The Modulation Of Mucosal Immune Markers, Urs, And Psychological Parameters Following Acute And Chronic Exercise

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THE MODULATION OF MUCOSAL IMMUNE MARKERS, URS, AND PSYCHOLOGICAL PARAMETERS FOLLOWING ACUTE AND CHRONIC EXERCISE

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DEDICATION

This work is dedicated to my mother, Ann France, for her unconditional love of me and my family and to my father, Richard Lyle France. If it were not for his belief that I could accomplish anything and his example of courage, I would never have attempted and accomplished such a task as a Ph.D. I hope I have passed his confidence and enthusiasm for adventure to my children. This will be his legacy.

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Equally significant to the success of my project was Dr. James Ritchie, Director of the Emory Clinical Translational Research Laboratory, who let me move into his lab for two weeks and trained me in the procedure for the analysis of SIgA and SAA. This laboratory experience was instrumental in helping me to become confident in my skills as a mucosal immune researcher.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

Adults suffer an average of 2- 4 colds/year resulting in an estimated 40 billion dollars in health care costs and 22 million days of work lost annually (Adams, Hendershot, & Marano, 1999). Symptoms of colds begin 2-3 days after infection and last 2-14 days with two-thirds of people recovering within 7 days. Great effort has been undertaken to find a cure for the common cold but these efforts have been unsuccessful because the challenge researchers have is there are over 200 varieties of viruses that can cause an infection. As a result, research efforts have shifted away from a cure to determining what can be done to prevent the common cold. To contract a cold, an individual must have exposure to a quantity of virus that is large enough to do harm and must have a slightly weakened immune system. Factors that weaken the immune system include: age, smoking, mental stress, energy deficient nutrition, lack of sleep, obesity, and a sedentary lifestyle (Bennet & Reade, 1982; Heath et al., 1991). Participants in exercise programs believe in the health benefits associated with moderate physical activity for the prevention and control of cardiovascular disease, diabetes, and weight management because these benefits are widely acknowledged by the medical community (ACSM, 2013). Additionally, exercise enthusiasts usually think that exercise has a broader effect on health including enhancement of their immune system and reducing their number of colds; however, this is equivocal (Simon, 1984).

Survey data has consistently reported more upper respiratory tract infections (URTI) and upper respiratory symptoms (URS) in individuals who are sedentary and in athletes following intensive vigorous training or competition (Shephard, 2000) compared to individuals who perform moderate exercise regularly (Kostka, Berthouze, Lacour, & Bonnefoy, 2000; Matthews et al., 2002). The cold is the most common occurring illness in men and women and a cause

many people give for missing work or athletes have for missing a practice or competition (Woods, 2009). Research that demonstrates the positive role exercise may have in strengthening immunity and decreasing the incidence, severity, or symptoms of the common cold could contribute significantly to decreased health costs and improved work productivity (Woods, 2009).

Salivary Immunoglobulin A

Salivary immunoglobulin A (SIgA) is the dominant antibody in the mucosal system providing the body with its first line of defense against an URTI. When the concentrations of SIgA are depressed, a person is more susceptible to URTI as demonstrated in individuals with immunoglobulin A deficiency (Cunningham-Rundles, 2001; Gleeson, 2000b). SIgA has been shown to prevent the invasion of pathogens into the body by neutralizing viruses and toxins, removing the pathogen, preventing the pathogen's replication, and blocking the pathogen's movement into the mucosal epithelium (Allgrove, Gomes, Hough, & Gleeson, 2008; Mazanec, Nedrud, Kaetzel, & Lamm, 1993). As a result, SIgA levels are widely recognized as a major physiological biomarker in examinations of the integrity of the human mucosal immune system (Bishop & Gleeson, 2009).

Though the research has not been consistent, there is general agreement that acute intense exercise often results in a suppression of SIgA levels (Fahlman, Engels, Morgan, & Kolokouri, 2001; Pacque, Booth, Ball, & Dwyer, 2007) and that chronic moderate exercise results in an elevation of SIgA levels (Akimoto et al., 2003; Klentrou, Cieslak, MacNeil, Vintinner, & Plyley, 2002; Sloan, Engels, Fahlman, Yarandi, & Davis, 2013).

Salivary Alpha Amylase

Salivary alpha amylase (SAA), is a protein found in saliva that may provide protection from URTI due to its ability to inhibit bacterial adherence and growth of bacteria in the airway (Arhakis, Karagiannis, & Kalfas, 2013). Following acute exercise of moderate to vigorous intensity, SAA has been found to increase (Allgrove et al., 2008; Li & Gleeson, 2004). There is strong evidence of a "vulnerable period" immediately following acute vigorous exercise when mucosal immunity is suppressed (Walsh et al., 2011). The anti-microbial properties of SAA may provide a defense against this immune suppression. It is unclear how SAA changes as a result of a chronic moderate intensity aerobic exercise program or a low intensity exercise program such as yoga because no research, to date, has been reported on this topic.

Upper Respiratory Tract Infections

The relationship between exercise and URTI has historically been explained by a "J curve" whereby regular moderate exercise may reduce the risk of URTI below that of individuals who are sedentary, while high intensity exercise may increase the risk of URTI (Nieman, 1994). Several studies have observed moderate levels of physical activity to be associated with a reduced number, duration, severity, or symptomatology of URTI (Nieman, Henson, Austin, & Sha, 2011; Nieman et al., 1990; Schouten, Verschuur, & Kemper, 1988). For example, Nieman and colleagues (2011) found the number of days with URTI was significantly reduced in adults reporting exercising aerobically five or more days per week compared to subjects that were sedentary. Additionally, the study found reduced severity and symptomatology of URTI with near daily physical activity.

Chronic Exercise

A review of literature on chronic exercise revealed significant increases in SIgA when adults are engaged in aerobic exercise programs at an exercise intensity equivalent to walking briskly over a 12 – 16 week time period (Akimoto et al., 2003; Klentrou et al., 2002; Sloan et al., 2013). A significant negative relationship was demonstrated between SIgA concentration and cold symptoms in response to a 12 week moderate exercise training program in previously sedentary men and women (Klentrou et al., 2002).

The increased popularity of yoga as an exercise program for young adults makes it important to examine the potential benefits of yoga (Field, 2011; Su & Li, 2011). Research has documented that yoga improves flexibility (Tran, Holly, Lashbrook, & Amsterdam, 2001) and muscular fitness, (Dash & Telles, 2001) but it is not strenuous enough to elicit improvements in VO_{2max} or weight loss (Chaya, Kurpad, Nagendra, & Nagarathna, 2006; Clay, Lloyd, Walker, Sharp, & Pankey, 2005). The research on the relationship between a low intensity exercise program such as yoga and mucosal immunity and URS is unclear. Determining if a low intensity yoga program elicits improved mucosal immunity and fewer URS, similar to a moderate aerobic exercise program is an important research question as yoga increases in popularity.

Acute Exercise

There is consensus in the research literature of an "open window" or vulnerability period when an individual is at greater risk for infection due to reduced resistance to infections following intense acute exercise (Walsh et al., 2011). Research demonstrated that intense acute exercise such as running a marathon lowers SIgA levels up to 48 hours following a race resulting in more URS when compared to runners who train for the race but do not compete (Peters & Bateman, 1983) . Elite kayakers report decreased secretion rates of SIgA from 27 - 38%

following intense interval exercise (MacKinnon & Jenkins, 1993). Even intense activity of lesser duration such as completing three Wingate tests or a maximal graded exercise test demonstrated a decrease in SIgA and saliva flow rates following the exercise (Fahlman et al., 2001; McDowell et al., 1992).

Limited research has been conducted on the acute effects of moderate or low intensity exercise such as the effects of a yoga or aerobics class on SIgA. A low intensity 20 minute exercise session resulted in significantly elevated SIgA levels in an elderly population (Sakamoto et al., 2005). However, walking for 30 minutes did not alter SIgA levels in a group of mildly obese women (Nieman, Henson, Austin, & Brown, 2005). Additional research is needed to clarify the effects of a single bout of moderate or low-intensity exercise on mucosal markers.

Mood States

There is broad agreement of a positive relationship between physical activity and improved mental health for adult men and women (Stephens, 1988). The field of psychoneuroimmunology studies the interactions of mental state and immunology and more recently the interactions of exercise with these variables. A meta-analysis on psychological intervention and the immune system found modest evidence for interventions such as hypnosis and relaxation resulting in an enhanced mucosal immune system, based largely on observed changes in SIgA (Miller, 1989). Some studies have shown mental relaxation procedures can lead to increases in SIgA while others have not (Arora & Bhattacharjee, 2008; Kugler, 1991; Reid, Mackinnon, & Drummond, 2001; Rood, Bogaards, Goulmy, & Houwelingen, 1993). For example, one study examined the acute effects of "Abbreviated Progressive Relaxation Training" on SIgA and found that SIgA levels were significantly higher post-intervention (Pawlow & Jones, 2005). Improved psychological mood states have occurred as a result of chronic exercise

(Cox et al., 2008; Kennedy & Newton, 1997; Rao et al., 2008). If yoga alters mood state positively, it appears worthwhile to examine if yoga may also alter mucosal immune status.

Few studies have examined how popular exercise classes that are commonly offered at universities and colleges throughout the United States may affect mucosal markers and URS in college students. Many college students participate in aerobic exercise classes of moderate intensity and with the increased popularity of yoga, many are taking up the ancient art of yoga. Claims are made that exercise will enhance immunity and prevent colds (Paul, 1993). Students might be more motivated to exercise regularly if research can document that participation in college exercise classes will strengthen mucosal immunity, decrease the symptoms, severity, and incidences of URTI, and improve mood states.

The primary scope of this dissertation is to evaluate the effect of a moderate intensity aerobic exercise class and a low intensity yoga exercise class on the acute and chronic mucosal immune responses of SIgA and SAA, as well as the symptoms, severity, and incidences of URTI. A secondary purpose is to evaluate the changes in mood states between groups. More specifically, this project will evaluate the

- effects of an acute 50 minute low intensity yoga and moderate intensity aerobic exercise class on the mucosal markers when compared to non-exercising subjects from a first aid class;

- changes in mucosal markers following participation in a 12 week low intensity yoga and a 12 week moderate intensity aerobic exercise class compared to non-exercising subjects from a first aid class;

- incidences of illness, symptoms, and severity of URTI in the exercise groups compared to non-exercising subjects from a first aid class; and

- alteration in mood states following the acute and chronic exercise programs compared to non-exercising subjects from a first aid class.

Research Hypotheses

1. There will be no difference between groups at the beginning of training (homogeneous) relative to food intake, habitual physical activity, and baseline physiological data (age, height, weight, body mass index (BMI), waist circumference, predicted maximal oxygen uptake (VO_{2max}), and flexibility measures).

Acute.

2. Comparing data collected before the yoga and aerobic exercise class with data collected at the end of the classes (50 minutes), the SIgA, secretion rate of SIgA, and saliva flow rate (SFR) will be significantly lower when compared to the same measures in the non-exercising group that is sedentary for 50 minutes.

3. Comparing data collected before the yoga and aerobic exercise class with data collected at the end of the classes (50 minutes), the SAA will be significantly higher when compared to the same measures in the non-exercising group that is sedentary for 50 minutes.

4. Comparing data collected before the yoga and aerobic exercise class with data collected at the end of the classes (50 minutes), subjects from the yoga and aerobic exercise classes will have significantly improved scores for the total mood disturbance and the six categories of the Profile of Mood States (POMS) (tension-anxiety, anger-hostility, fatigue-inertia, depression-dejection, vigor-activity, confusion-bewilderment) when compared to the same scores in the non-exercising group that is sedentary for 50 minutes.

Chronic.

5. Comparing data collected pre intervention with data collected post intervention (12 weeks), resting measures of SIgA, secretion rate of SIgA, SFR, SAA, and the secretion rate of SAA will be significantly higher in the yoga and exercise class treatment groups when compared to the non-exercising group.

6. Comparing data collected pre intervention with data collected post intervention (12 weeks), subjects from the yoga and aerobic exercise classes will have significantly improved scores for the total mood disturbance and the six categories of POMS (tension-anxiety, anger-hostility, fatigue-inertia, depression-dejection, vigor-activity, confusion-bewilderment) when compared to the same scores in the non-exercising group.

URTI.

7. The subjects in the yoga and aerobic exercise classes will report fewer incidences, symptoms, severity of symptoms, and sick days with symptoms of URTI following 12 weeks of exercise training when compared to the non-exercising group.

Assumptions

1. Subjects who participated in the exercise and yoga classes followed the exercise prescription as outlined and administered by the instructor of the class during class sessions.

2. Subjects did not change their eating patterns or habitual physical activity during the study.

3. Subjects are able to understand the terms low, moderate, and vigorous intensity when completing the habitual physical activity assessment.

Limitations

1. Randomization of subjects was not possible limiting the generalization that can be made from the study results and posing a threat to the study's internal validity (Campbell & Stanley, 1963).

This study used a convenience sample so subjects may not have been equal at the beginning of treatment.

2. Costs prohibit the validation of URTIs with a pathology exam as a measure to confirm infections. There can be confusion between asthma attacks and colds and whether a sore throat is an infection or caused by dehydration (Cox et al., 2008; Helenius, Lumme, & Haahtela, 2005). Physician diagnosis is commonly used as the gold-standard when a pathology exam is not available however, even physician diagnosed exams have been criticized because upper respiratory symptoms in 30-40% of the cases were attributed to asthma, allergy, autoimmune disorders, vocal cord dysfunction, and unresolved non-respiratory infections (Reid, Gleeson, Williams, & Clancy, 2004).

CHAPTER 2
REVIEW OF LITERATURE

The field of exercise immunology began in the 1980s with a small number of labs and has grown exponentially over the last 30 years (Shephard, 2010). Technical developments made by exercise immunologists have enabled researchers to monitor the immune system's response to exercise by making it easier to measure variables in plasma or saliva. Saliva is chosen frequently with exercise because of its ease of collection in field and laboratory conditions (Bishop & Gleeson, 2009). A peer reviewed research journal dedicated to exercise immunology research, the *Exercise Immunology Review*, was first published in 1995. Additionally, an international society on exercise immunology research (*International Society on Exercise and Immunology*) has been formed to support research in the field and to provide a venue for the exchange of ideas.

Human Immune System

The human immune system is an exquisitely complex system responsible for the body's response to an infinite number of challenges from pathogens such as fungi, protozoans, viruses and bacteria (Paul, 1993). Immunity involves communication among diverse cells, tissues, and messenger molecules throughout the body through the use of *innate immunity* and the *adaptive immune system*. Innate immunity occurs immediately in the presence of a pathogen with the use of phagocytes, whereas the adaptive immune system responds to specific antigens that it recognizes from previous exposure and typically takes a few days to build a defense and respond (Paul, 1993).

The innate and adaptive immune system respond to foreign material by a phagocyte engulfing the pathogen and degrading it to protein; recognizing a foreign protein appearing on the surface of a phagocyte and with helper T cells, stimulates other immune cells to proliferate and secrete substances that combat the pathogen; producing antibodies against the foreign

protein neutralizing some of the pathogens; and generating "memory" T and B cells that respond quickly to subsequent infection by the same agents. Usually these processes will eliminate the threat of an infection within a week (Paul, 1993).

The human immune system is unique compared to other body systems in that it is not isolated to several organs but found throughout the body (spleen, lymph nodes and Peyer's patches) and in its fluids (Janeway, 1993). This makes it one of the most widely studied and complex system of the human body (Weissman & Cooper, 1993). Originally, it was believed that an individual was born with the wide range of immune receptors necessary to defend against any pathogen a person would encounter in life. This theory was inaccurate as it was discovered that new antigens are created daily (Janeway, 1993). Research in the 1980s revealed that antibody genes are inherited as gene fragments. These fragments are joined together to form a complete gene in lymphocytes as they develop. New genes, each encoding a different protein chain, are formed by combining gene segments and random DNA bases to the ends of pieces being joined. Antibodies are made from two pairs of protein chains: a heavy chain and a light chain. The heavy chains are connected to form a Y with the light chains located on the upper branches, alongside the heavy chains. Each B cell produces just one kind of light chain and one kind of heavy chain, so that each B cell makes a unique antibody receptor. One thousand different chains of each type can in theory form a million combinations. These random joining creates more distinct antibody molecules than there are B cells in the body (Janeway, 1993). Fortunately, the respiratory pathogens are quickly recognized and controlled by a coordinated response involving the innate and adaptive arms of the immune system within the common mucosal immune system (Kohlmeier & Woodland, 2009).

Common Mucosal Immune System

The common mucosal immune system is a network of immune structures at mucosal surfaces throughout the body that provide protection to 400 m^2 of surface area in an adult from invasion by antigens (Brandtzaeg, Farstad, & Haraldsen, 1999). This network summarized in Figure 1 includes structures in the digestive system, urogenital tracts, lacrimal glands (tears), lactating mammary glands, bronchus, salivary glands and nasal lymphoid tissue (Gleeson, 2000a). This system forms the first line of defense against pathogens because mucosal surfaces of the respiratory system are in direct contact with a wide range of pathogens posing a serious threat to infection. In fact, 95% of all infections begin on the mucosal surfaces (Bosch, Ring, de Geus, Veerman, & Amerongen, 2002).
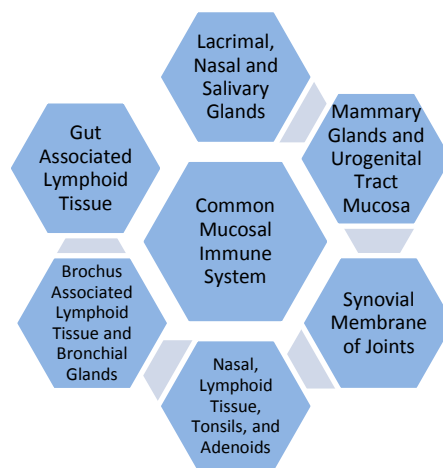
Figure 1: Structures that receive input from the common mucosal immune system

Humans produce about 750ml of saliva each day secreted from three major pairs of salivary glands. Saliva is a complex colorless body fluid comprised of > 99% water. The most significant inorganic constituents of saliva include Na$^+$, K$^+$, and Ca^{++}. The organic substances are more abundant including maltase, serum albumin, urea, uric acid, creatinine, mucin, ascorbic acid (vitamin C), several amino acids, lactate and hormones such as testosterone and cortisol. Gases such as CO_2, O_2, and N_2 are part of saliva in addition to immunoglobulins IgA, IgM, and

IgG. Further, alpha amylase is produced by the salivary glands and found in saliva in levels exceeding what is found in serum (Chicharro, Lucia, Perez, Vaquero, & Urena, 1998).

Salivary Immunoglobulin A (SIgA)

Dr. Thomas Tomasi is credited with discovering that immunoglobulin A is the predominate immunoglobulin in saliva. He observed that SIgA is different from plasma IgA because they are regulated separately and because SIgA has a higher molecular weight than plasma IgA. Tomasi also found that there was an additional polypeptide component (later isolated as secretory component) that was associated with SIgA (Tomasi, 1992).

SIgA is the predominate antibody located in the respiratory system that provides the first line of defense in upper respiratory infections. The B cells are responsible for the production of SIgA in the mucosa-associated lymphoid tissue (Proctor & Carpenter, 2002). After exposure to an antigen, the B cells migrate to the exocrine glands, including the salivary glands, where they reside in the interstitium as IgA-producing plasma cells (Teeuw, Bosch, Veerman, & Amerongen, 2004). All immunoglobulins share a common basic structure consisting of four polypeptide chains composed of two identical light chains and two identical heavy chains (Gleeson, 2000a). The molecule has two functions; one end is responsible for antibody binding and the opposite end is involved with specific complementary binding to an antigen (Brandtzaeg et al., 1999).

Transportation of IgA into the mucosal fluids is by polymeric immunoglobulin receptor (pIgR) and secretory component which internalizes the IgA to transport it across the epithelial wall via endocytosis (ingestion by the cell) and trancytosis (transport of molecule through the inside of the cell) (Teeuw et al., 2004). Partial proteolysis (breakdown of protein) of secretory component leaves one portion attached to IgA, forming secretory IgA which is secreted into the

mucosal fluids. The remaining secretory component is recycled for subsequent IgA transport (Mackinnon, 1999). Secretory component protects the SIgA molecule from degradation by bacterial and digestive enzymes (Tsujita & Morimoto, 1999). There are other protective proteins, other than IgA, secreted in the mucosae that include:

Mucins – a group of 14 large glycoproteins that consist mainly of carbohydrate. They give mucous its slimy characteristic and form an adhesive gel-like film on the mucosal epithelium that protects the epithelium against noxious substances, prevents dehydration, and traps microorganisms. This binding inactivates these microorganisms and prevents their attachment to the mucosal surfaces.

Cystatins – present in every cell and body fluid, these inhibit cysteine proteinases, a class of enzymes involved in virus replication and tissue invasion by bacteria.

Lactoferrin –binds iron which deprives microorganisms of this essential substrate as well as other protective functions.

Lysozyme – is known for its capacity to lyse bacteria by cleaving the polysaccharide component of their cell walls.

Alpha Amalyse – affects the adherence and growth of various bacteria and may play a role in regulating the normal microflora.

SIgA antibodies provide multilayered mucosal protection at mucosal surfaces by exclusion, neutralization, and elimination mechanisms (Allgrove et al., 2008). Specifically, SIgA prevents a pathogen from becoming an infection by binding with the pathogen which prevents its ability to adhere to and penetrate the mucosal epithelium, neutralizing viruses within the epithelial cells during transcytosis, and binding to antigens, the IgA carries it across the epithelium into the luminal contents. These methods operate concurrently to prevent infections

(Bishop & Gleeson, 2009). The protection has been described as a three-tier approach in that the IgA is able to attack the antigen in the lumen, inside the epithelial cell, and in the lamina propria (the loose connective tissue underlying the epithelial basement membrane) (Mazanec et al., 1993).

The production of saliva and saliva proteins, specifically SIgA is controlled by the autonomic nerves and the hypothalamic-pituitary-adrenal axis (Allgrove et al., 2008). Because exercise is associated with the parasympathetic and sympathetic nervous system activity, the activity from either system will alter the production of the proteins in saliva and the saliva flow rate (Leicht, Bishop, & Goosey-Tolfrey, 2011). The parasympathetic system exerts primary control but sympathetic innervation will contribute at different times and situations, particularly during exercise and times of stress (Proctor & Carpenter, 2002). During acute exercise, the production of SIgA is related to the activation of the sympathetic nervous system resulting in changes in salivary protein exocytosis and IgA transcytosis (Walsh et al., 2011). The autonomic nervous system's role in regulating SIgA and SAA production during exercise was evaluated in a study comparing tetraplegic, paraplegic, and non-spinal cord-injured athletes. Leicht and colleagues (2011) hypothesized that the tetraplegic athletes, who have reduced sympathetic output, would not experience the typical decrease in SIgA and increase in SAA seen with able-bodied or paraplegic athletes. However, the hypothesis was rejected because the three groups of athletes experienced similar effects of exercise on these markers of mucosal immune function. The authors concluded that tetraplegic athletes may compensate for the lack of sympathetic activity with a spinal reflex (Leicht et al., 2011).

The variability of SIgA is quite high (Mackinnon, 2000) which has contributed to the inconsistencies seen in the literature on SIgA and exercise. Numerous factors influence SIgA

including lack of euhydration (Bishop & Gleeson, 2009; Oliver et al., 2007) different methods of saliva collection (Strazdins et al., 2005), the presence or absence of salivary stimulation (Bishop & Gleeson, 2009), and nutritional status (Chandra, 1992). Additionally, sleep deprivation, altitude, and psychological stress can contribute to SIgA variability (Bishop & Gleeson, 2009; Konig, Grathwohl, Weinstock, Northoff, & Berg, 2000; Mazzeo, 2005). The humoral arm and cellular arm of the immune system function with varying frequencies demonstrating a circadian rhythm of about 24 hours (Haus & Smolensky, 1999). Controlling for as many of these variables will contribute to more consistent results in mucosal immunity research.

Mechanisms Underlying Changes in SIgA

Since the response of SIgA to exercise varies based on the intensity, duration, fitness level, and other variables, many theories have been suggested to explain the mechanisms that cause alterations in mucosal immunity. There is not one single mechanism but a combination of mechanisms that cause changes in SIgA seen as a result of exercise (Mackinnon, 1999). The rate of secretion of SIgA is dependent on the production of IgA by the plasma cells and/or the rate of IgA transcytosis across the epithelial cell which is determined by the availability of the polymeric immunoglobulin receptor (pIgR) and secretory component (SC) (Mackinnon, 1999). Changes in these variables (IgA production, transcytosis, and availability of pIgR and SC) have been observed in subjects following exercise and help explain how exercise alters the secretion of SIgA (Walsh et al., 2011). Bishop and Gleeson (2009) suggest that the parasympathetic and sympathetic nervous signals to the salivary glands change the rate of production of SIgA by the plasma cell, the rate of transport of IgA molecules though the membrane, and contribute to a decrease in PIgR.

Since the secretion of saliva is regulated by the autonomic nervous system, the secretion of SIgA can be altered by both the sympathetic and parasympathetic nervous systems (Proctor & Carpenter, 2002). Sympathetic control may influence migration of IgA-secreting B cells to the oral submucosa via vasoconstriction of blood vessels, effectively reducing the number of cells synthesizing and secreting IgA (Mackinnon, 1999). Gleeson and Bishop (2000) suggest that there is a mechanism that depresses secretion of IgA into saliva during periods of intense training linked to altered activity of the hypothalamic-pituitary-adrenal axis which has been shown to inhibit IgA synthesis and/or transcytosis. The observation that increased mobilization of the pIgR only occurred above a certain threshold frequency of stimulation could account for the finding of little change in saliva SIgA levels at more moderate intensities of exercise. It may be that repeated mobilization of the pIgR could deplete the available formed IgA pool, leading to decreases in saliva SIgA output (Walsh et al., 2011). These theories are illustrated in Figure 2.
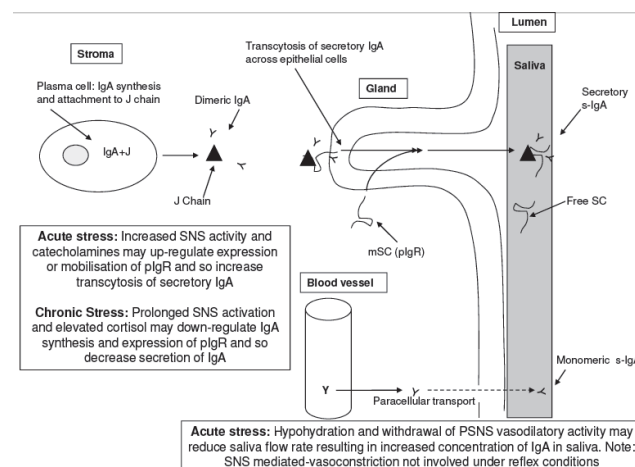
Figure 2: Mechanisms underlying change in SIgA as a result of acute and chronic stress (Walsh et al., 2011).

All cells need an energy source in order to survive, including cells of the immune system. The 5'AMP-activated protein kinase (AMPK) is an agent activated in cells to increase the cells intake of energy. One hypothesis is that intense exercise disrupts the function of AMPK's ability

to assist the cell in meeting its energy needs resulting in immunosuppression. This research reveals that AMPK becomes inactivated during and immediately following a brief bout of acute exercise (Moir et al., 2008).

Another theory proposed to explain the physiological changes to the immune response at exercise of varying intensity uses a neuroendocrine model suggesting that, during exercise, release of immunomodulatory hormones combine to exert either immunostimulation or suppression, depending on exercise intensity (Mackinnon, 1999). Moderate exercise causes release of immunostimulatory hormones such as growth hormone, prolactin, and endorphins/encephalins as well as stimulatory cytokines. As exercise intensity increases above a critical threshold, however, the immunosuppressive arm of the hypothalamic-pituitary-adrenal axis is activated with release of immunosuppressive hormones such as catecholamines, cortisol, and adrenocorticotropic hormone, (Mackinnon, 1999).

Intensive exercise training may deplete certain factors necessary for optimal function of the immune system, such as glutamine, or vitamin C (Krieger, Crowe, & Blank, 2004; Palmer et al., 2003; Pedersen et al., 1999). Psychological stress associated with training and competition may also contribute to altered immune function by altering circulating levels of stress hormones (Mackinnon, 1999). A growing field of study is evaluating whether the alterations in immune function seen with exercise occur by altering gene expression. This research will continue to enhance our understanding of the mechanisms involved with immunity (Mackinnon, 1998).

Salivary Alpha Amylase (SAA)

Most mucosal immunity studies to date have focused on SIgA as a marker of immunity changes related to exercise. Other antimicrobial proteins in saliva including alpha amylase, lacctoferrin, and lysozyme are gaining greater recognition as possibly having roles in mucosal

immunity (Walsh et al., 2011). SAA accounts for 10-20% of the total protein content in saliva (Arhakis et al., 2013; Baum, 1993) and is produced by the differentiated epithelial acinar cells in the parotid glands (Castle & Castle, 1998). SAA's primary role is as an enzyme used in the hydrolysis of starch to glucose and maltose. Additionally, SAA has anti-bacterial properties that prevent the adherence of bacteria to the oral surfaces and the removal of bacteria from the mouth (Arhakis et al., 2013). The ability of SAA to alter URTI may not seem relevant because its immunity properties are bacterial, however, bacterial infections account for 5% of URTI (Trochimiak & Huebner-Wozniak, 2012).

SAA has been used as a surrogate marker for autoimmune activity because it is secreted into saliva by parasympathetic and sympathetic stimulation of the saliva glands (Arhakis et al., 2013; Diaz, Bocanegra, Teixeira, Soares, & Espindola, 2012) and because its activity correlates with noradrenaline concentrations (Allgrove, Chapman, Christidies, & Smith, 2012). This evidence has resulted in SAA use in behavioral studies that monitor SAA response to adverse environments as well with physical stress such as walking on a treadmill (Arhakis et al., 2013). A high concentration of SAA indicates greater stress and is associated with a greater mood disturbance (Arhakis et al., 2013). SAA may be a viable alternative to more common measures of stress such as heart rate and blood pressure (Lee, Za'aba, Madzhi, & Ahmad, 2009).

Research studies documenting that SAA increases with the intensity of physical activity have been consistent (Allgrove et al., 2008; Bishop & Gleeson, 2009; Li & Gleeson, 2004; West, Pyne, Renshaw, & Cripps, 2006). A study evaluating the effects of cycling at 50% VO_{2max}, 75% VO_{2max} and exhaustion, reported that the secretion of SAA increased significantly immediately following the 75% VO_{2max} and exhaustive trials (Allgrove et al., 2008). Wheelchair athletes completing a one hour time trial on a track reported significant increases in SAA from pre

exercise to end exercise (Allgrove et al., 2012; Leicht et al., 2011). Since SAA has antimicrobial properties, these studies demonstrate that intense exercise may evoke upregulation of this antimicrobial protein and provide benefits to immunity. To date no studies have evaluated the chronic changes of SAA with moderate or low intensity exercise.

Saliva Flow Rate (SFR)

A factor that may explain changes in saliva antimicrobial proteins such as SIgA and SAA is the saliva flow rate (SFR); a measure of the volume of saliva produced per unit of time (Chicharro et al., 1998). Understanding saliva production is important for understanding the changes in flow rate that occur with exercise. Regulation of saliva flow is complex involving input from the sympathetic and parasympathetic nervous systems and any alterations in these systems may result in changes in salivary production (Trochimiak & Huebner-Wozniak, 2012). Parasympathetic control is generally recognized as having the primary control over saliva output resulting in vasodilation of the vessels supplying the salivary glands (Chicharro et al., 1998). An increase in sympathetic control reduces saliva flow or volume by limiting the water content of saliva and by vasoconstricting arterioles in the salivary glands (Proctor & Carpenter, 2002). An increase or decrease in saliva volume potentially could alter the concentration of SIgA and SAA (Kugler, Hess, & Haake, 1992). Hence, the most widely accepted method of expressing SIgA and SAA is as a secretion rate which describes the total amount of protein available on the mucosal surface (Blannin et al., 1998; Mackinnon, 1999). Early mucosal research expressed SIgA in relation to salivary proteins but since salivary proteins are highly variable this method of reporting is no longer recommended (Bishop & Gleeson, 2009).

The contribution of saliva from each of the salivary glands varies depending on stimulation which helps explain the variation of SFR levels among individuals (Gleeson, 2000a).

SFR is altered by a variety of factors including food ingestion, sensory stimulation, smoking, body positioning, stress, sore throat, toothache, alcohol consumption, and circadian rhythms (Dawes, 1974; Enberg, Alho, Loimaranta, & Lenander-Lumikari, 2001; Miletic, Schiffman, Miletic, & Sattely-Miller, 1996). A number of studies have demonstrated that unstimulated saliva is affected by hydration status (Bishop, Blannin, Armstrong, Rickman, & Gleeson, 2000; Oliver et al., 2007). Exercise-induced dehydration and fluid restriction resulted in decreased SFR, SAA secretion rates, and the concentration of SIgA but not the secretion of SIgA (Fortes, Diment, Di Felice, & Walsh, 2012). Exercise induced increases in vasopressin (a hormone that promotes water retention) may result in a lowered SFR (Chicharro et al., 1998). Supramaximal interval exercise has produced decreases in SFR attributed to increased beta-adrenergic activity, dehydration, and evaporation of saliva through hyperventilation (MacKinnon & Jenkins, 1993). Another study reported significantly lower secretion rates for SIgA following a single bout of moderate intensity exercise (30 minutes at 71% of heart rate reserve) in cold and thermo neutral environments when compared to the pretest in a thermo neutral environment (Mylona, Fahlman, Morgan, Boardley, & Tsivitse, 2002). Exercise in the cold environment resulted in significant increases in the secretion rate of SIgA and SFR 30 minutes post exercise. The changes in the secretion rate of SIgA were attributed to the changes in SFR (Mylona et al., 2002). Ensuring that subjects are adequately hydrated is important for studies measuring mucosal markers.

Stress tends to decrease SFR via sympathetic activation and relaxation increases SFR via parasympathetic activation (Bosch, De Geus, Veerman, Hoogstraten, & Amerongen, 2003). It can be difficult to determine if mucosal protein changes occur because of SFR changes or a specific stressor. Arhakis and colleagues (2013) developed a mathematical model that demonstrated that SFR does alter SAA activity in healthy young adults. Because SAA can be

altered by SFR, it is recommended that SAA be expressed as a secretion rate (Salimetrics, 2012b).

Abnormal dryness of the mouth, xerostomia, is a common complaint in the elderly. Manipulation of flow rate can increase the secretion of SIgA and could enhance immunity (Miletic et al., 1996). A study of postmenopausal women found significant increases in the secretion rate of SIgA following 12 weeks of a moderate aerobic exercise program, attributed to SFR increases (Sloan et al., 2013). Subjects with xerstomia who participate in a chronic moderate exercise program may benefit from a higher SFR as well as strengthened immunity.

URTI and Chronic Exercise

An argument made for the creation of the discipline of exercise immunology was the lack of treatments and cures for the common cold. The health of the general public would benefit greatly if exercise effects can be maximized to prevent the occurrence or lessen the severity of URTIs (Shephard, 2010). There is reasonable evidence to accept that reduced levels of SIgA are associated with an increased risk of URTI (Walsh et al., 2011). A greater number of URTI are reported in individuals with selective deficiency of SIgA and with low SFR (Cunningham-Rundles, 2001; Gleeson, 2000a). Conversely, high levels of SIgA are associated with fewer URTI (Hanson, Bjorkander, & Oxelius, 1983; Kostka et al., 2000; Matthews et al., 2002). Epidemiological research and anecdotal reports have credited exercise with decreasing the incidence and severity of URTI (Shephard, 2000). Research studies have generally reported improved immune function with moderate intensity exercise resulting in fewer URS or fewer URTI (Akimoto et al., 2003; Klentrou et al., 2002). Sometimes results demonstrate changes in symptomatology or duration of infections without changes in the number of infections. For example, a randomized 15-week trial of moderate exercise in mildly obese women found the

number of symptom days with URTI to be 50% lower in women who followed a brisk walking program at 60% heart rate reserve for five 45-min sessions/week compared with sedentary controls (Nieman et al., 1990) but found no variation in the number of incidences. A group of adults who performed aerobic exercise ≥ 5 days per week reported significantly fewer days with URTI and reduced symptomatology and severity of URTI compared to a sedentary group that exercised ≤ 1 day per week (Nieman et al., 2011).

Defining the relationship between URTI and SIgA is a challenge when subjects exhibit a low SIgA but do not exhibit symptoms of URTI. This is because of the wide interindividual variability of SIgA, making it difficult to determine an absolute reference range for SIgA at which all individuals are at increased risk of URTI (Dwyer, Booth, Pacque, & Ball, 2010). It has been suggested that a more meaningful way to monitor SIgA is to use an individual calculated biological variance which is determined by several measures over a period of days or weeks (Bishop & Gleeson, 2009; Dwyer et al., 2010). When this value falls a certain percentage below the baseline (e.g. 20% below baseline), that puts the individual at increased susceptibility for URTI (Hanson & Brandtzaeg, 1993). If the measurement of mucosal markers were to become a standard for assessing the effect exercise may have on mucosal immunity and risk for infection, it is better to follow changes on an individual basis rather than compare to normative values (Francis, Gleeson, Pyne, Callister, & Clancy, 2005).

Though the literature on the exercise and URTI relationship is equivocal, the "J curve" theory is widely used to explain this relationship. The "J curve" (Figure 3) illustrates that incidences of colds are higher in people who are sedentary, lower in people who have a regular habit of chronic moderate exercise and higher in individuals who train heavily either short or long term (Walsh et al., 2011).
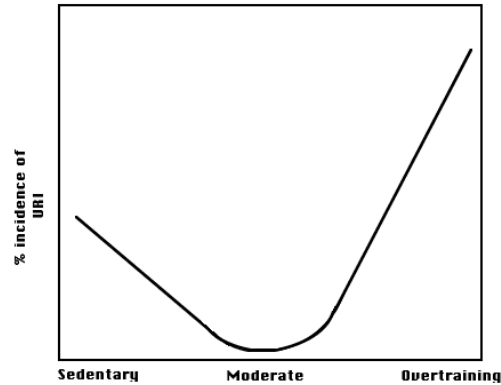
Figure 3: J curve (Nieman, 1994)

Research on URTI and exercise has been criticized because of the lack of verification of infections in subjects and because URS may have multiple causes (Walsh et al., 2011). A study on elite athletes found only 11 out of 37 URTI actually had a pathogenic cause. Most of the illnesses were shorter in duration and less severe than infectious illness leading researchers to question if the symptoms were a measure of URTI (Bermon, 2007). A concern is that some URTI may be caused by inflammation due to hyperventilation not due to infection (Bermon, 2007).

Exercise positively alters the function of the circulatory, respiratory, and muscular systems but it is unclear the extent to which exercises alters the immune system (ACSM, 2013). Just as exercise can positively and negatively impact musculature, immunity can be enhanced and depressed depending on the type, intensity and duration of the exercise. A literature review on the benefits of aerobic exercise and the immune system in older individuals concluded that healthy older adults may benefit from delayed immunosenescence (Haaland, Sabljic, Baribeau, Mukovozov, & Hart, 2008). Given the clear benefits of exercise to other body systems and the possible benefits of exercise to immunity provides a strong argument to continue to recommend exercise to most populations (Walsh et al., 2011).

Acute Exercise

Experimental research has reported transient mucosal immune changes following acute intense exercise and an increase in URTI in athletes following periods of heavy training (Neville, Gleeson, & Folland, 2008; Pyne & Gleeson, 1998). This has led to the formation of the "open-window" theory of immunosuppression (Walsh et al., 2011). This theory suggests that individuals involved in rigorous training are more susceptible to illness due to the downregulation of the immune system. Previous research has produced conflicting results as some studies have found decreases in SIgA with acute intense exercise such as with swimming (Tharp & Barnes, 1990), repeated Wingate tests (Fahlman et al., 2001), intense exercise (MacKinnon & Jenkins, 1993), and marathon running (Nieman et al., 2002). Others have not reported changes such as with running on a treadmill at varying intensities (McDowell, Chaloa, Housh, Tharp, & Johnson, 1991) and at least one study reported increases in SIgA following a game of basketball (Tharp, 1991). However, a recent position statement on immunity and exercise concludes that there is strong evidence to support the presence of a period of "vulnerability" or "open window" immediately following exercise, for up to 48 hours, when SIgA levels decrease possibly creating a time when pathogens can more easily invade the upper respiratory system (Walsh et al., 2011). During these times of increased vulnerability, it is wise for athletes to follow steps to avoid infection and to adjust exercise programs to decrease these periods of vulnerability.

Though the research on mucosal changes pertaining to acute intense exercise seems to be conclusive, acute exercise of moderate intensity has not been widely researched. A study on acute exercise of 20 minutes at intensities of $50 - 80\%$ of VO_{2max} (an intensity of exercise recommended by ACSM) had no effect on SIgA levels (McDowell et al., 1991). Concluding that

this level of exercise can be safely followed as it does not detrimentally lower SIgA or impair immunity. Women walking at 60% of VO_{2max} for 30 minutes had no significant changes in SIgA production (Nieman et al., 2005). Finally, a study evaluating the acute effect of one low intensity exercise class reported significant changes in SFR and the secretion of SIgA (Sakamoto et al., 2005). Further research is needed to evaluate the effects of one session of low intensity or moderate intensity exercise on mucosal markers such as SIgA and SAA.

Yoga

Yoga is an ancient form of exercise and meditation that involves specific postures in conjunction with deep abdominal breathing and typically concludes with a period of relaxation and meditation. Though there are numerous claims made for the benefits of yoga, a recent literature review summarizes the psychological benefits to include reduction in anxiety and depression, reduced sleep problems, and decreased pain associated with the low back, osteoarthritis, and headaches (Field, 2011). Further, the physiological benefits of yoga include decreased blood pressure and heart rate and increased muscular strength, balance, and flexibility (Dash & Telles, 2001; Field, 2011; Su & Li, 2011; Tran et al., 2001). Cardiovascular performance does not improve with yoga training, probably because yoga does not require the energy expenditure required of aerobic exercise, hence, it may not carry the same immunological benefits as seen with cardiovascular exercise (Chaya et al., 2006; Clay et al., 2005). Since the popularity of yoga has increased recently, more research is needed to determine additional physiological benefits of yoga particularly as they relate to immunity improvement (Roland, Jakobi, & Jones, 2011).

Generally, research studies have demonstrated mucosal immunity enhancements and decreases in URTI with activities that involve meditation and relaxation – activities that are often

part of a yoga practice (Pawlow & Jones, 2005; Reid, Mackinnon, et al., 2001). Basal SIgA levels have been shown to increase significantly following four weeks of integrative body-mind training compared to a control group (Fan, Tang, Ma, & Posner, 2010). An exercise study involving the ancient training of Qigong found increases in SFR and SIgA following 10 weeks of daily practice (Bayat-Movahed et al., 2008). Further, massage following three Wingate tests demonstrated a favorable effect on the recovery of immunosuppression that often accompanies intense exercise of short duration (Arroyo-Morales et al., 2009). These studies document support for the use of relaxation, meditation, and other low intensity exercise similar to yoga in chronic and acute settings as a viable approach to immunity enhancement.

Mood State

It is widely accepted in the field of psychoneuroimmunology that negative mood states can result in an increase in respiratory infections and colds (Cohen, Tyrrell, & Smith, 1991; Evans, Bristow, Hucklebridge, Clow, & Walters, 1993). For example, a prospective study that inoculated volunteers with one of five different cold viruses or a placebo found that rates of URTI increased in a dose-response manner with increases in psychological stress (Cohen et al., 1991). A study with medical students who received three hepatitis B vaccines following an academic stress found that the students with greater social support and less stress seroconverted (produced an antibody response) after the first injection whereas students with greater stress did not seroconvert until after the second vaccine demonstrating that the immune response in young healthy adults can be modulated by relatively minor stressors (Kiecolt-Glaser & Glaser, 1992). Most research that evaluates the effect of stress on immunity has observed immunosuppression (Kiecolt-Glaser & Glaser, 1992). In fact, the stress response may mimic the immune response exhibited with acute strenuous exercise (Shephard, 2010).

In contrast, regular exercise, improved fitness, and various types of relaxation lead to enhanced mood states and strengthened immunity (Cramer, Nieman, & Lee, 1991; Stewart et al., 2003). A randomized controlled trial found meditation and exercise to be effective in reducing the burden (decreases in global severity, total days of illness, and work day missed related to the illness) of acute respiratory infections (Barrett et al., 2012). Additionally, SIgA increased following various types of relaxation such as deep breathing, massage, and progressive relaxation (Dillon, Minchoff, & Baker, 1985; Green & Green, 1987; Reid, Mackinnon, et al., 2001). Specifically, twenty minutes of relaxation response, visualization, and massage resulted in significantly improved SIgA compared to a control group (Green & Green, 1987). Finally, community-recruited adults who participated in "mindfulness meditation" at weekly 2.5 hour group sessions and 45 minutes of daily at-home practice reported a mean global severity score for URTI of 144 compared to a score of 358 for the control group (Barrett et al., 2012). Using regular exercise and relaxation may be an important psychosocial variable related to improving immune functioning.

The Profile of Mood States (POMS) has successfully been used in psychological research to evaluate mood states of people before and after exercise. One hundred college students, taking a beginning or intermediate swimming class or a lecture-control class, completed the POMS before and after class. Results revealed that swimmers had significantly less tension, depression, anger, confusion, and more vigor after exercising than before. Both novice and intermediate swimmers changed significantly more than did the controls on all scales except fatigue, while none of the controls' pre- post-instruction mood changes were significant (Berger & Owen, 1983). To examine the exercise effect of one Taekwondo session, subjects completed the POMS questionnaire and had a significant improvement with respect to the control group in

scores of tension, depression, anger, fatigue, confusion, and vigor. Also, total mood disturbance significantly improved after the dynamic Taekwondo session (Toskovic, 2001). In contrast, another study found no change in POMS Global Mood State following moderate exercise for 30 minutes in cold and thermal neutral environments (Mylona et al., 2002) and no change was reported in the POMS variables during 21 days of intense training for male cyclists except for a decrease in vigor from day 1 to day 4 (Slivka, Hailes, Cuddy, & Ruby, 2010).

Some of the discrepancy related to observations in mood state is that POMS may lack the sensitivity to measure changes in mood state when subjects begin with normal mood states (Wadden, Steen, Wingate, & Foster, 1996). Researchers in the psychoneuroimmunology literature have pointed out that if an immune system is functioning adequately, it may not be desirable to enhance immune function as this could lead to autoimmune disease (Kiecolt-Glaser & Glaser, 1992).

College students enroll in exercise classes such as aerobics and yoga for a variety of reasons including improving health and strengthening immunity to prevent illness. Determining if a regular exercise pattern of aerobic or yoga exercise followed twice a week has the ability to provide immunoenhancement and a decrease in URS is unclear at this time. However, if exercise of this type could provide a benefit by decreasing the incidence, severity, and symptoms of colds and infections, and improve mood states, it could have a significant impact on the public health of college students.

This review highlights the complexity of the immune system and the difficulty in understanding the relationship between exercise and immunity. Because immune function is essential to survival, the immune system has evolved a large safety net and redundancy. It is difficult to determine how much immune function is lost or gained with exercise and how these

changes ultimately affect URTI (Walsh et al., 2011). To keep the immune system functioning at its optimum, a person needs to get adequate sleep, eat a healthy diet, keep stress to a minimum, limit exposures to infection, provide adequate time for recovery following training and avoid fatigue (Mackinnon, 2000). There is consensus among exercise immunologist that there is a benefit to using exercise to enhance immunity. Understanding the potential role that yoga and aerobic exercise may have in enhancing the immune system in college students is important.

CHAPTER 3
METHODOLOGY

Study Participants

Study subjects were a convenience sample of healthy college age students enrolled in two aerobic exercise classes (N = 48), two beginning yoga classes (N = 60) and two first aid classes (N=48) offered at similar times of the day (Monday and Wednesday from 7:00 – 8:00 am and 8:00 – 9:00 am or on Tuesday and Thursday from 7:00 – 8:00 am and 8:00 – 9:00 am) at a large (16,000 student population) metropolitan community college in the mid-west USA during the winter semester of 2013. For inclusion into the study, subjects were required to attend the formal class at least 80% of the scheduled class meetings (19 of 24 class meetings) as documented by attendance records kept by the instructor, be non-smoking as this can alter biochemical values of saliva samples collected with this study (Bennet & Reade, 1982), and be healthy as indicated on the PAR-Q evaluation (Jamnik et al., 2011). All subjects were asked to maintain their usual diets and physical activity habits outside of the intervention throughout the study period. At the conclusion of the study, the principal investigator met with each subject to review the results of their 24 hour diet recall as a token of appreciation for their participation in the study. Students were free to choose whether to participate in the research study after receiving an explanation of the purpose, possible risks, and benefits associated with participating. University Internal Review Board approval was obtained and all participants provided written informed consent in accordance with the guidelines established by the Human Investigations Committee of the University.

Intervention Protocols

Yoga Exercise Group (YEG).

Each yoga session consisted of 10 minutes of warm-up exercises, 30 minutes of *asanas*, (yoga postures), 5 minutes of cool-down exercises and 5 minutes of relaxation in *savasana*. The warm-up program focused on slow movements progressing to more dynamic muscular movements, consisting of the frog pose, dynamic lunges, shoulder/arm circles, neck rolls, standing forward bend, and two cycles of "sun salutation". The *asanas* taught in the class included head to knee *(maha mudra)*, cat *(vidalasana)*, forward bend *(pascimottanasana)*, spinal twist *(vakrasana)*, tree *(vrksasana)*, warrior *(virabhadrasana)*, triangular *(trikonasana)* variations one and two, superman, and pigeon *(eka pada rajakapotasana)*. The focus of the *asanas* was on breathing, flexibility, strength, balance, and concentration. Each class ended with 5 minutes of *savasana* to relax and cool down. The warm-up protocols were practiced in their entirety at each class session, but due to time constraints only a limited number of *asanas* were completed during each yoga class. Subjects were encouraged to follow appropriate yoga technique while maintaining their breath control throughout the class (Tran et al., 2001). A certified and experienced hatha yoga instructor taught the yoga classes.

Aerobic Exercise Group (AEG).

The aerobic exercise class protocol included a 10 minute warm-up including walking around the gymnasium, and 4-5 standing stretching exercises, 30 minutes of moderately intense aerobic exercise at target heart rate, and 10 minutes of active cool down exercises designed to lower the heart rate below 100 beats/min before dismissing the class. Target heart rates were established for each individual using percentage of maximal heart rate (220 – age) at 70 to 85% as prescribed by the American College of Sports Medicine (ACSM, 2013). This is equivalent to

moderate intensity exercise or $50 - 70\%$ of $\%VO_{2max}$ reserve (Katch, McArdle, & Katch, 2011). A progressive increase in exercise volume every 2 weeks was accomplished by increasing exercise intensity to provide for a suitable overload training stimulus (Garber et al., 2011). The same ACSM group exercise certified and experienced instructor led both classes of the aerobic program from the front of a gymnasium with a microphone using exercise that was choreographed to music with tempos ranging from 120-140 counts per minute.

Non-exercise Group (NEG).

The non-exercising group was students enrolled in a First Aid class taught by a certified Basic EMT and American Red Cross (ARC) instructor. The material taught in the First Aid class is dictated by the ARC because most students, at the completion of the class, receive Emergency Response and Basic Life Support certifications from the ARC. Sample topics covered in the class were assessing a scene; head, neck, and back injuries; infant, children, and geriatric emergencies; and environmental emergencies. The class included a lecture teaching format interspersed with practicing various skills such as cardio-pulmonary resuscitation, bandaging and splinting, and measuring blood pressure. To evaluate the change in the physical fitness of subjects, assessments of predicted VO_{2max}, anthropometric measurements, and flexibility were conducted.

Physical Assessments

Prediction of VO_{2max}.

Before and after the 12 week intervention period, each subject performed a battery of standardized tests that included evaluations for the prediction of VO_{2max}. The Queen's College Step Test for predicting aerobic capacity in college-aged individuals was used to predict the maximal oxygen uptake (VO_{2max} mL$\cdot$ kg$^{-1}\cdot$ min^{-1}) of subjects pre and post intervention

(Weidner, 1994). The test has been evaluated for validity and reliability for men ($r = -0.96$) (Chatterjee, Chatterjee, Mukherjee, & Bandyopadhyay, 2004) and women ($r = -0.75$) (McArdle, Katch, Pechar, Jacobson, & Ruck, 1972). Standardized procedures for the test included:

- Attachment of heart rate monitors (Polar Electro; Lake Success, New York)

- Subjects were asked to step up and down onto a 41 cm bench with a stepping pace of 22 steps/minute for women and 24 steps/minute for men using a metronome set at 88 and 96 beats, respectively.

- Subjects used a four-step cadence, 'UP-UP-DOWN-DOWN' continuously for 3 minutes.

- The technician took a post heart rate reading at 5 seconds, 10 seconds and 20 seconds after the cessation of exercise with the subject standing.

- The average of the three measures was used to calculate predicted VO_{2max} mL$\cdot$ kg$^{-1}\cdot$ min^{-1} with the following formulas:

  Males = 111.33 – (0.42 X heart rate/minute)

  Females = 65.8 – (0.1847 X heart rate/minute)

- All subjects were encouraged to cool down following the step test by walking around and stretching.

Anthropometric Measurements.

Physiological testing of height, weight, waist circumference, and flexibility were measured the week prior to and the week immediately following the 12 week treatment period. The subjects were measured for standing height to the nearest cm and body weight to the nearest 0.5 kg on a physician's balance beam (Health-O-Meter; Buffalo Grove, IL) calibrated scale with subjects dressed in shorts and shirts with no shoes. The BMI, or Quetelet index, was used to assess weight relative to height and was calculated by dividing body weight in kilograms by

height in meters squared (kg/m^2) (Gallagher et al., 2000). In addition, waist girth was measured with the subject standing upright and relaxed; a horizontal measure was taken at the greatest anterior extension of the abdomen, usually at the level of the umbilicus. Procedures for waist girth measurements included (Heath et al., 1991):

   • To assure the measurement was taken with the same compression on all subjects, a Gulick spring-loaded measuring tape (Power Systems; Knoxville, TN) was used and the handle was extended to the same marking point with each trial.

   • Duplicate measures were taken at each site until two measurements were within 5 mm.

Flexibility Testing.

Measurements of shoulder and hamstring flexibility were assessed to document the changes in flexibility as a result of the exercise programs. The sit-and-reach test was administered to document changes in hamstring flexibility (criterion validity, r = .89) (Bozic, Pazin, Berjan, Planic, & Cuk, 2010; Trochimiak & Huebner-Wozniak, 2012). Initial collected data was not available to the evaluator on subsequent assessments to prevent bias. Prior to each flexibility test, a warm-up of four exercises was given where the stretches were statically held for 20 seconds to the point of slight discomfort with a 10 second interval between stretches. Each stretch was executed twice; fast, uncontrolled bouncing movements were avoided (Garber et al., 2011).

The sit-and-reach test is a common flexibility test designed to measure the flexibility of the lower back and hamstrings (ACSM, 2013). The Acuflex sit-and-reach box (Novel Products; Rockton, Il) was used for the measurement. Subjects were instructed to:

   • Sit with back and head against a wall, without shoes, the soles of the feet were placed against the Acuflex with the legs straight.

- With arms extended in front, the subject was instructed to slowly reach forward with both hands as far as possible, holding this position for 2 seconds. The subject did not jerk or bounce in an attempt to reach further. Care was taken to ensure that the subjects' hands were parallel and that he/she did not lead with one hand. Fingertips overlapped and came in contact with the measuring portion of the sit-and-reach box. The score was the most distant point reached with the fingertips and the best of three trials were recorded.

- The subject was instructed to exhale and drop the head between the arms when reaching. Testers ensured that the knees of the participant stayed extended without hyperextension.

- Scoring was recorded to the nearest centimeter.

The stretches preceding the sit-and-reach measurement included ("Mayo's Clinic Guide to Ten Basic Stretches," 2012):

- Sitting hamstring stretch – sitting on the floor with the legs extended in front, subjects bent at the waist attempting to reach the knees, shin, or ankles for a stretch.

- Hip flexor stretch – from a lunge position (with one foot forward and one foot back), subjects were encouraged to lean into the lunge keeping the hips square.

- Knee extensors – from a standing position, subjects brought the knee to the chest and held for a stretch.

- Gastrocnemius stretch – with one foot forward and one foot back, subjects bent the forward knee and extended the back leg for a stretch of the calf.

Shoulder flexion flexibility was assessed to measure changes in shoulder flexion as a result of the exercise program using a goniometer test. The protocol for range-of-motion assessment as described in the ACSM's Health-Related Physical Fitness Assessment Manual was followed (ACSM, 2005). The procedures for the measurement included:

- Subjects began standing with the scapula stabilized to prevent tilting, rotation, or elevation of the shoulder. Subject began with the glenohumeral joint (shoulder joint) in 0 degrees of flexion, extension, abduction, or adduction, with the head in a neutral position, and the palm of the hand facing the body. The elbow was completely extended. The subject performed glenohumeral flexion as high as possible while still maintaining proper form.

- A measurement was taken with the fulcrum on the lateral aspect of the greater tubercle and with the stabilization arm perpendicular to the floor. The movement arm of the goniometer (an instrument used to measure the angle of a joint) was aligned with the midline of the humerus and referenced with the lateral epicondyle.

- The subject moved slowly through the proper range of motion, as the goniometer was kept aligned with the body segment. The best of three measurements were recorded for the right and left arms.

The stretches preceding the shoulder elevation measurement included (Anderson & Anderson, 2010; Liem & Kasten, 2012):

- Anterior shoulder stretch – from a standing position, subjects clasped both hands behind the back. The clasped hands were lifted keeping the posture upright. The stretch was stopped at the point of discomfort and held for 20 seconds.

- Posterior deltoid stretch – bringing the right hand to the left shoulder with the elbow elevated, the left palm, presses on the right elbow, stretching the shoulder. The stretch was repeated on the opposite shoulder.

- Supraspinatus stretch – placing hands on the hips and bringing the elbows to the front of the body, keeping hands stationary, subjects stretched the supraspinatus muscle.

At the conclusion of each testing session, test results for estimation of cardiovascular endurance, anthropometric measures, and flexibility were shared with subjects including educational materials interpreting the tests and a classification of disease risk based on both BMI and waist circumference developed by the Expert Panel on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults (Cohen et al., 1991).

Saliva Collection

To assess the changes in mucosal immunity, saliva was collected from subjects four times during the study period. Each time, the subjects were instructed to swallow and then unstimulated whole saliva was collected over a four minute period into 10-mL plastic sterile vials (Fisher Scientific, Pittsburg, PA). Subjects allowed saliva to dribble into the collecting tubes unaided by spitting. All saliva collections were made with the subjects seated, leaning forward with their heads down avoiding any deliberate movements of the oral musculature (e.g., chewing, etc.) during the collection period. Subjects were allowed to drink water ad libitum during and after exercise with the exception of the 10 minute period before each saliva sample collection. Saliva volume was recorded to the nearest 0.1 ml and samples were stored in a -20 degree Celsius freezer (Revco Scientific, Garden Grove, CA) until analysis (Papacosta & Nassis, 2011).

The first and second saliva collection was on the first day of the 12 week study period at 4 minutes before beginning class and again immediately following the end of the class. Two additional samples were collected, before exercise at the midpoint week (week 7) and one before exercise on the last day of the 12 week study period. Using a permanent marker each collection tube was marked with the subjects' research ID number and tubes were color coded to indicate pre, acute post, midpoint, or final collection. The saliva flow rate (SFR) was calculated by

dividing the total volume of saliva obtained in each sample (ml) by the time taken to produce each sample (4 minutes). The secretion rates of SIgA (µg/min) and SAA (U/min) were calculated by multiplying absolute concentration (µg/ml) by the SFR (ml/min) (Fahlman & Engels, 2005). The technician collecting the saliva samples and administering the pre and post intervention tests was trained in appropriate data gathering techniques and was the same technician for all subject data collection. One technician entered all data results into the software for later analysis.

Saliva samples were shipped on dry ice via Federal Express overnight delivery for biochemical analysis of SIgA and SAA to be performed at the Emory Clinical Translational Research Laboratory, a clinical trial testing lab, which is certified through the Clinical Laboratory Improvements Ammendments, using assay kits from Salimetrics (State College, PA). Following training and practice, in conjunction with lab personnel, the principal investigator led the evaluation of the assays in the lab. The test procedure for the biochemical analysis of each assay was as follows:

Salivary Immunoglobulin A

SIgA was measured using a Salimetrics immunoassay kit Catalog No. 1-1602. The principle of the test for SIgA is that a constant amount of goat anti-human SIgA conjugated to horseradish peroxidase is added to tubes containing specific dilutions of standards or saliva. The antibody-conjugate binds to the SIgA in the standard and saliva samples. The amount of free antibody remaining is inversely proportional to the amount of SIgA present (Figure 4). After incubation and mixing, an equal solution from each tube is added in duplicate, to microtitre plate coated with human SIgA. The free or unbound antibody conjugate binds to the SIgA on the plate. After a second incubation, unbound components are washed away. Bound conjugate is measured

by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction. Optical density is read on a standard plate reader at 450 nm. The amount of peroxidase is inversely proportional to the amount of SIgA present in the sample (Chard, 1990; Salimetrics, 2012a).
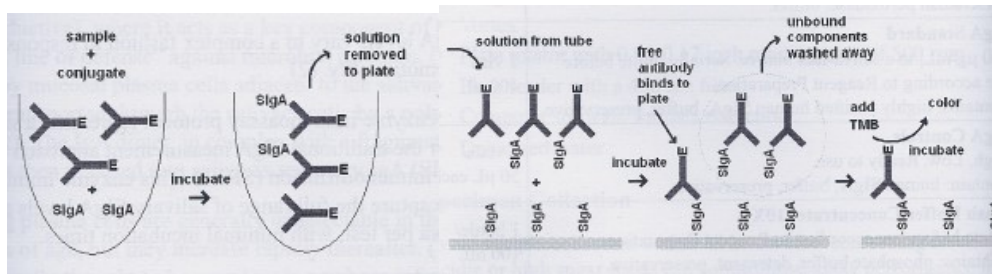
Figure 4: Test Principle

To avoid interassay variability, all samples from each subject were assayed on the same microplate. For quality control, the Salimetrics' high and low salivary SIgA controls were run with each assay and standards of known concentrations of purified IgA were included on each microplate, and absolute concentrations were calculated from the standard curve (Salimetrics, 2012a).

The specific procedure followed for the SIgA analysis included (Salimetrics, 2012a):

1) On the day of the assay, all reagents and saliva samples were brought to room temperature, vortexed (Thermolyne Maxi Mixer; Watham, MA), and centrifuged (Eppendorf Centrifuge; Hamburg, Germany) at 1500 x g for 15 minutes.

2) Serial dilution of standards was completed by pipetting (Eppendorf; Hamburg, Germany) 30 µL of 1X SigA diluent in tubes 2 through 6 and adding 15 µL of standard 3X (from tube 1) to tube 2. After a pipette change, 15 µL from tube 2 was put into tube 3. This process was continued for tubes 4, 5, and 6. Each tube was mixed well following introduction.

3) Saliva samples were prepared by labeling one small microcentrifuge tube for each saliva sample.

4) Using a Repeater Plus pipette (BrandTech Scientific; Essex, CT), 100 µL of 1X SIgA diluent was added to each tube.

5) 25 µL of saliva was added to the appropriate tube and lightly vortexed to mix.

6) Glass 12 x 75 mm capped tubes were labeled making one tube for standard 1-6, control high and low, zero value, and unknown samples.

7) Using the repeater pipette 4 mL of 1X SIgA diluent was added to each tube.

8) 10 µL of standard, control, or diluted unknown saliva sample was added to each tube and mixed.

9) An antibody-enzyme conjugate in the amount of 50 µL was added to each tube using a repeater pipette

10) Each tube was gently inverted and allowed to incubate for 90 minutes at room temperature.

11) Tubes were inverted gently again and 50 µL from each tube was placed into the microtitre plate, supplied with the assay kit, according to the predetermine layout.

12) Plate was covered with an adhesive plate sealer and was continuously mixed at 400 rpm for 90 minutes at room temperature.

13) Microplate washer (Biotek ELX50; Winooski, VT) was used to wash plate 6 times with 1X wash buffer.

14) The substrate tetramethylbenzidine (TMB) in the amount of 50 µL was added to each well using a multichannel pipette (Rainin; Columbus, OH).

15) Plate was set on plate rotator for 5 minutes at 500 rpm and incubated in the dark at room temperature for 40 minutes.

16) A stop solution of 50 µL was added to each well using a multichannel pipette.

17) Plate was rotated for 3 minutes at 500 rpm.

18) All plates were read within 10 minutes by a microplate reader (Molecular Devices, Center Valley, PA) at 450 nm.

19) Concentrations of unknown saliva were multiplied by 5 to obtain the final concentration of SIgA in µg/mL.

Salivary Alpha Amylase (SAA)

SAA was measured using a *Salimetrics* immunoassay kit Catalog No. 1-1902. This method utilizes a chromagenic substrate, 2-chloro-p-nitrophenol linked with maltotriose. The enzymatic action of alpha amylase on this substrate yields 2-chloro-p-nitrophenol, which can be spectrophotometrically measured at 405 nm. The amount of alpha amylase activity present in the sample is directly proportional to the increase in absorbance at 405 nm (Mazzeo, 2005). For ease of use, the reaction was read using half a 96-well microtiter plate. For quality control, the *Salimetrics'* high and low salivary alpha amylase controls were run with each assay. Currently, *Salimetrics* (Salimetrics, 2012b) and other researchers (Arhakis et al., 2013; Beltzer et al., 2010) recommend that the SFR be used to express SAA per unit of time (U/min). The specific procedure for the SAA analysis included the following steps (Salimetrics, 2012b):

1) All reagents and saliva samples were brought to room temperature. On the day of the assay, the saliva samples were thawed completely, vortexed, and centrifuged at 1500 x g for 15 minutes.

2) Saliva samples were diluted with the alpha amylase diluent by pipetting 10 µL of saliva into 90 µL of alpha amylase diluent. This was further diluted by pipetting 10 µL of the first solution into 190 µL alpha amylase diluent. Final dilution was 1:200.

3) The alpha amylase substrate solution is heated to 37^0C in a trough using a preheated microtiter plate incubator.

4) 8 µL of controls and diluted saliva samples was placed into individual wells. Reverse pipetting was used to avoid introducing any bubbles into the well.

5) A volume of 320 µL of preheated alpha amylase substrate solution was added to each well simultaneously using a multichannel pipette. Used pipette tips were discarded after each introduction.

6) The plate was immediately placed into a programmable 37^0 C microplate reader (Molecular Devices, Center Valley, PA) and mixed for three minutes. Mixing was interrupted for an optical density reading at one minute and three minutes.

7) The one minute reading was subtracted from the three minute reading and multiplied by a conversion factor of 328. The conversion factor takes the 1:200 sample dilutions into account for the prediluted controls and samples.

Upper Respiratory Symptom Survey

Symptoms of URTI were assessed with a short one-page survey that subjects completed each day of the study. The short form of the Wisconsin Upper Respiratory Symptom Survey (WURSS-21) is an evaluative illness-specific quality of life instrument, designed to assess the symptoms, severity, and incidences of upper respiratory infection (Barrett et al., 2002). The short WURSS-21 version has been found to be reliable with composite reliability coefficients ranging from 0.87 to 0.97 and Cronbach's alpha ranging from 0.76 to 0.96 and contains broad based construct validity (R = 0.85) (Barrett et al., 2009). The survey was administered each day for the duration of the project and scored as described below:

- Two or more consecutive days of feeling sick = illness

- Severity score = sum of the daily severity score (0 = not sick, 1 = very mild URTI to 7 = severe) for the days that an illness is recorded.

- Symptom score = sum of the 10 symptom scores for the days (0 = do not have this symptom, 1 = very mild to 7 = severe) that interferes with the subject's ability to accomplish a task during a period of illness.

Habitual Physical Activity Questionnaire

To determine the habitual physical activity of subjects at the onset of the research project, the Baecke Habitual Physical Activity Assessment was administered. Additionally, the assessment was administered at the conclusion of the project to confirm that the subjects had adhered to the request not to change their physical activities outside the prescribed exercise program. At the study's conclusion, subjects were instructed to complete the questionnaire without considering the activity of their respective class. Completion of the survey took about five minutes and produced estimates of: physical activity at work, sport during leisure time, physical activity during leisure time excluding sport and a total activity index calculated from a sum of the three previous indices. This questionnaire consisted of a scale of one to five (five representing the most active) with eight questions pertaining to occupation, four addressing athletic activities, and four addressing habitual leisure habits. Double Labeled Water validation of the questionnaire has been conducted revealing $r = 0.69$, $p < 0.001$ (Philippaerts, Westerterp, & Lefevre, 1999). If a question was left blank or answered unclearly, the principal investigator contacted the subject immediately following the test for clarification.

Profile of Mood States (POMS) Questionnaire

Mood assessments were given to all subjects to determine changes in mood following acute exercise of yoga or aerobics or sitting in class. Chronic changes in mood were assessed at

the beginning, midpoint (7 weeks), and at the conclusion of the study (12 weeks). The POMS, 65 -point adjective rating scale was administered to determine if the treatment alters the subjects' mood state. POMS is a psychological test designed to measure a person's affective states that is easy to administer and completed within 3-5 minutes. The POMS is based upon strong and well documented psychometric qualities designed to assess transient and fluctuating moods, and enduring states of affect (McNair, Lorr, & Droppelman, 1981). Reliability coefficients from 0.779-0.926 for six mood scales have been reported as well as good criterion validity in many subject groups including college students (Arroyo-Morales et al., 2009). The POMS allows for the measurement of tension-anxiety (defined as heightened musculoskeletal tension), depression-dejection (defined as personal inadequacy), anger-hostility (defined as anger and antipathy towards others), fatigue-inertia (defined as weariness and low-energy level), confusion-bewilderment (defined as muddleheadedness), vigor-activity (vigorousness, ebullience, and high energy), and total mood disturbance (sum of all six scores, weighting vigor negatively). Subjects were assigned numerical codes to ensure anonymity and to encourage honest responses. To control for day of week effects on mood, all mood evaluations took place on Monday.

The following instructions were recited to subjects as recommended in the POMS manual (Arroyo-Morales et al., 2009) to assure reliable and valid responses.

"Below is a list of words that describe feelings people have. Please read each one carefully. Then fill in ONE space under the answer to the right which best describes HOW YOU HAVE BEEN FEELING DURING THE PAST WEEK (HOUR was substituted for the acute posttest measure) INCLUDING TODAY. The numbers refer to the following descriptive phrases.
0 = not at all
1 = a little
2 = moderately
3 = quite a bit
4 = extremely"

All paper-pencil research surveys where immediately check for completeness, and entered into an excel data base.

24-Hour Dietary Recall

To verify that all study groups had homogeneous food intakes, a 24-hour dietary recall record was collected from all subjects at the beginning of the study. Students were instructed in the proper procedures for completing the 24-hour food record using the Automated Multiple-Pass Method of Dietary Recall (Haskell, 1984). The results from the recall were analyzed using the Food Processor II Program (ESHA Research, Salem, OR, USA) to estimate total kilocalorie intake and percentage of kilocalories from fat, carbohydrate, and protein. Subjects were asked to maintain their current dietary habits for the duration of the study.

Statistical Analysis

This study used a pretest-posttest control group design (Campbell & Stanley, 1963). The baseline and physical assessment data from the subjects were characterized using descriptive data analysis and a one-way ANOVA, respectively. A two-way ANOVA with repeated measures was used to compare acute and chronic physiological and biochemical data. A post hoc analysis using Duncan's Multiple Range Test was applied when appropriate. The URS data that was evaluated by Pearson's chi-square included: 1) the total of the number of illnesses (defined as 2 or more days of feeling sick) for each study group, 2) the total number of days sick with symptoms for each incident; 3) symptom and severity scores for each subject. Additionally, a coefficient of variation for SIgA and SAA was calculated. A probability value of $p < 0.05$ was accepted as significant. All statistical analyses were performed using Statistical Analysis Systems (SAS Institute, SAS Version 9.2, Cary, North Carolina, USA).

CHAPTER 4
RESULTS

This study evaluated the chronic (6 and 12 weeks) and acute (50 minutes) effects of participating in an aerobic exercise class, yoga exercise class, and a non-exercise class on salivary immunoglobulin A (SIgA), salivary alpha amylase (SAA), and changes to the incidence, severity, symptoms, and number of days sick with symptoms of URTI in a college student population. Additionally, this study served to evaluate the chronic and acute changes in mood state that occurred across groups. The group (aerobic, yoga, and non-exercise) served as a between-subjects variable and test was a within-subjects variable. Of the 136 subjects who were evaluated at baseline, 93 completed the acute study and 88 participated in the chronic study. All data, with the exception of the illness data and baseline data, were analyzed using a two-way ANOVA with repeated measures. The illness data were evaluated using a Pearson's chi-square test and baseline data were evaluated with a one-way ANOVA. Post hoc analysis, when appropriate, was performed using Duncan's Multiple Range Test. A probability value of $p < 0.05$ was accepted as significant. The statistical analysis was performed using Statistical Analysis Systems (SAS version 9.2, SAS Institute, Cary, North Carolina, USA).

Baseline Descriptive Data

Students enrolled in two aerobic classes, three yoga classes, and two non-exercise classes were invited to participate in the study. Enrollment in each class was 24 students. Of the possible 168 students eligible to enroll in the study, 136 students (46 from the aerobic exercise group (AEG), 52 from the yoga exercise group (YEG), and 38 from the non-exercise group (NEG) agreed to participate in the study and completed baseline testing of height, age, and food records for the evaluation of total kilocalories and kilocalories from lipid, carbohydrate, and protein. The average daily energy intake for the total study population was estimated at 1816 ±

58 kcal/day. On average, 49% of the energy was derived from carbohydrate sources, 30% from lipid, and 18% from protein. Analysis revealed no significant differences between groups for all baseline characteristics (p > 0.05). Mean ± SEM for the baseline characteristics of the three groups and a total for all participants combined are detailed in Table 1.

Table 1

Baseline Descriptive Data (N = 136)

	Aerobic (*n* = 46)	Yoga (*n* = 52)	Non-exercise (*n* = 38)	All Participants	*p*
Gender	Males = 4 Females = 42	Males = 8 Females = 44	Males = 17 Females = 21	Males = 29 Females = 107	
Age	29.96 ± 1.54	26.33 ± 1.47	25.71 ± 1.88	27.38 ± 0.94	0.139
Height (cm)	166.49 ± 1.09	166.24 ± 1.12	168.44 ± 1.73	166.94 ± 0.74	0.448
Total kcal	1923 ± 107	1755 ± 65	1770 ± 135	1816 ± 58	0.419
Carbohydrate kcal	962 ± 63(50%)	908 ± 47 (52%)	778 ± 65 (44%)	890 ± 34 (49%)	0.092
Lipid kcal	594 ± 43 (31%)	512 ± 32 (29%)	558 ± 64 (32%)	553 ± 26 (30%)	0.412
Protein kcal	331 ± 21 (17%)	298 ± 14 (17%)	346 ± 32 (20%)	323 ± 12 (18%)	0.276

df = 2, 135

Physical Assessments

Anthropometric measurements were evaluated pre intervention and again at 12 weeks, post intervention. There were no group or test differences for weight or BMI. Though there were no significant test differences for percent fat, there were significant group differences for percent fat (p < 0.01). Post hoc analysis of the group factor revealed that the NEG had significantly lower percent fat compared to the AEG at the pretest and posttest. Additionally, significant test differences were observed for waist circumference (p < 0.01) but no significant group differences. Post hoc analysis of the test factor revealed lower waist circumference scores on the

posttest compared to the pretest for all participants combined. Table 2 details the mean ± SEM for the assessments of weight, BMI, waist circumference, and percent fat for the three treatment groups and a total for all participants combined.

Table 2

*Anthropometric Data (*N = 88)

		Aerobic ($n = 30$)	Yoga ($n = 29$)	Non-exercise ($n = 29$)	All Participants
Weight (kg)[1]					
	Pre	77.37 ± 3.59	72.58 ± 3.79	71.02 ± 3.67	73.70 ± 3.69
	Post	76.36 ± 3.27	73.24 ± 3.84	72.08 ± 3.68	73.93 ± 3.59
BMI (kg/m^2)[2]					
	Pre	27.64 ± 1.27	25.81 ± 1.14	24.55 ± 1.02	26.02 ± 1.14
	Post	27.48 ± 1.08	25.69 ± 1.08	24.83 ± 1.00	26.02 ± 1.06
Waist Circumference (cm)[3]					
	Pre	79.81 ± 2.77	76.38 ± 2.59	77.50 ± 2.67	77.37 ± 2.67$_a$
	Post	79.20 ± 2.90	74.22 ± 2.44	76.12 ± 2.84	76.56 ± 2.74$_a$
Percent Fat[4*]					
	Pre	28.72 ± 1.91	26.20 ± 1.65	20.59 ± 1.39	25.21 ± 1.65
	Post	28.57 ± 1.80	25.31 ± 1.51	21.08 ± 1.38	25.03 ± 1.57

[1]Group	$F(2, 85) = 0.58, p > 0.05$	[2]Group	$F(2, 85) = 1.785, p > 0.05$
Test	$F(1, 175) = 0.29, p > 0.05$	Test	$F(1, 175) = 0.00, p > 0.05$
Group x Test	$F(2, 175) = 2.05, p > 0.05$	Group x Test	$F(2, 175) = 0.45, p > 0.05$
[3]Group	$F(2, 85) = 0.64, p > 0.05$	[4]Group	$F(2, 85) = 5.07, p < 0.05$
Test	$F(1, 175) = 7.09, p < 0.05$	Test	$F(1, 175) = 0.06, p > 0.05$
Group x Test	$F(2, 175) = 0.77, p > 0.05$	Group x Test	$F(2, 175) = 0.97, p > 0.05$

Note: Means in a column sharing subscripts are significantly different from each other.
*Significant group difference between the AEG and NEG on the pretest and posttest.

Physical fitness assessments for flexibility and predicted VO_{2max} were evaluated pre intervention and post intervention. Significant pretest and posttest differences were observed for right shoulder and left shoulder flexibility test scores ($p < 0.01$). Post hoc analysis of the test

factor revealed greater shoulder flexibility for the right and left shoulders on the posttest compared to the pretest for all participants combined. Regarding sit-and-reach flexibility, significant group differences were observed between the pre intervention and post intervention measurement ($p < 0.01$). Post hoc analysis of the group factor revealed significant differences in sit-and-reach flexibility between the NEG and the AEG and significant differences between the NEG and the YEG.

Analysis of the predicted VO_{2max} data indicated significant group differences ($p < 0.01$). Post hoc analysis of the group factor revealed significantly higher predicted VO_{2max} scores for the NEG compared to the AEG and compared to the YEG. No significant differences were found between the AEG and the YEG. Finally, the results of the analysis of the recovery heart rate measured following the Queens College Step Test detected significant group differences ($p < 0.05$) and significant group x test interactions ($p < 0.05$). Post hoc analysis of the group x test interaction found that the AEG had a significantly lower recovery heart rate on the Queens College Step posttest compared to the pretest. Additionally, the group x test interactions found that the AEG and the YEG had higher recovery heart rates during the pretest compared to the NEG. Table 3 details the mean ± SEM for the assessments of right and left shoulder flexibility, sit-and- reach flexibility, VO_{2max}, and recovery heart rate measured at the conclusion of the Queens College Step Test for all study groups and a total for all participants combined.

Table 3

*Physical Fitness Data (*N = 88)

		Aerobic ($n = 30$)	Yoga ($n = 29$)	Non-exercise ($n = 29$)	All Participants
Right Shoulder Flexibility (°)[1]					
	Pre	173.33 ± 1.91	173.10 ± 1.19	171.10 ± 1.55	172.52 ± 1.56$_a$
	Post	178.20 ± 1.15	177.17 ± 1.20	175.28 ± 0.98	176.90 ± 1.11$_a$
Left Shoulder Flexibility(°)[2]					
	Pre	177.47 ± 1.89	172.90 ± 1.43	172.83 ± 1.40	174.43 ± 1.58$_b$
	Post	178.47 ± 1.24	178.07 ± 1.09	175.52 ± 1.02	177.36 ± 1.12$_b$
Sit-and-Reach Flexibility (cm)[3]*					
	Pre	47.52 ± 1.33	46.79 ± 1.59	41.95 ± 1.43	45.44 ± 1.45
	Post	47.37 ± 1.43	46.84 ± 1.50	41.69 ± 1.32	45.31 ± 1.42
Predicted VO_{2max} (ml/kg/m)[4]*					
	Pre	36.46 ± 1.23	38.13 ± 1.48	44.03 ± 1.99	39.51 ± 1.56
	Post	38.31 ± 1.20	38.33 ± 1.16	42.65 ± 1.75	39.75 ± 1.37
Queens College Step Test Recovery HR[5]					
	Pre	164.27 ± 4.13$_{ab}$	160.07 ± 3.87$_c$	145.86 ± 3.56$_{bc}$	156.82 ± 3.86
	Post	155.90 ± 4.10$_a$	157.07 ± 3.14	152.45 ± 3.87	155.15 ± 3.71

[1]Group	$F (2, 85) = 1.27, p > 0.05$	[2]Group	$F (2, 85) = 2.71, p > 0.05$
Test	$F (1, 175) = 31.04, p < 0.05$	Test	$F (1, 175) = 12.6, p < 0.05$
Group x Test	$F (2, 175) = 0.10, p > 0.05$	Group x Test	$F (2, 175) = 2.13, p > 0.05$
[3]Group	$F (2, 85) = 5.07, p < 0.05$	[4]Group	$F (2, 85) = 5.49, p < 0.05$
Test	$F (1, 175) = 0.06, p > 0.05$	Test	$F (1, 175) = 0.11, p > 0.05$
Group x Test	$F (2, 175) = 0.03, p > 0.05$	Group x Test	$F (2, 175) = 1.89, p > 0.05$
[5]Group	$F (2, 85) = 3.46, p < 0.05$		
Test	$F (1, 175) = 0.44, p > 0.05$		
Group x Test	$F (2, 175) = 3.31, p < 0.05$		

Note: Means in a column/row sharing subscripts are significantly different from each other.
*Significant group difference between the NEG and the AEG/YEG on the pretest and posttest.

Habitual Physical Activity

Habitual physical activity was evaluated with the Baecke Habitual Physical Activity questionnaire before and after the study to determine if the physical activity that the subjects participated in outside of class changed throughout the study period.   This questionnaire measures physical activity at work, sport during leisure time, and physical activity during leisure time excluding sport. A total habitual physical activity score was calculated from the sum of the work, sport, and leisure indices. There were no group or test differences for total physical activity or for any of its subcategories indicating that the habitual physical activity did not change over the study period for all test subjects. Table 4 details the mean ± SEM for the three groups and for all participants combined on the pretest and posttest assessments of total habitual physical activity and its three subcategories.

Table 4

*Habitual Physical Activity (*N = 88)*

	Aerobic ($n = 30$)	Yoga ($n = 29$)	Non-exercise ($n = 29$)	All Participants
Physical Activity at Work[1]				
Pre	3.06 ± 0.11	3.02 ± 0.10	2.96 ± 0.12	3.02 ± 0.11
Post	3.06 ± 0.11	3.01 ± 0.11	2.98 ± 0.15	3.02 ± 0.13
Sport During Leisure Time[2]				
Pre	2.28 ± 0.16	2.34 ± 0.14	2.49 ± 0.17	2.37 ± 0.16
Post	2.31 ± 0.11	2.47 ± 0.12	2.64 ± 0.15	2.47 ± 0.13
Physical Activity During Leisure Time Excluding Sport[3]				
Pre	2.61 ± 0.13	2.64 ± 0.12	2.80 ± 0.16	2.68 ± 0.13
Post	2.33 ± 0.11	2.69 ± 0.12	2.54 ± 0.13	2.52 ± 0.12
Total Habitual Physical Activity[4]				
Pre	7.95 ± 0.28	8.00 ± 0.23	8.25 ± 0.31	8.07 ± 0.28
Post	7.70 ± 0.25	8.17 ± 0.25	8.16 ± 0.30	8.01 ± 0.27

[1]Group — $F_{(2, 85)} = 0.18, p > 0.05$
Test — $F_{(1, 175)} = 0.00, p > 0.05$
Group x Test — $F_{(2, 175)} = 0.03, p > 0.05$

[2]Group — $F_{(2, 85)} = 1.20, p > 0.05$
Test — $F_{(1, 175)} = 1.64, p > 0.05$
Group x Test — $F_{(2, 175)} = 0.19, p > 0.05$

[3]Group — $F_{(2, 85)} = 1.15, p > 0.05$
Test — $F_{(1, 175)} = 3.79, p > 0.05$
Group x Test — $F_{(2, 175)} = 1.74, p > 0.05$

[4]Group — $F_{(2, 85)} = 0.64, p > 0.05$
Test — $F_{(1, 175)} = 0.70, p > 0.05$
Group x Test — $F_{(2, 175)} = 0.48, p > 0.05$

Chronic Biological Data Results

Chronic biological measurements for SIgA concentration and secretion, SAA concentration and secretion, and SFR were evaluated at the pretest, midpoint (6 weeks), and posttest evaluation (12 weeks). SIgA was chosen because it is a common biological marker evaluated in mucosal immune research. Whereas, SAA was included in this research study because it is a relatively new anti-microbial marker that is being evaluated in mucosal immune research. Finally, SFR was monitored to evaluate flow rate changes following exercise and because it is necessary for the calculation of SIgA and SAA secretion rates.

Salivary Immunoglobulin A.

There were no group or test differences for SIgA concentration between the pretest, midpoint test (6 weeks), and posttest evaluations (12 weeks). The coefficient of variation for SIgA was 15% at 174.45 (n = 12).

The SIgA secretion had significant test differences ($p < 0.01$). Post hoc analysis of the test factor revealed lower SIgA secretion at the pretest compared to the midpoint test and pretest differences compared to the posttest for all participants combined. Table 5 details the mean ± SEM for the chronic measures of SIgA concentration and secretion for each group and a total for all participants combined.

Table 5

*Chronic Biological Data for SIgA Concentration and Secretion (*N = *93)*

	SIgA Concentration ($\mu g \cdot mL^{-1}$)[1]				SIgA Secretion ($\mu g \cdot min^{-1}$)[2]			
	Aerobic (*n* = 32)	Yoga (*n* = 29)	Non-exercise (*n* = 32)	All Partici-pants	Aerobic (*n* = 32)	Yoga (*n* = 29)	Non-exercise (*n* = 32)	All Partici-pants
Pretest								
Mean	144.46	153.38	172.37	156.84	70.10	73.79	49.34	64.11ab
SEM	14.36	20.67	36.4	23.91	7.07	12.78	7.06	8.85
Midpoint								
Mean	184.32	214.91	167.17	187.96	87.27	88.69	75.09	83.52a
SEM	22.62	41.47	21.15	27.99	9.70	15.89	16.69	14.04
Post Test								
Mean	148.94	186.56	140.12	157.64	87.87	118.71	66.75	90.22b
SEM	11.46	25.44	16.57	17.58	9.60	18.08	11.28	12.82

[1]Group	$F_{(2, 90)} = 0.72, p > 0.05$	[2]Group	$F_{(2, 90)} = 2.49, p > 0.05$
Test	$F_{(2, 180)} = 1.99, p > 0.05$	Test	$F_{(2, 180)} = 5.97, p < 0.05$
Group x Test	$F_{(4, 180)} = 0.76, p > 0.05$	Group x Test	$F_{(4, 180)} = 1.16, p > 0.05$

Note: Means in a column sharing subscripts are significantly different from each other.

Salivary Alpha Amylase.

There was a significant group x test interaction for SAA concentration ($p < 0.01$). Post hoc analysis revealed a significant difference between the pretest and posttest for all participants combined. The coefficient of variation for SAA was 3.3% at 146.47 (n = 11).

Regarding SAA secretion, group x test significance was revealed ($p = 0.02$) but further testing resulted in no significant differences. An inspection of the group specific means demonstrated some variation however; the mean secretion rates for all participants combined remained relatively similar at the three test points. Table 6 details the means ± SEM for the chronic measures of SAA concentration and secretion for each group and a total for all participants combined.

Table 6

Chronic Biological Data for SAA Concentration and Secretion (N = 93)

	SAA Concentration(U · mL^{-1})[1]				SAA Secretion (U · min^{-1})[2]			
	Aerobic *(n = 32)*	Yoga *(n = 29)*	Non-exercise *(n = 32)*	All Partici-pants	Aerobic *(n = 32)*	Yoga *(n = 29)*	Non-exercise *(n = 33)*	All Partici-pants
Pretest								
Mean	47.45	54.77	44.90	48.86$_a$	34.28	26.26	16.49	25.66
SEM	7.61	8.53	8.21	8.10	8.46	4.29	3.41	5.42
Midpoint								
Mean	50.19	29.07	58.93	46.61	28.75	15.51	35.37	26.90
SEM	6.55	4.20	9.33	6.77	4.99	2.45	9.69	5.82
Post Test								
Mean	40.05	33.09	46.44	40.08$_a$	29.68	20.80	22.79	24.54
SEM	5.78	6.28	7.39	6.49	5.39	4.02	4.90	4.79

[1]Group	$F_{(2, 90)} = 0.86, p > 0.05$	[2]Group	$F_{(2, 90)} = 1.25, p > 0.05$
Test	$F_{(2, 180)} = 2.46, p > 0.05$	Test	$F_{(2, 180)} = 0.16, p > 0.05$
Group x Test	$F_{(4, 180)} = 3.87, p < 0.05$	Group x Test	$F_{(4, 180)} = 3.06, p < 0.05$

Note: Means in a column sharing subscripts are significantly different from each other.

Saliva Flow Rate.

The SFR was calculated by dividing the volume of saliva by the saliva collection time of four minutes. Significant test differences were observed for SFR (p = 0.01). Post hoc analysis of the test factor revealed significantly lower SFR at the pretest compared to the posttest for all participants combined. A trend (p = 0.07) toward significance was observed for the group factor. Table 7 details the means ± SEM for the SFR for each group and a total for all participants combined.

Table 7

Chronic Biological Data for Saliva Flow Rate (N = 93)

Saliva Flow Rate (mL· min^{-1})

		Aerobic *(n = 32)*	Yoga *(n = 29)*	Non-exercise *(n = 32)*	All Participants
Pretest					
	Mean	0.58	0.52	0.37	0.49$_a$
	SEM	0.06	0.06	0.05	0.06
Midpoint					
	Mean	0.59	0.58	0.46	0.54
	SEM	0.06	0.08	0.08	0.07
Post Test					
	Mean	0.63	0.67	0.49	0.59$_a$
	SEM	0.06	0.06	0.07	0.06
Group	$F_{(2,\ 90)} = 2.70, p > 0.05$				
Test	$F_{(2,\ 180)} = 4.56, p < 0.05$				
Group x Test	$F_{(4,\ 180)} = 0.48, p > 0.05$				

Note: Means in a column sharing a subscript are significantly different from each other.

Acute Biological Results

The SIgA concentration and secretion, SAA concentration and secretion, and the SFR were measured prior to the onset of each class and again 50 minutes later to determine the changes in the acute biological measurements.

Salivary Immunoglobulin A.

SIgA concentration increased from pretest to posttest for the AEG and the YEG. Specifically, statistical analysis found significant test differences ($p < 0.01$) and significant group x test interaction ($p = 0.04$) for SIgA concentration. Post hoc analysis of the test factor revealed increased SIgA concentration at the posttest compared to the pretest. A simple main effects analysis of the interaction revealed that the AEG and the YEG differed at these test points.

There were significant test differences ($p < 0.01$) for SIgA secretion. Post hoc analysis of the test factor revealed increased SIgA secretion at the posttest compared to the pretest for all participants combined. Table 8 details the averages ± SEM for the acute measures of the SIgA concentration and secretion for each group and a total for all participants combined.

Table 8

*Acute Biological Data for SIgA Concentration and Secretion (*N = *129)*

Test	SIgA Concentration ($\mu g \cdot mL^{-1}$)[1]				SIgA Secretion ($\mu g \cdot min^{-1}$)[2]			
	Aerobic ($n = 41$)	Yoga ($n = 52$)	Non-Exercise ($n = 36$)	All Partici-pants	Aerobic ($n = 41$)	Yoga ($n = 52$)	Non-Exercise ($n = 36$)	All Partici-pants
Pretest								
Mean	157.22$_a$	145.65$_a$	166.00	155.01	79.74	69.94	52.13	68.08$_a$
SEM	17.12	16.30	32.56	21.10	7.55	9.66	7.07	8.27
Post Test								
Mean	196.06$_a$	221.18$_a$	159.55	196.01	99.75	97.80	72.26	91.59$_a$
SEM	28.64	25.73	18.07	24.52	11.98	9.17	12.90	11.10
[1]Group	$F (2, 127) = 0.25, p > 0.05$				[2]Group	$F (2, 127) = 2.38, p > 0.05$		
Test	$F (1, 126) = 7.32, p < 0.05$				Test	$F (1, 126) = 17.4, p < 0.05$		
Group x Test	$F (2, 126) = 3.26, p < 0.05$				Group x Test	$F (2, 126) = 0.30, p > 0.05$		

Note: Means in a column sharing subscripts are significantly different from each other.

Salivary Alpha Amylase.

No significant tests or group differences for the acute measurement for SAA concentration were observed. Directionally, the AEG was the only group to demonstrate a mean increase in SAA concentration. The YEG and NEG demonstrated decreases in the SAA concentration including an overall decrease for all participants combined.

Similar results were observed for SAA secretion. No significant test or group differences were noted and the pattern of change was similar to what was observed in the SAA concentration.   Table 9 details the averages ± SEM for the acute measures of SAA concentration and secretion for each group and a total for all participants combined.

Table 9

*Acute Biological Data for SAA Concentration and Secretion (*N = *129)*

	SAA Concentration $(U \cdot mL^{-1})^{1}$				SAA Secretion $(U \cdot min^{-1})^{2}$			
Test	Aerobic ($n = 41$)	Yoga ($n = 52$)	Non-Exercise ($n = 36$)	All Partici-pants	Aerobic ($n = 41$)	Yoga ($n = 52$)	Non-Exercise ($n = 36$)	All Partici-pants
Pretest								
Mean	52.64	49.71	61.30	53.88	13.16	12.43	22.66	15.52
SEM	6.85	6.56	13.88	8.69	1.71	1.64	6.45	3.00
Post Test								
Mean	59.96	46.82	52.22	52.50	14.99	11.71	13.06	13.13
SEM	6.66	6.48	7.16	6.73	1.67	1.62	1.79	1.68

[1]Group	$F (2, 127) = 0.65, p > 0.05$	[2]Group	$F (2, 127) = 2.10, p > 0.05$
Test	$F (1, 126) = 0.09, p > 0.05$	Test	$F (1, 126) = 1.80, p > 0.05$
Group x Test	$F (2, 126) = 0.55, p > 0.05$	Group x Test	$F (2, 126) = 2.32, p > 0.05$

Saliva Flow Rate.

The acute SFR had significant group differences (p = 0.049). Post hoc analysis of the group factor revealed lower SFR for the NEG compared to the AEG on pretest and posttest. Table 10 details the means ± SEM for the SFR for each group and a total for all participants combined.

Table 10

Acute Biological Data for Saliva Flow Rate (N = 129)

Saliva Flow Rate (mL· min^{-1})

		Aerobic$_a$ (n = 41)	Yoga (n = 52)	Non-exercise$_a$ (n = 36)	All Participants
Pretest					
	Mean	0.64	0.58	0.40	0.55
	SEM	0.06	0.06	0.05	0.06
Post Test					
	Mean	0.64	0.59	0.47	0.57
	SEM	0.06	0.06	0.05	0.06
Group	$F_{(2, 127)} = 3.09$, $p < 0.05$				
Test	$F_{(2, 180)} = 1.29$, $p > 0.05$				
Group x Test	$F_{(4, 180)} = 1.19$, $p > 0.05$				

Note: Group names sharing a subscript are significantly different from each other.

Symptom, Severity, and Incidence of URTI

The Wisconsin Upper Respiratory Symptom Survey (WURSS) is a survey that measures the health-related symptoms that are negatively affected by the common cold. The three indices evaluated by the survey include the symptoms, severity, and incidences of upper respiratory infection. Additionally, the number of days with symptoms was tabulated for each incidence.

Pearson's chi-square analysis revealed significant differences between observed and expected scores for the symptom score in the YEG and the NEG. Specifically, the observed symptom score for the YEG was significantly lower (O = 66.87; E = 116.03) than the expected symptom score. On the other hand, the observed symptom score was significantly higher than expected for the NEG (O = 185.99; E = 131.5). Non-significant differences were noted for the other variables including incidences of infection, severity of symptoms, and numbers of days sick with symptoms. The highest number of incidences was recorded for the NEG (n = 39) while

the YEG reported the fewest number of incidences (n = 27). The number of days sick with symptoms was highest with the YEG (n = 6.73) and lowest for the NEG (n = 5.19). Finally the severity score was similar for the AEG and the YEG (108.59 and 108.15, respectively) and 93.73 for the NEG. Table 11 details the observed and expected scores for each category on the WURSS survey and for the number of days sick with symptoms.

Table 11

WURSS Data (N = 103)

	Aerobic (n = 39)	Yoga (n = 30)	Non-exercise (n = 34)		
	n	*n*	*n*	$\chi^2(1)$	*p*
Incidences					
Observed	30.00	27.00	39.00		
Expected	36.00	28.00	32.00	2.83	0.243
Number of Days Sick					
Observed	5.87	6.73	5.19		
Expected	6.74	5.18	5.87	0.65	0.721
Severity Score					
Observed	108.59	108.15	93.73		
Expected	117.59	90.43	102.49	4.91	0.086
Symptom Score					
Observed	145.50	66.87$_a$	185.99$_b$		
Expected	150.84	116.03$_a$	131.50$_b$	43.60	< 0.001

Note: Subscripts sharing a column indicates significant difference in observed and expected scores.

Profile of Mood States

The POMS survey was administered pre intervention and post intervention for the acute and chronic study to evaluate changes in mood state following participation in the aerobic, yoga, and first aid class.

Chronic Mood State.

For the chronic assessment of mood state, the POMS questionnaire was administered pretest, at the midpoint (week six), and at the end of the study (12 weeks). The POMS survey was administered at the beginning of each group's activity or lecture. No significant differences were noted for the total mood disturbance score or for its subcategories with the exception of fatigue-inertia. Significant test differences were found for fatigue-inertia. Post hoc analysis of the significant test differences for fatigue-inertia revealed that the posttest was significantly lower than the pretest and that the posttest was significantly lower than the midpoint test for all participants combined. Table 12 details the mean ± SEM for the six chronic POMS categories and for the total mood disturbance score for all groups and a total for all participants combined.

Table 12

*Chronic POMS Results (*N = 85)

		Aerobic (*n* = 32)	Yoga (*n* = 22)	Non-exercise (*n* = 31)	All Participants
Tension-Anxiety[1]					
	Pre	8.64 ± 1.00	9.96 ± 1.37	9.00 ± 1.17	9.04 ± 1.11
	Midpoint	9.50 ± 1.30	8.95 ± 1.10	9.23 ± 1.25	9.18 ± 1.23
	Post	8.53 ± 1.15	7.09 ± 1.21	8.81 ± 1.16	8.3 ± 1.17
Depression-Dejection[2]					
	Pre	7.31 ± 1.78	9.04 ± 1.95	9.20 ± 1.81	8.62 ± 1.81
	Midpoint	8.69 ± 1.85	5.82 ± 1.56	7.68 ± 1.78	7.58 ± 1.75

		Aerobic ($n = 32$)	Yoga ($n = 22$)	Non-exercise ($n = 31$)	All Participants
	Post	6.06 ± 1.39	5.68 ± 1.14	8.13 ± 1.86	6.72 ± 1.50
Anger-Hostility[3]					
	Pre	6.92 ± 1.25	9.23 ± 1.91	8.34 ± 1.37	8.03 ± 1.45
	Midpoint	8.84 ± 1.97	8.05 ± 1.56	8.39 ± 1.64	8.47 ± 1.74
	Post	6.94 ± 1.49	7.05 ± 1.50	9.42 ± 1.73	7.87 ± 1.58
Vigor-Activity[4]					
	Pre	15.58 ± 1.01	15.38 ± 1.16	15.34 ± 1.25	15.09 ± 1.07
	Midpoint	14.38 ± 1.29	12.55 ± 1.55	15.42 ± 1.20	14.29 ± 1.32
	Post	15.69 ± 1.21	14.36 ± 1.39	13.61 ± 1.05	14.58 ± 1.20
Fatigue-Inertia[5]					
	Pre	6.42 ± 0.87	7.69 ± 1.25	6.2 ± 0.84	6.69 ± 0.93[a]
	Midpoint	6.16 ± 1.08	8.36 ± 1.42	6.16 ± 1.09	6.73 ± 1.17[b]
	Post	4.88 ± 0.89	5.77 ± 1.13	5.26 ± 0.89	5.25 ± 0.95[ab]
Confusion-Bewilderment[6]					
	Pre	5.50 ± 0.77	7.35 ± 1.06	6.57 ± 0.71	6.44 ± 0.80
	Midpoint	6.97 ± 0.82	6.73 ± 1.00	6.97 ± 0.95	6.91 ± 0.91
	Post	5.69 ± 0.67	6.09 ± 0.66	6.42 ± 0.81	6.07 ± 0.72
Total Mood Disturbance[7]					
	Pre	19.19 ± 5.47	27.88 ± 7.71	23.97 ± 5.93	23.24 ± 6.58
	Midpoint	25.78 ± 7.08	25.36 ± 6.03	23.00 ± 6.47	24.66 ± 6.59
	Post	16.41 ± 5.13	17.32 ± 4.70	24.42 ± 6.19	19.56 ± 5.41

[1]Group $F_{(2, 94)} = 0.02, p > 0.05$ [2]Group $F_{(2, 94)} = 0.14, p > 0.05$
Test $F_{(2, 164)} = 1.56, p > 0.05$ Test $F_{(2, 164)} = 1.16, p > 0.05$
Group x Test $F_{(4, 164)} = 0.65, p > 0.05$ Group x Test $F_{(4, 164)} = 0.93, p > 0.05$

[3]Group $F_{(2, 94)} = 0.17, p > 0.05$ [4]Group $F_{(2, 94)} = 0.42, p > 0.05$
Test $F_{(2, 164)} = 0.28, p > 0.05$ Test $F_{(2, 164)} = 0.81, p > 0.05$
Group x Test $F_{(4, 164)} = 0.74, p > 0.05$ Group x Test $F_{(4, 164)} = 1.57, p > 0.05$

[5]Group $F_{(2, 94)} = 1.01, p > 0.05$ [6]Group $F_{(2, 94)} = 0.31, p > 0.05$
Test $F_{(2, 164)} = 4.68, p < 0.05$ Test $F_{(2, 164)} = 1.83, p > 0.05$
Group x Test $F_{(4, 164)} = 0.45, p > 0.05$ Group x Test $F_{(4, 164)} = 0.95, p > 0.05$

[7]Group $F_{(2, 94)} = 0.16, p > 0.05$
Test $F_{(2, 164)} = 1.45, p > 0.05$
Group x Test $F_{(4, 164)} = 0.83, p > 0.05$

Note: Means in a column sharing subscripts are significantly different from each other.

Acute Mood State.

Comparing mood state at the onset of class with mood state 50 minutes later, analysis of the acute POMS data revealed significant test score decreases (indicating improved mood state) for all measured mood states except for the vigor-activity subscale for all participants combined,. The categories that registered significance included: tension-anxiety, depression-dejection, anger-hostility, fatigue-inertia, confusion-bewilderment, and total mood disturbance. Post hoc analysis of the mood states that had significant test differences revealed test differences for all participants between the pretest and the posttest. Mean values for vigor-activity increased for the AEG and YEG while the NEG reported decreases in vigor-activity. Table 13 details the mean ± SEM for the six acute POMS categories and for the total mood disturbance for all groups and a total for all participants combined.

Table 13

*Acute POMS Results (*N = 97)

		Aerobic (*n* = 36)	Yoga (*n* = 26)	Non-exercise (*n* = 35)	All Participants
Tension-Anxiety[1]					
	Pre	8.64 ± 1.00	9.96 ± 1.37	9.00 ± 1.17	9.12 ± 1.16[a]
	Post	5.36 ± 0.72	4.88 ± 1.21	5.97 ± 1.10	5.45 ± 0.99 [a]
Depression-Dejection[2]					
	Pre	7.31 ± 1.78	9.04 ± 1.95	9.20 ± 1.81	8.46 ± 1.84[b]
	Post	2.72 ± 1.15	3.96 ± 1.39	5.66 ± 1.73	4.11 ± 1.42 [b]
Anger-Hostility[3]					
	Pre	6.92 ± 1.25	9.23 ± 1.91	8.34 ± 1.37	8.05 ± 1.47[c]
	Post	2.58 ± 0.85	4.65 ± 1.28	4.86 ± 1.28	3.97 ± 1.12 [c]
Vigor-Activity[4]					
	Pre	15.58 ± 1.01	15.38 ± 1.16	15.34 ± 1.25	15.44 ± 1.14
	Post	16.19 ± 1.07	15.69 ± 1.27	14.09 ± 1.25	15.30 ± 1.19
Fatigue-Inertia[5]					
	Pre	6.42 ± 0.87	7.69 ± 1.25	6.2 ± 0.84	6.68 ± 0.96[d]
	Post	5.47 ± 0.90	3.31 ± 0.87	3.54 ± 0.87	4.19 ± 0.88 [d]
Confusion-Bewilderment[6]					
	Pre	5.50 ± 0.77	7.35 ± 1.06	6.57 ± 0.71	6.38 ± 0.83[e]
	Post	4.44 ± 0.76	5.04 ± 0.88	4.97 ± 0.83	4.79 ± 0.82 [e]
Total Mood Disturbance[7]					
	Pre	19.19 ± 5.47	27.88 ± 7.71	23.97 ± 5.93	23.24 ± 6.58[f]
	Post	4.39 ± 3.97	6.15 ± 5.82	10.91 ± 5.74	7.21 ± 5.10[f]

[1]Group	$F_{(2, 94)} = 0.08, p > 0.05$	[2]Group	$F_{(2, 94)} = 0.72, p > 0.05$
Test	$F_{(1, 94)} = 37.34, p < 0.05$	Test	$F_{(1, 94)} = 27.69, p < 0.05$
Group x Test	$F_{(2, 94)} = 0.98, p > 0.05$	Group x Test	$F_{(2, 94)} = 0.29, p > 0.05$
[3]Group	$F_{(2, 94)} = 1.01, p > 0.05$	[4]Group	$F_{(2, 94)} = 0.36, p > 0.05$
Test	$F_{(1, 94)} = 38.98, p < 0.05$	Test	$F_{(1, 94)} = 0.03, p > 0.05$
Group x Test	$F_{(2, 94)} = 0.25, p > 0.05$	Group x Test	$F_{(2, 94)} = 0.98, p > 0.05$
[5]Group	$F_{(2, 94)} = 0.48, p > 0.05$	[6]Group	$F_{(2, 94)} = 0.69, p > 0.05$
Test	$F_{(1, 94)} = 26.80, p < 0.05$	Test	$F_{(1, 94)} = 14.87, p < 0.05$
Group x Test	$F_{(2, 94)} = 3.60, p > 0.05$	Group x Test	$F_{(2, 94)} = 0.68, p > 0.05$
[7]Group	$F_{(2, 94)} = 0.39, p > 0.05$		
Test	$F_{(1, 94)} = 33.84, p < 0.05$		
Group x Test	$F_{(2, 94)} = 0.79, p > 0.05$		

Note: Means in a column sharing subscripts are significantly different from each other.

This chapter presented analyses of the data for the chronic and acute measurements of dependent variables including age, height, total kilocalories, and kilocalories for carbohydrate, lipid, and protein; physical and anthropometric assessments of BMI, waist circumference, percent fat, weight, sit-and-reach flexibility measurements, recovery heart rate, and VO_{2max}; habitual physical activity scores for work, leisure, sport, and a total activity score; mucosal markers for the concentration and secretion of SIgA and SAA, including SFR; incidence, severity, symptoms, and days sick with symptoms of URTI; and six different mood states in addition to a total mood disturbance score for the AEG, YEG, and NEG participants that were evaluated in this study.

CHAPTER 5
DISCUSSION

Mucosal immune research, an area of concentration in exercise immunology, has focused on changes in salivary immunoglobulin A (SIgA) because it is recognized as the dominate antibody found in the airway and considered to be the body's first line of defense against URTI (Bishop & Gleeson, 2009). The role chronic and acute exercise may have in altering antimicrobial proteins and the influence these proteins may have on URS is an interest of exercise immunologists (Walsh et al., 2011). Generally, people participating in chronic moderate exercise believe a benefit of exercise is enhanced immunity (Simon, 1984). However, the evidence is inconclusive because some investigations support this idea (Akimoto et al., 2003; Klentrou et al., 2002) while others do not (Mackinnon, 1999, 2000). Additionally, yoga, a form of low intensity exercise that is increasing in popularity has not been evaluated for its effect on mucosal immunity (Field, 2011).

Research on the acute effects of vigorous exercise has resulted in a consensus (Walsh et al., 2011) that SIgA decreases immediately following acute high intensity exercise (Fahlman et al., 2001; Pacque et al., 2007). Conflicting results on the effect of acute moderate aerobic exercise or low intensity exercise on mucosal markers have been reported (McDowell et al., 1991; Nieman et al., 2005; Sakamoto et al., 2005), and is a question deserving more research.

To broaden the understanding of mucosal immunity, investigators have begun to evaluate other microbial markers, beyond SIgA, with greater interest (Walsh et al., 2011). As an additional mucosal marker, this study evaluated SAA because of its ability to prevent the adherence and growth of certain bacteria in the airway (Arhakis et al., 2013; He, Bishop, Handzlik, Muhamad, & Gleeson, 2014). Previous research that assessed SAA following acute exercise has suggested that its level increases with exercise intensity (Allgrove et al., 2012; Li &

Gleeson, 2004; West et al., 2006). Limited data is available on chronic exercise and SAA in a college population and warrants additional research (Berndt, Strahler, Kirschbaum, & Rohleder, 2012).

The link between exercise and mood state may be important in protecting against URTI. Since negative mood states can contribute to making a person more vulnerable to URTI (Cohen et al., 1991) and since moderate and low intensity exercise improve mood states (Berger & Owen, 1983; Toskovic, 2001; Yoshihara, Hiramoto, Oka, Kubo, & Sudo, 2014), a final outcome may be enhanced mucosal immunity.

The mucosal immune response to exercise and the change in URS associated with exercise have been studied across a wide range of study populations (Akimoto et al., 2003; Nehlsen-Cannarella et al., 2000; Novas, Rowbottom, & Jenkins, 2003). Limited mucosal research has focused on college students enrolled in a chronic exercise program of moderate and low intensity exercise. Therefore, the aims of this study were to evaluate the acute and chronic effects of SIgA, SAA, URS, and mood state associated with students participating in a college based aerobic exercise class, yoga exercise class, and non-exercise class that meets two times per week.

Baseline Evaluations and Physical Assessments

At the beginning of the study, no significant differences in age, height, total kilocalorie intake, and intake of kilocalories for carbohydrate, lipid, and protein were found among the three study groups. Additionally, there were no significant differences among groups in weight, BMI, waist circumference, shoulder flexibility, and habitual physical activity. Differences in groups were observed at the study's onset for percent fat, sit-and-reach flexibility, predicted VO_{2max}, and recovery heart rate following the Queens College Step test. It is likely that these differences may

be attributed to the larger number of males in the NEG (n = 17 males) compared the AEG (n = 4 males) and the YEG (n = 8) as males typically score higher on VO_{2max}, lower on sit-and-reach tests, and have less body fat (ACSM, 2005).

Anthropometric Measurements.

At the end of this study, participants were posttested for anthropometric measurements of BMI, waist circumference, percent fat, and weight. Small but significant decreases were noted for waist circumference for all participants combined. As expected, non-significant changes in BMI, percent fat, and weight were measured with this cohort. College students are often motivated to enroll in an exercise program in an effort to alter body composition. Many are unaware that it is difficult to improve anthropometric characteristics by following an exercise program without also making dietary modifications (Miller, Koceja, & Hamilton, 1997). These results agree with similar research on college students engaged in moderate and low intensity exercise programs (Boyle, Mattern, Lassiter, & Ritzler, 2011).

Habitual Physical Activity Questionnaire.

Subject's habitual physical activity was estimated on the basis of scores from the questionnaire by Baecke et al. (Baecke, Burema, & Frijters, 1982) which were obtained pre intervention and post intervention. No differences were observed for either estimated total habitual physical activity level or for any of its three categories (work, sport, and leisure). Thus, the results of the Baecke Habitual Physical Activity Questionnaire confirmed that subjects maintained their usual physical activity level outside of class. Based on available normative data, study participants were a sedentary population (Baecke et al., 1982; Engels & Wirth, 1997; Guezennec et al., 1998).

Flexibility Testing.

Significant mean group differences in sit-and-reach flexibility for the AEG and YEG compared to the NEG were noted at the study's onset and were maintained throughout the study as the same group significance was found during the posttest.

This study recorded significant improvements in the flexibility of the right (+ 2.5%) and left (+ 1.7%) shoulder for all participants combined between the pretest and the posttest. Though specific group significance was not noted, each cohort did improve between the pretest and the posttest. Improvements in flexibility are expected for yoga participants (Vogler, O'Hara, Gregg, & Burnell, 2011) and have also been observed in participants of a moderate aerobic exercise program (Arazi, Faraji, Moghadam, & Samadi, 2011; D'Alonzo, Stevenson, & Davis, 2004) but flexibility improvements would not be expected for subjects in the NEG and may be attributed to a learning effect (Campbell & Stanley, 1963).

Predicted VO$_{2max}$.

Predicted VO$_{2max}$ values would be expected to improve as the exercise intensity and duration of a training program increase. This study collaborates prior findings that yoga exercise is not intense enough to improve cardiorespiratory endurance (Chaya et al., 2006; Clay et al., 2005; Field, 2011; Gharote & Ganguly, 1979). By contrast, this study noted significantly improved recovery heart rates following the Queens College Step test in the AEG indicating improvement in cardiorespiratory endurance and non-significant mean increases for VO$_{2max}$ in the AEG of +1.85 ml/kg $\cdot$ min^{-1} or +5.1%. This study's results provide support for an aerobic exercise program of moderate intensity, practiced two days per week may improve cardiorespiratory endurance in college students. This may be beneficial since exercise classes at colleges meet twice per week.

In summary, subjects were exposed to the same college environment and shared similar baseline characteristics and fitness parameter changes as is typically observed in an exercise program of moderate and low intensity (ACSM, 2005). Though there were some differences in groups at the onset of the study, these differences may be explained by the larger number of males in the NEG compared to the AEG and the YEG. This study used a quasi-experimental design where subjects self-selected to the treatment groups. This is the current practice of college students when selecting classes and may be reflective of the typical college class population (Cholewa & Irwin, 2008).

Chronic Biological Results and URTI

This section describes changes in the concentration and secretion rates of SIgA and SAA at the midpoint and end of the 12 week study period. Additionally, the results for SFR and WURSS are discussed.

Salivary Immunoglobulin A.

This study found notable mean differences in SIgA concentration. When comparing the cohorts at two time points, the AEG realized increases from pretest to midpoint of +27.6% but returned within +3% of baseline on the posttest. The YEG realized increases in SIgA concentration of +40.1% between pretest to midpoint and ended the study +21.6% above baseline values. The NEG exhibited inverse results to the AEG and YEG documenting a –3% decrease from pretest to midpoint and an overall decrease in SIgA concentration from baseline to posttest of -19%. Though these results were not significant, they are meaningful because elevated levels of SIgA result in fewer URTI and lower concentrations are associated with a greater number of infections (Cunningham-Rundles, 2001; Walsh et al., 2011).

The present study echoes similar results to a study that was conducted for 16 weeks with subjects that exercised aerobically at a moderate intensity, three days per week for 45 minutes producing no significant changes in the SIgA concentration (Martins et al., 2009). The exercise group did not experience the same decrease in SIgA concentration as seen with the control group. Martins and colleagues (2009) concluded that regular aerobic exercise may protect against the deterioration in SIgA concentration levels that were observed in the control group.

In contrast, another study reported a significant 36.5% increase in SIgA concentration following a 12 week moderate intensity exercise program that met three times per week (Klentrou et al., 2002). Similarly, a study following 12 months of moderate exercise training found significant increases in the SIgA concentration of 36.8% (Akimoto et al., 2003). A study of Qigong exercise with college students who exercised independently for a year, noted significant increases in SIgA concentration of 48.2% (Bayat-Movahed et al., 2008). However, a limitation of the Bayat-Movahed et al. (2008) and Akimoto et al. (2003) studies is that they did not have a control group. Also, the subjects in the Akimoto et al. (2003) study were older (mean age = 64.9) and the immune system of the elderly may be weaker and may more readily respond to exercise training (Haaland et al., 2008; Lowenthal, Kirschner, Scarpace, Pollock, & Graves, 1994; Walsh et al., 2011). Researchers have suggested that the discrepancy in studies on SIgA and exercise may be related to the different fitness level of subjects, the intensity and duration of the exercise, and the habitual physical activity level of subjects (Akimoto et al., 2003; MacKinnon & Jenkins, 1993; Nehlsen-Cannarella et al., 1991).

Current mucosal research on SIgA regularly reports SIgA as a secretion rate because a change in SFR may alter levels of SIgA concentration (Kugler et al., 1992). SIgA secretion

indicates the total amount of protein available on the mucosal surface (Bishop & Gleeson, 2009; Blannin et al., 1998; Mackinnon, 1999).

Regarding SIgA secretion changes, significant increases were noticed for all participants combined from pretest to midpoint test (+30.3%) and from pretest to posttest (+40.7%). Specifically, the mean increase in SIgA secretion was highest for the YEG from pretest to posttest (YEG = 60.9%, NEG = 35.5%, AEG = 25.4%). This result is similar to a study by Sloan and colleagues (2013) that found significant increases in SIgA secretion in postmenopausal women participating in a moderate walking exercise program, five days per week for 12 weeks.

These SIgA secretion rate increases appear to be attributed to significant SFR increases, as the changes in SIgA concentration from pretest to posttest were only + 0.5%. SFR for the same time period increased +20.4% and were significant for all participants combined. SIgA secretion increases can be caused by an increase in the absolute SIgA concentration or by an increase in SFR. For example, the increases in the secretion of SIgA in Akimoto's et al. (2003) study were attributed primarily to increases in SIgA concentration because no changes were recorded in SFR. However, other researchers have reported that the changes in the secretion of SIgA were attributed to changes in SFR (Mylona et al., 2002; Sloan et al., 2013). This is noteworthy because SFR may explain changes in antimicrobial proteins like SIgA and SAA (Chicharro et al., 1998).

The spectrum of variability for SIgA is high partly due to the numerous factors that influence it (Bishop & Gleeson, 2009) making it more challenging to establish statistical significance (Mackinnon, 2000). It is well known that SIgA is affected by a multitude of confounding variables including exposure to pathogenic viruses, sleep deprivation, diet, and stress. (Chandra, 1992; Konig et al., 2000).

Additionally, a combination of factors contributes to changes in SIgA. The parasympathetic and sympathetic nervous systems influence the rate of SIgA production by the plasma cells as well as its transcytosis (Walsh et al., 2011), exercise increases the release of immunomodulatory hormones (Mackinnon, 1999) and psychological stress may alter circulating levels of stress hormones which may contribute to SIgA changes (Mackinnon, 1999). Continued research is needed to elucidate the mechanisms of change for SIgA.

Salivary Alpha Amylase.

For SAA concentration, a significant decrease of -18% was reported for all participants combined between the pretest and the posttest. Specifically, the AEG and YEG experienced decreases in the means for SAA from pretest to posttest of -15.6% and -39.6% respectively, and the NEG reported an increase of +3.4%.

Previously, much of the research on SAA has been on its role as a marker of stress because its activity is stimulated by the sympathetic nervous system (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996). With recent interest in investigating additional antimicrobial markers beyond SIgA (Walsh et al., 2011), some research on the acute effects of exercise on SAA have been published (Allgrove et al., 2008; Leicht et al., 2011) but limited research is available on the chronic effects of exercise on SAA (Berndt et al., 2012). Determining if SAA has similar protective functions seen in SIgA is relevant because SAA accounts for $10 - 20\%$ of the total protein in saliva and has anti-bacterial properties (Arhakis et al., 2013; Leicht et al., 2011).

Regarding SAA secretion, non-significant changes were recorded for all cohorts and for all participants combined. The current recommendation (Salimetrics, 2012b) is to report SAA as a secretion rate in addition to its absolute value because SAA increases observed in stressful and non-stressful situations have been attributed primarily to SFR increases (Arhakis et al., 2013;

Bosch et al., 2003). SFR may be a confounding factor that modifies SAA concentration. As more research on the effects of chronic exercise on SAA concentration and secretion is published, the clinical relevance of these changes will become clear (Allgrove et al., 2012).

Saliva Flow Rate.

Between pre intervention and post intervention, significant increases in SFR of +20.4% were reported for all participants combined. Specifically, all groups increased from pretest to midpoint and continued to increase from midpoint to posttest. The SFR increases from pretest to posttest were +8.6%, +28.8%, and + 32.4% for the AEG, YEG, and NEG, respectively. Saliva has roles not only in preventing infection, but also in protecting and maintaining the integrity of the oral mucous membrane by promoting lubrication and soft tissue repair (Ben-Aryeh, Miron, Szargel, & Gutman, 1984). A reduction in SFR results in oral dryness and can increase the incidence of URTI (Fox, van der Ven, Sonies, Weiffenbach, & Baum, 1985). Protection from URTI may not only depend on SIgA but also on SFR (Lu, Ceddia, Price, Ye, & Woods, 1999). These results are similar to a study on elderly men and women that found significant increases in SFR following chronic moderate exercise training with no changes in SIgA concentration (Shimizu et al., 2007). The significant SFR increases observed with this study may be relevant for individuals suffering from xerostomia (Miletic et al., 1996; Sloan et al., 2013).

Incidence, Severity, and Symptoms of URTI.

Evaluation of the WURSS data found significantly lower symptom scores in the YEG compared to expected scores and significantly higher symptom scores in the NEG compared to expected scores. The two to five URTI the average adult has each year accounts for the majority of days missed at work and the majority of visits to a physician (Graham, 1990). A better understanding of the role exercise may have in preventing or lessening the severity of URTI

could contribute to decreasing health care costs and improving worker productivity.   A study that reported similar results as is reported for the YEG in this study found men and women who exercised moderately for 12 weeks had significantly fewer light symptoms of URTI compared to a control group (Klentrou et al., 2002).

The numbers of days sick with symptoms for the subjects in this study were 5.87, 6.73, and 5.19 for the AEG, YEG, and the NEG, respectively,which were not statistical significant. Previous research studies have reported significant differences in the number of days sick with symptoms (Nieman et al., 2011; Nieman et al., 1990).   Specifically, an exercise group that trained for 15 weeks, five days per week for 45 minutes per session had significantly fewer symptom days (3.6 ± 0.7) compared to a non-exercise group (7.0 ± 1.4 days) (Nieman et al., 1990).

This study recorded substantive reduction in incidences of infection in the exercise groups (AEG = 30 incidences; and YEG = 27 incidences) compared to the non-exercise group (NEG = 39 incidences), though these changes were not significant. Incidences were 30% lower in the AEG and 44% lower in the YEG compared to the NEG. A recent eight week investigation with 149 subjects that included a study group that meditated and a group that exercised moderately compared to a non-exercise control group reported incidences that were 29% lower in the exercise group (incidences = 26) and 39% lower in the meditation group (incidences = 27) compared to the non-exercise control group (incidences = 40) (Barrett et al., 2012). Another study, with similar results, reported non-significant differences of 5.3 ± 1.5 infections in a moderate exercise group and 6.3 ± 2.2 infections in a control group (n = 32) during a 16 week home based walking program (Sloan et al., 2013).

Additionally, non-significant differences were found in the severity score for the AEG and YEG, 108.59 and 108.15, respectively with the NEG reporting an overall severity score for the study period of 93.73. Although methodology varies, other exercise training studies have reported a reduction of URTI incidence or risk of 18 – 67% (Chubak et al., 2006; Ciloglu, 2005; Nieman et al., 2011).

The chronic SIgA results in conjunction with the WURSS results reported in this study suggest a strengthening of mucosal immunity with yoga exercise. The SIgA concentration and secretion for the YEG increased the greatest (21.6% and 60.9% respectively), compared to the AEG and the NEG, between the pretest and posttest. Similar findings demonstrated that a mindfulness meditation group experienced the greatest improvement in URS compared to an exercise group and a control group (Barrett et al., 2012). Studies often evaluate mucosal markers without collecting URS data (Akimoto et al., 2003; Bayat-Movahed et al., 2008; Martins et al., 2009) and others collect URS data without tracking mucosal markers (Barrett et al., 2012; Nieman et al., 2011). The few studies that report both variables reveal an inverse relationship between SIgA and URTI (Gleeson et al., 2012; Klentrou et al., 2002). Collectively, these results provide initial support for the hypothesis that a chronic low intensity physical activity program such as yoga may enhance mucosal immunity and decrease URS.

Acute Biological Results

This section describes acute changes in the concentration and secretion rates of SIgA and SAA between the pretest and posttest administered after 50 minutes of aerobic exercise, yoga exercise, or sitting in a first aid class.

Salivary Immunoglobulin A.

Comparing the outcomes of SIgA concentration to acute exercise, the AEG and YEG demonstrated significant increases following 50 minutes of the group's respective exercise program. No significant changes were observed for the NEG after sitting for 50 minutes in the first aid class. Early studies have reported decreases in SIgA following acute exercise at prolonged high intensity exercise (>1 hour) (Mackinnon, Chick, van As, & Tomasi, 1987; Nieman et al., 2002; Pistilli et al., 2002). Similar research at a much shorter duration (< 15 minutes) suggested that a long duration of high intensity exercise is not needed for mucosal immune markers to be depressed (Fahlman et al., 2001; McDowell et al., 1992). It has been proposed, that after an acute bout of aerobic exercise there is an "open window" period of impaired immunity (Walsh et al., 2011). The degree and duration of the immunosuppression is related to the intensity and duration of the exercise and the risk of infection from viruses and bacteria is increased during this time (Walsh et al., 2011).

While this study confirms some prior research, no conclusions can be reached about the link between acute exercise and SIgA. This current study's results agree with a study by Tharp (1991) that reported acute increases in SIgA concentration after playing basketball games and a study by Reid (2001) that reported increased SIgA concentration following 30 minutes of guided imagery. These results contradict research that did not find changes in SIgA concentrations in women walking on a treadmill at 60% VO_{2max} (Nieman et al., 2005) and a study by McDowell and colleagues (1991) that demonstrated no significant change in SIgA concentration levels following acute treadmill running at intensities between 50% - 80% of VO_{2max}.

The largest percent mean increase in acute SIgA concentration of +51.9% were observed in the YEG compared to +24.7% for the AEG and -3.9% for the NEG. Though there are no

research studies that specifically evaluate the effects of a single yoga session on SIgA, a study that evaluated Abbreviated Progressive Relaxation has found significant increases in SIgA concentration (42%) and secretion (42.5%) (Pawlow & Jones, 2005). Secretion rates provide further insight into the mucosal immune response to exercise.

This study noted significant increases (+34.5%) in SIgA secretion for all participants combined. Specifically, the increases recorded for each group were +39.8% for the YEG, +38.6% for the NEG, and +25.1% for the AEG between the pretest and posttest. A previous study reported significant increases in SIgA secretion rates of +31% in subjects following participation in low intensity exercise for 20 minutes (Sakamoto et al., 2005).

These biological results provide support for a low intensity exercise program such as yoga and a moderate intensity exercise program such as aerobic exercise for the enhancement of SIgA concentration immediately following 50 minutes of exercise. Neiman et al. (2011) suggests that although the immune system may return to pre-exercise levels following exercise, each exercise session may improve immunosurveillance against pathogens reducing overall URTI incidence and symptomatology. The underlying mechanisms for the reduction in URTI risk with exercise training is unclear but if each session of exercise increases SIgA, an aggregate effect of exercise may reduce URS (Walsh et al., 2011). The "open window" theory of vulnerability that has been postulated for vigorous intensity exercise may not be applicable to situations with moderate and low intensity exercise. Further research is needed to determine how quickly values return to baseline and the possible presence of a cumulative effect.

Salivary Alpha Amylase.

No significant changes were observed in the SAA concentration and SAA secretion following aerobic exercise, yoga exercise, or sitting in a first aid class for 50 minutes. The AEG, which was exercising at the greatest intensity of the three study groups, was the only study group to record increases in the mean SAA concentration (+13.9%) and secretion (+13.9%) compared to the YEG and the NEG which reported decreases of − 5.8% and -14.8% in concentration, respectively and a decrease of -5.8% and − 42.4% in secretion, respectively. Previous studies have reported that acute exercise consistently increases SAA and that this increase is related to exercise intensity (Li & Gleeson, 2004; West et al., 2006). Similarly, a study in healthy active men reported significant increases in SAA secretion with acute exercise above 75% VO_{2max} and non-significant increases in SAA concentration and secretion at 50% VO_{2max} (Allgrove et al., 2008).

Significant mean group differences in SFR for the AEG compared to the NEG were noted at the study's onset and were maintained throughout the study as the same group significance was found during the posttest.

Mood State

This section describes chronic and acute changes in mood state assessed by the Profile for Mood States (POMS) for the three groups evaluated in this study.

Chronic Mood State.

Significant decreases in the fatigue-inertia mood state were recorded for all participants combined between the pretest and posttest and between the midpoint test and the posttest. The reductions in all other categories of mood states were statistically nonsignificant. The significant decrease in fatigue-inertia, defined as weariness and low energy level (McNair et al., 1981), is a

positive outcome which could benefit college students who may be tired from the demands of college and work life.

It is widely recognized that chronic moderate exercise and relaxation affect mood state positively (Crews & Landers, 1987; Stewart et al., 2003). Kennedy and Newton (1997) suggest that physical activities that follow the ACSM recommendations for the development and maintenance of cardiorespiratory fitness will produce psychological benefits. This current study investigated whether positive mood benefits can be attained at an exercise frequency of two days per week which is less than the ACSM recommended minimum frequency of three days per week. Also, the intensity of exercise for participants in the YEG evaluated in this study was below current ACSM recommendations of 60% to 90% of maximum heart rate (Garber et al., 2011). Taking an exercise class may not have been a great enough lifestyle change to alter mood state since college students are often busy trying to juggle full-time jobs and families (Cramer et al., 1991).

This study's results are similar to findings in studies that did not produce improvement in mood state following a chronic moderate exercise training program (Cramer et al., 1991; Hughes, Casal, & Leon, 1986). Some of the discrepancy related to observations in mood state is that POMS may lack the sensitivity to measure changes in mood state when subjects begin with normal mood states (Wadden et al., 1996). This study's subjects exhibited scores close to the mean for all the mood states at baseline, therefore, it is not unexpected that the majority of mood states did not exhibit significant changes.

Acute Mood State.

Significant decreases in acute mood state for tension-anxiety, depression-dejection, anger-hostility, fatigue-inertia, confusion-bewilderment, and total mood disturbance were

measured for all participants in this study (decreases reflect movement toward a more positive mood) (McNair et al., 1981). Vigor-activity was the only mood state not to change significantly reporting small increases for the AEG and YEG and a small decrease in the NEG. Vigor-activity suggests a mood of vigorousness, ebullience, and high energy (McNair et al., 1981) and typically improves following moderate exercise (Berger & Owen, 1998; Motl, Berger, & Davis, 1997). It has been demonstrated that acute exercise may produce short-term improvements in mood state which could be an additional benefit of exercise at moderate and low intensity (Toskovic, 2001). Research evaluating the dose-response relationship between acute exercise intensity and mood state found that high intensity exercise does not positively alter mood state (Motl et al., 1997). However, moderate aerobic exercise and low intensity exercise such as yoga are associated with mood benefits (Berger & Owen, 1998; Steptoe & Cox, 1988; Treasure & Newbery, 1998). A study of college students reported significantly less tension-anxiety, depression-dejection, anger-hostility, and confusion-bewilderment in novice and intermediate swimmers following 40 minutes of continuous swimming compared to a control group (Berger & Owen, 1983). Though Berger and Owen (1983) did not report changes in fatigue-inertia and this study did report a decrease in fatigue-inertia. It can be argued that fatigue-inertia could increase or decrease (McNair et al., 1981) depending on the intensity of exercise which may explain the different results. A study evaluating the effects of one session of Taekwondo reported significant improvement on all six mood states and total mood disturbance score compared to a control group (Toskovic, 2001). Large decreases in total mood disturbance score were noted in the current study (-69%) and were also observed for the treatment subjects in the Toskovic (2001) study (-84%). Finally, some research has reported no changes in mood state following acute exercise (Mylona et al., 2002; Slivka et al., 2010), possibly due to the difficulty in finding mood

state changes in subjects that are already near the mean for a particular mood state (Wadden et al., 1996).

Summary

Comprehensive assessments of the mucosal immune modulations following an acute and chronic exercise program at moderate and low levels of intensity were evaluated in this study as well as mood states. Not only did this study assess two important antimicrobial markers, SIgA and SAA, it also measured URS on all participants each day over the 84-day study.

A main finding of the present study was that aerobic exercise of moderate intensity and yoga exercise of low intensity when practiced over 50 minutes produce significant improvements in SIgA concentration. This is a meaningful contribution to the mucosal immune research because little research has been published on the acute effects of moderate and low intensity exercise.

Yoga exercise has increased in popularity but lacks research as to its possible immune benefits. The analysis of the results of this study found that participants in the yoga exercise program had significantly fewer symptoms of URTI indicating that exercise of low intensity may affect mucosal immunity positively. The growth of yoga as a popular choice of exercise for Americans warrants further investigation to help determine the immune benefits that may be associated with this form of exercise. Additionally, the NEG reported significantly more symptoms of URTI supporting the hypothesis of a "J curve" in subjects that are not participating in an exercise class. Confirmation of these results with future studies could lead to important implications for public health policy and socioeconomic benefit.

The SIgA secretion rate and SFR for all participants combined increased significantly over the 12 week study period. These results indicated that the SIgA secretion changes were

attributed to SFR increases that were experienced by each cohort. These results are confirmed by other similar studies (Mylona et al., 2002; Sloan et al., 2013).

Though no significant changes were recorded for the acute changes in SAA concentration or secretion for all groups and all participants combined, this indicates that moderate and low intensity exercise do not have the same effects on SAA as demonstrated with acute exercise of higher intensity (Allgrove et al., 2012; Allgrove et al., 2008; Leicht et al., 2011).

Finally, the acute mood state changes measured in this study confirm other research of positive mood changes immediately following moderate and low intensity exercise (Berger & Owen, 1983; Toskovic, 2001). The chronic mood changes resulted in improvements in the fatigue-inertia mood state for all participants combined between the pretest and posttest.

Mucosal immunity will continue to be an interesting and relevant field of exercise immunology. Due to the wide inter-individual variability of SIgA (Mackinnon, 1999), more research needs to focus on determining easier and less expensive methods for measuring more frequent SIgA levels in subjects to record the change over time that occurs with infection. If a specific threshold below an individualized norm is likely to produce an infection, this would be useful information. More antimicrobial markers in addition to salivary alpha amylase need to be evaluated including lactoferrin and lysosome (Walsh et al., 2011). The criticism of studies that do not measure the exact pathogenic cause of an URTI has continued to be a concern with mucosal immunity research. There is a shift away from evaluating subjects URTI, and instead focusing on URS. Since there are many causes of URS, determining which of these symptoms can be aided by exercise would be useful. The acceptance of the WURSS as an instrument to assess symptoms of URTI is a valuable tool for continued immune research (Barrett et al., 2009).

The exercise immunology field is fertile with possibilities for future study. This research study contributes to the body of research evaluating mucosal immunity of SIgA and SAA, factors associated with URS, and changes in mood state related to aerobic exercise, yoga exercise, and a non-exercise class. Given that few lifestyle strategies other than hand washing have received consistent scientific support for the prevention of URTI, determining ways to enhance immunity with exercise has the possibility of great public health consequences and warrants continued study.

APPENDIX A: IRB APPROVAL LETTER

WAYNE STATE UNIVERSITY

IRB Administration Office
87 East Canfield, Second Floor
Detroit, Michigan 48201
Phone: (313) 577-1628
FAX: (313) 993-7122
http://irb.wayne.edu

NOTICE OF EXPEDITED APPROVAL

To: Carole Sloan
Kinesiology, Health and Sport Studies

From: Virginia Delaney-Black, M.D., M.P.H. or designee _____ *D. Bielawski Ph.D (Rmp)*
Chairperson, Medical/Pediatric Institutional Review Board (MP4)

Date: November 28, 2012

RE: IRB #: 111612MP4E

Protocol Title: Modulation of Mucosal Immune Markers, URS, and Mood Following Acute and Chronic Exercise

Funding Source: Unit: Kinesiology, Health and Sport Studies

Protocol #: 1210011426

Expiration Date: November 27, 2013

Risk Level / Category: Research not involving greater than minimal risk

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The above-referenced protocol and items listed below (if applicable) were **APPROVED** following *Expedited Review* (Category (3)*) by the Chairperson/designee *for* the Wayne State University Institutional Review Board (MP4) for the period of 11/28/2012 through 11/27/2013. This approval does not replace any departmental or other approvals that may be required.

- Receipt of Protocol Summary Form (revisions received 11/26/2012)
- Receipt of Protocol (revisions received 11/26/2012)
- Medical Records are not being created or used for data collection and therefore HIPAA does not apply.
- Research Informed Consent (dated 11/26/2012)
- Data Collection - Profile of Mood States Inventory; Wisconsin Upper Respiratory Symptom Survey; Baecke Questionnaire of Habitual Physical Activity; Daily Food Log; and Physical Activity Readiness Questionnaire (PAR-Q, revised 2002)

° Federal regulations require that all research be reviewed at least annually. You *may* receive a *"Continuation Renewal Reminder"* approximately two months prior to the expiration date; however, it is the Principal Investigator's responsibility to obtain review and continued approval *before* the expiration date. Data collected during a period of lapsed approval is unapproved research and can never be reported or published as research data.

° All changes or amendments to the above-referenced protocol require review and approval by the IRB **BEFORE** implementation.

° Adverse Reactions/Unexpected Events (AR/UE) must be submitted on the appropriate form within the timeframe specified in the IRB Administration Office Policy (http://www.irb.wayne.edu//policies-human-research.php).

NOTE:

1. Upon notification of an impending regulatory site visit, hold notification, and/or external audit the IRB Administration Office must be contacted immediately.
2. Forms should be downloaded from the IRB website at **each** use.

*Based on the Expedited Review List, revised November 1998

APPENDIX B: INFORMED CONSENT

Modulation of mucosal immune markers, URS, and mood following acute and chronic exercise

Medical Research Informed Consent
Title of Study: Modulation of mucosal immune markers, URS, and mood following acute and chronic exercise

Principal Investigator (PI):   Carole Sloan, M.Ed.
Henry Ford Community College
Office Number: PE11
313.845.6318
csloan@hfcc.edu

Purpose
You are being asked to be in a research study that examines how participation in different types of classes (2 exercise classes and 1 first aid class) affects a person's immune system and the likelihood of upper respiratory tract infections such as the common cold. This study is being conducted, as part of a dissertation project, at Henry Ford Community College by a student in the PhD program in Kinesiology at Wayne State University. Approximately 180 students from aerobic exercise, yoga, and advanced first aid classes are being recruited as study participants. **Please read this form and ask any questions you may have before agreeing to be in the study.**

In this research study, we are interested in determining if participation in exercise classes (yoga and/or aerobic exercise) or taking a first aid class affects the levels of certain proteins that are found in saliva and used to assess the strength of a person's immune system and susceptibility to upper respiratory tract infections.

Study Procedures
If you agree to take part in this research study, you will be asked to provide a sample of saliva four times during the semester. A pre and post exercise saliva sample will be taken some time during the week of January 21 and a pre exercise sample will be collected during the week of February 25 and the week of April 15. To give the sample, you will drool into a tube for 4 minutes.

The duration of this research study will be 15 weeks with pre testing taking place during the first 2 weeks of class and post testing taking place during the last week of classes. As much as possible, test measurements will be conducted during class and will take minimal time from regular class activities. You may be asked to attend class early or stay late to complete some study materials.

You will be asked to complete a 24-hour food recall record which will be analyzed for total calories and the spread of calories between carbohydrate, fat, and protein. These results will be shared with you at the completion of the project. You will be asked to complete a short questionnaire twice during the semester to evaluate your habitual physical activity and you will be asked to complete a questionnaire that evaluates your mood state three times during the semester. Finally, you will be asked to complete a short questionnaire that takes about one minute to complete that evaluates your symptoms of upper respiratory tract infections (e.g. runny nose, watery eyes, etc.) each day for a total of 12 weeks.

Modulation of mucosal immune markers, URS, and mood following acute and chronic exercise

Fitness assessments of flexibility and predicted VO_{2max} will be assessed with two simple flexibility measures involving the range of motion of the right and left shoulder and a sit and reach test designed to evaluate the flexibility of the hamstring muscles and low back. The measurement of predicted VO_{2max} will involve a 3-minute test stepping up and down on a 16-inch bench to a preset cadence of about 23 – 24 steps per minute. Heart rate will be measured from a monitor that you will strap across your chest which will transmit a heart rate reading to a monitor held by the investigator. All fitness testing results will be shared with you immediately following testing.

Benefits
As a participant in this research study, there may be no direct benefit for you; however, information from this study may benefit other people if it is determined that exercise improves immunity now or in the future. The data collected from this study will be analyzed and submitted for publication. The fitness testing (flexibility and VO_{2max}) results will be shared with you and interpreted for you. Additionally, everyone who completes the requirements of the project (class attendance of 80% and completion of all evaluations) will receive an individualized interpretation of their 24-hour diet analysis record as a token of appreciation for participating in the study.

Risks
As with any exercise class, there is an inherent risk of injury during exercise. By participating in this study your risk of injuring yourself during exercise is no different than if you had not participated in the project.

You may feel socially uncomfortable drooling into a tube for the saliva collection. All saliva collections will follow established procedures, including the use of gloves and hand sanitizers. If drooling into a tube in front of classmates makes you feel uncomfortable, the saliva collection can occur in a private office.

There may also be risks involved from taking part in this study that are not known to the researchers at this time.

Alternatives
The alternative is not to participate in the study.

Study Costs
Participation in this study will be of no cost to you.

Compensation
You will not be paid for taking part in this study.

Research Involving the Future Use of Biological Specimens
All saliva samples will be disposed of at the conclusion of the research study which will be approximately one year after the saliva's collection.

Submission/Revision Date: 11/26/2012
Protocol Version: 1

_____
Participant's Initials

HIC Date: 08/11

Modulation of mucosal immune markers, URS, and mood following acute and chronic exercise

Research Related Injuries
In the event that this research related activity results in an injury, treatment will be made available including first aid, emergency treatment, and follow-up care as needed. Cost for such care will be billed in the ordinary manner to you or your insurance company. No reimbursement, compensation, or free medical care is offered by Wayne State University or Henry Ford Community College. If you think that you have suffered a research related injury, contact the PI right away at 313.845.6318.

Confidentiality
All information collected about you during the course of this study will be kept confidential to the extent permitted by law. You will be identified in the research records by a coded number. The Principal Investigator will be the only person who can link your name with the data collection. No other participants in the study will have access to this link. Information that identifies you personally will not be released without your written permission. However, the study sponsor, the Institutional Review Board (IRB) at Wayne State University, or federal agencies with appropriate regulatory oversight [e.g., Food and Drug Administration (FDA), Office for Human Research Protections (OHRP), Office of Civil Rights (OCR), etc.) may review your records.

When the results of this research are published or discussed in conferences, no information will be included that would reveal your identity.

Voluntary Participation/Withdrawal
Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you decide to take part in the study you can later change your mind and withdraw from the study. You are free to only answer questions that you want to answer. You are free to withdraw from participation in this study at any time. Your decisions will not change any present or future relationship with Wayne State University or Henry Ford Community College, or other services you are entitled to receive. Your participation or lack of participation will in no way affect your final grade in the class.

The Principal Investigator may stop your participation in this study without your consent. The Principal Investigator will make the decision and let you know if it is not possible for you to continue. The decision that is made is to protect your health and safety, or because you did not follow the instructions to take part in the study.

Questions
If you have any questions about this study now or in the future, you may contact Carole Sloan at the following phone number (313.845.6318). If you have questions or concerns about your rights as a research participant, the Chair of the Institutional Review Board can be contacted at (313) 577-1628. If you are unable to contact the research staff, or if you want to talk to someone other than the research staff, you may also call (313) 577-1628 to ask questions or voice concerns or complaints.

Modulation of mucosal immune markers, URS, and mood following acute and chronic exercise

Consent to Participate in a Research Study
To voluntarily agree to take part in this study, you must sign on the line below. If you choose to take part in this study you may withdraw at any time. You are not giving up any of your legal rights by signing this form. Your signature below indicates that you have read, or had read to you, this entire consent form, including the risks and benefits, and have had all of your questions answered. You will be given a copy of this consent form.

_____   _____
Signature of participant   Date

_____   _____
Printed name of participant   Time

_____   _____
Signature of person obtaining consent   Date

_____   _____
Printed name of person obtaining consent   Time

APPROVAL PERIOD

NOV 2 8 '12   NOV 2 7 '13

WAYNE STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD

Submission/Revision Date: 11/26/2012
Protocol Version: 1

_____

Participant's Initials

HIC Date: 08/11

APPENDIX C: DAILY FOOD LOG

Daily Food Log   *Name*_____

Day	Food Eaten	Amount/Serving Size	Meal
			APPROVED
			NOV 2 8 2012
			WAYNE STATE UNIVERSITY INSTITUTIONAL REVIEW BOARD

APPENDIX D: PAR-Q AND YOU

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition _and_ that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of _any other reason_ why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:
- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:
- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

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NAME _____

SIGNATURE _____   DATE _____

NOV 2 8 2012

SIGNATURE OF PARENT _____   WITNESS _____   WAYNE STATE UNIVERSITY
or GUARDIAN (for participants under the age of majority)

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Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

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APPENDIX E: BAECKE QUESTIONNAIRE OF HABITUAL PHYSICAL ACTIVITY

Baecke Questionnaire of Habitual Physical Activity

ID# _____

1. What is your main occupation? _____

	Never	Seldom	Sometimes	Often	Always
2. At work I sit	☐	☐	☐	☐	☐
3. At work I stand	☐	☐	☐	☐	☐
4. At work I walk	☐	☐	☐	☐	☐
5. At work I lift heavy loads	☐	☐	☐	☐	☐
	Very often	Often	Sometimes	Seldom	Never
6. After working I am tired	☐	☐	☐	☐	☐
7. At work I sweat	☐	☐	☐	☐	☐
	Much heavier	Heavier	As heavy	Lighter	Much lighter
8. In comparison with others my own age, I think my work is physically	☐	☐	☐	☐	☐

9. Do you play a sport?	Yes ☐	No ☐
Which sport do you play most frequently?		
How many hours per week?		
How many months per year?		
Do you play a second sport:	Yes ☐	No ☐
Which sport do you play most frequently?		
How many hours per week?		
How many months per year?		

	Much more	More	The same	Less	Much less
10. In comparison with others my own age, I think my physical activity during leisure time is	☐	☐	☐	☐	☐
	Very often	Often	Sometimes	Seldom	Never
11. During leisure time I sweat	☐	☐	☐	☐	☐
	Never	Seldom	Sometimes	Often	Very Often
12. During leisure time I play sport	☐	☐	☐	☐	☐
13. During leisure time I watch television	☐	☐	☐	☐	☐
14. During leisure time I walk	☐	☐	☐	☐	☐
15. During leisure time I cycle	☐	☐	☐	☐	☐
16. How many minutes do you walk and/or cycle per day to and from work, school, and shopping?		

APPENDIX F: PROFILE OF MOOD STATES

NAME _____ DATE _____

SEX: Male ⓜ   Female ⒡

Below is a list of words that describe feelings people have. Please read each one carefully. Then fill in ONE circle under the answer to the right which best describes HOW YOU HAVE BEEN FEELING DURING THE PAST WEEK INCLUDING TODAY.

The numbers refer to these phrases.

0 = Not at all
1 = A little
2 = Moderately
3 = Quite a bit
4 = Extremely

Col ⓒ   O.P. ⓞ

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	Not at all	A little	Moderately	Quite a bit	Extremely
1. Friendly	0	1	2	3	4
2. Tense	0	1	2	3	4
3. Angry	0	1	2	3	4
4. Worn out	0	1	2	3	4
5. Unhappy	0	1	2	3	4
6. Clear-headed	0	1	2	3	4
7. Lively	0	1	2	3	4
8. Confused	0	1	2	3	4
9. Sorry for things done	0	1	2	3	4
10. Shaky	0	1	2	3	4
11. Listless	0	1	2	3	4
12. Peeved	0	1	2	3	4
13. Considerate	0	1	2	3	4
14. Sad	0	1	2	3	4
15. Active	0	1	2	3	4
16. On edge	0	1	2	3	4
17. Grouchy	0	1	2	3	4
18. Blue	0	1	2	3	4
19. Energetic	0	1	2	3	4
20. Panicky	0	1	2	3	4
21. Hopeless	0	1	2	3	4
22. Relaxed	0	1	2	3	4
23. Unworthy	0	1	2	3	4
24. Spiteful	0	1	2	3	4
25. Sympathetic	0	1	2	3	4
26. Uneasy	0	1	2	3	4
27. Restless	0	1	2	3	4
28. Unable to concentrate	0	1	2	3	4
29. Fatigued	0	1	2	3	4
30. Helpful	0	1	2	3	4
31. Annoyed	0	1	2	3	4
32. Discouraged	0	1	2	3	4
33. Resentful	0	1	2	3	4
34. Nervous	0	1	2	3	4
35. Lonely	0	1	2	3	4
36. Miserable	0	1	2	3	4
37. Muddled	0	1	2	3	4
38. Cheerful	0	1	2	3	4
39. Bitter	0	1	2	3	4
40. Exhausted	0	1	2	3	4
41. Anxious	0	1	2	3	4
42. Ready to fight	0	1	2	3	4
43. Good natured	0	1	2	3	4
44. Gloomy	0	1	2	3	4
45. Desperate	0	1	2	3	4
46. Sluggish	0	1	2	3	4
47. Rebellious	0	1	2	3	4
48. Helpless	0	1	2	3	4
49. Weary	0	1	2	3	4
50. Bewildered	0	1	2	3	4
51. Alert	0	1	2	3	4
52. Deceived	0	1	2	3	4
53. Furious	0	1	2	3	4
54. Efficient	0	1	2	3	4
55. Trusting	0	1	2	3	4
56. Full of pep	0	1	2	3	4
57. Bad-tempered	0	1	2	3	4
58. Worthless	0	1	2	3	4
59. Forgetful	0	1	2	3	4
60. Carefree	0	1	2	3	4
61. Terrified	0	1	2	3	4
62. Guilty	0	1	2	3	4
63. Vigorous	0	1	2	3	4
64. Uncertain about things	0	1	2	3	4
65. Bushed	0	1	2	3	4

MAKE SURE YOU HAVE ANSWERED EVERY ITEM.

POM 021

APPENDIX G: WISCONSIN UPPER RESPIRATORY SYMPTOM SURVEY

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

Day:	Date:	Time:	ID:

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

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

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

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

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

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

Compared to yesterday, I feel that my cold is...

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

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ABSTRACT

THE MODULATION OF MUCOSAL IMMUNE MARKERS, URS, AND PSYCHOLOGICAL PARAMETERS FOLLOWING ACUTE AND CHRONIC EXERCISE

by

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Major: Exercise and Sport Science

Degree: Doctor of Philosophy

The purpose of this dissertation was to evaluate changes in two antimicrobial markers, upper respiratory symptom (URS) variables, and mood state following participation in an acute (N = 93) and chronic (N = 88) observational study of college aerobic exercise, yoga exercise, and non-exercise classes.

METHODS: Unstimulated whole saliva was collected pre/post-acute (50 minutes) and pre/midpoint/post-chronic (12 weeks) during the study period. Saliva was analyzed for salivary immunoglobulin A (SIgA) and salivary alpha amylase (SAA) using protocols and assay kits by *Salimetrics*, upper respiratory symptoms (URS) were evaluated using the Wisconsin Upper Respiratory Symptom Survey (WURSS), and mood states were evaluated by the Profile of Mood States (POMS) questionnaire.

RESULTS: Analysis of the acute data revealed significant improvements for SIgA concentration in the aerobic exercise group (AEG) and the yoga exercise group (YEG) as well as SIgA secretion increases for all participants combined. No significant acute SAA concentration or secretion improvements were noted.

Chronic measurements revealed significant SIgA secretion (pretest to midpoint and pretest to posttest) and SFR increases (pretest to posttest) for all participants combined with no changes in SIgA concentration. The WURSS data revealed a significant decrease in the symptom score for the YEG and a significant increase in the symptom score for the non-exercise group (NEG) with notable but non-significant decreases in the incidence of infections of 39, 30, and 27 for the NEG, AEG, and YEG, respectively. Significant chronic SAA concentration decreases were noted for all participants combined between the pretest and posttest.

Analysis of the POMS scores found significant acute improvements for all cohorts in all POMS categories and Total Mood Disturbance except for vigor-activity. Additionally, the entire cohort had significant chronic improvements for fatigue-inertia.

CONCLUSIONS: This study's results provide meaningful contributions to the field of mucosal immune research indicating that a 12 week yoga exercise class may decrease symptomatology of URS. The chronic SIgA secretion increases were attributed to SFR increases and the significant SFR increases may be a benefit for people suffering from xerostomia. Additionally, participation in an acute exercise session of aerobic or yoga exercise may elevate SIgA concentration immediately following exercise indicating strengthened mucosal immunity.

Since SAA is a relatively new antimicrobial marker, this study provides meaningful observations about the pattern of change seen in SAA following acute and chronic exercise of low and moderate intensity.

Collectively, these results provide support for the continued encouragement of individuals to use moderate and low intensity exercise such as aerobics and yoga to improve mucosal immunity.

AUTOBIOGRAPHICAL STATEMENT

Carole Sloan earned a B.Ed. in Physical Education and a M.Ed. in Exercise Physiology at Georgia State University, and will complete her Ph.D. (magna cum laude) this summer from the Graduate School at Wayne State University in Exercise and Sport Science with concentrations in nutrition and statistics.

While pursuing her Ph.D. at Wayne State University, Carole was a 2013 recipient of the Summer Dissertation Fellowship award. Carole published data from a NIH funded study on postmenopausal women, exercise, and mucosal immunity in the *International Journal of Sports Medicine* and gave poster presentations at the Midwest American College of Sports Medicine, 2010 and at the American College of Sports Medicine, 2011, receiving the Outstanding Doctoral Student Poster Presentation Award and the Regional Chapter Poster Award Winner, respectively. She taught classes through the Division of Nutrition and Food Science and the Division of Kinesiology, Health, and Sport Studies at Wayne State University while pursuing her Ph.D. studies.

Carole is a full-time faculty member at Henry Ford Community College and has served as Department Chair of Health and Physical Education, Faculty Senate Chair, and Director of the Certificate and Associate Degree Programs in Fitness Leadership. Additionally, she was the Director of Wellness for faculty and staff at the college for over 15 years. She has enjoyed a professional work environment for 31 years that has provided her with meaningful experiences for leadership and growth.

Carole enjoys most any form of exercise, loves to read, sew, but most of all enjoys spending time with her family. She and her husband John, welcomed their first granddaughter, Audrey Josephine, this fall.