cells). It is possible that cytokines from cells of the immune system signal the brain (MacNeil et al., 1997) perhaps via the vagal effects (Maier et al., 1998), to increase norepinephrine concentrations in the spleen. The elevation in tissue levels of norepinephrine would stimulate the mobilization of

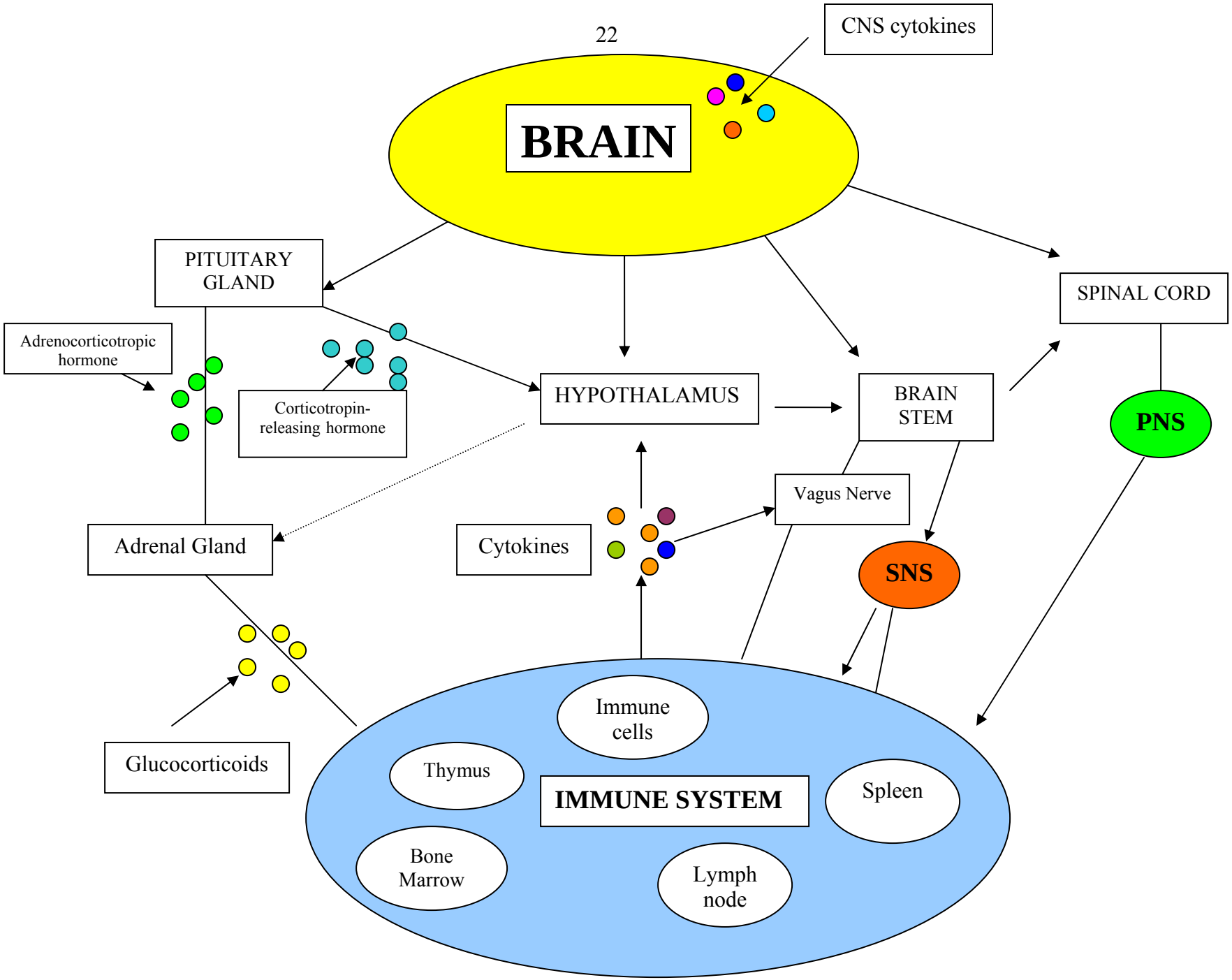


Figure 2: Connections between the nervous and immune systems

Adapted from: (Sternberg, 2006)

cells, as well as antigen trapping. Both consequences could be beneficial when mounting an optimal antibody response (Fleshner, 2000). Rogausch et al. (1999) reported that norepinephrine regulates splenic lymphocyte mobilization via β-adrenergic receptors and that mobilization can occur independently of sympathetic effects on smooth muscle.

It has also been demonstrated that when B-cells and T_H cells (which control B-cell proliferation and differentiation) are exposed to antigens in the lymphoid tissue, NE, through stimulation of β2 adrenergic receptors, exerts an enhancing effect on B-cell antibody production (Sanders, 1995). The proposed mechanism through which NE elevates antibody production is via interaction with the β2 adrenergic receptors on B-cells, which increases the frequency of B-cells differentiating into antibody-secreting cells (Sanders, 1998). Research has shown a two-fold increase in the frequency of pre-cursor B-cells differentiating into IgM-secreting B-cells after treatment with a β2 adrenergic agonist (Sanders, 1998). Stimulation of the β2 adrenergic receptors on B-cells is thought to increase intracellular cAMP, a second messenger, which mediates B-cell activation and antibody production (Elenkov et al., 2000). The elevation in cAMP, initiated by the β2 adrenergic receptor, also enhances the antibody response through two additional mechanisms. Firstly, there is an increased expression of the B7 molecule on B-cells that, along with CD40, determines the effectiveness of a T-cell-B-cell interaction for a B-cell activation (Watts et al., 1993). Secondly, it has been shown that a critical threshold level of intracellular cAMP must be obtained before B-cells can be activated (Pollok et al., 1991). Therefore, β2 adrenergic receptor stimulation during the critical Th/B-cell interaction, may augment cAMP in those B-cells that did not reach a critical threshold level of cAMP (Sanders, 1998). The evidence suggests that an optimal antibody response requires the interaction with the SNS.

After exposure to physical or psychological stress, blood and splenic tissue concentrations of norepinephrine increase (Mazzeo and Grantham, 1989) and the in vivo antibody is suppressed (Fleshner, 2000). This has stimulated research, which has aimed to determine whether stimulation of the SNS suppresses the antibody response to antigen. Research has provided evidence supporting the role of the SNS in stress-induced suppression of the antibody response. Central stimulation of the SNS suppresses the antibody response (Irwin,

1993), and the stress-induced suppression in the development of antigen-specific B-cells requires intact splenic sympathetic innervation (Watts et al., 1993).

With a basic understanding of some of the components of the immune system, mucosal immunity and regulation of the systemic antibody response, the next section will discuss exercise as a medium for influencing the immune system. Two of the research studies for this dissertation collected salivary analytes that provided insight into how exercise and CRF affected mucosal immunity and sympathetic activation in children.

2.8 Exercise Immunology

Interest in the field of exercise immunology has been stimulated in the past three decades by reports from coaches and athletes observing an increase in illness and infections following periods of intense training or acute bouts of strenuous exercise (Matthews et al., 2002, Gabriel et al., 1992). Studies have also looked at the critical levels of PA in relationship to stimulating immune function and the impact on the development of upper respiratory tract infections (URTIs) (Matthews et al., 2002).

The "J-shaped" model (Figure 3) proposes that the risk of URTI may decrease below that of a sedentary individual when one engages in moderate exercise training, while the risk may rise above average during periods of excessive amounts of high-intensity exercise (thus the "J-shaped" curve) (Nieman, 1997). The research providing support for this model has focused on the effect of heavy exertion on increased susceptibility to URTI, while there have been few studies that have examined the relationship between moderate exercise and lowered URTI risk (Nieman, 2000). However, some literature suggests that moderate PA may improve mucosal immune function (sIgA) in the elderly (Shimizu et al., 2007) and in children (Dorrington et al., 2003) that may reduce susceptibility to URTIs

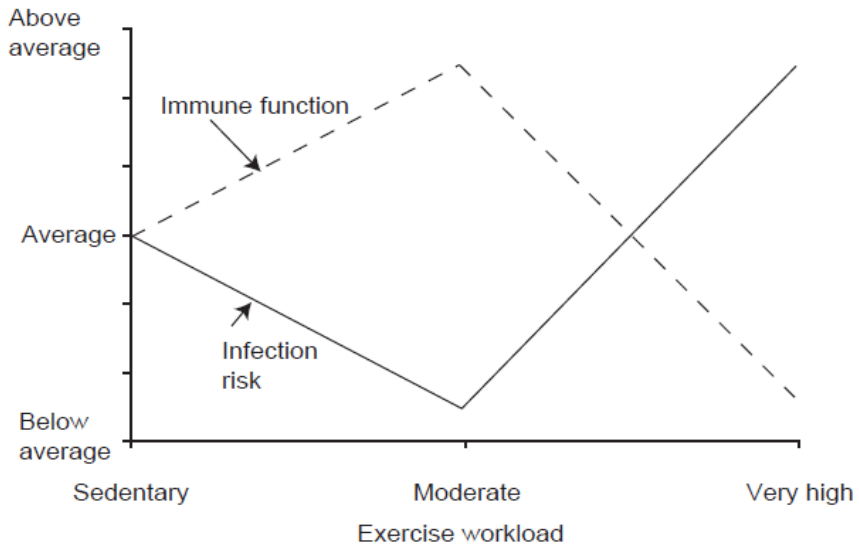


Figure 3: **Infection risk and immune function in relationship to exercise workload.**

Source: (Nieman, 2000)

Despite the historical longevity of the field of exercise immunology and the more recent advances in the past three decades, very little research has been focused on obese adults and there is limited research on the obese paediatric population (Naidoo et al., 2012). The number of investigations devoted to the paediatric population in general remains low, and our understanding of the interaction between acute and chronic exercise and the immune system in pre-pubescent youth has not been fully investigated. The earliest published data in regards to paediatric exercise immunology reported a leukocytosis of PA in children published in 1979 (Christensen and Rothstein, 1979). In a way, this study was the first to determine the effect of exercise intensity on immune changes, by comparing the total leukocytosis and neutrophilia in newborn babies in response to three conditions: circumcision, chest physical therapy, and heel puncture. The greatest increases in circulating total leukocyte (~46%) and neutrophil (~48%) counts were observed following the "most violent physical activity" (i.e., circumcision), with a full recovery of cell counts to resting levels by 60 min following the procedure (Christensen and Rothstein, 1979). Later in 1987, the same primary author documented exercise-induced changes in blood leukocyte subsets in teenage athletes (Christensen and Hill, 1987). Since this time, the research involving children and adolescents

has grown, but there is still a gap in the body of knowledge for studies including pre-pubescent children.

2.9 Mucosal Immunity Biomarker: Salivary Immunoglobulin A (sIgA)

Salivary immunoglobulin A is frequently known as the "first-line of defence" against pathogenic microorganisms, viruses, and bacteria within the immune system and is the dominant immunoglobulin in external secretions that bathe respiratory and intestinal mucosal surfaces (Tomasi, 1976). Salivary IgA is undetectable at birth but then consistently increases with age (Salimetrics, 2006, Kugler et al., 1992). The levels of sIgA reach their approximate peak by seven years of age and remain consistently high during mid-life and then decline during old age (Salimetrics, 2006, Kugler et al., 1992). Gender differences in sIgA levels have been reported in healthy young men and women (Van Anders, 2010). Serum levels of IgA have not been shown to have direct relationship with those found in saliva (Kugler et al., 1992, Smith et al., 1987, Ventura, 1991, Ben-Aryeh et al., 1990). In children and the elderly, both who are at increased risk of a compromised immune system, a lower concentration of IgA in saliva has been conceptualized as a risk factor for URTI (Ben-Aryeh et al., 1990, Smith et al., 1987, Ventura, 1991). Additional studies have also linked mood, academic stress and social support with altered levels of sIgA (Jemmot III and Magloire, 1988, Kugler, 1991). Lower levels of sIgA have also been shown to be associated with increased risk for periodontal disease and caries (Gregory et al., 1992, Ruan, 1990).

Salivary immunoglobulin A is a polypeptide complex comprising two IgA monomers, the connecting J chain, and the secretory component (Snoeck et al., 2006). The first mechanism of protection by sIgA occurs at the stromal side of the epithelium (Snoeck et al., 2006). At this location, sIgA can complex with antigens present locally in the underlying tissue. Salivary IgA has been shown to have an early morning acrophase followed by a decline to a stable base some six hours after awakening (Hucklebridge et al., 1998).

2.9.1 Synthesis and Transport of sIgA into the Lumen

Lymphoid tissue in the sub mucosal areas throughout the CMIS network is collectively termed mucosa-associated lymphoid tissue (MALT). This area contains long-lived plasma B-cells that have migrated specifically to the MALT (Mackinnon and Hooper, 1996). The structure of IgA differs depending on whether it is synthesized by plasma B-cells in the circulation or in the sub mucosa.

Secretion of IgA into the mucosal fluids requires specialized transport of dimeric IgA across the mucosal epithelial barrier, since tight intercellular junctions prevent the diffusion of large molecules between epithelial cells (Mackinnon and Hooper, 1996, Tomasi, 1992) (Figure 3). Transport is achieved through the attachment of dimeric IgA to a molecule (termed the polyimmuno-globulin receptor, poly-IgR), synthesized by epithelial cells, on the basolateral surface of the mucosal epithelium. The IgA-poly-IgR complex is endocytosed (internalized), and transported through the epithelial cells (transcytosis) to the luminal surface. Secretion of IgA involves partial cleavage (proteolysis) of the IgA-poly-IgR complex. This releases IgA and remnant of poly-IgR, termed the secretory component (SC) (Mackinnon and Hooper, 1996, Janeway et al., 2001, Roitt et al., 2001, Salvi and Holgate, 1999). The SC is crucial for the survival of sIgA in the lumen. It increases the stability of sIgA by helping to hold the IgA monomers together. SC also protects sIgA from proteolysis by pathogens found in the lumen by masking proteolytic sites (Brandtzaeg, 1981, Salvi and Holgate, 1999).

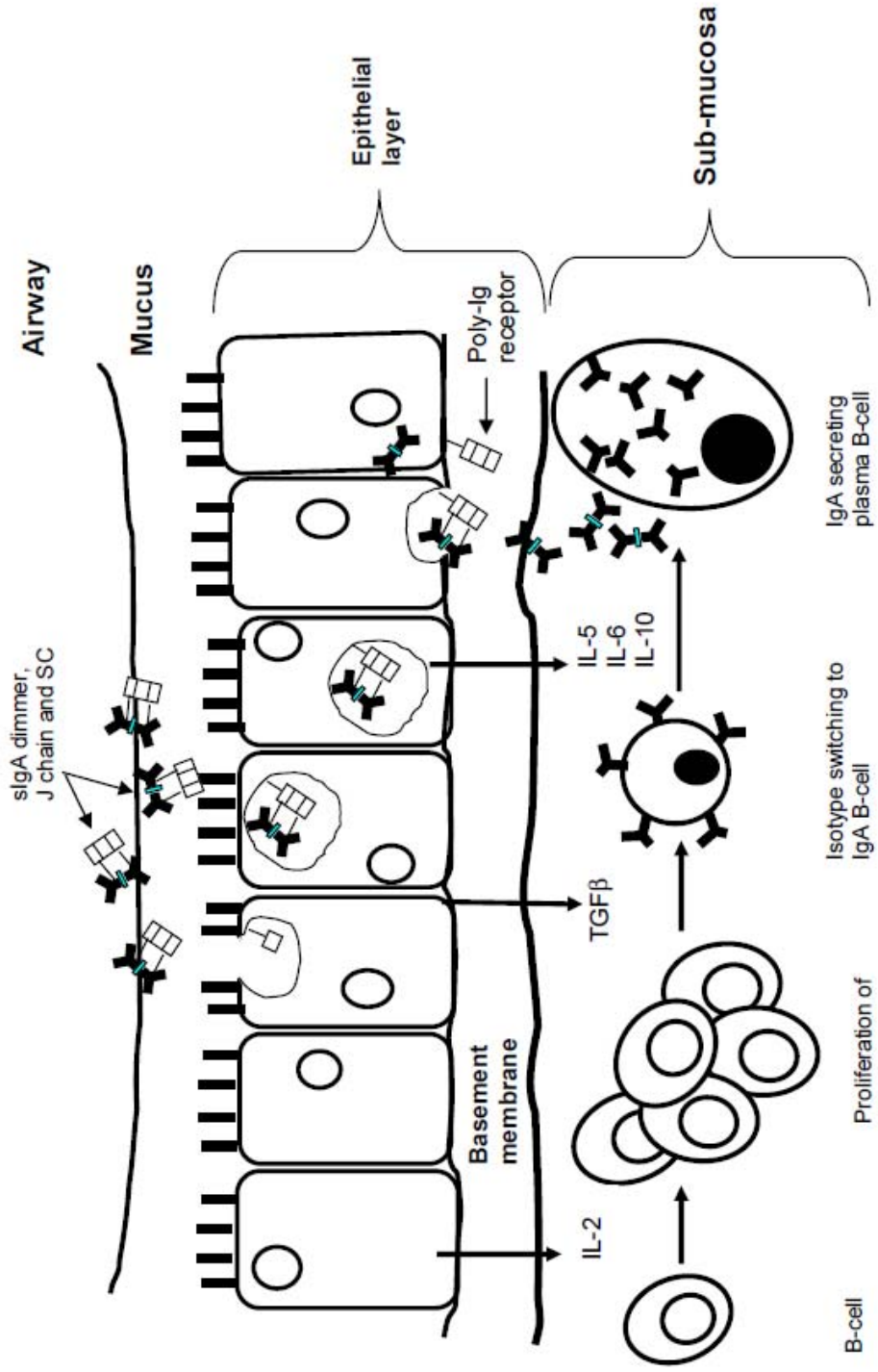


Figure 4: Secretory IgA synthesis and transport across the epithelium.

Adapted from: (Janeway et al., 2001, Salvi and Holgate, 1999)

2.9.2 Cytokines Mediate IgA Synthesis by B-cells in the Mucosa

Immunoglobulin A in the mucosa is produced locally by long-lived plasma B-cells (Lamm, 1997, Salvi and Holgate, 1999). Cytokines play a role in the activation, differentiation and proliferation of plasma B-cells into Ig/antibody secreting cells. These cytokines can be divided into two groups with respect to their biological effects on B-cells (Salvi and Holgate, 1999). The first group consists of Ig isotope switching cytokines while the second group functions as maturation cytokines, which induce proliferation and differentiation of isotope switched B-cells into antibody-secreting plasma cells (Salvi and Holgate, 1999). The cytokines responsible for B-cell switching to the IgA isotype, as well as those required for the growth and differentiation of these cells into IgA-secreting plasma B-cells, are shown in Table II.

TGFβ plays a central role in modulating mucosal cell growth, differentiation, migration, extra-cellular matrix synthesis, wound repair and immune responses (Clark and Coker, 1998). It is the most important IgA isotype switch factor known (Salvi and Holgate, 1999). In addition to inducing IgA isotype switch in B-cells, TGFβ induces the up-regulation of IL-5 receptor expression on B-cells (Sonoda et al., 1992).

Table II: Cytokines mediating mucosal IgA production (Salvi and Holgate, 1999)

CYTOKINE	**EFFECT**
TGFβ	IgA isotype switch
IL-5 and IL-6	Differentiation of IgA switched B-cells into IgA secreting plasma cell
IL-2	Clonal proliferation of B-cells
IL-10	IL-2 receptor up regulation Differentiation of IgA switched B-cells into IgA secreting plasma cell

Both IL-5 and IL-6 have been shown to be the most effective cytokines that promote the differentiation of IgA-committed B-cells to becoming IgA-producing plasma cells (Sonoda et al., 1992). Research has demonstrated that IL-5 also plays an important role in IgA synthesis

(Salvi and Holgate, 1999). Interleukin-6 is a growth and differentiation factor for B-cells (Van Snick, 1990) and has been shown to induce IgA secretion from B-cells in Peyer's Patch (lymphoid tissue found in the sub-mucosa of the small intestine) (Beagley et al., 1989).

Interleukin-10 is a potent growth and differentiation factor for activated B-cells and stimulates the production of IgG, IgM and IgA (Rousset et al., 1992). Interleukin-10 has been shown to stimulate B-cell production of IgA and also up-regulates the high affinity IL-2 receptor on B-cells. IL-2 does not induce Ig switching or the differentiation of B-cells (Gleeson, 2007). Through interaction with its receptor on B-cells, IL-2 plays an important role in the initial clonal expansion and proliferation of activated IgA isotype B-cells (Gleeson, 2007).

Due to the relatively short half-life (5-6 days) for the majority of IgA plasma B-cells, it is necessary that many IgA plasma B-cells must develop daily from B-cells to guarantee a continuous supply of sIgA antibodies in the mucosa (Salvi and Holgate, 1999). For this to occur, surrounding cells must provide a constant supply of the above cytokines to ensure B-cell isotype switching, growth and differentiation into IgA-secreting plasma B-cells (Salvi and Holgate, 1999).

2.9.3 Dendritic Cells Stimulate IgA Secretion by B-Cells

Dendritic cells are present within the airway epithelium and possess interdigitating fibers that cover the epithelial cell surface (Salvi and Holgate, 1999). It has been demonstrated that dendritic cells can capture and retain unprocessed antigen, transfer them directly to naïve B-cells, and provide them with signals that influence the isotype of antibody they subsequently secrete (Wykes et al., 1998). Specifically, dendritic cells were reported to directly modulate B-cell growth and differentiation, and stimulated B-cells to undergo isotype switching to IgA_1 and IgA_2, with the help of TGFβ and IL-10 (Fayette et al., 1997). IgA_1 is the predominant IgA subclass found in serum. Most lymphoid tissues have a predominance of IgA-producing cells (Delacroix et al., 1982). In IgA_2, the heavy and light chains are not linked with disulfide, but with noncovalent bonds. In secretory lymphoid tissues (e.g., gut-associated lymphoid tissue, or GALT), the share of IgA_2 production is larger than in the non-

secretory lymphoid organs (e.g. spleen, peripheral lymph nodes) (Delacroix et al., 1982). Both IgA_1 and IgA_2 have been found in external secretions like colostrum, maternal milk, tears and saliva, where IgA_2 is more prominent than in the blood (Simell et al., 2006)

2.9.4 Epithelium Secretes Cytokines Specific for IgA Synthesis

B-cell isotype switching to IgA isotype B-cells, as well as the differentiation of these into IgA antibody-secreting cells therefore occurs at local sites, specifically the sub mucosa (Salvi and Holgate, 1999). This switching depends on cytokines generated by surrounding cells. In the lungs, the IgA-secreting plasma B-cells are mainly found in close proximity to the epithelium and have been shown to lie within the epithelium (Soutar, 1976, GOODMAN et al., 1981).

Similarly, IgA memory B-cells are mainly found within intraepithelial areas, and not B-cell follicles (Schmedtje and Batts, 1973) as occurs with IgG memory B-cells. B-cells are also found within the epithelium of the mucosal region of the intestines (Spencer et al., 1985) and nose (Graeme-Cook et al., 1993). Traditionally, the epithelial lining of the respiratory tract was considered to be merely a mechanical barrier to antigen and pathogen penetration (Salvi and Holgate, 1999). However, research has focused on its metabolic activity and the way in which these cells interact with other elements of host defence (Salvi and Holgate, 1999). The airway epithelium has been shown to produce a wide range of cytokines, which play an important role in maintaining airway homeostasis (Stadnyk, 1994).

Interleukin-2, a B-cell growth factor, plays an important role in the clonal expansion of activated B-cells and is produced constitutively by airway epithelial cells (Aoki et al., 1997). TGFβ is produced by a variety of cells in the normal human lung, however, it is preferentially generated by bronchial and bronchiolar epithelial cells (Magnan et al., 1994). Both ciliated and mucus human airway epithelial cells constitutively produce TGFβ (Magnan et al., 1994). Human intestinal epithelial cells also produce TGFβ constitutively, and this is thought to account for the polyclonal production and secretion of IgA at the intestinal mucosal surface (Chen and Li, 1990).

It has been shown that airway (bronchial and nasal) epithelial cells constitutively express IL-5 mRNA and that the healthy human bronchial epithelium produces IL-5 constitutively in vivo (Salvi and Holgate, 1999). Healthy human bronchial epithelial cells also produce IL-6 (Cromwell et al., 1992) and IL-10 (Bonfield et al., 1995) mRNA and protein constitutively in vivo, similar to normal gut epithelium (Panja et al., 1995).

Cytokines normally act over relatively short distances in an autocrine/paracrine fashion to regulate various tissues homeostatic mechanisms (Salvi and Holgate, 1999). The close proximity of B-cells to the airway epithelium and the constitutive production of IL-2, TGFβ, IL-5, IL-6 and IL-10 by airway and gut epithelial cells is a crucial adaptation. This ensures that there is a constant supply of growth factors, necessary for mucosal sIgA production (Salvi and Holgate, 1999).

2.9.5 Salivary Immunoglobulin A and Exercise

There is limited research on the effect of moderate intensity training on sIgA, namely in children. Dorrington et al. (2003) reported that sIgA levels were enhanced in children following moderate intensity exercise, but suppressed following high intensity exercise. This is of significance for coaches working with young athletes who need to ensure that the volume and intensity of exercise sessions do not compromise the immune system leaving the athlete more prone to illness. An additional study on moderate intensity exercise with the elderly regarding PA and sIgA found that a moderated amount of PA of moderate-intensity at home, at work, and during walking might enhance salivary sIgA levels (Shimizu et al., 2007). A study by Tharp (1991) on 27 prepubescent boys aged 10-12y, and 23 post-pubescent boys aged 16-18y examined sIgA before and after three games and three practice sessions during the basketball season. Results showed that sIgA levels were significantly elevated following basketball practice and games, suggesting that basketball exercise can increase sIgA levels and that chronic exercise over the basketball season may increase the resting levels of sIgA. These changes may give athletes more protection against respiratory infections both after exercise and in the resting state later in the season. Filaire et al. (2004) examined the effects of physiological and psychological stress on sIgA in young female gymnasts. A significant reduction in sIgA concentration was found following acute exercise and resting sIgA levels

did not seem to be affected by periods of training. They also found no relationship between sIgA and cortisol (Filaire et al., 2004). A study of adolescent female tennis players (included 17 subjects up to the age of 21) examined the incidence of URTIs and sIgA and found that resting sIgA levels do not seem to be affected by periods of training. In this study, those with the greatest exercise-induced reduction in sIgA secretion rate, but not concentration, had the highest incidence of URTI (Novas et al., 2003).

Research has also examined the relationship between PA, obesity and sIgA, URTIs and cortisol. Cislak et al. (2003) examined the effect of PA, body fat and salivary cortisol on mucosal immunity in children using a 20 m shuttle run for prediction of aerobic fitness in 29 boys, 32 girls ages 10-11y. The authors found that sIgA was significantly correlated with reported URTIs and that children who spent more time in sport activities had a higher aerobic fitness and reported fewer "sick" days. Children with body fat >25% reported more sick days. There was no correlation between sIgA and cortisol (Cieslak et al., 2003).

A study examining the incidence of infections in 10-12y old children participating in sports found that participation in more than 5 sports activities per week increased the occurrence of the common cold, cough, fever symptoms, where three to four activities per week lowered occurrence (Waku et al., 1998). This result suggested a protective mechanism whereby moderate exercise may enhance the immune system, and overexertion may increase susceptibility to illness in children. Jedrychowski et al. (1998) examined childhood respiratory infections in terms of lifestyle factors and found that overweight children (BMI > 20) experienced twice as high a risk of respiratory infections than children with low BMI, independent of PA levels, compared to normal weight children. However, they did not include any information regarding immune function, such as sIgA level (Jedrychowski et al., 1998). An additional study by Jedrychowski et al. (2001) examined the relationship between PA and URTIs in preadolescent children and found that preadolescent children who had low PA levels had an increased risk of recurrent acute respiratory infections, compared to those that were moderately active and highly active (Jedrychowski et al., 2001). Pallaroa et al. (2002) examined the total salivary IgA, serum C3c and IgA in obese and lean school children between 6-13y of age and found that obese compared to lean children had a compromised secretory immune system, as indicated by lower levels of total sIgA, without an increased incidence of clinical symptoms and infections (Pallaroa et al., 2002). This study further

suggested the importance of regular PA for children to not only assist in achieving an optimum weight for their age, but also improve the protective mechanism on the immune system. In conclusion, the limited research examining the effect of *moderate* intensity exercise on immune function in normal and overweight children highlights the need to perform further research with this age group.

2.9.6 Mechanisms Underlying Alterations in Ig and Antibody

While studies have reported exercise-induced alterations in Ig or antibody level, few have examined the possible mechanisms that underlie these changes. Systemic and mucosal Ig and antibody secretion are mediated independently and by different mechanisms (Mackinnon, 1999). These will be discussed in the following sections.

2.9.6.1 Neuro-Endocrine System

Research examining the relationship between exercise-induced alterations in the neuro-endocrine system and Ig/antibody suppression in elite athletes has been confounded by the possible influence of the psychological stress on the neuro-endocrine and immune system.

Psychological stress has been shown to suppress T-cell-dependent antibody production and this may be regulated by alterations in the HPA axis or SNS in response to psychological stress (Silberman et al., 2003). Training and competing at the elite level involves both physical and psychological stress. Therefore the literature continues to investigate the primary stimulus relating to exercise-induced suppression of Ig/antibody as physical or psychological (Mackinnon, 1999).

2.9.6.2 Sympathetic Nervous System, Exercise and Enhanced Ig/Antibody Synthesis

The primary and secondary lymphoid organs are innervated by norepinephrine-releasing adrenergic nerve fibers, and B- and T-cells express β-adrenergic receptors for norepinephrine. This suggests that input from the sympathetic nervous system may be the

mechanism through which exercise exerts acute and chronic effects on Ig and antibody synthesis (Mackinnon, 1999, Kohut et al., 2004, Nieman and Nehlsen-Cannarella, 1991).

Acute exercise increases sympathetic input in a dose-dependent manner, with large increases in norepinephrine and lesser increases in epinephrine released in direct proportion to exercise intensity above a threshold of about 50-65% VO_2 max (Wilmore and Costill, 1994). In addition, exercise training results in a dampening of the catecholamine response to acute exercise at the same intensity (Wilmore and Costill, 1994). It has also been shown that lymphocyte β-adrenergic receptor density and sensitivity are also influenced by exercise (Maisel et al., 1990). Specifically, acute exercise has been shown to increase β-adrenergic receptor number, due to recruitment into the circulation of lymphocytes expressing this receptor (Maisel et al., 1990). In contrast, exercise training appears to down regulate β-adrenergic density and sensitivity. Norepinephrine has been shown to enhance specific antibody synthesis in response to antigen by increasing the number of antigen specific B-cells that differentiate in to IgM, IgG and IgA antibody-secreting plasma cells (Sanders, 1995).

Mackinnon (1999) proposed a mechanism for how norepinephrine may enhance the response of Ig and antibody to acute prolonged exercise and exercise training. Acute exercise causes a rapid migration of lymphocytes from lymphoid tissue, under the influence of norepinephrine (Maisel et al., 1990). This lymphocytosis is temporary and possibly therefore does not influence serum Ig levels (Mackinnon, 1999). During exercise, however, repeated elevation in the number of B-cells, together with increases in norepinephrine, may lead to enhanced antibody synthesis over time as shown in the training studies (Eskola et al., 1978, Smith et al., 2004, Whitham and Blannin, 2003).

2.9.6.3 Mechanisms Underlying the Mucosal Ig Response to Exercise

The mechanisms responsible for changes in the sIgA response to intensive exercise and training continue to be investigated (Mackinnon, 1999). Possible mechanisms include alterations in the oral mucosal surfaces due to high ventilatory flow during exercise, suppression of sIgA secretion and/or SC-mediated transport across the mucosal epithelium,

and changes in the homing of IgA secreting plasma B-cells to the airway sub mucosal regions (Mackinnon, 1999, Reid et al., 2001).

The decrease in sIgA in saliva may be due to a decrease in saliva flow (Mackinnon, 1999). The regulation of saliva flow is complex, involving input from the PNS and the SNS. Increased sympathetic output reduces saliva flow or volume by limiting the water content of saliva and by vasoconstricting arterioles in the salivary gland. The sympathetic control may also influence the migration of IgA-secreting plasma B-cells to the airway sub mucosa via vasoconstriction of blood vessels, effectively reducing the number of cells synthesizing and secreting IgA (Mackinnon, 1999).

Exercise-induced elevations in catecholamines and cortisol do not alter sIgA responses to intense exercise and training (Mackinnon, 1999). However, similar to serum Ig/antibody, psychological stress, the perception of stress and personality attributes, have been shown to lower mucosal sIgA levels (Mackinnon, 1999). It seems, then, that the effect of intense exercise, training and/or competition on Ig/antibody responses should take into the account the possible influence of psychological stress on results.

2.10 Neuro-Endocrine Stress Response of the Hypothalamic Pituitary Adrenal Axis - Sympathetic Nervous System

The psychobiology of the stress response has been defined as having two principal components. The first involves activation of the HPA axis (Kivlighan and Granger, 2006). The HPA axis involves multiple steps, including the release of corticotropin releasing hormone (CRH) from the hypothalamus, triggering the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, causing the release of the steroid hormone cortisol from the adrenal glands (Gordis et al., 2008). The second, and faster acting component, involves activation of the sympathetic branch of the locus ceruleus/ autonomic nervous system (SNS) and the release of catecholamines (Chrousos and Gold, 1992). The SNS is responsible for effects often referred to as the “fight or flight” response, which include increased cardiovascular tone, faster breathing rate, and increased blood flow to muscles (Cannon, 1914).

The HPA axis and the SNS are connected at multiple neural levels, and thus activity in these two systems should demonstrate some degree of symmetry (Chrousos and Gold, 1992). Factors leading to individual differences in the degree of symmetry between these systems remain unclear. One possible factor may be different habituation rates of these systems given previous exposure to stress (Gordis et al., 2008).

While it is clear that the HPA axis and the SNS work in coordination to generate the physiologic changes associated with the stress response, the exact nature of the coordination (e.g. additive or interactive; opposing or complementary) is a subject of debate (Kivlighan and Granger, 2006). One author speculated that SNS activity increases in response to challenges that are perceived as manageable or controllable, whereas an HPA response is more likely during emotionally distressful or uncontrollable situations (Henry, 1992).

The multisystem measurement approach has been endorsed as the gold standard for assessing the physiology of stress (Kivlighan and Granger, 2006). Despite a clear theoretical rationale, individual differences in, and consequences of, joint or dissociated activation of the HPA and SNS has only rarely been examined empirically (Bauer et al., 2002). There are a few recent exceptions (Granger et al., 2006). Some researchers have reported that dissociation in children's sAA and cortisol reactivity to challenge is associated with lower levels of cognitive function, poor teacher ratings of academic performance, and poorer performance on standardized achievement tests (Buckhalt et al., 2006). Others have noted that during adolescence, a pattern of joint inactivation in sAA and cortisol reactivity is associated with aggressive behaviour (Gordis et al., 2006). Examination of both the independent and interactive effects of the SNS and the HPA axis in the context of competition may be key in advancing the understanding of the role of stress in gaining or maintaining of status (Kivlighan and Granger, 2006).

2.11 Salivary Alpha-Amylase

2.11.1 Salivary Alpha-Amylase and the Adrenergic Component of the Stress Response

The SNS is an important branch of the stress response. Catecholamines are a part of the acute stress response (as part of the SNS) but are difficult to assess in saliva because of the low concentrations and rapid degradation of epinephrine and norepinephrine and the difficulty of stabilizing these hormones in the sample (Groschl, 2008). Other substances co-secreted with catecholamines can serve as an alternative index of adrenergic activity within the SNS and can be reliably measurable in saliva owing to their greater stability (Groschl, 2008).

One of these such alternatives or surrogates for adrenal medulla activity is alpha-amylase, which, although not a hormone, shows the same excretion patterns as catecholamines (Groschl, 2008). Therefore if catecholamines are elevated there is a corresponding increase in alpha-amylase in the saliva that indicates increased SNS activity. Because assays for amylase are more easily available in smaller clinical laboratories, saliva analysis of this enzyme may offer an interesting alternative for SNS activity testing (Groschl, 2008). Assessment of salivary alpha-amylase (sAA) as a non-invasive biomarker for the SNS offers a multitude of possibilities in different research areas and may well become an important parameter in stress research (Nater and Rohleder, 2009).

2.11.2 Production of Alpha-Amylase

Alpha-amylase is one of the major salivary enzymes in humans, and is secreted from the salivary glands in response to SNS stimuli. In acinar cells, release of salivary components is under the control of neuronal stimuli where classic neurotransmitters and specific bioactive peptides serve as the main stimuli for alpha-amylase secretion (Nater and Rohleder, 2009) Neurotransmitters exert their activity at the cell membrane; they communicate with intracellular second messengers that have direct control of secretory processes (Smith, 1996). Released in response to secretory stimuli, they bind to specific receptor proteins on the basolateral membrane, causing acute elevation of intracellular calcium (Nater and Rohleder,

2009). This results in large-scale fluid and electrolyte transport, and modest exocytosis of stored protein (Nater and Rohleder, 2009) (Figure 5). In contrast to many analytes present in oral fluid, alpha-amylase is not actively transported, nor does it passively diffuse into saliva from the general circulation (Granger et al., 2007). Under conditions of normal oral health, alpha-amylase is present in saliva in relatively high concentrations (Granger et al., 2007). Determining sAA levels and responses to stressors can provide information about the differences in SNS stress response. Normal sAA has a pronounced and distinct diurnal rhythm with a strong drop in activity in the first hour after awakening, and a steady increase towards the evening (Nater et al., 2007).

2.11.3 Biological Properties of Alpha-Amylase

Salivary alpha-amylase is unique among salivary biomarkers because it is an enzyme, officially classified as family 13 of the glycosyl hydrolases. The structure is an 8-stranded α - β barrel containing the active site, interrupted by a ~ 70 a.a. calcium-binding domain protruding between β strand 3 and α helix 3, and a carboxyl-terminal Greek key β−barrel domain (Granger et al., 2007).

The structure of the two families of isoenzymes is comprised of one set that is glycosylated and the other contains no carbohydrate (Nater and Rohleder, 2009). The molecular weight of the glycosylated form is about 57,000; that of the non-glycosylated form is about 54,000 (Nater and Rohleder, 2009). Salivary alpha-amylase accounts for 40% to 50% of the total salivary gland-produced protein, with most of the enzyme being synthesized in the parotid gland (80% of the total) (Zakowski and Bruns, 1985, Makinen, 1989). It is a calcium-containing metalloenzyme that hydrolyzes the a-1,4 linkages of starch to glucose and maltose (Nater and Rohleder, 2009).

A primary biological function of sAA is the digestion of macromolecules (e.g., carbohydrates and starch) (Granger et al., 2007). A secondary role of sAA is immune in nature and involves bacterial clearance from the mouth and prevention of bacterial attachment to oral surfaces (Marcotte and Lavoie, 1998). Salivary AA is the most important and abundant protein in saliva, and is mostly synthesized by the parotid gland (Filaire et al., 2012). Salivary AA is an

enzyme that breaks down starch into maltose and is also important in host defenses, inhibiting the adherence and growth of certain bacteria (Filaire et al., 2012). Much of what is known about the "biobehavioral" implications of individual differences in sAA levels is documented in the literature on oral biology and disease. Higher sAA activity is associated with reduced risk for a variety of processes related to oral health (bacteria load, caries, and periodontal disease) (Granger et al., 2007). Atypically low sAA activity is associated with oral disease (Granger et al., 2007).

2.11.4 Production of Alpha-Amylase

At birth, alpha-amylase is not present in the oral or gastrointestinal compartment (O'Donnell and Miller, 1980). Salivary AA activity shows a sharp rise in the 0.9- to 1.9-yr period, reaching maximum levels by five to six years of age (O'Donnell and Miller, 1980, Susman et al., 2006). The age of onset in the rise of alpha-amylase levels parallels the timing of the introduction of solid foods in the diet and the emergence of dentition needed to chew those solids. During middle childhood (ages eight to nine years), (El-Sheikh et al., 2005) reported that pubertal status and age were positively associated with sAA activity. Recently a relationship between α-amylase reactivity and pubertal development (Tanner Stage) was reported in boys but not girls (ages eight to thirteen years) (Susman et al., 2006).

2.11.5 Salivary Alpha-Amylase and Physical Activity

A strong activator of the SNS is PA (Nater and Rohleder, 2009). Exercise is known to increase sympathetic activity, and the high protein level in saliva following exercise may be due to increased adrenergic activity in the salivary glands (Nater and Rohleder, 2009). It is suggested that sAA response patterns to both physical and psychological stressors correspond to the response patterns of the SNS (Nater and Rohleder, 2009). A variety of studies have examined the effects of exercise on sAA, however most of the studies have focused on the adult population. Salivary AA has been shown to be a valid measure of sympathetic activity via adrenergic receptors and they observed a significantly higher concentration of sAA during exercise than in a control period (Gilman et al., 1979).

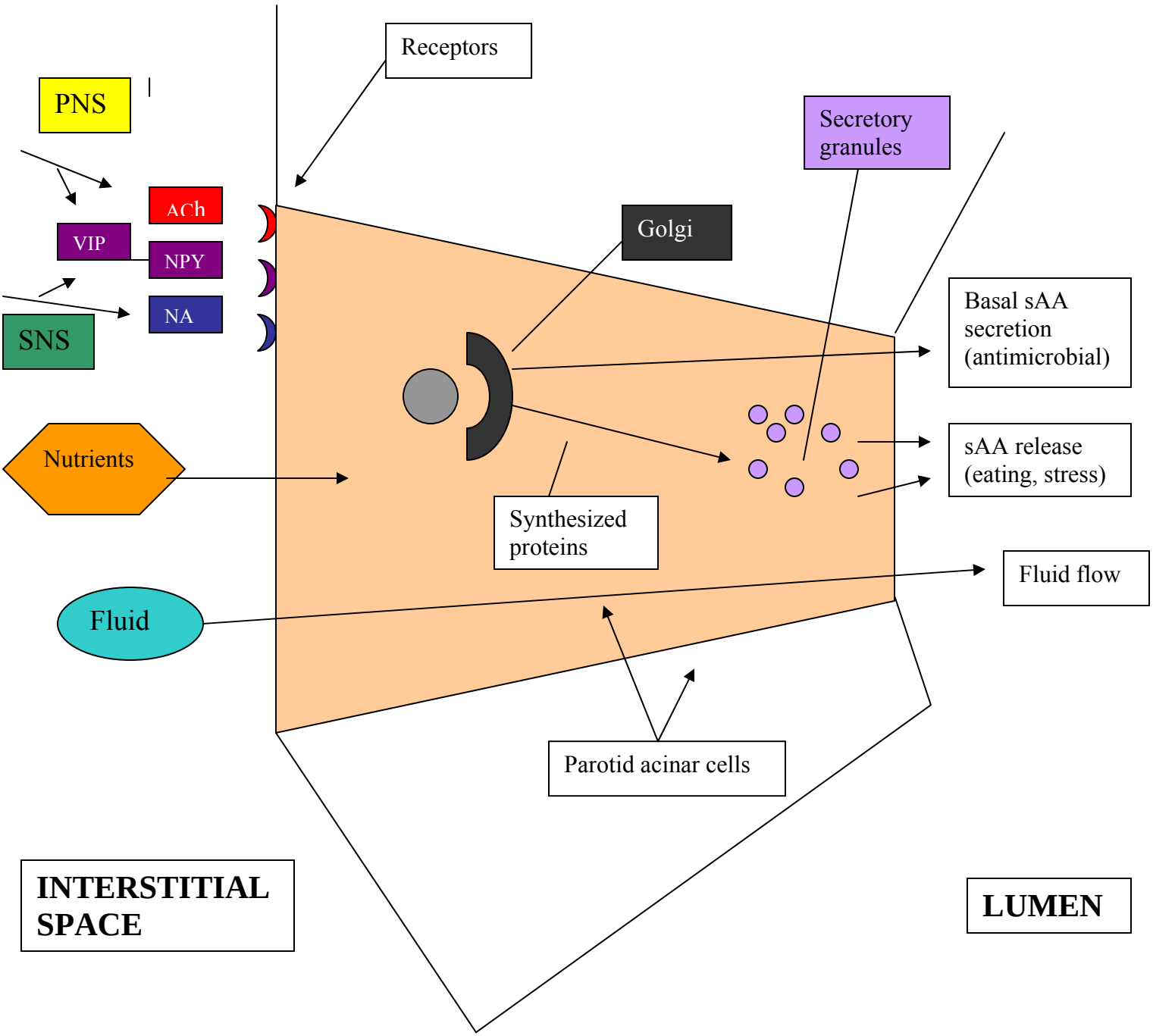


Figure 5: Nervous control of sAA and fluid secretion (Salimetrics, 2010)

Nexo et al. (1988) examined the impact of a 2-h long cross-country race, and found a marked increase in sAA after the race; the median level increased almost sevenfold compared to the beginning of the race. The measurement included collecting stimulated whole saliva before and after the race (Nexo et al., 1988). sAA also increased in response to bouts of running and cycling (Chatterton et al., 1996). An additional study investigated salivary components before, during and one hour after a marathon and found that sAA activity increased significantly due to the strenuous activity, and that the levels remained high at one hour after the marathon (Ljungberg et al., 1997). In a study of triathletes, mean sAA activity was found to be increased significantly after a race (Steerenberg et al., 1997). Another study examined eight well-trained athletes during a high-intensity 60-min cycle exercise task. Unstimulated whole saliva was collected before exercise, immediately after the task, and 1, 2.5, 5 and 24 h after exercise. A significant increase in sAA activity was found after exercise (Walsh et al., 1999). A study by Granger et al. (2006) examining the impact of exercise on a rowing ergometer on sAA levels showed increased levels after exercise as well as a positive association between sAA levels and performance. Therefore, these studies suggest that exercise can result in a marked increase in sAA as exercise is known to increase sympathetic activity (Granger et al., 2006).

A study by Capranica et al. (2012) on 12 young (10.4 ± 0.2 years) male taekwondo athletes evaluated the effects of an official taekwondo competition (three 1-min rounds with a 1-min recovery in-between) on heart rate (HR), sAA, and salivary cortisol. The authors found that taekwondo competitions impose a high stress on young athletes (Capranica et al., 2012). Peak sAA observed at the end of the match (169.6 ± 47.0 U/mL) was different ($P = 0.0001$) from the other samplings (pre-competition 55.0 ± 14.0 U/mL, 30-min recovery 80.4 ± 17.7 U/mL, 60-min recovery 50.5 ± 7.6 U/ml; 90-min recovery 53.2 ± 9.6 U/mL). The different sAA and salivary cortisol reactions in response to the physical stressor mirror the faster reactivity of the sympathetic-adrenomedullary system relative to the hypothalamic-pituitary-adrenocortical system (Capranica et al., 2012). Walsh et al. (1999) assessed the effect of an acute bout of high-intensity intermittent laboratory cycling exercise on sIgA concentration and sAA activity in eight well trained male games players and found a five-fold increase in sAA activity ($P<0.01$ compared with pre-exercise). The increased sAA activity after exercise may improve the protective effect of saliva, since this enzyme is known to inhibit bacterial

attachment to oral surfaces (Walsh et al., 1999). It is difficult to deduct the function of short-term increases in sAA while the biological meaning of transient rises in the anti-bacterial action of the enzyme remains unclear. However, such short-term increases may be useful to the body in that energy is made available by increased digestive action in response to stress (Nater and Rohleder, 2009). Physiological stress reactions comprise orchestrated actions throughout the body, putting the organism in a state of overall preparedness to engage in fight or flight. Thus, increases in amylase activity may be one of many actions involved in activating the body's resources to cope with stressful events or threats to homeostasis (Nater and Rohleder, 2009). Further studies are needed to examine long-term changes in sAA concentrations.

Additional research has shown that sAA levels rise in response to both physical and psychological stress including exercise, heat and cold and written examinations (Granger et al., 2007). Large sAA increases were also shown as a result of participation in collegiate level individually orientated athletic competition (Granger et al., 2007). The profile of stress-related change in sAA is distinct. In response to stressors, sAA reaches its peak and recovers to baseline faster than salivary cortisol, which is consistent with physiological differences in the timing of the SNS (quicker) and HPA (slower) stress responses (Granger et al., 2007). Greater intensities of exercise have been shown to be associated with greater increases in sAA concentrations. Studies have found increased sAA levels after prolonged events such as a 2-h marathon as well as triathlon (as mentioned above), but others have seen discrepancies, most likely due to exercise of low intensity or duration (Nater et al., 2007).

2.11.6 Salivary Alpha-Amylase and Eating Behaviour

Additionally, there appears to be a possible link between sAA and eating behaviour. One study reported a positive association between sAA activity and increased BMI (greater obesity) in adolescent males and females (Susman et al., 2006). An additional study found that BMI was negatively associated with average morning sAA, indicating a decrease in average sAA levels by 3.4% with each increasing point on the BMI scale (Nater et al., 2007). Salivary alpha-amylase stress reactivity across different age groups was investigated in 62 children (32 boys, 30 girls), 78 young adults (45 men, 33 women), and 74 older adults (37

men, 37 women) (Strahler et al., 2010). Children were aged 6 to 10y, young adults aged 20 to 31y, and older adults aged 59 to 61y. BMI, perceived stress scale, chronic stress screening scale as well as cortisol, HR, and root mean square of successive differences (RMSSD) response indices failed to predict stress-induced sAA initially with hierarchical linear regression until the group with children were included. Similar results with one exception occurred. Body mass index was now an even stronger predictor of stress induced alteration of sAA than age. All analyses revealed that age and BMI were the strongest predictors of sAA increases, whereas subjective stress levels as well as cortisol, HR, and RMSSD response indices failed to predict sAA stress responses. A further unexpected finding was that children had significantly higher baseline sAA concentrations than both groups of adult participants (Strahler et al., 2010). The research related to sAA, PA and obesity in children is very limited compared to the adult population and indicates that the chronic effect of training and the role of obesity have not been fully examined in prepubescent children.

2.12 Additional Salivary Analytes

2.12.1 Inflammation: Salivary C-Reactive Protein

It has been suggested that salivary C-reactive protein (CRP) may provide information relating to systemic health status associated with chronic disease (Tonetti and D'Aiuto, 2007). Up to half of all events associated with cardiovascular disease (CVD) are reported to occur in apparently healthy individuals who have few or none of the traditional risk factors, including hyperlipidemia (Clearfield, 2005). As a result, there has been increased focus on the role of other factors, such as inflammation, in the development of atherosclerosis and CVD (Clearfield, 2005). These efforts have led to the search for inflammatory biomarkers to improve the detection of coronary and cardiovascular risk among seemingly healthy individuals (Clearfield, 2005). Prominent among the possible candidates for a clinically useful biomarker of CVD risk is circulating CRP as measured by high-sensitivity (hs) assay. Studies have shown that elevated levels of CRP are associated with inflammation and increased cardiovascular risk (Patel and Robbins, 2001). Research has suggested a prognostic association between increased CRP production and outcome after acute myocardial infarction and in acute coronary syndromes (Pepys and Hirschfield, 2003).

2.12.1.1 Salivary C-Reactive Protein Production and Function

C-reactive protein, is a pentameric protein produced by the liver and is part of the nonspecific acute-phase response during inflammation, infection and tissue damage (Pepys and Hirschfield, 2003). C-reactive protein secreted by hepatocytes under the transcriptional control of the cytokine interleukin (IL)-6 (Pepys and Hirschfield, 2003), is likely to originate from the liver and to mirror serum CRP levels. Therefore, salivary CRP could offer a more accurate estimate of systemic inflammation than peripheral measures of cytokines, which are locally synthesized (Ouellet-Morin et al., 2011). The high-sensitivity commercially available enzyme-linked immunoassay (ELISA) adapted to measure CRP in human saliva may offer a valuable strategy to assess expected low CRP levels in various populations (Ouellet-Morin et al., 2011). One study provided initial evidence suggesting that non-invasive assessment of CRP in saliva allows for a valid prediction of serum CRP (Ouellet-Morin et al., 2011). The researchers found a moderate-to-strong association between CRP measured in saliva and in serum ($r = .72$, $P < .001$). The research suggested that salivary CRP may thus facilitate and promote research exploring the correlates of low-grade inflammation in epidemiological studies and make it feasible to expand immune, inflammatory and neuro-endocrine research in children (Ouellet-Morin et al., 2011).

2.12.1.2 C-Reactive Protein, Obesity and Exercise

CRP has been shown to be strongly related to all anthropometric and direct measures of total and central abdominal obesity, diastolic blood pressure, and apolipoprotein and lipid levels (Hack and Aarden, 1997) . In one particular study the observation made was that CRP levels were strongly and independently related to directly measured total and central obesity and this is consistent with the finding that adipocytes secrete interleukin-6, the main stimulus for CRP biosynthesis (Pepys and Hirschfield, 2003).

There have been numerous studies relating serum CRP and markers of inflammation with physical fitness and obesity measurements in children (Halle et al., 2004, Wannamethee and Lowe, 2002, McMurray et al., 2007, Isasi et al., 2003). Although a moderate-to-strong

association between CRP measured in saliva and in serum was found (Ouellet-Morin et al., 2011), this author has only observed 2 studies relating salivary CRP with measures of physical fitness, obesity or health status in children. The first study (Naidoo et al., 2012) examined the relationship between salivary CRP, CRF and body composition in 170 black South African children (age 9.41 ± 1.55y, 100 females, 70 males). Results indicated that poor CRF was independently associated with elevated salivary CRP concentration (OR 3.9, 95% CI 1.7-8.9, P =0.001). Poor CRF (OR 2.7, 95% CI 1.2-6.1, P =0.02) and overweight/obesity (BMI $\geq 85^{th}$ percentile) (OR 2.5, 95% CI 1.1-5.9, P =0.03) were independent predictors of elevated salivary CRP secretion rate. These results suggest a strong association between poor CRF and/or overweight/obesity and inflammatory status in children based on elevated salivary CRP levels. The second study (Krasteva et al., 2010) aimed to evaluate the oral inflammatory and humoral immune status in 32 children with allergic asthma and 20 control children. A significant correlation between total protein/haptoglobin and IgG/sIgA for children with allergic asthma was found. The results suggest that the higher salivary levels of CRP and haptoglobin may be an answer to allergic inflammation and severity of asthma. Further research is needed to better understand the role salivary CRP, as compared to serum CRP, and markers of inflammation have on physical fitness and obesity measurements in children.

2.12.2 Hypothalamic Pituitary Adrenal Axis: Salivary Cortisol

Salivary cortisol has emerged as an easy-to-collect, relatively inexpensive, biologic marker of stress (Hanrahan et al., 2006). Moreover, salivary cortisol levels reflect the biologically active (unbound) fraction of cortisol (Vining et al., 1983). Cortisol is lipid-soluble that enables the molecule to diffuse rapidly through the acinar cells of the salivary glands from the bloodstream into the saliva, without any influence of salivary flow rate (Pasquali et al., 2006). Although its absolute concentration in the saliva is approximately 30–50% lower than in the blood, its measurement may nonetheless be helpful in evaluating subtle alterations of the HPA axis in many pathophysiological conditions not classically dependent on relevant endogenous hypercortisolism (Pasquali et al., 2006). Researchers have developed methods for collecting salivary cortisol in children, and recent laboratory techniques have made it possible to detect very small concentrations of cortisol in plasma and saliva (Hanrahan et al.,

2006). Salivary and serum cortisol in children and adolescents have been shown to correlate strongly (Burke et al., 1985, Bober et al., 1988). Salivary cortisol thus enables the study of HPA axis function in epidemiological cohorts (Rosmalen et al., 2005).

2.12.2.1 Cortisol and Neuro-Endocrine Stress Response

The HPA axis is a central component of the body's neuro-endocrine response to stress. Its major end-product, cortisol, has profound effects on mood and behaviour as well as metabolism where exposure to increases in cortisol secretion can result in disruption of HPA axis regulation (Rosmond, 2005). The cortisol stress response is kept under control through a negative feedback loop including the pituitary, hypothalamus and hippocampus (Jacobson and Sapolsky, 1991). Cortisol influences a wide variety of processes, including cardiovascular function, fluid volume and haemorrhage, immunity and inflammation, metabolism, neurobiology, and reproductive physiology (Sapolsky et al., 2000). Normal circadian rhythm is comprised of high morning and low afternoon-evening cortisol levels and normal feedback control (Rosmond, 2003). However, when the final stage of chronic stress with 'burn-out' of central regulatory systems occurs, the result is a net decrease of cortisol output, a flattened diurnal secretory pattern, and inhibition of other endocrine axes, and can result in a metabolic syndrome (Rosmond, 2003). These observations highlight the importance of the HPA axis (and cortisol regulation) in the control of human health (Rosmond, 2003).

2.12.2.2 Cortisol, Exercise and Obesity

Increased levels of cortisol has a significant role to play in the body, and so if the HPA axis is not functioning appropriately this will alter obese individuals (including children's) ability to adapt and function metabolically. Numerous laboratories have reported an association between obesity, particularly central adiposity, and high cortisol concentrations in adults. During exercise, cortisol concentrations have been shown to remain higher in obese than in lean adults demonstrating a greater HPA response to the same exercise intensity with obesity (Hershberger et al., 2004). Although there is a large body of literature devoted to the neuro-endocrine response to exercise in adults, namely that increases in intensity and duration of exercise increase salivary cortisol, we do not fully understand the relationship between

exercise and the neuro-endocrine response in children. One study found that salivary cortisol concentration was decreased by 32% in obese children (8 – 11y) from pre- to post-exercise compared to lean children (Hershberger et al., 2004). Another study examined the associations between morning cortisol and adiposity in children (9.6 +/- 0.9y) at baseline and 9-month follow-up (Hill et al., 2011). Participants included 649 (301 males, 348 females) children for the cross-sectional analysis and 316 (153 males, 163 females) for the longitudinal analysis. A positive relationship was found between morning salivary cortisol and change in waist circumference over 9 months in overweight children. An additional study investigated whether 12 min of high-intensity exercise performed within a regular school break would lead to an increase in cortisol levels in 53, 4th grade (9-10y) primary school students (Budde et al., 2010). They observed a significant group by test interaction indicating a different pre-to-post-test development for the experimental group as compared to the control group. The interaction effect, however, was caused by an attenuated cortisol concentration in the control group. They argued that the control condition, in which the students watched a joyful movie, acted as a distractor, which led to a reduction of general school stress. Another study examining serum and salivary cortisol responses to cycling exercise in male children, 10.6 +/- 0.2y (mean +/- SE) and found that 30 min of submaximal exercise at 70% of VO_{2max} significantly increased serum cortisol level; and salivary and serum cortisol were correlated during and after exercise (del Corral et al., 1994). Further research measuring salivary cortisol as a marker of the neuro-endocrine stress response in normal and overweight children and associations with PA will assist in promoting understanding of the roles they play in the paediatric versus adult population.

2.13 Conclusion

Participation in regular moderate intensity PA or exercise appears to enhance mucosal immunity (increase sIgA) in preadolescent children. In contrast, poor fitness and inactivity as well as strenuous training appear to compromise the mucosal immune system thereby increasing the risk of URTIs. Children reporting higher levels of body fat and with greater BMI appear to have lower sIgA levels and a greater incidence of infections. The limited research with salivary CRP does suggest a strong association between poor cardio-respiratory fitness and/or overweight/obesity and inflammatory status in children based on elevated salivary CRP levels. Research surrounding sAA indicates that exercise can result in a marked

increase in sAA as seen by an increase sympathetic activity via increased adrenergic activity in the salivary glands. The limited research suggests exercise may also pose a high stress on young athletes as seen with an increase in sAA. Additionally it appears that BMI may be a strong predictor of stress-induced sAA increases in children. Greater HPA axis response, as seen by increases in salivary cortisol, appears to be influenced by increases in obesity. Higher salivary cortisol secretions have been observed in obese versus lean adults and children alike in response to exercise.

CHAPTER 3-5: SCIENTIFIC PAPER PUBLICATIONS

This section consists of three papers that have been submitted to accredited journals for publication. The first paper is a review of literature for salivary biomarkers in children as they relate to exercise, PA and obesity. The second paper examined mucosal immunity and sympathetic activation salivary biomarkers in African children as predicted by age, body composition and cardiorespiratory variables. The final paper investigated mucosal immunity and sympathetic activation of salivary biomarkers in black male African children in response to 12 weeks of soccer training

The first paper is a review titled: “Salivary biomarkers in children: Exercise, physical activity and obesity studies” and has been submitted to the South African Journal for Research in Sport, Physical Education and Recreation.

The second paper is original research titled: “Mucosal immunity and sympathetic activation salivary biomarkers in African children predicted by age, body composition and cardiorespiratory variables” and has been submitted to the South African Journal of Sports Medicine.

The third paper is original research titled: “Salivary biomarkers of mucosal immunity and sympathetic activation in black male African children after 12 weeks of soccer training” and has been submitted to the Journal of Strength and Conditioning Research.

SALIVARY BIOMARKERS IN CHILDREN: EXERCISE, PHYSICAL ACTIVITY AND OBESITY STUDIES

ABSTRACT

Worldwide, overweight/obesity and associated chronic diseases such as type 2 diabetes, have reached epidemic proportions. The current statistics show that overweight/obesity and chronic disease is prevalent amongst adults and children in South Africa. The aim of the review is to discuss current research investigating how overweight/obesity and inactivity impact on salivary biomarkers of immune and sympathetic activation in children and how these may change with weight-loss and increased activity. There is limited research regarding the effect that these factors have on salivary biomarkers of health status, especially in children. Further research is required to provide a clearer understanding of how salivary biomarkers may be used for understanding the impact of obesity and physical inactivity on paediatric health. This will play a role in the development of appropriate physical activity and exercise guidelines for children.

Keywords: Physical activity, obesity, immunity, neuro-endocrine, children, salivary biomarkers, sympathetic activation

3.1 Introduction

This paper aims to review the current research on salivary biomarkers of immune and sympathetic activation in the fields of physical activity (PA), exercise, obesity and health with a focus on children. The adult population has been the focus of previous research in this area. Overweight/obesity and/or lack of PA have been suggested to have negative effects on immune and neuro-endocrine function (Cieslak et al., 2003, Granger et al., 2007). Currently there is limited research worldwide and in South Africa as to the effect these factors have on salivary biomarkers of immune function and sympathetic activation in children. Understanding the relationship between these variables will help in the development of safe and effective PA and exercise prescription guidelines for health and exercise professionals, coaches and teachers. Such guidelines will be particularly relevant for children whose immune function is compromised and/or sympathetic activation is dysfunctional.

3.2 Saliva as a Diagnostic Tool

Saliva contains a certain level of previously circulating elements/molecules that are secreted consistently into the saliva and can be measured using biological assays (Lac, 2001). Over the past two decades, saliva has been advocated as an alternative to blood as a diagnostic fluid. In addition to being more straightforward and more economical to obtain than blood, saliva has the added advantage of being easier to handle for diagnostic purposes because it does not clot once it comes in to contact with ambient air (Gutie´rrez and Martı´nez-Subiela, 2009). Collection of saliva is non-invasive, painless and convenient and is an important research tool for assessing the health of children (Christodoulides and Mohanty, 2005). Immune, inflammatory, and neuro-endocrine biomarkers can be measured accurately and reliably in saliva. The following sections will provide an overview of the research examining these biomarkers in children.

3.3 Immune Biomarker: Salivary Immunoglobulin A

Salivary IgA (sIgA) is frequently known as the "first-line of defence" against pathogenic microorganisms, viruses, and bacteria within the immune system and is the dominant

immunoglobulin in external secretions that bathe respiratory and intestinal mucosal surfaces (Tomasi, 1976). Salivary IgA is undetectable at birth but then consistently increases with age (Kugler et al., 1992). The levels of sIgA reach their approximate peak by seven years of age and remain consistently high during mid-life and then decline during old age (Kugler et al., 1992). Gender differences in sIgA levels have been reported in healthy young men and women (Van Anders, 2010). Serum levels of IgA have not been shown to have direct relationship with those found in saliva (Ben-Aryeh et al., 1990, Smith et al., 1987, Ventura, 1991, Kugler et al., 1992). In children and the elderly, both who are at increased risk of a compromised immune system, a lower concentration of IgA in saliva has been conceptualized as a risk factor for upper respiratory tract infections (URTI) (Ben-Aryeh et al., 1990, Smith et al., 1987, Ventura, 1991). Additional studies have also linked mood, academic stress and social support with altered levels of sIgA (Jemmot III and Magloire, 1988, Kugler, 1991). Lower levels of sIgA have also been shown to be associated with increased risk for periodontal disease and caries (Gregory et al., 1992, Ruan, 1990).

Salivary IgA is a polypeptide complex comprising two IgA monomers, the connecting J chain, and the secretory component (Snoeck et al., 2006). The first mechanism of protection by sIgA occurs at the stromal side of the epithelium (Snoeck et al., 2006). At this location, sIgA can complex with antigens present locally in the underlying tissue. Salivary IgA has been shown to have an early morning acrophase followed by a decline to a stable base some 6 h after awakening (Hucklebridge et al., 1998).

There is limited research on the effect of moderate intensity training on sIgA, namely in children. sIgA levels were reported to be enhanced in children following moderate intensity exercise, but suppressed following high intensity exercise (Dorrington et al., 2003). This is of significance for coaches working with young athletes who need to ensure that the volume and intensity of exercise sessions do not compromise the immune system leaving the athlete more prone to illness. A study by Tharp (1991) on 27 prepubescent boys aged 10-12y, and 23 post-pubescent boys aged 16-18y examined sIgA before and after three games and three practice sessions during the basketball season. Results showed that sIgA levels were significantly elevated following basketball practice and games, suggesting that basketball exercise can increase sIgA levels and that chronic exercise over the basketball season may increase the resting levels of sIgA. These changes may give athletes more protection against respiratory

infections both after exercise and in the resting state later in the season (Tharp, 1991). Filaire et al. (2004) examined the effects of physiological and psychological stress on sIgA in young female gymnasts. A significant reduction in sIgA concentration was found following acute exercise and resting sIgA levels did not seem to be affected by periods of training. They also found no relationship between sIgA and cortisol. A study of adolescent female tennis players (included 17 subjects up to the age of 21) examined the incidence of URTIs and sIgA and found that resting sIgA levels were not affected by periods of training. The study showed that players with the greatest exercise-induced reduction in sIgA secretion rate, but not concentration, had the highest incidence of URTI (Novas et al., 2003).

Research has also examined the relationship between PA, obesity and sIgA, URTIs and cortisol. Cieslak et al. (2003) examined the effect of PA, body fat percentage and salivary cortisol on mucosal immunity in children using a 20 m shuttle run for prediction of aerobic fitness in 29 boys, 32 girls ages 10-11y. The authors found that sIgA was significantly correlated with reported URTIs and that children who spent more time in sport activities had a higher aerobic fitness and reported fewer "sick" days. Children with a body fat >25% reported more sick days. There was no correlation between sIgA and cortisol.

A study examining the incidence of infections in 10-12y old children participating in sports found that participation in greater than five sports activities per week increased the occurrence of the common cold, cough, fever symptoms, where three to four activities per week lowered occurrence (Waku et al., 1998). This result suggested a protective mechanism whereby moderate exercise may enhance the immune system, and overexertion may increase susceptibility to illness in children. Jedrychowski et al. (1998) examined childhood respiratory infections in terms of lifestyle factors and found that overweight children (BMI > 20) experienced twice as high a risk of respiratory infections than children with low BMI, independent of PA levels, compared to normal weight children. However, they did not include any information regarding immune function, such as sIgA level. An additional study examined the relationship between PA and URTIs in preadolescent children and found that preadolescent children who had low PA levels had an increased risk of recurrent acute respiratory infections, compared to those that were moderately active and highly active (Jedrychowski et al., 2001). Pallaroa et al. (2002) examined the total sIgA, serum C3c and IgA in obese and lean school children between 6-13y of age and found that obese compared

to lean children had a compromised secretory immune system, as indicated by lower levels of total sIgA, without an increased incidence of clinical symptoms and infections (Pallaroa et al., 2002). This study further suggested the importance of regular PA for children to not only assist in achieving an optimum weight for their age, but also improve mucosal immunity. In conclusion, the limited research examining the effect of moderate intensity exercise on immune function in normal and overweight children highlights the need to perform further research with this age group.

3.4 Inflammation: Salivary C-reactive Protein

It has been suggested that salivary C-reactive protein (CRP) may provide information relating to systemic health status associated with chronic disease (Tonetti and D'Aiuto, 2007). Up to half of all events associated with cardiovascular disease (CVD) are reported to occur in apparently healthy individuals who have few or none of the traditional risk factors, including hyperlipidemia (Clearfield, 2005). As a result, there has been increased focus on the role of other factors, such as inflammation, in the development of atherosclerosis and CVD (Clearfield 2005). These efforts have led to the search for inflammatory biomarkers to improve the detection of coronary and cardiovascular risk among seemingly healthy individuals (Clearfield 2005). Prominent among the possible candidates for a clinically useful biomarker of CVD risk is circulating CRP as measured by high-sensitivity (hs) assay. Studies have shown that elevated levels of CRP are associated with inflammation and increased cardiovascular risk (Patel and Robbins, 2001). Research has suggested a prognostic association between increased CRP production and outcome after acute myocardial infarction and in acute coronary syndromes (Pepys and Hirsch field 2003).

C-reactive protein, a pentameric protein produced by the liver and is part of the nonspecific acute-phase response during inflammation, infection and tissue damage (Pepys and Hirschfield, 2003). C-reactive protein secreted by hepatocytes under the transcriptional control of the cytokine interleukin (IL)-6 (Pepys and Hirschfield, 2003). Salivary CRP is likely to originate from the liver and to mirror serum CRP levels. Therefore, salivary CRP could offer an estimate of systemic inflammation (Ouellet-Morin et al., 2011). Recently, a high-sensitivity commercially available enzyme-linked immunoassay (ELISA) adapted to

measure CRP in human saliva has become available and may offer a valuable strategy to assess salivary CRP levels in various populations (Ouellet-Morin et al., 2011). These authors provided initial evidence suggesting that assessment of CRP in saliva allows for a valid prediction of serum CRP. The researchers found a moderate-to-strong association between CRP measured in saliva and in serum ($r = .72$, $P < .001$) (Ouellet-Morin et al., 2011). The research suggested that salivary CRP may thus facilitate and promote research exploring the correlates of low-grade inflammation in epidemiological studies and make it feasible to expand immune, inflammatory and neuro-endocrine research in children (Ouellet-Morin et al., 2011).

CRP has been shown to be strongly related to all anthropometric and direct measures of total and central abdominal obesity, diastolic blood pressure, and apolipoprotein and lipid levels (Hack and Aarden, 1997). In one particular study the observation made was that CRP levels were strongly and independently related to directly measured total and central obesity and this is consistent with the finding that adipocytes secrete interleukin-6, the main stimulus for CRP biosynthesis (Pepys and Hirschfield, 2003).

There have been numerous studies relating serum CRP and markers of inflammation with physical fitness and obesity measurements in children (Halle et al., 2004, Wannamethee and Lowe, 2002, McMurray et al., 2007, Kelly et al., 2004, Isasi et al., 2003). Although a moderate-to-strong association between CRP measured in saliva and in serum was found (Ouellet-Morin et al., 2011), there are only two studies relating salivary CRP with measures of physical fitness, obesity or health status in children. The first study (Naidoo et al., 2012) examined the relationship between salivary CRP, cardio-respiratory fitness and body composition in 170 black South African children (age 9.41 ± 1.55y, 100 females, 70 males). Results indicated that poor CRF was independently associated with elevated salivary CRP concentration (OR 3.9, 95% CI 1.7-8.9, P=0.001). Poor CRF (OR 2.7, 95% CI 1.2-6.1, P =0.02) and overweight/obesity (BMI $\geq 85^{th}$ percentile) (OR 2.5, 95% CI 1.1-5.9, P =0.03) were independent predictors of elevated salivary CRP secretion rate. These results suggest a strong association between poor cardio-respiratory fitness and/or overweight/obesity and inflammatory status in children based on elevated salivary CRP levels. The second study (Krasteva et al., 2010) aimed to evaluate the oral inflammatory and humoral immune status in 32 children with allergic asthma and 20 control children. A significant correlation between

total protein/haptoglobin and IgG/sIgA for children with allergic asthma was found. The results suggest that the higher salivary levels of CRP and haptoglobin may be markers of allergic inflammation and severity of asthma. Further research is needed to better understand the role salivary CRP, as compared to serum CRP, and markers of inflammation have on physical fitness and obesity measurements in children.

3.5 Sympathetic Nervous System: Salivary Alpha-Amylase

The sympathetic nervous system (SNS) is an important regulator of the stress response. Catecholamines, secreted as part of the acute SNS stress response, are difficult to assess in saliva because of the low concentrations and rapid degradation of epinephrine and norepinephrine and the difficulty of stabilizing these hormones in the sample (Groschl, 2008). Other substances co-secreted with catecholamines can serve as an alternative index of adrenergic activity within the SNS and can be reliably measurable in saliva owing to their greater stability (Groschl, 2008).

One of these such alternatives or surrogates for SNS activity is alpha-amylase, which, although not a hormone, shows the same excretion patterns as catecholamines (Groschl, 2008). Therefore if catecholamines are elevated there is a corresponding increase in alpha-amylase in the saliva that indicates increased SNS activity. Because assays for amylase are more easily available in smaller clinical laboratories, saliva analysis of this enzyme may offer an interesting alternative for SNS activity testing (Groschl, 2008). Assessment of salivary alpha-amylase (sAA) as a non-invasive biomarker for the SNS, offers a multitude of possibilities in different research areas and may well become an important parameter in stress research (Nater and Rohleder, 2009).

Alpha-amylase is one of the major salivary enzymes in humans, and is secreted from the salivary glands in response to SNS stimuli. In acinar cells, release of salivary components is under the control of neuronal stimuli where classic neurotransmitters and specific bioactive peptides serve as the main stimuli for sAA secretion (Nater and Rohleder, 2009). Determining sAA levels and responses to stressors can provide information about the differences in SNS stress response. Normal sAA has a pronounced and distinct diurnal

rhythm with a strong drop in activity in the first hour after awakening, and a steady increase towards the evening (Nater et al., 2007).

A strong activator of the SNS is PA (Nater and Rohleder, 2009). Exercise is known to increase sympathetic activity, and the high protein level in saliva following exercise may be due to increased adrenergic activity in the salivary glands (Nater and Rohleder, 2009). It is suggested that sAA response patterns to both physical and psychological stressors correspond to the response patterns of the SNS (Nater and Rohleder, 2009). A variety of studies have examined the effects of exercise on sAA, however most of the studies have focused on the adult population (Chatterton et al., 1996, Ljungberg et al., 1997, Steerenberg et al., 1997, Granger et al., 2006, Walsh et al., 1999). These studies suggest that exercise can result in a marked increase in sAA as exercise is known to increase sympathetic activity. High protein level in saliva following exercise is suggested (Nater and Rohleder, 2009) to be due to increased adrenergic activity in the salivary glands.

Capranica et al. (2012) evaluated the effects of an official taekwondo competition (three 1-min rounds with a 1-min recovery in-between) on heart rate (HR), sAA and salivary-free cortisol (sC) in 12 young (10.4 ± 0.2y) male taekwondo athletes. Peak sAA was observed at the end of the match (169.6 ± 47.0 U/mL) and was different (P = 0.0001) from the other samplings (pre-competition 55.0 ± 14.0 U/mL, 30-min recovery 80.4 ± 17.7 U/mL, 60-min recovery 50.5 ± 7.6 U/ml; 90-min recovery 53.2 ± 9.6 U/mL). These findings confirmed that taekwondo competitions pose a high stress on young athletes (Capranica et al., 2012). The different sAA and sC reactions in response to the physical stressor mirrored the faster reactivity of the sympathetic-adrenomedullary system relatively to the hypothalamic-pituitary-adrenocortical system (Capranica et al., 2012). An additional study (Walsh et al., 1999) assessed the effect of an acute bout of high-intensity intermittent laboratory cycling exercise on sIgA concentration and sAA activity in eight well trained games players and found a five-fold increase in sAA activity ($P<0.01$ compared with pre-exercise). The increased sAA activity after exercise may improve the protective effect of saliva, since this enzyme is known to inhibit bacterial attachment to oral surfaces (Walsh et al., 1999). It is difficult to deduce the function of short-term increases in sAA while the biological meaning of transient rises in the anti-bacterial action of the enzyme remains

unclear. However, such short-term increases may be useful to the body in that energy is made available by increased digestive action in response to stress (Nater and Rohleder, 2009). Physiological stress reactions comprise orchestrated actions throughout the body, putting the organism in a state of overall preparedness to engage in fight or flight. Thus, increases in amylase activity may be one of many actions involved in activating the body's resources to cope with stressful events or threats to homeostasis (Nater and Rohleder, 2009). Further studies are needed to examine long-term changes in sAA concentrations.

There appears to be a possible link between sAA and eating behaviour in children. One study reported a positive association between sAA activity and increased BMI (greater obesity) in adolescent males and females (Susman et al., 2006). Another study with men and women (average age 26.7y (8.8)) found that BMI was negatively associated with average morning sAA. Specifically, there was a 3.4% decrease in average sAA level with each increasing point on the BMI scale (Nater et al., 2007). sAA stress reactivity was investigated across different age groups, including 62 children (6 – 10y, 32 boys, 30 girls), 78 young adults (20 – 31y, 45 men, 33 women), and 74 older adults (59 – 61y, 37 men, 37 women) (Strahler et al., 2010). Body mass index, perceived stress scale, chronic stress screening scale as well as cortisol, heart rate, and root mean square of successive differences (RMSSD) response indices failed to predict stress-induced sAA initially with hierarchical linear regression until the children were included in the analysis. With the inclusion of the children, BMI became an even stronger predictor of stress induced alteration of sAA than age. All analyses revealed that age and BMI were the strongest predictors of sAA increases, whereas subjective stress levels as well as cortisol, HR, and RMSSD response indices failed to predict the sAA stress responses (Strahler et al., 2010). The research related to sAA, PA and obesity in children is very limited compared to the adult population and indicates that the chronic effect of training and the role of obesity have not been fully examined in pre-pubescent children.

3.6 Hypothalamic Pituitary Adrenal Axis: Salivary Cortisol

Salivary cortisol has emerged as an easy-to-collect, relatively inexpensive, biologic marker of stress (Hanrahan et al., 2006). In addition, salivary cortisol levels reflect the biologically active (unbound) fraction of cortisol (Vining et al., 1983). Cortisol is lipid-soluble, enabling

the molecule to diffuse rapidly from the circulation through the acinar cells of the salivary glands into the saliva, without any influence of salivary flow rate (Pasquali et al., 2006). Although its absolute concentration in the saliva is approximately 30–50% lower than in the blood, its measurement may nonetheless be helpful in evaluating subtle alterations of the HPA in many pathophysiological conditions not classically dependent on relevant endogenous hypercortisolism (Pasquali et al., 2006). Researchers have developed methods for collecting salivary cortisol in children, and recent laboratory techniques have made it possible to detect very small concentrations of cortisol in plasma and saliva (Hanrahan et al., 2006). Salivary and serum cortisol in children and adolescents have been shown to correlate strongly (r = 0.86 to 0.97) (Burke et al., 1985, Bober et al., 1988). Salivary cortisol thus enables the study of HPA axis function in epidemiological cohorts (Rosmalen et al., 2005).

The HPA-axis is a central component of the body's neuro-endocrine response to stress. Its major end-product, cortisol, has profound effects on mood and behaviour as well as metabolism where exposure to increases in cortisol secretion can result in disruption of HPA axis regulation (Rosmond, 2005). The cortisol stress response is kept under control through a negative feedback loop including the pituitary, hypothalamus and hippocampus (Jacobson and Sapolsky, 1991). Cortisol influences a wide variety of processes, including cardiovascular function, fluid volume and haemorrhage, immunity and inflammation, metabolism, neurobiology, and reproductive physiology (Sapolsky et al., 2000). Normal circadian rhythm is comprised of high morning and low afternoon-evening cortisol levels and normal feedback control (Rosmond, 2003). However, when the final stage of chronic stress with 'burn-out' of central regulatory systems occurs, the result is a net decrease of cortisol output, a flattened diurnal secretory pattern, and inhibition of other endocrine axes, and can result in the Metabolic Syndrome (Rosmond, 2003). These observations highlight the importance of the HPA axis (and cortisol regulation) in the control of human health (Rosmond, 2003).

Increased levels of cortisol has a significant role to play in the body, and so if the HPA axis is not functioning appropriately this will alter obese individuals (including children's) ability to adapt and function metabolically. Numerous laboratories have reported an association between obesity, particularly central adiposity, and high cortisol concentrations in adults. During exercise, cortisol concentrations have been shown to remain higher in obese than in

lean adults demonstrating a greater HPA response to the same exercise intensity with obesity (Hershberger et al., 2004). Although there is a large body of literature devoted to the neuro-endocrine response to exercise in adults, namely that increases in intensity and duration of exercise increase salivary cortisol, the relationship between exercise, PA, overweight/obesity and salivary cortisol in children is not fully understood. One study found that salivary cortisol concentration was decreased by 32% in obese children (8 – 11y) from pre- to post-exercise compared to lean children (Hershberger et al., 2004). Another study examined the associations between morning cortisol and adiposity in children (9.6 +/- 0.9y) at baseline and a 9-month follow-up (Hill et al., 2011). Participants included 649 (301 males, 348 females) children for the cross-sectional analysis and 316 (153 males, 163 females) for the longitudinal analysis. A positive relationship was found between morning salivary cortisol and change in waist circumference over 9-months in overweight children. An additional study (Budde et al., 2010) investigated whether 12 min of high-intensity exercise performed within a regular school break would lead to an increase in cortisol levels in 53, 4th grade (9-10y) primary school students. They observed a significant group by test interaction indicating a different pre-to-post-test development for the experimental group compared to the control group. However, the interaction effect was caused by an attenuated cortisol concentration in the control group. The authors argued that the control condition, where the students watched a joyful movie, acted as a distracter, which led to a reduction of general school stress (Budde et al., 2010). Another study examining serum and salivary cortisol responses to cycling exercise in male children, 10.6 +/- 0.2y found that 30 min of submaximal exercise at 70% of VO_{2max} significantly increased serum cortisol level; and salivary and serum cortisol were correlated during and after exercise (del Corral et al., 1994). Further research measuring salivary cortisol as a marker of the neuro-endocrine stress response in normal and overweight children and associations with PA will assist in promoting understanding of the roles they play in the paediatric versus adult population.

3.7 **Conclusion**

Participation in regular moderate intensity PA or exercise appears to enhance mucosal immunity (increase sIgA) in preadolescent children. However, research in this area is limited and currently not conclusive. In contrast, poor fitness and inactivity as well as strenuous

training appear to compromise the mucosal immune system thereby increasing the risk of URTIs. Children reporting higher levels of body fat and with greater BMI appear to have lower sIgA levels and a greater incidence of infections.

There is very limited research surrounding salivary CRP and PA, obesity and health status in children. The limited research does, however, suggest a strong association between poor CRF and/or overweight/obesity and inflammatory status in children based on elevated salivary CRP levels.

Research surrounding sAA indicates that exercise can result in a marked increase in sAA as seen by an increase sympathetic activity via increased adrenergic activity in the salivary glands. The limited research suggests exercise may also pose a high stress on young athletes as seen with an increase in sAA. Additionally it appears that BMI may be a strong predictor of stress-induced sAA increases in children.

Greater HPA axis response, as seen by increases in salivary cortisol, appears to be influenced greatly by increases in obesity. Higher salivary cortisol secretions have been observed in obese versus lean adults and children alike in response to exercise.

Current research surrounding salivary biomarkers in children highlights the vast gaps that are present with regard to PA and obesity**.** Table I summarises research studies that have focused on the relationships between physical activity, body weight, and salivary biomarkers of immune function, inflammatory status and neuroendocrine activation in children (and in some cases adolescents). The majority of research studies currently focus on the adolescent and adult population. However, parallels cannot always be drawn between pre-pubescent children and those individuals in the post-pubescent population. A limitation in current research is how “children” are defined, as Tanner stages are not always identified and pubescent status is not always readily available. Further research is also needed to examine the role that moderate intensity and chronic exercise and obesity have on salivary biomarkers in children as much of the current research on salivary biomarkers is surrounding higher intensity exercise in the athletic adult population. The current research has suggested that markers of immune function and sympathetic activation can be greatly affected by lack of PA and increases in obesity at a young age, which may continue into adulthood. Understanding

the relationship between these variables will help in the development of safe and effective PA and exercise prescription guidelines for health and exercise professionals, coaches and teachers. Such guidelines will be particularly relevant for children whose immune function is compromised and/or sympathetic activation is dysfunctional.

Table I: Research studies examining the relationships between physical activity, body weight, and salivary biomarkers of immune function, inflammatory status and neuroendocrine activation in children

Author, Year	Participants	Research Focus	Findings
(Tharp, 1991)	27 prepubescent boys (10-12y) and 23 postpubescent boys (16-18y) on basketball teams	Examined saliva levels of sIgA before and after three games and three practice sessions during the basketball season	sIgA levels were slightly but significantly elevated following basketball practice and game situations Indicate that basketball exercise can increase sIgA levels and that chronic exercise over the basketball season may increase the resting levels of sIgA
(Filaire et al., 2004)	12 young female gymnasts (12-15y)	Physiological and psychological stress and sIgA	Significant reductions in sIgA concentration following acute exercise, resting sIgA levels do not seem to be affected by periods of training ; also found no relationship between sIgA and cortisol
(Novas et al., 2003)	17, young female tennis players (14-21y)	Incidence of URTIs and sIgA	Resting sIgA levels do not seem to be affected by periods of training Those with greatest exercise-induced reduction in sIgA secretion rate, but not concentration, also had the highest incidence of upper respiratory tract infection.
(Dorrington et al., 2003)	15 boys and 14 girls (8-12y)	Effect of exercise intensity on sIgA in children	sIgA levels were reported to be enhanced following moderate intensity exercise, but depressed following high intensity
(Thomas et al., 2009)	17 old boys (15-16y)	Effect of repeated bouts of short-term, high-intensity cycling exercise on the sC, sT and sIgA concentrations All participants completed 6 × 8 s sprints, interspersed with 30 s recovery intervals on a cycle ergometer	The increases in sT and sC reported in this study confirm that repeated bouts of short-term, high-intensity exercise produces significant physiological hormonal responses in adolescent boys, but does not affect mucosal immune function.

(Cieslak et al., 2003)	29 boys, 32 girls (10-11y)	Effects if of PA, BF, and sC on mucosal immunity in children using 20 m shuttle run for prediction of aerobic fitness	SIgA sig correlated with reported URTIs. Children who spent more time in sport activities and had higher aerobic fitness reported fewer "sick" days. Children with BF >25% more sick days ; also no correlation between sIgA and sC.
(Pallaroa et al., 2002)	Obese and lean children (6-13y)	Examined the total sIgA, serum C3c and IgA levels	Obese children showed lower levels of sIgA than lean children
(Thomas et al., 2010)	19 girls (15-16y)	Completed 668 s sprints, interspersed with 30 s recovery intervals on a cycle ergometer.	Showed no changes in salivary testosterone, cortisol or IgA following repeated bouts of supra-maximal cycling ($P > 0.05$)
(Naidoo et al, 2012)	170 black South African children, 100 females, 70 males (7-12y)	Relationship between sCRP, CRF, and body composition	Strong association between poor CRF and/or overweight/obesity and inflammatory status with elevated sCRP
(Capranica et al, 2012)	12 male taekwondo athletes (10-11y)	Examined taekwondo competition on HR, sAA and sC	Peak sAA at end of match was different compared to pre and 30,60, 90 min. recovery values

Abbreviation key

sIgA	*Salivary Immunoglobulin A*
URTIs	*Upper Respiratory Tract Infections*
sC	*Salivary Cortisol*
sT	*Salivary Testosterone*
PA	*Physical Activity*
BF	*Body Fat*
CRF	*CardiorespiratoryFitness*
sCRP	*Salivary C-Reactive Protein*
sAA	*Salivary Alpha-Amylase*
HR	*Heart Rate*

MUCOSAL IMMUNITY AND SYMPATHETIC ACTIVATION SALIVARY BIOMARKERS IN AFRICAN CHILDREN PREDICTED BY AGE, BODY COMPOSITON AND CARDIORESPIRATORY VARIABLES

Abstract

The aim of this cross-sectional study was to examine whether cardiorespiratory fitness and body composition have an effect on resting levels of salivary IgA (sIgA) and salivary alpha-amylase (sAA) in children. One hundred and thirty-two black South African children (age 10.05 ± 1.68y, 74 females, 58 males) participated in the study. Resting saliva samples were collected 90 minutes after waking. Body fat percentage was determined using a 4 site skinfold and cardio-respiratory fitness (predicted VO_2max) was assessed using the 20 meter multi-stage shuttle run test. The outcomes of the one-way ANOVAs examining the differences by BMI categories showed there were significant differences in weight (F = 83.64, df = 2, 129, P < 0.0001), BMI (F = 193.36, df = 2, 129, P < 0.0001), waist-to-hip ratio (F = 193.36, df = 2, 129, P < 0.0001), body fat percentage (F = 336.98, df = 2, 129, P = 0.0001), SBP (F = 5.72, df = 2, 129, P = 0.0042), DBP (F = 291.76, df = 2, 129, P < 0.0001), VO_{2max} (F = 521.00, df = 2, 129, P < 0.0001), sAA concentration (F = 17.05, df = 2, 129, P < 0.0001), sAA secretion rate (F = 15.15, df = 2, 129, P < 0.0001), sIgA concentration (F = 11.30, df = 2, 129, P < 0.0001), and sIgA secretion rate (F = 8.08, df = 2, 129, P = 0.0005), between children of different BMI categories. Tukey's post hoc analyses revealed that obese children had significantly (P < 0.01) higher weight, BMI, body fat percentage, DBP, SBP, sAA concentration and secretion rate, compared to overweight and normal weight children, as well as a significantly lower aerobic capacity (VO_{2max}) than both normal (P < 0.001) weight and overweight (P < 0.05) children. In addition, sIgA concentration and secretion rate were significantly lower between normal weight and obese children (P < 0.01). Multiple linear regression revealed that BMI, DBP and VO_{2max} predict sAA. BMI (P = 0.04) and DBP (P = 0.04) were found to be independent predictors of sAA concentration. Age and BMI category predicted sIgA secretion rate. BMI category (P = 0.0006) was found to be an independent predictor of sIgA secretion rate. This study suggests that obesity, based on BMI, has a major role to play in mucosal immunity and sympathetic activation and that obese children have elevated sAA, lowered sIgA, and poor CRF.

Keywords: Physical activity, obesity, immunity, neuro-endocrine, children, salivary biomarkers, sympathetic activation

4.1 Introduction

Worldwide, obesity and associated diseases such as type 2 diabetes, have reached epidemic proportions. Statistics show that childhood obesity is prevalent in South Africa (Goedecke et al., 2005). Steyn et al. (2005) reported that 17.1% of South African children between the ages of 1-9y living in urban areas were overweight. Traditional and cultural perceptions regarding body size, urbanization, poor diet, low socioeconomic status and lack of physical activity (PA) are a few of the suggested contributing factors (Steyn, 2006, Puoane et al., 2002). The combination of obesity and lack of PA have been suggested to have negative effects on immune and neuro-endocrine function (Cieslak et al., 2003, Granger et al., 2007). Currently there is limited research worldwide and in South Africa as to the effect these factors have on salivary biomarkers of immune and sympathetic activation in the paediatric population.

Salivary immunoglobulin A (sIgA) is regarded as the "first-line of defence" against pathogenic microorganisms, viruses, and bacteria within the immune system and is the dominant immunoglobulin in external secretions that bathe respiratory and intestinal mucosal surfaces (Tomasi, 1976). The levels of sIgA reach their approximate peak by seven years of age and remain consistently high during mid-life and then decline during old age (Salimetrics, 2006, Kugler et al., 1992). Preliminary paediatric reference range for IgA in saliva using an enzyme immunoassay for children under 16y is 18 – 237 μg/ml (mean 128 μg/ml) (Hofman and Le, 2002). Gender differences in sIgA levels have been reported in healthy young men and women (Van Anders, 2010).

There is limited research examining the association between body composition, aerobic capacity and immunity in children. Cieslak et al. (2003) examined the effect of PA, body fat and salivary cortisol on mucosal immunity in children (29 boys, 32 girls ages 10-11y). The authors found that sIgA was significantly correlated with reported upper respiratory tract infections (URTIs) and that children who spent more time in sport activities had a higher aerobic fitness and reported fewer “sick” days. Children with body fat >25% reported more sick days. A study examining the incidence of infections in 10-12y old children participating in sports found that participation in greater than five sports activities per week increased the occurrence of the common cold, cough, fever symptoms, where three to four activities per week lowered occurrence (Waku et al., 1998). Jedrychowski et al. (1998) found that overweight children (BMI > 20) had double the risk of

URTI's compared to normal weight children that was independent of PA levels. Jedrychowski et al. (2001) found that preadolescent children who had low PA levels had an increased risk of recurrent acute respiratory infections, compared to those that were moderately and highly active. Pallaroa et al. (2002) examined the total sIgA, serum C3c and IgA in obese and lean school children between 6-13y of age and found that obese compared to lean children had a compromised secretory immune system, as indicated by lower levels of total sIgA, without an increased incidence of clinical symptoms and infections (Pallaroa et al., 2002).

The SNS is an important branch of the stress response. Catecholamines are a part of the acute stress response (as part of the SNS) but are difficult to assess in saliva because of the low concentrations and rapid degradation of epinephrine and norepinephrine and the difficulty of stabilizing these hormones in the sample (Groschl, 2008). Other substances co-secreted with catecholamines can serve as an alternative index of adrenergic activity within the SNS and can be reliably measurable in saliva owing to their greater stability (Groschl, 2008). One of these such surrogates for adrenal medulla activity is alpha-amylase, which, although not a hormone, shows the same excretion patterns as catecholamines (Groschl, 2008). Therefore if catecholamines are elevated there is a corresponding increase in alpha-amylase in the saliva that indicates increased SNS activity (Groschl, 2008).

A strong activator of the SNS is PA (Nater and Rohleder, 2009). Exercise is known to increase sympathetic activity, and the high protein level in saliva following exercise may be due to increased adrenergic activity in the salivary glands (Nater and Rohleder, 2009). It is suggested that salivary alpha-amylase (sAA) response patterns to both physical and psychological stressors correspond to the response patterns of the SNS (Nater and Rohleder, 2009). A variety of studies have shown that exercise increases sAA, however most of the studies have focused on the adult population (Chatterton et al., 1996, Ljungberg et al., 1997, Steerenberg et al., 1997, Granger et al., 2006, Walsh et al., 1999) with limited research performed on children. However, Capranica et al. (2012) examined the effects of an official taekwondo competition on heart rate (HR), sAA, and salivary-free cortisol (sC) in 12 young (10.4 ± 0.2y) male taekwondo athletes. Peak sAA observed at the end of the match was significantly different from the other time points (pre-competition, 30, 60 and 90 min recovery). The study found that taekwondo competitions pose a high stress on young athletes (Capranica et al., 2012). An additional study (Walsh et al., 1999) assessed the effect of an acute bout of high-intensity intermittent laboratory cycling exercise on sIgA concentration and sAA activity in eight well trained games players and found a five-

fold increase in alpha-amylase activity. The increased sAA activity after exercise may improve the protective effect of saliva, since this enzyme is known to inhibit bacterial attachment to oral surfaces (Walsh et al., 1999). It is difficult to deduce the function of short-term increases in sAA while the biological meaning of transient rises in the anti-bacterial action of the enzyme remains unclear. However, such short-term increases may be useful to the body in that energy is made available by increased digestive action in response to stress (Nater and Rohleder, 2009). The normal values for sAA range from 27±3.8 to 1440±160 U/ml where the wide variation in the concentration could be due to differences in the assay techniques, the reagents used, glandular contribution, and carbohydrate consumption (Makinen, 1989). Physiological stress reactions comprise orchestrated actions throughout the body, putting the organism in a state of overall preparedness to engage in fight or flight. Thus, increases in amylase activity may be one of many actions involved in activating the body's resources to cope with stressful events or threats to homeostasis (Nater and Rohleder, 2009). Further studies are needed to examine long-term changes in sAA concentrations.

Research has shown a possible link between sAA and BMI. Susman et al. (2006) reported a positive association between sAA activity and increased BMI in adolescent males and females. In addition, BMI was negatively associated with average morning sAA, with a decrease in average sAA levels by 3.4% with each increasing point on the BMI scale (Nater et al., 2007). Strahler et al. (2010) examined sAA stress reactivity across different age groups including 62 children, 78 young adults, and 74 older adults found that age and BMI were the strongest predictors of sAA increases and that children had significantly higher baseline sAA concentrations than both groups of adult participants (Strahler et al., 2010).

The research related to sIgA, sAA, PA and obesity in children is very limited compared to the adult population and the relationships between these variables are not fully understood in children. Therefore, the aim this study was to examine whether cardiorespiratory fitness and body composition have an effect on resting levels of sIgA and sAA in children.

4.2 Methodology

4.2.1 Participants

One hundred and thirty-two black South African children (74 females, 58 males) in grades 3-7 (age 10.05 ± 1.68y) participated in the study. Participants were recruited from an urban, combined junior and senior primary school in Pietermaritzburg, KwaZulu-Natal. Gate-keeper permission to perform the study was obtained from the KwaZulu-Natal Department of Education (Appendix I), the school's Headmaster (Appendix II) and Governing Body. The study was approved by the institution's Biomedical Ethics Research Committee (Appendix XIII). Once permission to continue was obtained, a meeting was held with parents/guardians and children to discuss the research details and expectations of the participants. Written informed consent was obtained at the meeting (Appendix III). The guardians/parents completed a medical history form (Appendix V) that included sections on infectious, immune and salivary gland disorders. The parents/guardians were trained in the salivary collection procedure and were provided with instructions regarding brushing teeth and the intake of food and drink on the morning of the saliva collection (Appendix IX). These standardized instructions are outlined below.

4.2.2 Saliva Collection

Each grade was tested on a separate morning over a week, starting with Grade 3 on Monday and ending with Grade 7 on Friday. Saliva samples were collected between 07:30 and 08:30, approximately 90 minutes after waking. The parents/guardians and children were requested to adhere as closely as possible to the following standardised saliva collection instructions (Salimetrics, 2010): The children should 1) not eat a major meal (breakfast) within 60 minutes of sample collection, 2) not brush their teeth prior to sample collection (this may cause the gums to bleed causing blood contamination of the saliva), 3) avoid dairy products for 20 minutes before sample collection, 4) avoid foods with high sugar or acidity, or high caffeine content, immediately before sample collection (these have all been shown to impact on the saliva pH altering assay results), 5) rinse their mouths with water to remove food residue before sample collection, and swallow to increase hydration, and 6) wait at least 10 minutes after rinsing before collecting saliva to avoid sample dilution.

Upon arriving in the school hall at 07h00 the children sat for 20 minutes. Based on completion of a short health questionnaire and interview with the researchers upon arrival, no participant reported symptoms suggesting that they were sick (e.g. fever, flu, diarrhoea) and there were no reports of "bleeding gums" or "tooth ache" on the day of data collection. Saliva samples were collected via unstimulated passive drool over a time period of five minutes. Whilst seated the children were asked to lean slightly forward and tilt their heads down and accumulate saliva in the floor of the mouth for a minute, which was subsequently swallowed. Following this there was a four minute collection where the children dribbled saliva through a 5 cm plastic straw into a pre-weighed polypropylene cryovial (5 ml capacity). Care was taken to allow saliva to dribble into the collecting tubes with minimal orofacial movement. After collection the cryovial was weighed in order to determine the saliva flow rate. Samples were refrigerated immediately in dry ice and kept frozen until reaching the laboratory, upon which they were stored at -70^{o}C until analysis.

Salivary IgA and alpha amylase concentrations were determined in duplicate by enzyme-linked immunoassays with the sIgA and alpha amylase kits (Salimetrics, State College, PA, USA). The coefficients of variation (CV) of all duplicate samples were less than 10%. The results were expressed as absolute concentration (µg sIgA or U/ml sAA) and salivary secretion rate (sIgA µg/min IgA or sAA U/min), or the total amount of sIgA and sAA appearing on the mucosal surface per unit of time. Salivary IgA and sAA secretion rate was calculated by multiplying absolute sIgA and sAA concentration by saliva flow rate (ml x min-^{1}). Saliva flow rate was calculated by dividing the total amount of saliva obtained in each sample (mL) by the time taken to produce the sample (4 minutes) (Novas et al., 2003).

4.2.3 Body Composition, Cardiovascular Measurements and Cardio-Respiratory Fitness

These measures were determined after the saliva collection. Body composition was assessed by measuring height and weight to calculate BMI, skinfolds to predict body fat percentage and lean muscle mass, and waist and hip circumferences to calculate waist-to-hip ratios. Stature was measured to the nearest millimetre (mm) using a portable stadiometer (Nagata bw-1122h) and mass was measured to the nearest 0.1 kilogram (kg) using a calibrated electronic scale (Nagata bw-1122h). Participants were asked to remove footwear and only wore their school physical education outfit that included shorts and a short sleeve T-shirt. Seated resting heart rate, measured to the nearest beat per minute (bpm) and resting blood pressure, measured to the nearest

millimetre per mercury (mmHg) were recorded after a resting period of ten minutes. Body fat percentage was determined using a 4 site skinfold method (Brook, 1971).Triceps, biceps, supra iliac and sub scapular skinfolds were measured on the right hand side of the body using Harpenden© (West Sussex, UK- Quality Measurement, Ltd) skinfold callipers. Each site was measured twice to the nearest mm and the mean value was recorded. Circumferences at the waist (narrowest part of the torso) and hip (level of maximum extension of the buttocks) were measured to the nearest mm with a tape measure and the waist-to-hip ratio was calculated

CRF was assessed using the 20 meter multi-stage shuttle run test that predicts an individual's maximal aerobic capacity (VO_{2max}) (Ledger and Lambert, 1982). This test has been shown to be an appropriate predictor of CRF for the age groups participating in the study (Tomkinson et al., 2003).

4.3 Statistical Analyses

The data were analyzed with a 1-sample nonparametric test (Kolmogorov-Smirnov test) to determine whether the data distribution was normal. Descriptive statistics (mean ± SD) were calculated. One-way ANOVA examined the differences in body composition, cardiovascular and CRF variables, sIgA and sAA concentration and secretion rates by BMI categories. The BMI categories were determined using the growth charts published by the Centre for Disease Control and Prevention (CDC) for BMI in boys and girls, 2 to 20y. These charts are percentiles showing the distribution of BMI at a given age and can be used to identify children who are at risk of overweight (BMI >85th percentile) or obese (BMI >95th percentile) (Ogden and Flegal, 2010). According to these CDC-BMI-for-age standards, the participants were grouped into the following CDC-BMI-for-age categories: normal weight (< 85th percentile), overweight (≥ 85th percentile to < 95th percentile), and obese (≥ 95th percentile) (Ogden and Flegal, 2010). These cut off points are unchanged from the 1998 expert committee recommendations and CDC and Institute of Medicine recommendations (Ogden and Flegal, 2010). Tukey's post hoc analysis was completed when appropriate.

In addition, the significance of associations between body composition, cardiovascular and CRF variables, sIgA and sAA were determined using multiple linear regression (Stepwise method) analyses. For the multiple linear regressions, sIgA and sAA concentration and secretion rate were set as the dependent variables and the independent variables included the body composition,

cardiovascular and CRF variables. All the independent variables were correlated against themselves and their R squared values were checked for multi-collinearity. Independent variables with R squared values >0.75 indicating high collinearity were removed from the regression analysis. Significance was set a $p \leq .05$. All statistics were run using the IBM SPSS version 19 (IBM, USA).

4.4 Results

Demographic, body composition, cardiovascular, CRF variables and sIgA, sAA concentration and secretion rates for the children were divided according to the three BMI categories (normal weight, overweight and obese) and are presented as means ± standard deviations in Table I.

The outcomes of the one-way ANOVAs examining the differences by BMI categories are indicated in Table I. There were significant differences in weight ($F = 83.64$, $df = 2, 129$, $P < 0.0001$), BMI ($F = 193.36$, $df = 2, 129$, $P < 0.0001$), waist-to-hip ratio ($F = 193.36$, $df = 2, 129$, $P < 0.0001$), body fat percentage ($F = 336.98$, $df = 2, 129$, $P = 0.0001$), SBP ($F = 5.72$, $df = 2, 129$, $P = 0.0042$), DBP ($F = 291.76$, $df = 2, 129$, $P < 0.0001$), VO_{2max} ($F = 521.00$, $df = 2, 129$, $P < 0.0001$), sAA concentration ($F = 17.05$, $df = 2, 129$, $P < 0.0001$), sAA secretion rate ($F = 15.15$, $df = 2, 129$, $P < 0.0001$), sIgA concentration ($F = 11.30$, $df = 2, 129$, $P < 0.0001$), and sIgA secretion rate ($F = 8.08$, $df = 2, 129$, $P = 0.0005$), between children of different BMI categories. Tukey's post hoc analyses revealed that obese children had significantly ($P < 0.01$) higher weight, BMI, body fat percentage, DBP, SBP, sAA concentration and secretion rate, compared to overweight and normal weight children, as well as a significantly lower aerobic capacity (VO_{2max}) than both normal ($P < 0.001$) weight and overweight ($P < 0.05$) children. In addition, sIgA concentration and secretion rate were significantly lower between normal weight and obese children ($P < 0.01$).

The multiple linear regression results are presented in Table II. The model that best predicted sAA and accounted for 16.82% (R square = .1682, Adjusted R square = .1487) of the variance was: sAA (U/ml) = 6.964 + 1.934*[BMI] + 0.9208*[DBP] - 0.7321*[VO_{2max}]. This model was significant ($F = 8.63$, $P < 0.0001$) and BMI ($P = 0.04$) and DBP ($P = 0.04$) were found to be independent predictors of sAA concentration.

The model that best predicted sAA secretion rate and accounted for 14.84% (R square = -1682, Adjusted R square = .1285) of the variance was sAA (U/min) = -5.314 + 2.351*[BMI] + 0.7135*[DBP] - 0.2394*[VO_{2max}]. This model was significant ($F = 7.44$, $P < 0.0001$) and BMI (P = 0.01) was found to be an independent predictor of sAA secretion rate.

Table I. **Demographic, body composition, CRF data for normal weight, overweight and obese children. Values are mean ± standard deviation.**

N=132	Normal (N = 74) (males 36; females 38)	Overweight (N = 22) (males 8; females 14)	Obese (N=36) (males 14; females 22)
Age (y)	10.26 (1.74)	9.59 (1.71)	9.97 (1.52)
Stature (cm)	140.25 (11.42)	141.20 (10.74)	143.53 (9.19)
Mass (kg)	34.10 (7.58)	40.83 (8.73)*	58.03 (11.85)$^{\#}$
BMI (kg/m^2)	17.11 (1.63)	20.20 (1.49)***	28.03 (4.55)$^{\#}$
Waist-Hip Ratio	0.77 (0.04)	0.79 (0.04)$^{\&\&}$	0.84 (0.07)$^{\&}$
Body fat %	19.56 (5.47)	28.18 (4.11)***	39.38 (5.37)$^{\#}$
Resting HR (b/min)	87.00 (12.57)	85.09 (11.21)	89.11 (12.01)
SBP (mmHg)	94.20 (12.20)	96.27 (9.67)$^{\&\&}$	107.28 (11.67)$^{\&}$
DBP (mmHg)	63.22 (8.33)	64.64 (8.17)	75.22 (10.00)$^{\#}$
VO_2max (ml/kg/min)	29.35 (5.67)	25.97 (4.01)^{a}	22.34 (3.02)$^{\&, aa}$
sAA (U/ml)	79.83 (43.12)	62.13 (36.06)	122.75 (46.50)$^{\#}$
sAA (U/min)	75.07 (42.64)	59.91 (41.97)	116.75 (46.21)$^{\#}$
sIgA (µg/ml)	243.95 (119.23)	167.92 (71.46)*	158.34 (56.03)$^{\&}$
sIgA (µg/min)	224.88 (118.45)	152.95 (54.91)*	150.05 (53.98)**

$^{*} = P < 0.01$ for overweight vs. normal weight; $^{**} = P < 0.01$ for obese vs normal weight; $^{***} = P < 0.001$ for overweight vs. normal weight, $^{\&} = P < 0.001$ obese vs. normal weight, $^{\&\&} = P < 0.01$ for obese vs. overweight, $^{\#}$ $P < 0.001$ for obese vs. normal weight and overweight; $^{a} = P < 0.05$ overweight vs. normal weight, $^{aa} = P < 0.05$ obese vs. overweight

Table II. Multiple linear regression results for sAA, sAA secretion rate, sIgA and sIgA secretion rate

Models		Coefficients		t	Sig.
		B	Std. Error		
sAA (U/ml)	(Constant)	6.964	38.75	0.18	.86
	BMI	1.934	0.92	2.09	.04*
	DBP	0.9208	0.46	2.00	.04*
	VO_{2max}	-0.7321	0.76	0.96	.34
sAA (U/min)	(Constant)	-5.314	39.24	0.14	.89
	BMI	2.351	0.94	2.51	.01*
	DBP	0.7135	0.47	1.53	-13
	VO_{2max}	-0.2394	0.73	0.31	.76
sIgA (µg/ml)	(Constant)	303.71	58.28	5.21	<.0001
	Age	-1.681	5.27	0.32	.75
	BMI Category (1 = normal, 2 = overweight, 3 = obese)	-45.737	10.09	4.54	<.0001*
sIgA (µg/min)	(Constant)	233.04	56.97	4.09	< .0001
	Age	2.210	5.15	0.43	.67
	BMI Category (1 = normal, 2 = overweight, 3 = obese)	-34.886	9.86	3.54	.0006*

The model that best predicted sIgA concentration and accounted for 13.86% (R square = .1386, Adjusted R square = .1251) of the variance was sIgA (µg/ml) = 303.71 - 1.681*[Age] - 45.737*[BMI Category]. This model was highly significant (F = 10.29, P < 0.0001) and BMI category (P = 0.01) was found to be an independent predictor of sIgA concentration.

The model that best predicted sIgA secretion rate and accounted for 9.32% (R square = .0932, Adjusted R square = .0791) of the variance was sIgA (µg/min) = 233.04 + 2.210*[Age] - 34.886*[BMI Category]. This model was highly significant (F = 6.58, P < 0.0019) and BMI category (P = 0.0006) was found to be an independent predictor of sIgA secretion rate.

4.5 Discussion

The combination of obesity and lack of PA have been suggested to have negative effects on immune and neuro-endocrine function (Cieslak et al., 2003, Granger et al., 2007). The limited research worldwide and in South Africa as to the effect these factors have on salivary biomarkers of immune and sympathetic activation in the paediatric population sparked interest for additional research and investigation into the subject.

The main findings for this study revealed that BMI, DBP and VO_2 predict sAA (with BMI and DBP as independent predictors) and that age and BMI category predict sIgA (with BMI as an independent predictor). This study suggests that obesity based on the BMI categories, as well as elevated DBP and poor CRF, have major roles to play in elevating resting sympathetic activation and lowering mucosal immunity.

Obese children were also shown to have significantly greater weight, BMI, body fat percentage, DBP, SBP, sAA concentration and secretion rate, compared to overweight and normal weight children, as well as a significantly lower aerobic capacity (VO_{2max}) than both normal weight and overweight children. In addition, sIgA concentration and secretion rate were significantly lower in obese compared with normal weight children.

The finding that BMI is associated with sAA is similar to that reported in the literature. Previous studies reported a positive association between sAA activity and increased body BMI (greater obesity) in adolescent males and females (Granger et al., 2007). However, an additional study found that BMI was negatively associated with average morning sAA, indicating a decrease in average sAA levels by 3.4% with each increasing point on the BMI scale (Nater et al., 2007). Importantly, results also indicate that DBP is related to sAA and that increased aerobic fitness plays a role in reducing sAA. The increased sAA activity after exercise was suggested to improve the protective effect of saliva, since this enzyme is known to inhibit bacterial attachment to oral surfaces (Walsh et al., 1999). Short-term increases in sAA may be useful to the body in that energy is made available by increased digestive action in response to stress (Nater and Rohleder, 2009). Physiological stress reactions comprise orchestrated actions throughout the body, putting the organism in a state of overall preparedness to engage in fight or flight. Thus, increases in amylase activity may be one of many actions involved in activating the body's resources to cope with stressful events or threats to homeostasis (Nater and Rohleder, 2009). Further studies are needed to examine long-term changes in sAA concentrations.

The results also indicated that there is a decrease in sIgA with increasing age and that the higher the BMI category the lower the sIgA, suggesting that mucosal immunity is reduced in obese children. This is in line with previous research as it has been shown that levels of sIgA reach their approximate peak by seven years of age and remain consistently high during mid-life and then decline during old age (Salimetrics, 2006, Kugler et al., 1992). Additionally, Jedrychowski et al. (1998) found that overweight children (BMI > 20) had double the risk of URTI's compared to normal weight children, independent of PA levels. Another study also found that obese compared to lean children had a compromised secretory immune system, as indicated by lower levels of total sIgA (Pallaroa et al., 2002).

There were also limitations in this study. Although salivary flow rate was controlled for and clear instructions were provided to the parents as well as the participants regarding brushing teeth as well as dietary and hydration practices prior to saliva collection, the health condition of the children's gums and teeth were not determined using standardised methods.

Additionally, examination for Tanner stages for sexual maturation were not able to be performed to ensure all children were in fact pre-pubescent.

4.6 Conclusion

The study provides continued support for a possible association between poor CRF and/or overweight/obesity and compromised mucosal immunity and sympathetic activation in children, based on the result that obese children have elevated sAA, lowered sIgA, and poor CRF. Replication of the study with larger samples is required together with longitudinal follow up of clinical outcomes. This may contribute to a better understanding of the pathways mediating the enhancement of mucosal immunity and control of sympathetic activation and chronic disease in children and subsequently adults.

MUCOSAL IMMUNITY AND SYMPATHETIC ACTIVATION SALIVARY BIOMARKERS IN BLACK MALE CHILDREN AFTER 12 WEEKS OF SOCCER TRAINING

ABSTRACT

The primary aim of this study was to determine the levels of salivary IgA (sIgA) and salivary alpha-amylase (sAA) of black male soccer players, before and after 12 weeks of training. Thirty-four children (11– 13y), who were part of a youth soccer development training academy, participated in the study. Resting saliva samples were collected between 07:30 and 08:30, 90 minutes after waking, 48 hours pre and post, the 12 weeks of training. After saliva sampling, body fat percentage was determined using a 4 site skinfold using triceps, biceps, supra iliac and sub scapular skinfolds and then cardio-respiratory fitness (predicted VO_2max) were assessed using the 20 meter multi-stage shuttle run test. Significant differences were found between pre vs. post for BMI (P =0.034), waist-to-hip ratio (P =0.046), age ($P <$ 0.0001), height ($P < 0.0001$), body fat % ($P < 0.0001$) and LMM ($P < 0.0001$). A significant difference was found between sIgA secretion rate pre vs. post (P =0.025). However, no significant differences were found in sAA concentration ($P = 0.088$), sAA secretion rate ($P =$ 0.103), sIgA concentration (P =0.067). The increase in sIgA secretion rate suggests an increase in the transport of sIgA across the mucosal epithelium, whereby exercise may have enhanced the mediators of IgA synthesis by B-cells in the mucosa.

Keywords: Physical activity, obesity, mucosal immunity, neuro-endocrine, children, salivary biomarkers, sympathetic activation

5.1 **Introduction**

Soccer is the most popular sport in the world and is performed by men and women, children and adults with different levels of expertise. Performance depends upon a myriad of factors such as technical/biomechanical, tactical, mental and physiological areas. Soccer is an intermittent sport that involves low-intensity running interspersed with high-intensity actions over 40 - 90 min depending on age (Stølen et al., 2005). It has been reported that youth soccer players cover distances of approximately 6-9 km during competition (Castagna et al., 2003, Helgerud et al., 2001) and success in youth soccer is therefore associated with good aerobic endurance performance (Vaeyens et al., 2006, Wong et al., 2011). Although there has been a great deal of research on the physiological demands of soccer, the relationship between physical conditioning and immune function and the sympathetic activation has not been extensively studied, especially in youth soccer players. Research has suggested that failure to recover fully between sessions and overtraining can cause immune-suppression that increases a player's susceptibility to illness as well as overall performance in practice and game situations (Sari-Sarraf et al., 2008).

Sari-Sarraf, et al. (2007) examined the effects of single and repeated bouts of soccer-specific exercise on salivary IgA (sIgA) a marker of mucosal immunity in ten adult males. They found that two 90-min exercise sessions performed at a moderate intensity with a 2.25h rest in between did not have adverse effects on sIgA levels (Sari-Sarraf et al., 2007). An additional study (Sari-Sarraf et al., 2008) examined at the effects of repeated bouts of soccer-specific intermittent exercise on sIgA in nine male subjects. Results suggested that performing two bouts of moderate intensity soccer-specific intermittent exercise 48 h apart does not suppress resting sIgA concentration significantly although a small progressive reduction in sIgA was observed (Sari-Sarraf et al., 2008).

The effect of moderate intensity training on sIgA in children has not been extensively studied. A study by Tharp (1991) on 27 prepubescent boys aged 10-12y, and 23 post-pubescent boys aged 16-18y examined sIgA before and after three games and three practice sessions during the basketball season. Results showed sIgA levels were significantly elevated following basketball practice and games, suggesting that basketball exercise can increase

sIgA levels and that chronic exercise over the basketball season may increase the resting levels of sIgA. Dorrington et al. (2003) reported that sIgA levels were enhanced in children following moderate intensity exercise, but suppressed following high intensity exercise. Filaire et al. (2004) examined the effects of physiological and psychological stress on sIgA in young female gymnasts. A significant reduction in sIgA concentration was found following acute exercise and resting sIgA levels did not seem to be affected by periods of training.

Physical activity (PA) is a strong activator of the SNS (Nater and Rohleder, 2009). Exercise is known to increase sympathetic activity, and the high protein level in saliva following exercise may be due to increased adrenergic activity in the salivary glands (Nater and Rohleder, 2009). It is suggested that sAA response patterns to both physical and psychological stressors correspond to the response patterns of the SNS (Nater and Rohleder, 2009). A variety of studies have examined the effects of exercise on sAA, however most of the studies have focused on the adult population (Granger et al., 2006, Ljungberg et al., 1997, Chatterton et al., 1996, Steerenberg et al., 1997, Walsh et al., 1999). These studies found that exercise can result in a marked increase in sAA. Capranica et al. (2012) evaluated the effects of an official taekwondo competition (three 1-min rounds with a 1-min recovery in-between) on heart rate (HR), salivary alpha-amylase (sAA), and salivary-free cortisol (sC) in twelve boys (10.4 ± 0.2y). Peak sAA was observed at the end of the match and was different from the other time points (pre-competition, 30, 60, 90 min recovery). They found that taekwondo competitions posed a high stress on young athletes (Capranica et al., 2012). An additional study (Walsh et al., 1999) assessed the effect of an acute bout of high-intensity intermittent laboratory cycling exercise on sIgA concentration and sAA activity in eight well trained games players and found a five-fold increase in sAA activity. The increased sAA activity after exercise may improve the protective effect of saliva, since this enzyme is known to inhibit bacterial attachment to oral surfaces (Walsh et al., 1999). Short-term increases may be useful to the body in that energy is made available by increased digestive action in response to stress (Nater and Rohleder, 2009). Increases in amylase activity may be one of many actions involved in activating the body's resources to cope with stressful events or threats to homeostasis (Nater and Rohleder, 2009). Further studies are needed to examine long-term changes in sAA concentrations.

Understanding how sIgA and sAA respond to training in children will provide information for coaches, strength and conditioning as well as health professionals regarding how exercise alters mucosal immunity and sympathetic activation. This will help these professionals provide exercise and PA prescriptions that do not negatively alter immunity and sympathetic activation but rather improve immune status and sympathetic activation in young athletes. Therefore the aim of the study was to examine whether 12-weeks of soccer specific training would have an impact on sIgA and sAA in males youths.

5.2 Methods

5.2.1 Participants

Fifty black South African males (11.74y ± 0.45 pre and 12.18y ± 0.79 post) volunteered to participate in the study. The children were from a youth soccer development training academy in Pietermaritzburg, Kwa-Zulu Natal (South Africa). Thirty-four children were included in the final data analysis. Exclusion criteria included failure to attend at least 80% of the training sessions, medical conditions, or inadequate saliva samples that could not be tested. The study was approved by the University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (Appendix XIII). Once ethical approval was obtained, a meeting was held with parents/guardians and children to discuss the research details and expectations of the participants. Written informed consent was obtained at the meeting (Appendix III). The guardians/parents completed a medical history form that included sections on infectious, immune and salivary gland disorders (Appendix V). The parents/guardians were trained in the salivary collection procedure and were provided with instructions regarding brushing teeth and the intake of food and drink on the morning of the saliva collection (Appendix IX). These standardized instructions are outlined below.

5.2.2 Tests

Data was collected 48 hours pre- as well as 48 hours post a 12- week pre-season training period. The training was preceded by a six week off-season period where the children had

performed no structured exercise. All measurements were taken in the morning in a designated area at the sports grounds of UKZN in Pietermaritzburg.

5.2.2.1 Saliva Collection and Salivary Alpha-Amylase and IgA Analysis

Saliva samples were collected between 07:30 and 08:30, approximately 90 minutes after waking. The parents/guardians and children were requested to adhere as closely as possible to the following standardised saliva collection instructions (Salimetrics, 2010): The children should 1) not eat a major meal (breakfast) within 60 minutes of sample collection, 2) not brush their teeth prior to sample collection (this may cause the gums to bleed causing blood contamination of the saliva), 3) avoid dairy products for 20 minutes before sample collection, 4) avoid foods with high sugar or acidity, or high caffeine content, immediately before sample collection (these have all been shown to impact on the saliva pH altering assay results), 5) rinse their mouths with water to remove food residue before sample collection, and swallow to increase hydration, and 6) wait at least 10 minutes after rinsing before collecting saliva to avoid sample dilution.

Saliva samples were collected via unstimulated passive drool over a time period of five minutes. Whilst seated the children were asked to lean slightly forward and tilt their heads down and accumulate saliva in the floor of the mouth for a minute, which was subsequently swallowed. Following this there was a four minute collection where the children dribbled saliva through a 5 cm plastic straw into a pre-weighed polypropylene cryovial (5 ml capacity). Care was taken to allow saliva to dribble into the collecting tubes with minimal orofacial movement. After collection the cryovial was weighed in order to determine the saliva flow rate.

Upon arriving in the change room at the sports field at 07h00 the children sat for 20 minutes. Based on completion of a short health questionnaire and interview with the researchers upon arrival, no participant reported symptoms suggesting that they were sick (e.g. fever, flu, diarrhoea) and there were no reports of “bleeding gums” or “tooth ache” on the day of data collection. Saliva samples were collected via unstimulated passive drool over a time period of five minutes. Whilst seated the children were asked to lean slightly forward and tilt their

heads down and accumulate saliva in the floor of the mouth for a minute, which was subsequently swallowed. Following this there was a four minute collection where the children dribbled saliva through a 5 cm plastic straw into a pre-weighed polypropylene cryovial (5 ml capacity). Care was taken to allow saliva to dribble into the collecting tubes with minimal orofacial movement. After collection the cryovial was weighed in order to determine the saliva flow rate. The sAA activity (U/ml) and sIgA concentration (μg/ml) were calculated and then expressed as the secretion rate of sAA (U/min) and sIgA (μg/min) or the total amount of sAA or sIgA appearing on the mucosal surface per unit time. sAA and SIgA rate were calculated by multiplying absolute sAA and sIgA concentrations (pg/mL) by saliva flow rate (mL/min), this latter value was calculated by dividing the total volume of saliva obtained in each sample (mL) by the time taken to produce each sample (4 min). Samples were placed immediately on dry ice and kept frozen until reaching the laboratory, upon which they were stored at -70^{o}C until analysis. sAA and sIgA concentrations were determined in duplicates by using sAA and sIgA ELISA Kits (Salimetrics, State College, PA, USA). The coefficients of variation (CV) of all duplicate samples were less than 10%.

5.2.2.2 Body Composition, Cardiovascular Measurements and Cardio-Respiratory Fitness

These measures were determined after the saliva collection. Body composition was assessed by measuring height and weight to calculate BMI, skinfolds to predict body fat percentage and waist and hip circumferences to calculate waist-to-hip ratios. Stature was measured to the nearest millimetre (mm) using a portable stadiometer (Nagata bw-1122h) and mass was measured to the nearest 0.1 kilogram (kg) using a calibrated electronic scale (Nagata bw-1122h). Participants were asked to remove footwear and only wore their school physical education outfit that included shorts and a short sleeve T-shirt. Seated resting heart rate, measured to the nearest beat per minute (bpm) and resting blood pressure, measured to the nearest millimetre per mercury (mmHg) were recorded after a resting period of ten minutes. Body fat percentage was determined using a 4 site skinfold method (Brook, 1971). Triceps, biceps, supra iliac and sub scapular skinfolds were measured on the right hand side of the body using Harpenden© (West Sussex, UK- Quality Measurement, Ltd) skinfold callipers. Each site was measured twice to the nearest mm and the mean value was recorded.

Circumferences at the waist (narrowest part of the torso) and hip (level of maximum extension of the buttocks) were measured to the nearest mm with a tape measure and the waist-to-hip ratio was calculated (Isasi et al., 2003).

CRF was assessed using the 20 meter multi-stage shuttle run test that predicts an individual's maximal aerobic capacity (VO_{2max}) (Ledger and Lambert, 1982). This test has been shown to be an appropriate predictor of CRF for the age groups participating in the study (Tomkinson et al., 2003).

5.2.3 Twelve-Week Training Programme

The 12-week training program was implemented at the UKZN Sports Ground in Pietermaritzburg (South Africa) for 50 participants in the soccer training group. The training included soccer specific speed, agility, and quickness (SAQ) drills as well as small-sided games, shooting, passing, dribbling, and tactical drills as part of their normal soccer training activities. The participants practiced 5 days per week, 90 minutes per session. Three SAQ sessions were included in the 90 minutes and were performed every Monday, Wednesday and Friday. A 60 minute practice game would be played on a Thursday. The duration of each SAQ session was 40 minutes and was broken down into the following phases: 5 minutes warm up, 30 minutes of moderate to high intensity aerobic activity and 5 minutes cool down. Each session was conducted by a qualified exercise instructor (investigator) and coach on site. The warm up and cool down phases included 'dynamic flex' movements and stretching. The aerobic phase included aspects of soccer, SAQ for juniors and youth as well as other foundational areas of endurance, strength, and balance of body movement (See Table I for sample and Appendix XII for full programme).

The SAQ portion of the program was developed specifically for children and youth aged 4-16 years old, which falls into the category of this study. Experts have developed the program to improve performance and participation in PA which features an innovative conditioning and training structure that has already revolutionized sport for all ages. The program is suitable for those who do not have a foundation of good movement skills, as well as those who are

gifted and talented at sports and games activities. The program is in line with National Curriculum (UK) guidelines for this age range (Pearson, 2005).

5.2.3.1 Description and Samples of the 12-week SAQ Training Programme

The programme was based on the following rules:

1) Start slowly
2) Gradually increase from simple to more complex movements (perfect simple movements before progressing onto more complex drills)
3) Always start a session with SAQ® Dynamic Flex®
4) Explosive work and sprints should be completed early in the session, before endurance work. Train fresh and fast!
5) Do not use fatigue as a punishment
6) Teach one skill at a time
7) Remember good mechanics at all times
8) Quality and consistency
9) Keep it simple
10) Keep training positive
11) Keep sessions short and sharp
12) Monitor recovery time between sets and reps

Progression of continuum phases and complexity/specificity over 12 week SAQ® programme

Week	**Specificity**
1-3	Foundation (with Transition)
4-6	Complex (with Transition)
7-9	Sport Specific (with Transition)
10-12	Position Specific

Table I: Samples of SAQ 12--week training programme: Weeks 1, 6, 12

Week 1

SAQ® Continuum	Drills	Sets and Reps	Time
Dynamic Flex® (Warm-up)	Walking on balls of feet Ankle flicks Small skips Lateral running Walking hamstring Wall drill – leg across body Wall drill – forward leg swing	Up and back each drill 10 on each leg 10 on each leg	5 mins
Mechanics	Arm mechanics – partner Running form – dead-leg run Running form – pre-turn Running form – leading leg run Running form – lateral stepping Running form – 1-2-3 lift	3 sets of 16 reps, with 1 min recovery between each set 1 set of 6 reps, leading leg should be alternated 1 set of 6 reps, 3 leading with the left leg and 3 leading with right	15 mins
Innervation	Fast foot ladder – single runs Fast foot ladder – single lateral step Fast foot ladder – double run Fast foot ladder – Crossover	3 sets of 4 reps, 1 min recovery between each set 3 sets of 6 reps, 1 min recovery between each set	15 mins
Warm-down	Knee-across skip Small skips Ankle flicks Walking hamstring Quadriceps stretch Adductors stretch Calf stretch	Up and back – each drill, and 10-second hold on static stretches	5 mins

Week 6

SAQ® Continuum	Drills	Sets and Reps	Time
Dynamic Flex®	Dynamic Flex® Ankle flicks, small skips, wide skip, knee across skip, walking lunge, walking hamstring Wall drill – leg across body Wall drill – forward leg swing Wall drill – knee across body	2 x 20 yards, 1 forwards, 1 backwards for each of the Dynamic Flex® and ball drills 10 on each leg	5 mins
Mechanics (2 min recovery before innervation)	Arm mechanics – partner, with hand weights Running form – with a ball, use various mechanics drills (e.g. dead leg run, single step, lateral step)	2 sets of 16 reps, with 1 min recovery between each set 3 sets of 6 reps	5 mins
Innervation (2 min recovery before accumulation of potential)	Fast foot ladder – single runs Fast foot ladder – "T" formation Fast foot ladder – with a ball	1 set of 6 reps, walk back recovery 1 set of 6 reps (2 moving to the left and 2 moving to the right) 1 min recovery between each set 3 sets of 6 reps, 1 min recovery between each set	5 mins
Accumulation of Potential (3min recovery before explosion)	Swerve-development run with ball, lateral Fast Foot® ladder with 180 degree turn in middle, lateral zig-zag run, ending with a jump to header a ball.	5 sets of 5 reps, with a 30 sec recovery between each rep	10 min
Explosion (3min recovery before expression)	Seated forward and backward get-up (turn, run forwards) with a ball Flexi Cord – Buggy Runs	2 sets of 5 reps, jog-back recovery between reps (1 set forwards, 1 set backwards 1 set of 6 reps plus a contrast run, 30 sec recovery between each rep	10 mins
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	5 mins

Week 12

SAQ® Continuum	Drills	Sets and Reps	Time
Dynamic Flex®	Dynamic Flex® small skips, wide skip, knee across skip, pre-turn, side lunge, walking hamstring Wall drill – leg across body Wall drill – forward leg swing Wall drill – knee across body Pair drill – lateral runs, passing ball hand-to-foot	2 x 20 yards, 1 forwards, 1 backwards for each of the Dynamic Flex® and ball drills 10 on each leg 2 x 20 yards, 1 left leg leading, 1 right leg leading	10 mins
Accumulation of Potential (3min recovery before explosion)	Soccer-specific run, with pressure from a defender	2 sets of 5 reps, with a 30 sec recovery between each rep	4 mins
Explosion (3min recovery before expression)	Side-stepper jockeying in pairs Flexi-cord – Vertical power Seated forward get-up	3 sets of 4 reps plus contrast set of 2 reps, 30-second recovery between each rep and 2 min recovery between each set 2 sets of 8 reps plus 1 contrast jump, with 3 minutes recovery between each set 2 sets of 5 reps, with a jog-back recovery between each rep and a 2-min recovery between each set	10 mins
Expression of Potential / Skills Session	Cone turns Midfielders – Ball control, feed, turn, receive, shoot Midfielders – Assisted and resisted arcing	Each game should last 60 seconds 1 set of 8 reps, with a walk-back recovery between each rep 1 set of 8 reps, i.e. 4 lead runs for each player, 90 seconds recovery between each rep	16 mins
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	5 mins

5.3 Statistical Analysis

The data were analysed with a 1-sample nonparametric test (Kolmogorov-Smirnov test) to determine whether the data distribution was normal. Descriptive statistics (mean ± SD) were calculated and the pre vs. post data were compared using a 2-tailed paired sample T-test.

Significance was set a $p \leq .05$. All statistics were run using the IBM SPSS version 19 (IBM, USA).

5.4 Results

Table II presents the physical characteristics data for pre and post testing and the results for the 2-tailed paired samples T-tests. Significant differences were found between pre vs. post for BMI (P =0.034), waist-to-hip ratio (P = 0.046), age ($P < 0.0001$), height ($P < 0.0001$), body fat % ($P < 0.0001$) and LMM ($P < 0.0001$).

Table II: Physical characteristics of the participants (N = 34)

N = 34	Mean (SD)	Range	Sig. (2-tailed) Pre vs. Post
Age Pre (y)	11.74 (.45)	11.00-12.00	< .000*
Age Post (y)	12.18 (.79)	11.00-13.00	
Height Pre (cm)	146.35 (7.59)	132.00-166.00	< .000*
Height Post (cm)	147.82 (7.84)	133.50-168.00	
Weight Pre (kg)	38.59 (6.78)	30.00-58.80	< .056
Weight Post (kg)	39.05 (6.54)	31.00-60.00	
BMI Pre (cm/kg^2)	17.91 (1.87)	14.70-21.80	< .034*
BMI Post (cm/kg^2)	17.47 (1.88)	14.00-21.30	
Waist to Hip Ratio Pre	.79 (.05)	0.68-0.91	< .046*
Waist to Hip Ratio Post	.81 (.04)	0.72-0.92	
BF% (Brook) Pre	20.02	12.16-32.26	< .000*
BF% (Brook) Post	18.12	11.30-30.14	
LMM (Bunc) Pre	30.72	21.68-45.62	< .000*
LMM (Bunc) Post	31.87	25.20-48.15	

*P <0 .005

Table III presents the physiological and salivary biomarker data for pre and post testing and the results for of the 2-tailed paired samples T-tests. Significant were found between sIgA secretion rate pre vs. post (P =0.025).

Table III: Physiological and biochemical data of soccer players (N = 34)

	Mean (SD)	Range	Sig. (2-tailed) Pre vs. Post
sAA (U/ml) Pre	92.00 (60.53)	6.23-246.33	.088
Post	109.13 (59.55)	17.06-237.47	
sAA secretion (U/min) Pre	84.86 (54.81)	4.99-221.08	.103
Post	108.69 (85.39)	10.87-336.99	
sIgA (µg/ml) Pre	162.83 (79.22)	83.03-378.15	.067
Post	211.52 (129.40)	66.09-714.65	
sIgA secretion (µg/min) Pre	147.21 (80.12)	45.75-357.35	.025*
Post	224.68 (172.86)	51.86-723.58	
VO_{2max} (ml/kg/min) Pre	43.16 (4.44)	37.11-50.85	.213
Post	45.04 (9.12)	39.87-55.42	
Resting Heart Rate (bpm) Pre	85.76 (12.35)	60-112	.378
Post	87.47 (10.48)	72-114	
SBP (mmHg) Pre	96.03 (10.06)	80-115	.085
Post	99.32 (7.96)	90-121	
DBP (mmHg) Pre	66.91 (7.59)	55-85	.562
Post	66.41 (6.66)	54-84	

*P <0.005

5.5 Discussion

The primary finding of this study was that 12 weeks of soccer specific training resulted in an increase in sIgA secretion rate. In addition, there were significant alterations in BMI, waist-to-hip ratio, height, body fat % and LMM.

Soccer performance depends upon a myriad of factors such as technical/biomechanical, tactical, mental and physiological areas. Coaches and players are continually looking ways to maximize the overall potential of an athlete to attain an elite level of performance. Assessment of physiological and performance measures in youth soccer are some important aspects to monitor an athlete's overall health and physical characteristics as well as the effectiveness of physical and training conditioning programs. Athletes in this study were assessed for a variety of physiological, biochemical and physical characteristic measures. Predicted VO_2max values in this cohort were lower and BF% higher than reported for elite youth soccer players in previous studies (Chamari et al., 2005b, Chamari et al., 2005a), while similar results were found by (Bunc and Psotta, 2001). Differences may be because of variability in age as well as differences in the modalities of testing for VO_2 max and BF%. The athletes in this study were from families with a low socioeconomic status which could potentially have impact on nutrition status, immune function and neuro-endocrine status. To the best of the author's knowledge, this was a novel group of athletes to assess because there is limited data available relating to children from this socioeconomic group.

The athletes who participated in the 12 week soccer specific programme showed significant differences between pre vs. post for BMI (P =0.034), waist-to-hip ratio (P = 0.046), age (P < 0.0001), height (P < 0.0001), body fat % (P < 0.0001) and LMM (P < 0.0001). This suggests that as growth and development occurs chronologically (with age), that participation in the programme also had potential health and performance benefits as well. Decreases in body composition, BMI and waist-to-hip ratio while also seeing increased LMM could have the potential to make a quicker, more agile athlete capable of better body control and speed, leading to enhanced performance. Additionally, these significant differences may also provide a possible protective mechanism for risks related to CVD and metabolic dysfunction.

There has been extensive research on the physiological demands of soccer. However, the relationship between physical conditioning and immune function and sympathetic activation has not been extensively studied, especially in youth soccer players. Although no significant differences were found in the measurements of sAA in the present study, the research is novel in that it examined sAA in youth soccer players from a low socioeconomic group. Previous research regarding PA and sAA was performed in the adult/post-pubescent

population from middle to high socioeconomic groups. Additionally, these studies used different modalities of exercise, namely prolonged, strenuous exercise (Nexo et al., 1988, Chatterton et al., 1996, Ljungberg et al., 1997, Steerenberg et al., 1997, Granger et al., 2006, Walsh et al., 1999). The current study implemented a SAQ program alongside regular soccer training in youth soccer players, which has not been studied previously.

The most significant finding in the present study was the increase in sIgA secretion rate after the 12 weeks of training. Previously an increase in resting sIgA was also found in boys aged 10-12 and 16-18y after three games and three practice sessions during a basketball season (Tharp, 1991). Dorrington et al. (2003) examined the effect of exercise intensity on sIgA in children and found that sIgA was enhanced following moderate intensity exercise, but depressed following high intensity exercise.

The finding of an increase in sIgA secretion rate suggests that soccer specific training in youth soccer players can elevate biomarkers of immune function. The increase in sIgA suggests an increase in the transport of sIgA across the mucosal epithelium. The increase in sIgA would enhance the "first-line of defence" against pathogenic microorganisms, viruses, and bacteria within the immune system. sIgA is the dominant immunoglobulin in external secretions that bathe mucosal surfaces, such as respiratory and intestines (Tomasi, 1976). The increase in sIgA transport may occur via enhanced IgA transcytosis by increased transepithelial transport/availability of the polymeric Ig receptor (pIgR)-IgA complex. IgA in the mucosa is produced by long-lived plasma B-cells (Salvi and Holgate, 1999, Lamm, 1997). The most effective cytokines that promote the differentiation of IgA-committed B-cells to becoming IgA-producing plasma cells are IL-5 and IL-6 (Sonoda et al., 1992). In addition to IL-5 and IL-6 being of importance in the mediation of growth and differentiation of B-cells, IL-10 and IL-2 are of importance in stimulating the production of IgA (Rousset et al., 1992). IL-10 mediates IgA production by promoting the differentiation of IgA switched B-cells into IgA secreting plasma cells as well as being an IL-2 receptor up regulator for clonal proliferation of B-cells (Salvi and Holgate, 1999). Therefore, due to the relatively short half-life (5 to 6 days) for the majority of IgA plasma B-cells, it is necessary that many IgA plasma B-cells must develop daily from B-cells to guarantee a continuous supply of sIgA antibodies in the mucosa (Salvi and Holgate, 1999). As the half-life requires daily development of B-cells, the findings in the present study of an increase of sIgA secretion rate

could also be a result of the athletes participating in regular training that may have enhanced the production of IgA in the mucosa via mediating cytokines. As the aforementioned cytokines are of importance for IgA synthesis, it is important that they are also readily available for mediating B-cell production. There is evidence that exercise influences natural immunity, T- and B-cell functions, and cytokine responses through hemodynamic changes and hormonal secretion in adults (Nieman et al., 1990, Gabriel et al., 1992), which may also explain the increases in IgA section rate by enhancing the mediators of IgA synthesis of B-cells in the mucosa.

There were limitations in this study. Although salivary flow rate was controlled for and clear instructions were provided to the parents as well as the participants regarding brushing teeth as well as dietary and hydration practices prior to saliva collection, the health condition of the children's gums and teeth were not determined using standardised methods. In the pre and post analysis there was no control group to compare intervention vs. non-intervention exercise groups. The sample size was also a limitation in this study as only 34 of the 50 participants completed all tests pre and post intervention tests and measurements for analysis. Although the 12 week intervention of soccer-specific exercise was appropriate for this age group, the exact intensity via individual HR monitors was not used for measurement of intensity during the training sessions. Finally, examination for Tanner stages for sexual maturation were not able to be performed to ensure all children were in fact pre-pubescent.

5.6 Practical Applications

Research in the area of paediatric exercise immunology and sympathetic activation in youth athletes has not been investigated as fully as their adult counterparts. Although the present study did not find any significant changes in sAA concentration or secretion rate, African children who participated in a 12 week soccer specific programme showed significant differences between pre vs. post for BMI (P =0.034), waist-to-hip ratio (P = 0.046), age (P < 0.0001), height (P < 0.0001), body fat % (P < 0.0001) and LMM (P < 0.0001). These findings suggest the potential for health and performance benefits for the athlete, increasing their opportunity to succeed on the field. Significant differences were also found between sIgA secretion rate pre vs. post (P =0.025). With the popularity of soccer worldwide, it is of

importance to note that youth participating at an elite level of fitness, regardless of socioeconomic status, can enhance their immune function status with participation in a 12 week soccer training program.

CHAPTER 6: CONCLUSIONS

6.1 Overview

The aim of this thesis was to investigate how salivary biomarkers of sIgA and sAA may be altered by overweight/obesity and CRF in a paediatric population. The sIgA response to acute exercise appears to be variable and suggests this may be influenced by exercise mode, intensity and duration as well as the fitness of the subjects, unstimulated versus stimulated saliva collection methods, and how sIgA is expressed (e.g. absolute concentration, as a secretion rate). Previous research focused on how sIgA levels influence incidence of URTIs, primarily in the athletic adult population. Additionally, the importance of other antimicrobial proteins in saliva such as alpha-amylase have been noted where exercise intensity may alter in alpha-amylase activity. Although research with these salivary biomarkers has increased, there seems to be a gap in research with understanding more fully how exercise and body composition influence immune function and sympathetic activation in children.

The studies on the school children and soccer players suggested that mucosal immunity and sympathetic activation appear to be affected by body composition, CRF and chronic exercise training. The finding of an increase in sIgA secretion rate in the soccer study suggested that a structured 12 week exercise programme can elevate mucosal immune function in youth soccer players. The underlying mechanism responsible may be an exercise-induced increase in the transport of sIgA across the mucosal epithelium and/or enhanced production of IgA in the mucosa via mediating cytokines. The main findings for the school study revealed that BMI, DBP and VO_2 predict sAA and that age and BMI category predict sIgA. This study suggests that obesity, based on the BMI categories when performing the regression analysis, has a major role to play and that obese children have elevated sAA, lowered sIgA, and poor CRF. The literature review article demonstrated that PA and overweight/obesity may have an impact on salivary biomarkers of mucosal immunity and sympathetic activation in children, however further research with regards to optimal intensity, duration and modality need to be assessed with this pre-pubescent population. The following sections include discussion and conclusions reflective of the papers produced as a

result of this research. Additionally, Table I includes a summary of findings for the school and soccer research.

6.2 Discussion (Review Article)

Participation in regular moderate intensity PA or exercise appears to enhance mucosal immunity (increase sIgA) in preadolescent children. However, research in this area is limited and currently not conclusive. In contrast, poor fitness and inactivity as well as strenuous training appear to compromise the mucosal immune system thereby increasing the risk of URTIs. Children reporting higher levels of body fat and with greater BMI appear to have lower sIgA levels and a greater incidence of infections.

There is very limited research surrounding salivary CRP and PA, obesity and health status in children. The limited research does, however, suggest a strong association between poor CRF and/or overweight/obesity and inflammatory status in children based on elevated salivary CRP levels.

Research surrounding sAA indicates that exercise can result in a marked increase in sAA as seen by an increase sympathetic activity via increased adrenergic activity in the salivary glands. The limited research suggests exercise may also pose a high stress on young athletes as seen with an increase in sAA. Additionally it appears that BMI may be a strong predictor of stress-induced sAA increases in children.

Greater HPA axis response, as seen by increases in salivary cortisol, appear to be influenced greatly by increases in obesity. Higher salivary cortisol secretions have been observed in obese versus lean adults and children alike in response to exercise.

Current research surrounding salivary biomarkers in children highlights the vast gaps that are present with regard to PA and obesity. A limitation in current research is how “children” are defined, as Tanner stages are not always identified and pubescent status is not always readily available. Research is also needed to look at the role that moderate intensity and chronic exercise and obesity have on salivary biomarkers in children. Much of the current research on

salivary biomarkers is surrounding higher intensity exercise in the athletic adult population. The current research has suggested that markers of mucosal immune function and sympathetic activation can be greatly affected by a lack of PA and increases in obesity at a young age, which may continue into adulthood. Understanding the relationship between these variables will help in the development of safe and effective PA and exercise prescription guidelines for health and exercise professionals, coaches and teachers. Such guidelines will be particularly relevant for children whose immune function is compromised and/or sympathetic activation is dysfunctional.

6.3 Discussion (Soccer Study)

The results of this study indicate that significant differences were found between pre vs. post for BMI (P =0.034), waist-to-hip ratio (P = 0.046), age ($P < 0.0001$), height ($P < 0.0001$), body fat % ($P < 0.0001$) and LMM ($P < 0.0001$). This suggests that as growth and development occurs chronologically (with age), that participation in the programme also had potential health and performance benefits as well. Decreases in body composition, BMI and waist-to-hip ratio while also seeing increased LMM could have the potential to make a quicker, more agile athlete capable of better body control and speed, leading to enhanced performance. Additionally, these significant differences may also provide a possible protective mechanism for risks related to CVD and metabolic dysfunction. The athletes in this group are, to this author's knowledge, a novel group of athletes to assess due to the limited data available in lower socioeconomic groups.

The findings of an increase in sIgA secretion rate suggests that SAQ incorporated into normal soccer training activities in youth soccer players can elevate biomarkers of immune function. This increase in sIgA indicates an increase in the transport of sIgA across the mucosal epithelium. The increase in sIgA would enhance the "first-line of defence" against pathogenic microorganisms, viruses, and bacteria within the immune system as the dominant immunoglobulin in external secretions that bathe mucosal surfaces, such as respiratory and intestines (Tomasi, 1976). The increase in sIgA transport is likely due to enhanced IgA transcytosis by increased transepithelial transport/availability of the polymeric Ig receptor (pIgR)-IgA complex. As the half-life requires daily development of B-cells, the findings in

the present study of an increase of sIgA secretion rate could also be a result of the athletes participating in regular SAQ sessions, in addition to their normal training activities, which may have enhanced this production of IgA in the mucosa via mediating cytokines. As the aforementioned cytokines are of importance for IgA synthesis, it is important that they are also readily available for mediating B-cell production. There is evidence that exercise influences natural immunity, T- and B-cell functions, and cytokine responses through hemodynamic changes and hormonal secretion in adults (Nieman et al., 1990, Gabriel et al., 1992), which may also explain the increases in IgA section rate by enhancing the mediators of IgA synthesis of B-cells in the mucosa.

There were also limitations in this study. Although salivary flow rate was controlled for and clear instructions were provided to the parents as well as the participants regarding brushing teeth as well as dietary and hydration practices prior to saliva collection, the health condition of the children's gums and teeth were not determined using standardised methods. In the pre and post analysis there was no control group to compare intervention vs. non-intervention exercise groups. The sample size was also a limitation in this study as only 34 of the 50 participants completed all tests pre and post intervention tests and measurements for analysis. Although the 12 week intervention of soccer-specific exercise was appropriate for this age group, the exact intensity via individual HR monitors was not used for measurement of intensity during the training sessions. Finally, examination for Tanner stages for sexual maturation were not able to be performed to ensure all children were in fact pre-pubescent.

6.3.1 Practical Applications (Soccer Study)

Research in the area of paediatric exercise immunology and sympathetic activation in youth athletes has not been investigated as fully as in their adult counterparts. Although the present study did not find any significant changes in sAA concentration or secretion rate, African children who participated in a 12 week soccer specific programme showed the potential for enhancement of health and performance variables as well as improvements in mucosal immunity. With the popularity of soccer worldwide, it is of importance to note that youth participating at an elite level of fitness, regardless of socioeconomic status, can enhance their immune function status with SAQ incorporated into a regular soccer training program.

6.4 Discussion (School Study)

The main findings for this study revealed that BMI, DBP and VO_2 predict sAA and that age and BMI category predict sIgA. This study suggests that obesity, based on BMI,has a major role to play and that obese children have elevated sAA, lowered sIgA, and poor CRF.

Importantly, results also indicate that DBP is related to sAA and that aerobic fitness plays a role in reducing sAA. Although sAA has an immune function as well as being a marker of SNS, it is also an enzyme that breaks down carbohydrate. This may suggest that greater CRF and lower weight in children assist sAA to break down carbohydrate with its enzymatic function. The increased sAA activity after exercise was suggested in a previous study to improve the protective effect of saliva, since this enzyme is known to inhibit bacterial attachment to oral surfaces (Walsh et al., 1999). Short-term increases may be useful to the body in that energy is made available by increased digestive action in response to stress (Nater and Rohleder, 2009). Physiological stress reactions comprise orchestrated actions throughout the body, putting the organism in a state of overall preparedness to engage in fight or flight. Thus, increases in amylase activity may be one of many actions involved in activating the body's resources to cope with stressful events or threats to homeostasis (Nater and Rohleder, 2009). Further studies are needed to examine long-term changes in sAA concentrations.

The results also indicated that there is a decrease in sIgA with increasing age and that the higher the BMI category the lower the sIgA, suggesting that mucosal immunity is reduced in obese children. This is in line with previous research as it has been shown that levels of sIgA reach their approximate peak by seven years of age and remain consistently high during mid-life and then decline during old age (Salimetrics, 2006, Kugler et al., 1992).

There were also limitations in this study. Although salivary flow rate was controlled for and clear instructions were provided to the parents as well as the participants regarding brushing teeth as well as dietary and hydration practices prior to saliva collection, the health condition of the children's gums and teeth were not determined using standardised methods.

Additionally, examination for Tanner stages for sexual maturation were not able to be performed to ensure all children were in fact pre-pubescent.

6.4.1 Conclusion (School Study)

The school study provides continued support for a possible association between poor CRF and/or overweight/obesity and compromised mucosal immunity and sympathetic activation in children, based on the result that obese children have elevated sAA, lowered sIgA, and poor CRF. Further interventions and programmes need to be implemented in schools, clubs, churches, etc. to increase the opportunity for children to participate in regular PA. Regular participation may lead to increases in CRF as well as aid in the fight against obesity amongst children.

Additionally, saliva sampling is non-invasive, stress-free, can easily be performed in a participant's natural setting and can be repeated over time. Furthermore, saliva collection has considerable economic and logistic advantages over venipuncture because it does not require immediate manipulations, access to specialised laboratory equipment and qualified personnel. Replication of the study with larger samples is required together with longitudinal follow up of clinical outcomes. This may contribute to a better understanding of the pathways mediating the enhancement of mucosal immunity and control of sympathetic activation and chronic disease in children and subsequently adults.

6.5 Future Research Recommendations

This research has shown that 12 weeks of soccer training can elevate sIgA secretion rate and thereby mucosal immune function in youth soccer players. Additionally, that obesity (based BMI) has a major role to play and that obese children have elevated sAA, lowered sIgA, and poor CRF. This and previous research has shown that PA and overweight/obesity have an impact on salivary biomarkers of mucosal immunity and sympathetic activation in children, however further research in the pre-pubescent population is needed to better understand the pathways mediating the response. Further research with in regards to optimal intensity, duration and modality of exercise is recommended to aid exercise scientists,

trainers, and coaches for implementation of safe and effective guidelines for enhancement of mucosal immunity and control of sympathetic activation. It is hoped that the results of the research performed for this study will add to the body of knowledge regarding mucosal immunity and sympathetic activation as it relates to PA and obesity in the pre-pubescent population.

Table I: Summary of findings for school and soccer research

Research study-subjects	Immune/Sympathetic Activation Response	Possible outcomes/conclusions	Practical application
School Study 132 black South African school children (74 females, 58 males) in grades 3-7 (age 10.05 ± 1.68y)	Obese children had significantly ($P < 0.01$) higher weight, BMI, body fat percentage, DBP, SBP, sAA con and SR, compared to overweight and normal weight children, as well as a significantly lower aerobic capacity (VO_{2max}) than both normal ($P < 0.001$) weight and overweight ($P < 0.05$) children. In addition, sIgA con and SR were significantly lower between normal weight and obese children ($P < 0.01$)	BMI, DBP and VO_2 predict sAA and that age and BMI category predict sIgA. This study suggests that obesity, based on BMI, has a major role to play and that obese children have elevated sAA, lowered sIgA, and poor CRF.	A possible association between poor CRF and/or overweight/obesity and compromised mucosal immunity and neuro-endocrine status in children, based on the result that obese children have elevated sAA, lowered sIgA, and poor CRF.
Soccer Study 34 black male South African soccer players between the ages of 11– 13y (pre/post intervention)	Sig. diff in pre to post measurements of sIgA secretion rate (p=0.025). No sig. diff were found with measurements of sAA con (p=0.088), sAA secretion rate (p=0.103) or sIgA con (p=0.067). Sig. decrease in BMI, WHR and BF %	Increase in sIgA indicates an increase in the transport of sIgA across the mucosal epithelium. Enhanced IgA transcytosis by increased transepithelial transport/availability of the polymeric Ig receptor (pIgR)-IgA complex. IgA in the mucosa is produced by long-lived plasma B-cells. Enhanced production of IgA in the mucosa via mediating cytokines.	Participation in a 12 week soccer specific programme was shown to sig. increase the secretion rate of sIgA as well as physical characteristics such as BMI, WHR, BF% in African children

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Appendix I

Consent Form to the Department of Education:

University of KwaZulu Natal
Private Bag X54001
Durban
4000

Regional Director
Department of Education
Durban Region
Durban
4000

Dear Sir/Madam

RE: Permission to Conduct Research at a School in KwaZulu Natal (Gateway Christian School Primary)

I humbly request permission to conduct a research project at a selected school in the Pietermaritzburg area of KwaZulu Natal. The project is entitled **"Effect of Physical Activity on Immune Function and Inflammatory Status in Normal and Overweight Children."**

This project will form part of my Ph.D degree in fulfilment for a Ph.D in Sport Science at The University of KwaZulu Natal. We aim to use a school in Pietermartizburg (Gateway Christian School) so as to obtain our necessary data. A sample of 240 students will be required. Should the participants, the headmaster of Gateway Christian School, and the participants parents/guardians agree to their involvement in this project, the participants will be asked to fill out forms about physical activity and to be involved in a one-day assessment for general fitness and health .

The total time spent participating in the study for each student will be approximately 3 hours, which includes measurement of height, weight, waist circumference, body fat %, resting heart rate and blood pressure. Students will also perform a 20-meter shuttle run test for cardiorepiratory fitness assessment. Saliva samples will be collected to be tested ***solely*** for SIgA, CRP and alpha-amylase. This study will not interfere with the school's education program. Once ethical clearance has been granted by the Research Committee of the University of KwaZulu Natal, the researcher will commence the study.

The proposed benefits to being a participant in this study will include gaining information about the health of children in SA and providing essential information about the role that physical activity has on immune function and inflammatory status in normal and overweight children. In addition, the information gathered will provide additional data to produce appropriate guideline and recommendations for school children in SA. The school will also be provided t-shirts, sports equipment and workshops for teachers to be able to continue appropriate physical activity programs in their school.

The policy of the University of KwaZulu-Natal does not provide for compensation or medical treatment for participants because of injury resulting from this research activity. However, every effort will be made to make the facilities safe, with little risk of injury;

Participants may decide not to take part in the project without any disadvantage to themselves of any kind. Participants may withdraw from participation in the project at any time and without any disadvantage to themselves of any kind.

The data from assessments will be collected and then compared. This will primarily allow the researcher to determine how physical activity affects immune and inflammatory status in normal and overweight children as well as baseline health and fitness information about school children in SA. Results of this project may be published but any data included will in no way be linked to any specific participant. Participants/Parents/Guardians as well as The Department of Education are most welcome to request a copy of the results of the project should they wish. The data collected will be securely stored in such a way that only those mentioned above will be able to gain access to it. The student data will not be collected by name, rather each student will be given a Study ID # so as to assure confidentiality of all information. At the end of the project any information will be destroyed immediately except that, as required by the University's research policy, any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed.

If there are any further questions or concerns, please feel free to contact me or my supervising Professor (Professor A. McKune) at the University of KwaZulu Natal (Westville Campus) on the numbers listed below.

I thank you in anticipation of a favourable response.

Yours sincerely,

Kristen Konkol (Mrs.)
Bachelor of Science, Exercise Science
Master of Arts, Kinesiology
Tel: 074-121-7774
E-mail: Konkol@ukzn.ac.za

Professor Andrew McKune
DTech, CSCS
Tel: 031-260 7985
mckunea@ukzn.ac.za

Appendix II

Consent Form to the Headmaster:

University of KwaZulu Natal
Private Bag X54001
Durban
4000

Dear Mr. Matemelo

RE: Request For Consent To Perform a PH.D Dissertation Using Students at Gateway Christian School

I humbly request permission to conduct a research project at a selected school in the Pietermaritzburg area of KwaZulu Natal. The project is entitled **"Effect of Physical Activity on Immune Function and Inflammation Status in Normal and Overweight Children."**

This project will form part of my Ph.D degree in fulfilment for a Ph.D in Sport Science at The University of KwaZulu Natal. We aim to use Gateway Christian School so as to obtain our necessary data. A sample of 240 students will be required. Should the participants, the headmaster of Gateway Christian School, and the participants parents/guardians agree to their involvement in this project, the participants will be asked to fill out forms about nutrition and physical activity and to be involved in a one-day assessment for general fitness and health.

The total time spent participating in the study for each student will be approximately 3 hours, which includes measurement of height, weight, waist circumference, body fat %, resting heart rate and blood pressure. Students will also perform a 20-meter shuttle run test for cardiorepiratory fitness assessment. Saliva samples will be collected to be tested ***solely*** for SIgA, CRP and alpha-amylase. This study will not interfere with the school's education program. Once ethical clearance has been granted by the Research Committee of the University of KwaZulu Natal, the researcher will commence the study.

The proposed benefits to being a participant in this study will include gaining information about the health of children in SA and providing essential information about the role that physical activity has on immune function and inflammatory status in normal and overweight children. In addition, the information gathered will provide additional data to produce appropriate guideline and recommendations for school children in SA. The school will also be provided t-shirts, sports equipment and workshops for teachers to be able to continue appropriate physical activity programs in the school.

The policy of the University of KwaZulu-Natal does not provide for compensation or medical treatment for participants because of injury resulting from this research activity. However, every effort will be made to make the facilities safe, with little risk of injury;

Participants may decide not to take part in the project without any disadvantage to themselves of any kind. Participants may withdraw from participation in the project at any time and without any disadvantage to themselves of any kind.

The data from assessments will be collected and then compared. This will primarily allow the researcher to determine how physical activity affects immune and stress response in normal and overweight children as well as baseline health and fitness information about school children in SA. Results of this project may be published but any data included will in no way be linked to any specific participant. Participants/Parents/Guardians as well as The Department of Education are most welcome to request a copy of the results of the project should they wish. The data collected will be securely stored in such a way that only those mentioned above will be able to gain access to it. The student data will not be collected by name, rather each student will be given a Study ID # so as to assure confidentiality of all

information. At the end of the project any information will be destroyed immediately except that, as required by the University's research policy, any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed.

If there are any further questions or concerns, please feel free to contact me or my supervising Professor (Professor A. McKune) at the University of KwaZulu Natal (Westville Campus) on the numbers listed below.

I thank you in anticipation of a favourable response.

Yours sincerely,

Kristen Konkol (Mrs.)
Bachelor of Science, Exercise Science
Master of Arts, Kinesiology
Tel: 074-121-7774
E-mail: konkol@ukzn.ac.za

Professor Andrew McKune
DTech, CSCS
Tel: 031-260 7985
mckunea@ukzn.ac.za

Appendix III

Consent Form to the Parents/Guardians:

RE: Parent/Guardian Consent Form

INFORMED CONSENT DOCUMENT FOR PARENT/GUARDIAN

CONSENT TO PARTICIPATE IN RESEARCH

Dear _____

Your child has been invited by Ph.D candidate Kristen Konkol and Associate Professor Andrew McKune from the Discipline of Sport Science, University of KwaZulu-Natal, to participate in a study examining the effect of physical activity on immune and inflammatory status in normal and overweight children.

The research will examine the effect of physical activity on immune function and inflammatory status in normal and overweight children. The study aims to determine whether exercise training in normal and overweight children have different effects on the immune system and inflammatory status. This information is important as it will allow exercise scientists to become more specific regarding the type of exercise that they recommend to children who would like to become fitter and healthier. The research will also provide information regarding "how" exercise actually "works" in regard to immune function and inflammatory status amongst normal and overweight children.

The research study will be divided into to parts, or phases. Phase I will include the collection of baseline data information (as listed below) on 240 participants total from the Gateway Christian School. Phase II will use 50 selected participants from a Pietermaritzburg Youth Football Development Academy, who will participate in a 12-week exercise intervention.

PLAN AND PROCEDURES:

Phase I (Baseline Data):

(a) Contact Details: I agree to allow my child to give their basic information to the researcher including my age (date of birth), teacher and school. I understand I they will be given a Study ID # for use throughout the study and their personal information will not be recorded.
(b) Medical History Form: I agree to allow my child to give information about their medical history. The purpose of completing this form is to ensure that they meet the medical requirements to be included in this study and that the researcher obtains information to declare them "apparently healthy" for inclusion as a participant.
(c) Baseline Data Collection: I agree to allow their height, weight, waist circumference, body fat %, resting heart rate and blood pressure to be measured. I also agree to allow them to perform a 20-meter shuttle run test. I also agree to allow them to provide saliva samples to be tested ***solely*** for SIgA, CRP and alpha-amylase and to have them adhere to the information given by the researcher as what to avoid doing prior to saliva collection.
(d) Nutrition and Physical Activity Information: I agree to allow them to complete a 1-day food diary and physical activity questionnaire with assistance from myself and/or teacher.

Phase II (12-week Intervention)

(a) Data Collection: I agree to allow my child to have the same information collected as aforementioned in Phase I ***before and after*** the 12-week intervention for the athletes in the Youth Football Development Academy.
(b) Exercise Intervention: I agree to allow them to participate in a 12-week exercise program 3 times per week for the duration of 60-90 minutes as part of the athlete's normal training session. I agree that as an exercise participant they may be asked to wear a heart rate monitor during the exercise session at various intervals during the intervention.

RISKS AND DISCOMFORTS

Baseline Measurements: Measurement of height, weight, waist circumference, heart rate, blood pressure and saliva samples will produce no physical discomfort. Skinfold (testing body fat %) may produce slight discomfort for a few seconds when the skin is pinched to get a measurement but will disappear immediately after releasing skin. The 20-meter shuttle run test may produce some discomfort as maximum effort is being performed to produce the highest stage/level possible.

Exercise Intervention: The same information as written above applies to all participants as we will take measurements before and after the 12-week intervention. This group will be asked to participate in 60-90 minutes of exercise 3 days per week as part of the normal training sessions in the Pietermaritzburg Youth Football Development Academy. Performing these exercises may produce muscle soreness and there is a chance of injury if the participant does not follow the instructor's directions or if an accident occurs.

POTENTIAL BENEFITS

The proposed benefits to being a participant in this study will include gaining information about the health of children in SA and providing essential information about the role that

physical activity has on immune function and inflammatory status in normal and overweight children. For those who from the Pietermaritzburg Youth Football Development Academy, the proposed benefits include the increase in physical fitness, and potential decreases in measurements such as BMI, resting heart rate and blood pressure as well as changes in salivary markers of immune function and stress response. In addition, the information gathered will provide additional data to produce appropriate guideline and recommendations for school children in SA. The school and football programs will also be provided t-shirts and/or sports equipment and workshops for teachers and coaches to be able to continue appropriate physical activity programs in their school and football training program.

TERMINATION OF PARTICIPATION

I understand that if the screening, data collection procedures and/or exercise phase provide evidence that the tests or activities cannot be safely performed, or if the participant has a pre-existing condition which will not allow them to participate in the study, I will be informed at that time and they will not be included in the study. I understand that the investigator will explain the reason for the exclusion to me.

COSTS/COMPENSTAION

The policy of the University of Kwa-Zulu Natal does not provide for compensation or medical treatment to participants who are injured as a result of this research study. However, every effort will be made to make the tests and activities as safe as possible, with little risk of injury.

CONFIDENTIALITY

All data and information collected in this study will be maintained in complete confidence and privacy will be protected as all information will be collected using a Study ID # and not by name. The participant will not be identified in any report or presentation by name as a result of this study.

You may contact the investigators in this study Ph.D candidate Kristen Konkol (033-396-5494), Associate Prof Andrew McKune (031-260-7985), any time if you have questions about the research or if the participant is injured as a result of the research.

You may contact the **Biomedical Research Ethics Office** on **031-260 4769 or 260 1074** if you have questions about the rights of a research participant.

The participation in this research is voluntary, and the participant will not be penalized or lose benefits if they refuse to participate or decide to stop at any time.

Upon agreement for your child to participate, you will be given a signed copy of this document and the participant information sheet which is a written summary of the research.

The research study, including the above information, has been described to me fully. I understand what my involvement in the study means and I voluntarily agree to allow my child to participate. I have been given an opportunity to ask any questions that I might have about their participation in the study.

_____ _____

Signature of Parent/Guardian **Date**

Appendix IV

Assent Form for Participants:

EFFECT OF PHYSICAL ACTIVITY ON IMMUNE FUNCTION AND INFLAMMATORY STATUS IN NORMAL AND OVERWEIGHT STUDENTS

I (and my parent/guardian) have been informed by the researcher and/or my school teacher or principal concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:-

1. My participation in the project is entirely voluntary;
2. I am free to withdraw from the project at any time without any disadvantage;
3. The data will be destroyed at the conclusion of the project but any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed;
4. I understand that I may experience little, if any, discomfort during the study.
5. The policy of the University of KwaZulu-Natal does not provide for compensation or medical treatment for participants because of injury resulting from this research activity. However, every effort will be made to make the facilities safe, with little risk of injury;
6. The results of the project may be published but my anonymity will be preserved.
7. All information will be collected using a Study ID # and not using my name.

I (with the consent from my parent/guardian) agree to take part in this project.

....

(Signature of participant) (Date)

....

(Signature of parent/guardian) (Date)

This project has been reviewed and approved by the Faculty Ethics Committee
Of the UNIVERSITY OF KWAZULU-NATA

Appendix V **STUDY ID #_____**

MEDICAL HISTORY QUESTIONNAIRE

Name_____Sex_____Age_____Date of Birth_____
Sport(s)_____Phone_____E-mail Address_____
In case of emergency, contact
Name_____Relationship_____Phone(H)_____(W)_____

Explain "Yes" answers on second page	**Y**	**N**
1. Has a doctor even denied or restricted your participation in sports for any reason?	☐	☐
2. Do you have an ongoing medical condition (like diabetes or asthma)?	☐	☐
3. Are you currently taking any prescription or nonprescription (over-the-counter) medicines or pills?	☐	☐
4. Do you have allergies to medicines, pollens, foods, or stinging insects?	☐	☐
5. Have you ever passed out or nearly passed out DURING exercise?	☐	☐
6. Have you ever passed out or nearly passed out AFTER exercise?	☐	☐
7. Have you ever had discomfort, pain, or pressure in your chest during exercise?	☐	☐
8. Does your heart race or skip beats during exercise?	☐	☐
9. Has a doctor ever told you that you have (check all that applies)? ☐ High Blood Pressure ☐ High Cholesterol ☐ A heart murmur ☐ A heart infection	☐	☐
10. Has a doctor ever ordered a test for your heart? (for example, ECG, echocardiogram)	☐	☐
11. Has anyone in your family died for no apparent reason?	☐	☐
12. Does anyone in your family have a heart problem?	☐	☐
13. Has any family member or relative died of heart problems or of sudden death before age 50?	☐	☐
14. Does anyone in your family have Marfan's syndrome?	☐	☐
15. Have you ever spent the night in a hospital?	☐	☐
16. Have you ever had surgery?	☐	☐
17. Have you ever used an inhaler or taken asthma medicine?	☐	☐
18. Were you born without or are you missing a kidney, an eye, a testicle, or any other organ?	☐	☐
19. Have you had infectious mononucleosis (mono) within the last month?	☐	☐
20. Do you have any rashes, pressure sores, or other skin problems?	☐	☐
21. Have you had a herpes skin infection?	☐	☐
22. Have you ever had a head injury or concussion?	☐	☐
23. Have you been hit in the head or been confused or lost your memory?	☐	☐
24. Have you ever had a seizure?	☐	☐
25. Do you have headaches with exercise?	☐	☐
26. Have you ever had numbness, tingling, or weakness in your arms or legs after being hit or falling?	☐	☐
27. Have you ever been unable to move your arms or legs after being hit or falling?	☐	☐
28. When exercising in the heat do you have severe muscle cramps or become ill?	☐	☐
29. Has a doctor told you that you or someone in your family has sickle trait or sickle cell disease?	☐	☐
30. Have you had any problems with your eyes or vision?	☐	☐
31. Do you wear glasses or contact lenses?	☐	☐
32. Do you wear protective eyewear, such as goggles or a face shield?	☐	☐
33. Are you happy with your weight?	☐	☐
34. Are you trying to gain or lose weight?	☐	☐
35. Has anyone recommended you change your weight or eating habits?	☐	☐

36. Have you ever had a stress fracture?	☐ ☐	42. Do you limit or carefully control what you eat?	☐ ☐
37. Have you been told that you have or have you had an x-ray for atlantoaxial (neck)?	☐ ☐	43. Do you have any concerns that you would like to discuss with a doctor?	☐ ☐
38. Do you regularly use a brace or assistive device?	☐ ☐	**FEMALES ONLY**	
39. Has a doctor ever told you that you have asthma or allergies?	☐ ☐	44. Have you ever had a menstrual period?	☐ ☐
40. Do you cough, wheeze, or have difficulty breathing during or after exercise?	☐ ☐	45. How old were you when you had your first menstrual period?	_____
41. Is there anyone in your family who has asthma?	☐ ☐	46. How many periods have you had in the last year?	_____

Explain "Yes" answers from previous page here:_____
_____
_____
_____
_____
_____

List all *previous injuries* and *approximate dates.* Check N/A if not applicable

N/A ☐ Shoulder/Elbow (dislocation, rotator cuff, AC separation):_____ Date:_____
N/A ☐ Arm/Wrist/Hand (fractures):_____ Date:_____
N/A ☐ Neck (burners, pinched nerve):_____ Date:_____
N/A ☐ Ribs/Abdomen:_____ Date:_____
N/A ☐ Low back pan (herniated disc):_____ Date:_____
N/A ☐ Leg (quadriceps, hamstring strain):_____ Date:_____
N/A ☐ Knee (ligament, meniscus, patella):_____ Date:_____
N/A ☐ Lower leg (shin splints, calf strain):_____ Date:_____
N/A ☐ Ankle/Calf/Foot (sprain, Achilles):_____ Date:_____
N/A ☐ Stress Fractures:_____ Date:_____
N/A ☐ Concussions:_____ Date:_____

If yes, have you ever been knocked out (unconscious)? Yes: ☐ No: ☐
How many times?_____
How long were you unconscious?_____
Have you ever lost your memory? Yes: ☐ No: ☐
How many times?_____
Did you have problems in the days afterward (confusion, headache, concentration)?
Yes: ☐ No: ☐
How long did it take you to recover?_____
Are you still having problems? Yes: ☐ No: ☐

Do you have any unhealed or chronic injuries? Yes: ☐ No: ☐
Please list:_____
_____
_____

I hereby state that, to the best of my knowledge, my answers to the above questions are complete and correct.

Signature of athlete_____ Date_____

Signature of parent/guardian_____Date_____

Appendix VI

STUDY ID#_____

DATA RECORDING SHEET

Basic Information and Contact Details:

Date:_____ Age:_____

Teacher/Coach:_____ School:_____

Gender:_____

DOB (dd/mm/yyyy):_____

Measurements

Height (m):_____

Weight (kg):_____ Resting HR (bpm):_____

BMI:_____ Resting BP (mmHg):_____

Waist Circumference (cm):_____ Bleep-Test Stage/Level:_____

Body fat %:_____ Estimated VO2:_____

Saliva Sample

Time of collection (am/pm):_____ Resting CRP:_____

Resting SIgA:_____ Resting alpha-amylase:_____

Date of analysis:_____

Appendix VII

Physical Activity Readiness Questionnaire (PAR-Q)

PAR-Q is designed to help you help yourself. Many health benefits are associated with regular exercise, and the completion of PAR-Q is a sensible first step to take if you are planning to increase the amount of physical activity in your life.

For most people, physical activity should not pose any problems or hazard. PAR-Q has been designed to identify the small number of adults for whom physical activity might be inappropriate or those who should have medical advise concerning the type of activity most suitable for them.

Common sense is your best guide in answering these few questions. Please read the carefully and check **YES** or **NO** opposite the question if it applies to you. If yes, please explain.

YES	**NO**	
____	____	1. Has your doctor ever said you have heart trouble? Yes, _____
____	____	2. Do you frequently have pains in your heart and chest? Yes, _____
____	____	3. Do you often feel fain or have spells of severe dizziness? Yes, _____
____	____	4. Has a doctor ever said your blood pressure was too high? Yes, _____
____	____	5. Has your doctor ever told you that you have a bone or joint problem(s), such as arthritis that has been aggravated by exercise, or might be made worse with exercise? Yes, _____
____	____	6. Is there a good physical reason, not mentioned here, why you should not follow an activity program even if you wanted to? Yes, _____
____	____	7. Do you suffer from any problems of the lower back, i.e., chronic pain, or numbness? Yes, _____
____	____	8. Are you currently taking any medications? If YES, please specify. Yes, _____
____	____	9. Do you currently have a disability or a communicable disease? YES, Please specify, _____

If you answered NO to all questions above, it gives a general indication that you may participate in physical and aerobic fitness activities and/or fitness evaluation testing. The fact

that you answered NO to the above questions, is no guarantee that you will have a normal response to exercise. If you answered Yes to any of the above questions, then you may need written permission from a physician before participating in physical and aerobic fitness activities and/or fitness evaluation testing for this research study.

_____	_____	_____
Print Name	Signature	Date

Appendix VIII

4- Site Skinfold Body Composition

Date:_____

Teacher/Coach:_____

STUDY ID#:_____

Sex: _____

Age: _____

Weight: _____ kg

Sites

1) Triceps: _____mm
2) Midaxilla: _____mm
3) Subscapula: _____mm
4) Suprailiac: _____mm

Appendix IX

COLLECTING UNSTIMULATED WHOLE SALIVA SAMPLES BY PASSIVE DROOL FROM HUMAN SUBJECTS (ages 5+)

Things to avoid:
1. Brushing teeth within 1 hour prior to collection.
2. Using salivary stimulants: chewing gum, lemon drops, granulated sugar, drink crystals.
3. Consuming a major meal within 1 hour prior to collection.
4. Consuming alcohol 12 hours prior to collection.
5. Consuming acidic or high sugar foods within 20 minutes prior to collection.

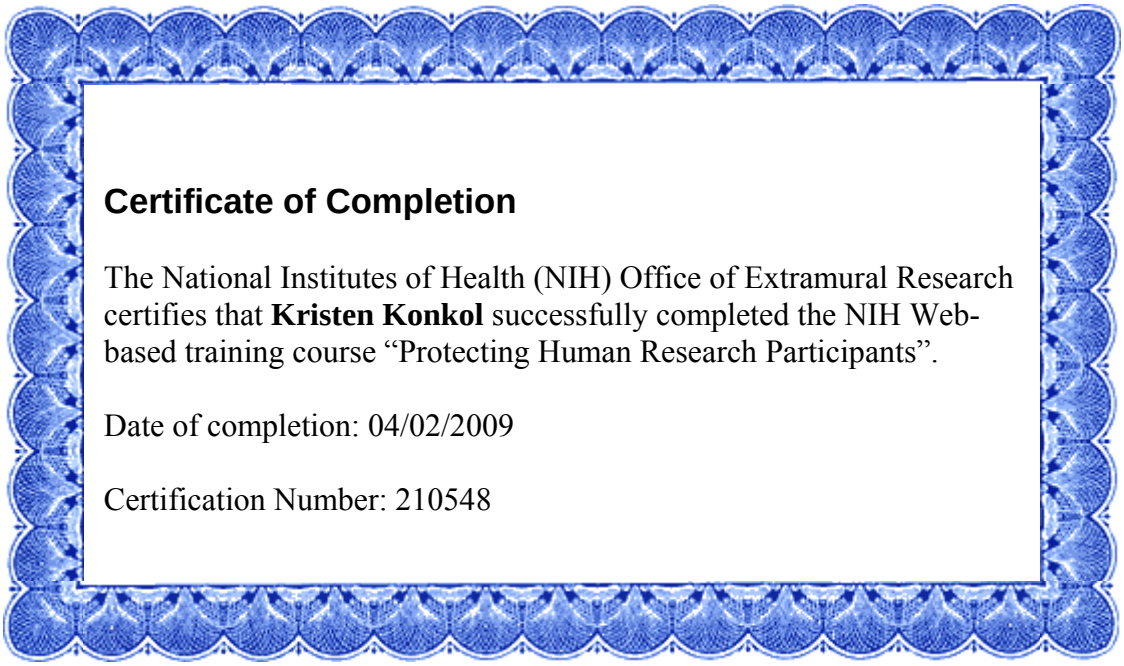
Suggested protocol:
1. Rinse mouth with water 10 minutes prior to sample collection
2. Document prescription and over-the-counter medications taken.
3. Record time of day sample is collected.

Materials required:
_ Plastic drinking straws
_ Scissors
_ Cryovials: polypropylene – 2mL capacity
_ Labels

Prior to Saliva Collection:
1. Cut plastic drinking straws into 2-inch (5 cm) pieces.
2. Give each subject one (1) straw piece and one (1) cryovial.
3. Have subjects rinse their mouth with water 10 minutes prior to collection.

Collecting saliva:
1. Instruct subject to imagine eating their favorite food and allow saliva to pool in the mouth. (Moving the jaw in a chewing motion is acceptable.)
2. With head tilted forward, subject should drool down the straw and collect saliva in the cryovial. (It is normal for saliva to foam.)
3. Repeat as often as necessary until sufficient sample is collected.
(1 mL - excluding foam - is adequate for most tests).
4. If subject's mouth is dry, instruct them to gently chew on the end of the straw. This will stimulate saliva production.
5. Keep samples cold after collection (4ºC) and freeze (-20º to -80ºC) as soon as possible.

Appendix X



Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Kristen Konkol** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 04/02/2009

Certification Number: 210548

Appendix XI

AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire

Assess your health needs by marking all true statements.

History

You have had:

___ A heart attack
___ Heart surgery
___ Cardiac catheterization
___ Coronary angioplasty (PTCA)
___ Pacemaker/implantable cardiac defibrillator/rhythm disturbance
___ Heart valve disease
___ Congenital heart disease
___ Heart failure
___ Heart transplantation

Other health issues

___ You have diabetes
___ You have or asthma other lung disease.
___ You have burning or cramping in your lower legs when walking short distances.
___ You have musculoskeletal problems that limit your physical activity.
.___ You have concerns about the safety of exercise.
___ You take prescription medication(s).
___ You are pregnant.

Symptoms

___ You experience chest discomfort with exertion.
___ You experience unreasonable breathlessness
___ You experience dizziness, fainting, blackouts.
___ You take heart medications.

Cardiovascular risk factors

___ You are a man older than 45 years.
___ You are a woman older than 55 years, you have had a hysterectomy, or you are postmenopausal.
___ You smoke, or quite within the previous 6 mo.
___ Your BP is greater than 140/90.
___ You don't know your BP.
___ You take BP medication.
___ Your blood cholesterol level is >200 mg/dL.
___ You don't know your cholesterol level.
___ You have a close blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister).
___ You are physically inactive (i.e., you get less than 30 min. of physical activity on at least 3 days per week).

___ You are more than 20 pounds overweight.

___ **None of the above is true**.

If you marked that none of the above is true, you should be able to exercise safely without consulting your physician or other healthcare provider in a self-guided program or almost any facility that meets your exercise program needs.

If you marked any of the statements in this section, consult your physician or other appropriate healthcare provider before engaging in exercise. You may need to use a facility with a **medically qualified staff**.

If you marked two or more of the statements in this section, you should consult your physician or other appropriate healthcare provider before engaging in exercise. You might benefit by using a facility with a **professionally qualified exercise staff** to guide your exercise program.

Balady et al. (1998). AHA/ACSM Joint Statement: Recommendations for Cardiovascular Screening, Staffing, and Emergency Policies at Health/Fitness Facilities. Medicine & Science in Sports & Exercise, 30(6).
(Also in: ACSM's Guidelines for Exercise Testing and Prescription, 7th Edition, 2005. Lippincott Williams and Wilkins http://www.lww.com)

www.acsm-msse.org/pt/pt-core/template-journal/msse/media/0698c.htm

Appendix XII

Outline of the 12-week SAQ® training programme

The programme was based on the following rules:

13) Start slowly
14) Gradually increase from simple to more complex movements (perfect simple movements before progressing onto more complex drills)
15) Always start a session with SAQ® Dynamic Flex®
16) Explosive work and sprints should be completed early in the session, before endurance work. Train fresh and fast!
17) Do not use fatigue as a punishment
18) Teach one skill at a time
19) Remember good mechanics at all times
20) Quality and consistency
21) Keep it simple
22) Keep training positive
23) Keep sessions short and sharp
24) Monitor recovery time between sets and reps

Progression of continuum phases and complexity/specificity over 12 week SAQ® programme

Week	*Specificity*
1-3	*Foundation (with Transition)*
4-6	*Complex (with Transition)*
7-9	*Sport Specific (with Transition)*
10-12	*Position Specific*

SAQ® 12--week training programme:

Weeks 1-3

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex® (Warm-up)	Walking on balls of feet Ankle flicks Small skips Lateral running Walking hamstring Wall drill – leg across body Wall drill – forward leg swing	Up and back each drill 10 on each leg 10 on each leg	Cones	Outdoor grid, 20 yards in length and 40 yards in width. Perform Dynamic Flex® drill forwards over 20 yards. Return to the start by performing the drill backwards.	5 mins
Mechanics	Arm mechanics – partner Running form – dead-leg run Running form – pre-turn Running form – leading leg run Running form – lateral stepping Running form – 1-2-3 lift	3 sets of 16 reps, with 1 min recovery between each set 1 set of 6 reps, leading leg should be alternated 1 set of 6 reps, 3 leading with the left leg and 3 leading with right	16 hurdles, 4 cones	Outdoor area. Work in pairs for arm mechanics. Place the hurdles in 2 lines of 8 with 18 inches between each hurdle and 3 yards between each line. 1 min recovery between drills	15 mins
2 minute recovery before next stage of continuum					
Innervation	Fast foot ladder – single runs Fast foot ladder – single lateral step Fast foot ladder – double run Fast foot ladder – Crossover	3 sets of 4 reps, 1 min recovery between each set 3 sets of 6 reps, 1 min recovery between each set	4 X Fast Foot Ladder (6), cones.	Outdoor area. Place the ladders next to each other with approximately 3 yards between them. 1 min recovery between drills Cross-over drill: see description of drills manual	15 mins
Accumulation of Potential					
Explosion					
Expression of Potential					
Warm-down	Knee-across skip Small skips Ankle flicks Walking hamstring Quadriceps stretch Adductors stretch Calf stretch	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Weeks 1-3

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Ankle flicks Wide skips Knee-across skip Lateral running Walking lunge Walking hamstring Wall drill – leg across body Wall drill – forward leg swing	Up and back each drill 10 on each leg 10 on each leg	Cones	Outdoor grid, 20 yards in length and 40 yards in width. Perform Dynamic Flex® drill forwards over 20 yards. Return to the start by performing the drill backwards.	5 mins
Mechanics	Arm mechanics – buttock bounces Running form – dead-leg run Running form – pre-turn Running form – leading leg run Running form – lateral stepping	3 sets of 6 reps. Rep = 6-8 explosive arm drives, 1 min recovery between sets 1 set of 6 reps, leading leg should be alternated	16 hurdles, 4 cones	Outdoor area. Place the hurdles in 2 lines of 8 with 18 inches between each hurdle and 3 yards between each line. 1 min recovery between drills	20 mins
		2 minute recovery before next stage of continuum			
Innervation	Fast foot ladder – single runs Fast foot ladder – single lateral step Fast foot ladder – double run Line drills Fast foot ladder – Crossover	3 sets of 4 reps, 1 min recovery between each set 3 sets of 20 reps, with 1 minute's recovery between each set 3 sets of 6 reps, 1 min recovery between each set	4 X Fast Foot Ladder (6), cones.	Outdoor area. Place the ladders next to each other with approximately 3 yards between them. 1 min recovery between drills Cross-over drill: see description of drills manual	7 mins
Accumulation of Potential					
		3 minute recovery before next stage of continuum			
Explosion	Seated forward get-up	1 set of 5 reps, jog-back recovery between reps		Outdoor area of 20 square yards	3 mins
Expression of Potential					
Warm-down	Knee-across skip Small skips Ankle flicks Walking hamstring Quadriceps stretch Adductors stretch Calf stretch	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Weeks 1-3

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Ankle flicks (perform laterally) Knee-out skip Knee-across skip Lateral running (zig-zag runs) Walking lunge Walking hamstring Wall drill – leg across body Wall drill – forward leg swing Sprints	Up and back each drill 10 on each leg 10 on each leg 1 set of 5 sprints, varying the start position	Cones	Outdoor grid, 20 yards in length and 40 yards in width. Perform Dynamic Flex® drill forwards over 20 yards. Return to the start by performing the drill backwards.	5 mins
Mechanics	Arm mechanics – partner Running form – pre-turn Running form – leading leg run with change of direction Running form – lateral stepping Jumping – two-footed singles	3 sets of 16 reps, with 1 min recovery between each set 1 set of 6 reps, leading leg should be alternated 2 sets of 8 reps, 1 min recovery between each set	Hand weights, hurdles	Outdoor area. Place the hurdles in 2 lines of 8 with 18 inches between each hurdle and 3 yards between each line. 1 min recovery between drills Arm mechanics: use light hand weights for the first 2 sets, controlling the movement carefully on the upswing Leading leg run: Change direction after running in a straight line down the hurdles. Place 3 cones at the end of the hurdles and at different angles, approximately 2-3 yards away. On leaving the last hurdle, the player sprints out to the cone nominated by the coach.	20 mins
2 minute recovery before next stage of continuum					
Innervation	Fast foot ladder – single runs Fast foot ladder – single lateral step Fast foot ladder – in and out Line drills – two footed side jumps with 180 degree twist Fast foot ladder – Crossover with side step	3 sets of 4 reps, 1 min recovery between each set 3 sets of 20 reps, with 1 minute's recovery between each set 3 sets of 6 reps, 1 min recovery between each set	4 X Fast Foot Ladder (6), cones.	Outdoor area. Place the ladders next to each other with approximately 3 yards between them. 1 min recovery between drills In and out: moving sideways along the ladder, stepping into and out of each ladder space (i.e. both feet in and both feet out) Cross-over drill: At the end of the first ladder, side-step to the right or left and single-step down the appropriate adjacent ladder	5 mins
3 minute recovery before next stage of continuum					
Explosion	Seated forward get-up Ball drops	1 set of 5 reps, jog-back recovery between reps 2 sets of 5 reps, with a 2-minute recovery between each set	Balls	Outdoor area of 20 square yards	5 mins
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Weeks 1-3

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Ankle flicks (perform laterally) Knee-out skip (linear coach) Knee-across skip Carioca (lateral performed on curve of circle) Walking lunge Walking hamstring Wall drill – leg across body Wall drill – forward leg swing Sprints	40 flicks (20 each leg) leading with left and right shoulder Forwards middle, backwards to outer circle 2 sets of 5 reps, with a 2-minute recovery between each set Forwards middle, backwards to outer circle 10 on each leg 10 on each leg 1 set of 5 sprints, varying the start position	Cones	An outdoor circle grid with 6 cones making up a circle approximately 3 yards in diameter. Nine cones form a larger circle around this circle, approximately 40 yards in diameter. The players are positioned around the larger circle and the coach is positioned in the centre circle. The players move in towards the coach and away form the coach (moving backwards) or move sideways along the curve of the larger circle. The linear or lateral movement depends on the drill.	5 mins
Mechanics (2 min recovery before innervation)	Arm mechanics – partner Running form – leading leg run with change of direction Running form – lateral stepping Running form – hurdle mirror drills (partner) Jumping – two-footed singles Running form – complex mechanics dead leg	3 sets of 16 reps, with 1 min recovery between each set 1 set of 6 reps, leading leg should be alternated 2 sets of 30-second work periods (1 linear and 1 lateral). 30 sec. recovery 2 sets of 8 reps, 1 min recovery between each set 2 sets of 4 reps with a 30 sec rest between sets	Hand weights, hurdles	Outdoor area. Place the hurdles in 2 lines of 8 with 18 inches between each hurdle and 3 yards between each line. 1 min recovery between drills Arm mechanics: use light hand weights for the first 2 sets, controlling the movement carefully on the upswing Leading leg run: Change direction after running in a straight line down the hurdles. Place 3 cones at the end of the hurdles and at different angles, approximately 2-3 yards away. On leaving the last hurdle, the player sprints out to the cone nominated by the coach.	15 mins
Innervation (2 min recovery before accumulation of potential)	Fast foot ladder – single lateral step Fast foot ladder – icky shuffle side-stepping movement into and out of each ladder space whilst moving forwards Fast foot ladder – "T" formation Fast foot ladder – Crossover with side step	3 sets of 4 reps, 1 min recovery between each set 2 sets of 4 reps (2 moving to the left and 2 moving to the right) 1 min recovery between each set 3 sets of 6 reps, 1 min recovery between each set	4 X Fast Foot Ladder (6), cones.	Outdoor area. Place the ladders next to each other with approximately 3 yards between them. 1 min recovery between drills Cross-over drill: At the end of the first ladder, side-step to the right or left and single-step down the appropriate adjacent ladder	5 mins
Accumulation of Potential (3min recovery before explosion)	Swerve-development runs	1 set of 5 reps, with a 30 sec recovery between each rep	8 – 12 poles and cones	Set out 8-12 poles or cones in a "zig-zag" formation. The distance between the poles/cones should be 2-4 yards at varying angles. The total length of the run is 30 yards.	5 min
Explosion (3min recovery before expression)	Seated backward get-up (turn, run forwards) Ball drops	1 set of 5 reps, jog-back recovery between reps 2 sets of 5 reps, with a 2-minute recovery between each set	Balls	Outdoor area of 20 square yards	5 mins
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Weeks 4-6

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Ankle flicks (perform laterally) Wide-skip (linear coach) Knee-across skip Carioca (lateral performed on curve of circle) Walking hamstring Wall drill – leg across body Wall drill – forward leg swing Wall drill – knee across body Sprints	40 flicks (20 each leg) leading with left and right shoulder Forwards middle, backwards to outer circle 2 sets of 5 reps, with a 2-minute recovery between each set Forwards middle, backwards to outer circle 10 on each leg 1 set of 5 sprints, varying the start position	Cones	An outdoor circle grid with 6 cones making up a circle approximately 3 yards in diameter. Nine cones form a larger circle around this circle, approximately 40 yards in diameter. The players are positioned around the larger circle and the coach is positioned in the centre circle. The players move in towards the coach and away form the coach (moving backwards) or move sideways along the curve of the larger circle. The linear or lateral movement depends on the drill.	5 mins
Mechanics (2 min recovery before innervation)	Arm mechanics – buttock bounces Running form – leading leg run with change of direction Running form – lateral short-stepping hurdle mirror drills (partner) Running form – complex mechanics lateral hurdle drill	3 sets of 16 reps, with 1 min recovery between each set 1 set of 6 reps, leading leg should be alternated 2 sets of 30-second work periods 30 sec. recovery 2 sets of 4 reps with a 30 sec rest between sets	Hand weights, hurdles	Outdoor area. Place the hurdles in 2 lines of 8 with 18 inches between each hurdle and 3 yards between each line. 1 min recovery between drills Arm mechanics: use light hand weights for the first 2 sets, controlling the movement carefully on the upswing Leading leg run: Change direction after running in a straight line down the hurdles. Place 3 cones at the end of the hurdles and at different angles, approximately 2-3 yards away. On leaving the last hurdle, the player sprints out to the cone nominated by the coach. Hurdle mirror drills: Perform the drill laterally. Players work in pairs with only 2 hurdles per player. This drill is used for improving short-stepping, lateral marking skills. Complex lateral drill: Perform the drill laterally, moving both forwards and backwards to cross the centre 4 hurdles.	12 mins
Innervation (2 min recovery before accumulation of potential)	Fast foot ladder – single lateral step Fast foot ladder – icky shuffle side-stepping movement into and out of each ladder space, moving forwards Fast foot ladder – "T" formation Fast foot ladder – Crossover with lateral run and side step	3 sets of 4 reps, 1 min recovery between each set 2 sets of 4 reps (2 moving to the left and 2 moving to the right) 1 min recovery between each set 3 sets of 6 reps, 1 min recovery between each set	4 X Fast Foot Ladder (6), cones.	Outdoor area. Place the ladders next to each other with approximately 3 yards between them. 1 min recovery between drills Cross-over drill: Lateral run down the first ladder, at the end, side-step to the right or left and lateral step down the appropriate adjacent ladder	5 mins
Accumulation of Potential (3min recovery before explosion)	Swerve-development run with lateral zig-zag	2 set of 5 reps, with a 30 sec recovery between each rep	21 cones	Set out 8-12 poles or cones in a "zig-zag" formation. The distance between the poles/cones should be 2-4 yards at varying angles. In addition, 4 yards after the last cone, add an additional 2 lines of 6 cones. The cones must be staggered so that the line is in a zig-zag formation. The total length of the run is 44yards.	7 min
Explosion (3min recovery before expression)	Seated forward and backward get-up (turn, run forwards) Hand-weight drops	2 set of 5 reps, jog-back recovery between reps (1 set forwards, 1 set backwards 1 sets of 5 reps	Balls, cones, hand weights	Outdoor area of 20 square yards See description of drills manual for hand-weight drops	6 mins
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Weeks 4-6

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Dynamic Flex® Ankle flicks, small skips, wide skip, knee across skip, Walking hamstring Fast Feet®, alternate single runs with single lateral step Wall drill – leg across body Wall drill – forward leg swing Wall drill – knee across body	5 times out and back 10 on each leg	Cones	Combination warm-up grid. Players perform Dynamic Flex® drills over the first 10 yards marked by beacons spaced 2 yards apart, then Fast Foot® drills through ladder (6) (starting slow but building up speed). Followed by a swerve run through 5 beacons, spaced 2 yards apart. The players then perform Dynamic Flex® drills back to the first line of beacons where they perform another swerve run back to the start	5 mins
Mechanics (2 min recovery before innervation)	Arm mechanics – buttock bounces, with hand weights Running form – stride frequency and stride length Running form – complex mechanics lateral hurdle drill	3 sets of 16 reps, with 1 min recovery between each set 2 set of 4 reps 2 sets of 4 reps with a 30 sec rest between sets	Hand weights, hurdles, 12 coloured sticks	Outdoor area. See description of drills manual for stride frequency and stride length drill	8 mins
Innervation (2 min recovery before accumulation of potential)	Fast foot ladder – single lateral step Fast foot ladder – icky shuffle side-stepping movement into and out of each ladder space, moving forwards Fast foot ladder – "T" formation Fast foot ladder – Crossover with lateral run and side step	3 sets of 4 reps, 1 min recovery between each set 2 sets of 4 reps (2 moving to the left and 2 moving to the right) 1 min recovery between each set 3 sets of 6 reps, 1 min recovery between each set	4 X Fast Foot Ladder (6), cones.	Outdoor area. Place the ladders next to each other with approximately 3 yards between them. 1 min recovery between drills Cross-over drill: Lateral run down the first ladder, at the end, side-step to the right or left and lateral step down the appropriate adjacent ladder	8 mins
Accumulation of Potential (3min recovery before explosion)	Swerve-development run with lateral zig-zag	3 sets of 5 reps, with a 30 sec recovery between each rep	21 cones	Set out 8-12 poles or cones in a "zig-zag" formation. The distance between the poles/cones should be 2-4 yards at varying angles. In addition, 4 yards after the last cone, add an additional 2 lines of 6 cones. The cones must be staggered so that the line is in a zig-zag formation. The total length of the run is 44yards.	10 min
Explosion (3min recovery before expression)	Seated forward and backward get-up (turn, run forwards) Hand-weight drops	2 sets of 5 reps, jog-back recovery between reps (1 set forwards, 1 set backwards 1 set of 5 reps	Cones, hand weights	Outdoor area of 20 square yards See description of drills manual for hand-weight drops	4 mins
Expression of Potential					
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Weeks 4-6

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Dynamic Flex® Ankle flicks, small skips, wide skip, knee across skip, Walking hamstring Fast Feet®, alternate single runs with single lateral step Wall drill – leg across body Wall drill – forward leg swing Wall drill – knee across body	5 times out and back 10 on each leg	Cones	Combination warm-up grid. Players perform Dynamic Flex® drills over the first 10 yards marked by beacons spaced 2 yards apart, then Fast Foot® drills through ladder (6) (starting slow but building up speed). Followed by a swerve run through 5 beacons, spaced 2 yards apart. The players then perform Dynamic Flex® drills back to the first line of beacons where they perform another swerve run back to the start	5 mins
Mechanics (2 min recovery before innervation)	Arm mechanics – buttock bounces, with hand weights Running form – stride frequency and stride length Running form – complex mechanics dead leg drill	2 sets of 16 reps, with 1 min recovery between each set 2 set of 4 reps 1 sets of 6 reps with a 30 sec rest between sets	Hand weights, hurdles, 12 coloured sticks	Outdoor area. See description of drills manual for stride frequency and stride length drill	7mins
Innervation (2 min recovery before accumulation of potential)	Fast foot ladder – single runs Fast foot ladder – "T" formation Fast foot ladder – Crossover with a 360 degree turn in the centre square	2 sets of 4 reps, 1 min recovery between each set 2 sets of 4 reps (2 moving to the left and 2 moving to the right) 1 min recovery between each set 2 sets of 6 reps, 1 min recovery between each set	4 X Fast Foot Ladder (6), cones.	Outdoor area. Place the ladders next to each other with approximately 3 yards between them. 1 min recovery between drills Cross-over drill: Single run down the first ladder. In the centre square, include a 360 degree turn. This will help develop positional awareness.	6 mins
Accumulation of Potential (3min recovery before explosion)	Swerve-development run with Fast Foot® ladder and lateral zig-zag run	4 sets of 5 reps, with a 30 sec recovery between each rep	Cones, Fast Foot Ladder	Set out 8-12 cones in a "zig-zag" formation. The distance between the poles/cones should be 2-4 yards at varying angles. In addition, 4 yards after the last cone, add a Fast Foot® ladder. Four yards after the ladder add an additional 2 lines of 6 cones. The cones must be staggered so that the line is in a zig-zag formation. The approximate total length of the run is 50yards.	10 min
Explosion (3min recovery before expression)	Seated forward and backward get-up (turn, run forwards) Flexi Cord – Buggy Runs	2 sets of 5 reps, jog-back recovery between reps (1 set forwards, 1 set backwards 1 set of 6 reps plus a contrast run, 30 sec recovery between each rep	Cones, Viper belts, flexi-cord attached at both ends	Outdoor area, work in pairs, ensure health and safety is priority. Especially with regards to length of flexi-cord and attachments	7 mins
Expression of Potential					
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Weeks 4-6

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Dynamic Flex® Ankle flicks, small skips, wide skip, knee across skip, walking lunge, walking hamstring Wall drill – leg across body Wall drill – forward leg swing Wall drill – knee across body	2 x 20 yards, 1 forwards, 1 backwards for each of the Dynamic Flex® and ball drills 10 on each leg	Cones	Soccer specific split grid. Mark out 20 yards length with additional 10 yards. Use different coloured cones. Place ball after 20 yards for each player who has completed dynamic flex drill. Perform Dynamic Flex® over first 20 yards. On reaching additional 10 yards, perform ball skills up and back over 10 yards. Oon completion pass ball to another player completing Dynamic Flex® drill. Precision and quality of pass is NB.	5 mins
Mechanics (2 min recovery before innervation)	Arm mechanics – partner, with hand weights Running form – with a ball, use various mechanics drills (e.g. dead leg run, single step, lateral step)	2 sets of 16 reps, with 1 min recovery between each set 3 sets of 6 reps	Hand weights hurdles, ball	Outdoor area.	5 mins
Innervation (2 min recovery before accumulation of potential)	Fast foot ladder – single runs Fast foot ladder – "T" formation Fast foot ladder – with a ball	1 set of 6 reps, walk back recovery 1 set of 6 reps (2 moving to the left and 2 moving to the right) 1 min recovery between each set 3 sets of 6 reps, 1 min recovery between each set	Balls, Fast Foot ladders (6), cones.	Outdoor area. Place the ladders next to each other with approximately 3 yards between them. 1 min recovery between drills	5 mins
Accumulation of Potential (3min recovery before explosion)	Swerve-development run with ball, lateral Fast Foot® ladder with 180 degree turn in middle, lateral zig-zag run, ending with a jump to header a ball.	5 sets of 5 reps, with a 30 sec recovery between each rep	Cones, Fast Foot ladders, balls	Set out 8-12 cones in a "zig-zag" formation. The distance between the poles/cones should be 2-4 yards at varying angles. Dribble a ball through these cones. Leave the ball and perform a lateral Fast Foot® drill down a ladder, 4 yards after the last cone. Half way down the ladder perform a 180 degree turn and perform lateral stepping leading with the opposite shoulder. Four yards after the ladder add an additional 2 lines of 6 cones. The cones must be staggered so that the line is in a zig-zag formation. Perform lateral zig-zag. An assistant positioned 10 yards after the last zig-zag cone should then throw a ball for the player to jump up and head. The approximate total length of the run is 50yards.	10 min
Explosion (3min recovery before expression)	Seated forward and backward get-up (turn, run forwards) with a ball Flexi Cord – Buggy Runs	2 sets of 5 reps, jog-back recovery between reps (1 set forwards, 1 set backwards 1 set of 6 reps plus a contrast run, 30 sec recovery between each rep	Cones, balls, Viper belts, flexi-cord attached at both ends	Outdoor area, work in pairs for get-ups an have competitions chasing a ball. The player that reaches the ball first then has to dribble the ball a further 10 yards with the other player trying to get the ball back.	10 mins
Expression of Potential					
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Weeks 7-9

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Dynamic Flex® Ankle flicks, small skips, wide skip, knee across skip, walking lunge, walking hamstring Wall drill – leg across body Wall drill – forward leg swing Wall drill – knee across body	2 x 20 yards, 1 forwards, 1 backwards for each of the Dynamic Flex® and ball drills 10 on each leg	Cones	Soccer specific split grid. Mark out 20 yards length with additional 10 yards. Use different coloured cones. Place ball after 20 yards for each player who has completed dynamic flex drill. Perform Dynamic Flex® over first 20 yards. On reaching additional 10 yards, perform ball skills up and back over 10 yards. On completion pass ball to another player completing Dynamic Flex® drill. Precision and quality of pass is NB.	10 mins
Mechanics (2 min recovery before innervation)	Arm mechanics – partner, with hand weights Running form – with a ball, use various mechanics drills (e.g. dead leg run, single step, lateral step)	2 sets of 16 reps, with 1 min recovery between each set 3 sets of 6 reps	Hand weights hurdles, ball	Outdoor area. Variation / progression for running form: On clearing the final hurdle, the ball is fed to the player at chest height. The player controls the ball and executes a side-foot volley to the coach, who lays the ball off for the player to accelerate on to.	5 mins
Innervation (2 min recovery before accumulation of potential)	Fast foot ladder – single runs Fast foot ladder – 'Ipswich Town grid" Fast foot ladder – with a ball	1 set of 6 reps, walk back recovery 2 sets of 4 reps, with 1 min recovery between each set 3 sets of 6 reps, 1 min recovery between each set	Balls, Fast Foot ladders (6), cones.	Outdoor area.	5 mins
Accumulation of Potential (3min recovery before explosion)	Swerve-development run with ball, lateral Fast Foot® ladder with 180 degree turn in middle, lateral zig-zag run, ending with a jump to header a ball.	5 sets of 5 reps, with a 30 sec recovery between each rep	Cones, Fast Foot ladders, balls	Set out 8-12 cones in a "zig-zag" formation. The distance between the poles/cones should be 2-4 yards at varying angles. Dribble a ball through these cones. Leave the ball and perform a lateral Fast Foot® drill down a ladder, 4 yards after the last cone. Half way down the ladder perform a 180 degree turn and perform lateral stepping leading with the opposite shoulder. Four yards after the ladder add an additional 2 lines of 6 cones. The cones must be staggered so that the line is in a zig-zag formation. Perform lateral zig-zag. An assistant positioned 10 yards after the last zig-zag cone should then throw a ball for the player to jump up and head. The approximate total length of the run is 50yards.	10 min
Explosion (3min recovery before expression)	Seated forward and backward get-up (turn, run forwards) with a ball Flexi Cord – Out and Back	1 set of 6 reps, jog-back recovery between reps (3 reps forwards, 3 reps backwards 2 set of 6 reps plus a contrast run, 30 sec recovery between each rep	Cones, balls, Viper belts, flexi-cord attached at both ends	Outdoor area, work in pairs for get-ups an have competitions chasing a ball. The player that reaches the ball first then has to dribble the ball a further 10 yards with the other player trying to get the ball back.	10 mins
Expression of Potential					
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Weeks 7-9

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Dynamic Flex® Ankle flicks, wide skip, pre-turn, hurdle walk, walking hamstring Wall drill – leg across body Wall drill – forward leg swing Wall drill – knee across body Pair drill – lateral runs	2 x 20 yards, 1 forwards, 1 backwards for each of the Dynamic Flex® and ball drills 10 on each leg 2 x 20 yards, 1 left leg leading, 1 right leg leading	Cones	Soccer specific split grid. Mark out 20 yards length with additional 10 yards. Use different coloured cones. Place ball after 20 yards for each player who has completed dynamic flex drill. Perform Dynamic Flex® over first 20 yards. On reaching additional 10 yards, perform ball skills up and back over 10 yards. On completion pass ball to another player completing Dynamic Flex® drill. Precision and quality of pass is NB.	10 mins
Mechanics (2 min recovery before innervation)	Running form – with a ball, use various mechanics drills (e.g. dead leg run, single step, lateral step)	2 sets of 6 reps, with 1 min recovery between each set 3 sets of 6 reps	Hurdles, ball	Variation / progression for running form: The player performs lateral mechanics drills with their back to the coach; the coach also works laterally approximately 2 yards away from the player. The coach feeds the ball to the player who must then turn to the left or right as instructed, gather, control and return the ball.	5 mins
Innervation (2 min recovery before accumulation of potential)	Fast foot ladder – 'Ipswich Town grid" Fast foot ladder – with a ball Line drills: two footed side jumps with 180º twist with ball work	2 sets of 4 reps, with 1 min recovery between each set 2 sets of 6 reps, 1 min recovery between each set 2 sets of 20 reps, 1 min recovery between sets	Balls,4 Fast Foot ladders (6), cones.	Outdoor area. Ipswich variation/progression: Start players on the ladders next to each other on either the left or right of the grid, and work them across the 4 – ladders. Line drill variation: introduce the ball either at the end of the drill so that the player explodes on to it, or during the drill so that the players passes it back before continuing the drill	5 mins
Accumulation of Potential (3min recovery before explosion)	Swerve-development run with ball, lateral Fast Foot® ladder with 180 degree turn in middle, lateral zig-zag run, sprinting onto a ball which is then passed through two cones	4 sets of 5 reps, with a 30 sec recovery between each rep	Cones, Fast Foot ladders, balls	Set out 8-12 cones in a "zig-zag" formation. The distance between the poles/cones should be 2-4 yards at varying angles. Dribble a ball through these cones. Leave the ball and perform a lateral Fast Foot® drill down a ladder, 4 yards after the last cone. Half way down the ladder perform a 180 degree turn and perform lateral stepping leading with the opposite shoulder. Four yards after the ladder add an additional 2 lines of 6 cones. The cones must be staggered so that the line is in a zig-zag formation. Perform lateral zig-zag. The player then sprints onto a ball 5 yards away from the last zig-zag cone, and passes it through 2 cones. The approximate total length of the run is 55yards.	10 min
Explosion (3min recovery before expression)	Flexi Cord – Buggy Runs Flexi Cord – Out and Back	1 set of 6 reps plus a contrast run, 30 sec recovery between each rep 2 sets of 6 reps, plus 1 contrast run per set, 3 min recovery between each set	Cones, balls, Viper belts, flexi-cord attached at both ends	Outdoor area	10 mins
Expression of Potential	British Bulldog	4 mins	Cones, balls	The player in the middle uses a ball to touch other players in order to capture them. The ball is kicked or thrown	4 mins
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Weeks 7-9

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Dynamic Flex® Ankle flicks, wide skip, pre-turn, carioca Wall drill – leg across body Wall drill – forward leg swing Wall drill – knee across body Pair drill – jockeying	2 x 20 yards, 1 forwards, 1 backwards for each of the Dynamic Flex® and ball drills 10 on each leg 2 x 20 yards, 1 left leg leading, 1 right leg leading	Cones	Soccer specific split grid. Mark out 20 yards length with additional 10 yards. Use different coloured cones. Place ball after 20 yards for each player who has completed dynamic flex drill. Perform Dynamic Flex® over first 20 yards. On reaching additional 10 yards, perform ball skills up and back over 10 yards. On completion, pass ball to another player completing Dynamic Flex® drill. Precision and quality of pass is NB.	10 mins
Mechanics (2 min recovery before innervation)	Running form – hurdle mirror drill	3 sets of 30-second work periods, with 30 second recovery between each work period	Hurdles, ball	Variation / progression: First-to-the-ball drill – same as the usual hurdle mirror drill, except a ball is placed between the 2 lines of hurdles. The proactive partner commences the drill as normal then accelerates to the ball, collects it and dribbles to an end cone. The reactive player attempts to beat the proactive player to the ball	4 mins
Innervation (2 min recovery before accumulation of potential)	Fast foot ladder – with passing	2 sets of 6 reps, with 1 minute recovery between each set	Balls, 4 Fast Foot ladders (6), cones.	Outdoor area. 3 reps as player 1 and 3 reps as player 2. Variation / progression: Vary the quick-foot ladder drills performed linearly and laterally by the players.	4 mins
Accumulation of Potential (3min recovery before explosion)	Soccer-specific runs	2 sets of 5 reps, with a 30 sec recovery between each rep. Circuit takes 60 seconds to complete. 1 minute recovery between sets	Cones, Fast Foot ladders, hurdles, balls	Balls are introduced randomly by coach and assistants at different points of the circuit. Player has to control ball, thigh, chest, head and return the ball along the ground.	10 min
Explosion (3min recovery before expression)	Flexi Cord – Out and Back Flexi-cord – Vertical power	2 sets of 6 reps, plus 1 contrast run per set, 3 min recovery between each set 2 sets of 8 reps plus 1 contrast jump, with 3 minutes recovery between each set	Cones, balls, Viper belts, flexi-cord attached at both ends	Outdoor area	6 mins
Expression of Potential	British Bulldog Circle ball	4 mins Each pair to stay in centre for 45 seconds	Cones, balls	Outdoor area, Variation/progression for circle ball: Players in the middle have to hold on to each other.	6 mins
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Weeks 7-9

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Dynamic Flex® small skips, wide skip, knee across skip, pre-turn, side lunge, walking hamstring Wall drill – leg across body Wall drill – forward leg swing Wall drill – knee across body Carioca – perform drill with a partner (mirror drills)	2 x 20 yards, 1 forwards, 1 backwards for each of the Dynamic Flex® and ball drills 10 on each leg 2 x 20 yards, 1 left leg leading, 1 right leg leading	Cones	Soccer specific split grid. Mark out 20 yards length with additional 10 yards. Use different coloured cones. Place ball after 20 yards for each player who has completed dynamic flex drill. Perform Dynamic Flex® over first 20 yards. On reaching additional 10 yards, perform ball skills up and back over 10 yards. On completion, pass ball to another player completing Dynamic Flex® drill. Precision and quality of pass is NB. Carioca variation/progression – one partner initiates/leads the movement while the other attempts to follow	10 mins
Mechanics (2 min recovery before innervation)					
Innervation (2 min recovery before accumulation of potential)	Fast foot ladder – with passing and shooting	2 sets of 6 reps, with 1 minute recovery between each set. 3 reps as player 1 and 3 reps as player 2.	Balls, 4 Fast Foot ladders (6), cones. Soccer goal with keeper	Outdoor area. Variation / progression: Vary the quick-foot ladder drills performed linearly and laterally by the players. The drill must be performed in front of goal. Different from the previous session, the players must shoot at goal instead of passing the ball between the cones.	4 mins
Accumulation of Potential (3min recovery before explosion)	Soccer-specific runs	2 sets of 8 reps, with a 20 sec recovery between each rep. Circuit takes 60 seconds to complete. 45 second recovery between sets	Cones, Fast Foot ladders, hurdles, balls	Balls are introduced randomly by coach and assistants at different points of the circuit. Player has to control ball, thigh, chest, head and return the ball along the ground.	4 min
Explosion (3min recovery before expression)	Flexi Cord – lateral ball work Flexi-cord – Vertical power	2 sets of 6 reps, plus 1 contrast run per set, 3 min recovery between each set 2 sets of 8 reps plus 1 contrast jump, with 3 minutes recovery between each set	Cones, balls, Viper belts, flexi-cord attached at both ends	Outdoor area. For lateral ball work, work both the left and right sides – i.e. just turn the belt around on the player's waist	10 mins
Expression of Potential	British Bulldog Circle ball Robbing the nest	4 mins Each pair to stay in centre for 45 seconds Each pair defends for approximately 45 seconds	Cones, balls	Outdoor area	6 mins
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	6 mins

Weeks 10-12 – Position specific transition – Midfielders, passing under pressure, turning, moving into space, core and upper body strength

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Dynamic Flex® small skips, wide skip, knee across skip, pre-turn, side lunge, walking hamstring Wall drill – leg across body Wall drill – forward leg swing Wall drill – knee across body Pair drill – lateral runs, passing ball hand-to-foot	2 x 20 yards, 1 forwards, 1 backwards for each of the Dynamic Flex® and ball drills 10 on each leg 2 x 20 yards, 1 left leg leading, 1 right leg leading	Cones	Soccer specific split grid. Mark out 20 yards length with additional 10 yards. Use different coloured cones. Place ball after 20 yards for each player who has completed dynamic flex drill. Perform Dynamic Flex® over first 20 yards. On reaching additional 10 yards, perform ball skills up and back over 10 yards. On completion, pass ball to another player completing Dynamic Flex® drill. Precision and quality of pass is NB.	10 mins
Mechanics (2 min recovery before innervation)					
Innervation (2 min recovery before accumulation of potential)	Fast foot ladder – giant crossover	2 sets of 6 reps, with 1 minutes recovery between each set.	Balls, 4 Fast Foot ladders (6), cones.	Outdoor area. Two touches allowed, control and then pass the ball	4 mins
Accumulation of Potential (3min recovery before explosion)	Soccer-specific run, with pressure from a defender	4 sets of 5 reps, with a 30 sec recovery between each rep	Cones, Fast Foot ladders, hurdles, balls	After the last obstacle, the player is passed a ball by the coach. At the same time a defender puts pressure on the player who has to pass the ball back to the coach and then move into space behind the defender to receive the ball again from the coach and then pass it through two beacons	4 min
Explosion (3min recovery before expression)	Side-stepper– lateral runs Flexi-cord – Vertical power Jelly ball workout, chest pass and single-arm pass	3 sets of 6 reps, plus 1 contrast run per set, no recovery time between each rep. 3 min recovery between each set 2 sets of 8 reps plus 1 contrast jump, with 3 minutes recovery between each set 1 set of 12 reps for each drill, with a 1 minute recovery between each drill	Cones, balls, Viper belts, flexi-cord attached at both ends, Jelly balls 5 lb and 10 lb	Outdoor area. Lateral runs: Include a ball, which is controlled and then passed to the coach and received again at the end of each rep.	16 mins
Expression of Potential	British Bulldog Circle ball Robbing the nest	4 mins Each pair to stay in centre for 45 seconds Each pair defends for approximately 45 seconds	Cones, balls	Outdoor area	6 mins
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Weeks 10-12 Position specific – Midfielders, passing under pressure, turning, moving into space, core and upper body strength

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Dynamic Flex® small skips, wide skip, knee across skip, pre-turn, side lunge, walking hamstring Wall drill – leg across body Wall drill – forward leg swing Wall drill – knee across body Pair drill – lateral runs, passing ball hand-to-foot	2 x 20 yards, 1 forwards, 1 backwards for each of the Dynamic Flex® and ball drills 10 on each leg 2 x 20 yards, 1 left leg leading, 1 right leg leading	Cones	Soccer specific split grid. Mark out 20 yards length with additional 10 yards. Use different coloured cones. Place ball after 20 yards for each player who has completed dynamic flex drill. Perform Dynamic Flex® over first 20 yards. On reaching additional 10 yards, perform ball skills up and back over 10 yards. On completion, pass ball to another player completing Dynamic Flex® drill. Precision and quality of pass is NB.	10 mins
Mechanics (2 min recovery before innervation)	Running form – hurdle mirror drill	3 sets of 30 second work periods. 30 second's recovery between each work period	Hurdles, cones, ball	Variation/progression First-to-the-ball drill.. Ball is placed between 2 lines of hurdles. The proactive partner commences the drill as normal then accelerates to the ball, collects it and dribbles to an end cone. The reactive player attempts to beat the proactive player to the ball	4 mins
Innervation (2 min recovery before accumulation of potential)	Fast Foot ladder – Long pass	3 sets of 6 reps, with 1 minutes recovery between each set	4 ladders, balls	After passing the ball the player sprints forward to a cone 10 yards in front slows down and then does a 180 turn as fast as possible and sprints to the back of his line.	4 mins
Accumulation of Potential (3min recovery before explosion)					
Explosion (3min recovery before expression)	Side-stepper– Jockeying in pairs Flexi-cord – Vertical power Jelly ball workout, chest pass and single-arm pass, twist pass	3 sets of 4 reps, plus contrast set of 2 reps, with 30 second recovery between each rep and 2 minutes recovery between each set 2 sets of 8 reps plus 1 contrast jump, with 3 minutes recovery between each set 1 set of 12 reps for each drill, with a 1 minute recovery between each drill	Cones, balls, Viper belts, flexi-cord attached at both ends, Jelly balls 5 lb and 10 lb	Outdoor area. Jockeying in pairs, attacking player must use a ball	10 mins
Expression of Potential / Skills Session	British Bulldog Midfielders - Palmer Drill Midfielders – Backward Turn and Cover	4 mins 1 set of 8 reps, with a 30 second recovery between reps 1 set of 6 reps, with a walk-back recovery between each rep	Cones, balls, 2 ladders	Outdoor area. For Palmer Drill, for nominated cones call 4 to the left and 4 to the right	12 mins
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Weeks 10-12 Position specific – Midfielders, passing under pressure, turning, moving into space, core and upper body strength

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Dynamic Flex® small skips, wide skip, knee across skip, pre-turn, side lunge, walking hamstring Wall drill – leg across body Wall drill – forward leg swing Wall drill – knee across body Pair drill – lateral runs, passing ball hand-to-foot	2 x 20 yards, 1 forwards, 1 backwards for each of the Dynamic Flex® and ball drills 10 on each leg 2 x 20 yards, 1 left leg leading, 1 right leg leading	Cones	Soccer specific split grid. Mark out 20 yards length with additional 10 yards. Use different coloured cones. Place ball after 20 yards for each player who has completed dynamic flex drill. Perform Dynamic Flex® over first 20 yards. On reaching additional 10 yards, perform ball skills up and back over 10 yards. On completion, pass ball to another player completing Dynamic Flex® drill. Precision and quality of pass is NB.	10 mins
Mechanics (2 min recovery before innervation)					
Innervation (2 min recovery before accumulation of potential)					
Accumulation of Potential (3min recovery before explosion)	Soccer-specific run, with pressure from a defender	2 sets of 5 reps, with a 30 sec recovery between each rep	Cones, Fast Foot ladders, hurdles, balls	After the last obstacle, the player is passed a ball by the coach. At the same time a defender puts pressure on the player who has to pass the ball back to the coach and then move into space behind the defender to receive the ball again from the coach and then pass it through two beacons	4 mins
Explosion (3min recovery before expression)	Ball drops Flexi-cord – Vertical power Jelly ball workout, chest pass and single-arm pass, twist pass	3 sets of 10 reps with a 2 minute recovery between each set 2 sets of 8 reps plus 1 contrast jump, with 3 minutes recovery between each set 1 set of 12 reps for each drill, with a 1 minute recovery between each drill	Cones, balls, Viper belts, flexi-cord attached at both ends, Jelly balls 5 lb and 10 lb	Outdoor area. Ball drops variation/progression: Working in groups of 3, with 2 players at different angles alternately dropping a ball for the third player to trap. On achieving this, the player turns and accelerates away to trap the second ball.	10 mins
Expression of Potential / Skills Session	Cone turns Midfielders – Turn and attack Midfielders – Backward Turn and Cover	Each game should last 60 seconds 2 sets of 6 reps, with a walk back recovery between each rep, 2 min recovery between each set 1 set of 6 reps, with a walk-back recovery between each rep	Cones, balls, light hand weights	Outdoor area. Backward turn and cover variation/progression: On player 1's release, the coach delivers a ball to an outer marker. The player must explode to the ball and retrieve it	16 mins
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Weeks 10-1 Position specific – Midfielders, passing under pressure, turning, moving into space, core and upper body strength

SAQ® Continuum	Drills	Sets and Reps	Equipment	Plan	Time
Dynamic Flex®	Dynamic Flex® small skips, wide skip, knee across skip, pre-turn, side lunge, walking hamstring Wall drill – leg across body Wall drill – forward leg swing Wall drill – knee across body Pair drill – lateral runs, passing ball hand-to-foot	2 x 20 yards, 1 forwards, 1 backwards for each of the Dynamic Flex® and ball drills 10 on each leg 2 x 20 yards, 1 left leg leading, 1 right leg leading	Cones	Soccer specific split grid. Mark out 20 yards length with additional 10 yards. Use different coloured cones. Place ball after 20 yards for each player who has completed dynamic flex drill. Perform Dynamic Flex® over first 20 yards. On reaching additional 10 yards, perform ball skills up and back over 10 yards. On completion, pass ball to another player completing Dynamic Flex® drill. Precision and quality of pass is NB.	10 mins
Mechanics (2 min recovery before innervation)					
Innervation (2 min recovery before accumulation of potential)					
Accumulation of Potential (3min recovery before explosion)	Soccer-specific run, with pressure from a defender	2 sets of 5 reps, with a 30 sec recovery between each rep	Cones, Fast Foot ladders, hurdles, balls, goals	After the last obstacle, the player is passed a ball by the coach. At the same time a defender puts pressure on the player who has to pass the ball back to the coach and then move into space behind the defender to receive the ball again from the coach and shoot it at the goals	4 mins
Explosion (3min recovery before expression)	Side-stepper jockeying in pairs Flexi-cord – Vertical power Seated forward get-up	3 sets of 4 reps plus contrast set of 2 reps, 30-second recovery between each rep and 2 min recovery between each set 2 sets of 8 reps plus 1 contrast jump, with 3 minutes recovery between each set 2 sets of 5 reps, with a jog-back recovery between each rep and a 2-min recovery between each set	Cones, side-steppers Viper belts, flexi-cord attached at both ends	Outdoor area. Side-steppers variation/progression: Both players perform the drill laterally, with one player leading and the other trying to mirror their movements	10 mins
Expression of Potential / Skills Session	Cone turns Midfielders – Ball control, feed, turn, receive, shoot Midfielders – Assisted and resisted arcing	Each game should last 60 seconds 1 set of 8 reps, with a walk-back recovery between each rep 1 set of 8 reps, i.e. 4 lead runs for each player, 90 seconds recovery between each rep	Cones, balls, light hand weights, 2 Viper Belts	Outdoor area.	16 mins
Warm-down	Dynamic Flex and static stretching	Up and back – each drill, and 10-second hold on static stretches	Cones	Work over a 20-yard grid for the dynamic flexibility, gradually decreasing the intensity of the drills	5 mins

Appendix XIII

Biomedical Research Ethics Approval

UNIVERSITY OF
KWAZULU-NATAL

RESEARCH OFFICE
Biomedical Research Ethics Administration
Westville Campus, Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

16 November 2010

Ms KF Konkol
School of Physiotherapy,Sport Science & Optometry
Westville Campus.
University of Kwa-Zulu Natal

Dear Ms Konkol

PROTOCOL: The effect of physical activity on immune and stress responses in normal and overweight children. REF: BE254/09.

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application dated 12 November 2009.

The study was provisionally approved pending appropriate responses to queries raised. Your responses dated 11 November 2010 to queries raised on 11 November 2010 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 16 November 2010.

This approval is valid for one year from **16 November 2010.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines

(2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/ResearchEthics11415.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** at a full sitting of the Biomedical Research Ethics Committee meeting to be held on **14 December 2010.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor D.R Wassenaar
Chair: Biomedical Research Ethics Committee

Appendix XIV

DECLARATION

I, ...Kristen Konkol..... declare that:

(i) The research reported in this dissertation/thesis, except where otherwise indicated, is my original research.

(ii) This dissertation/thesis has not been submitted for any degree or examination at any other university.

(iii) This dissertation/thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

(iv) This dissertation/thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
a) their words have been re-written but the general information attributed to them has been referenced;
b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced.

(v) Where I have reproduced a publication of which I am author, co-author or editor, I have indicated in detail which part of the publication was actually written by myself alone and have fully referenced such publications.

(vi) This dissertation/thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation/thesis and in the References sections.

Signed: ..... Date: FEB. 19, 2013