Effects of group exercise on salivary biomarkers of mucosal immunity and hypothalamic-pituitary adrenal axis activation in older persons living in aged care facilities

By

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DECLARATION

I, Prathna Abhimun Dudhrajh, student number 9800408, hereby declare that the dissertation entitled:

Effects of group exercise on salivary biomarkers of mucosal immunity and hypothalamic-pituitary adrenal axis activation in older persons living in aged care facilities

Is the result of my own investigation and research and that it has not been submitted in part or full for any other degree or to any other University or tertiary institution. Where use was made of the work of others, it is duly acknowledged in the text. The research done in this study was carried out under the supervision of Prof AJ McKune and Dr SS Ramklass.

30 Nov 2015

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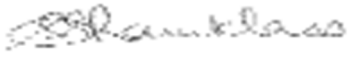
Prof AJ McKune Date



30 Nov 2015

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Dr SS Ramklass Date

DEDICATION

This dissertation is dedicated to my parents and late grandparents whom have instilled in me the importance of education and for always allowing me to follow my dreams. It is through them that I am the person I have become and for this I will forever be grateful.

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GLOSSARY

ACQUIRED IMMUNITY

Refers to the type of specific immunity that develops after exposure to a suitable antigen or is produced after antibodies are transferred from one individual to another.

ADRENOPAUSE

The decline of the production levels of DHEA with age.

ANTIBODY

A glycoprotein produced in response to the introduction of a foreign body (antigen). It has the ability to combine with the antigen that stimulated its production. Also known as an immunoglobulin.

ANTIGEN

A foreign substance to which lymphocytes respond to.

ANTIGEN PRESENTING CELLS (APC)

Cells that take in protein antigens, process them and present antigen fragments to B and T cells in conjunction with class II MHC molecules so that cells are activated. B cells may act as APCs.

B-CELL OR B LYMPHOCYTE

A type of lymphocyte derived from bone marrow stem cells that matures into an immunologically competent cell under the influence of the bone marrow. Following

interaction with antigen, it becomes a plasma cell, which synthesizes and secretes antibody molecules involved in humoral immunity.

COMPLEMENTARY DETERMINING REGIONS (CDR)

Are part of the variable chains in immunoglobulins (antibodies) and T cell receptors, generated by B-cells and T-cells respectively, where these molecules bind to their specific antigen.

CORTISOL AWAKENING RESPONSE (CAR)

It is an increase of about 50% in cortisol levels occurring 20–30 minutes after awakening in the morning in some people. This rise is superimposed upon the late-night rise in cortisol which occurs before awakening.

DIMER

A molecule or molecular complex consisting of two identical molecules linked together.

IMMUNOGLOBULINS

Any of a group of largely co-proteins that are secreted by plasma cells and that function as antibodies in the immune response by binding with specific antigens. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM.

IMMUNOSENESCENCE

The decline in the efficiency of the immune response with age.

INNATE IMMUNITY

Possessed by a group that is present in an individual at birth prior to exposure to a pathogen or antigen and that includes components which provide an initial response against infection

ISOTYPE

A variant form of an immunoglobulin that occurs in every normal individual of a particular species. Usually the antigenic determinant is in the constant region of H and L chains.

MUCOSAL-ASSOCIATED LYMPHOID TISSUE (MALT)

Comprises all lymphoid cells in the epithelia and in the lamina propria lying below the body's mucosal surfaces.

PLASMA CELLS

Terminally differentiated B lymphocytes and are the main antibody-secreting cells of the body.

POLYMERIC IMMUNOGLOBULIN RECEPTOR (pIgR)

Is expressed on several glandular epithelia including those of liver and breast. It mediates transcellular transport of polymeric immunoglobulin molecules.

SECRETORY COMPONENT

Is a component of immunoglobulin A (IgA) which consists of a portion of the polymeric immunoglobulin receptor. Polymeric IgA binds to the polymeric immunoglobulin receptor on the basolateral surface of epithelial cells and is taken up into the cell via transcytosis.

SECRETORY IMMUNOGLOBULIN A (sIgA)

The primary immunoglobulin of the secretory immune system.

T-CELL OR T LYMPHOCYTE

A type of lymphocyte derived from bone marrow stem cells that matures into an immunologically competent cell under the influence of the thymus. T cells are involved in a variety of cell mediated immune reactions.

TRANSCYTOSE

It is the process by which various macromolecules are transported across the interior of a cell. Macromolecules are captured in vesicles on one side of the cell, drawn across the cell, and ejected on the other side.

LIST OF ABBREVIATIONS

6MWT- 6 Minute walk test

APC- Antigen presenting cells

CAR- Cortisol awakening response

CDR- Complementarity-determining regions

CI- Confidence intervals

CNS- Central nervous system

CR- Category ratio

DHEA- Dehydroepiandrosterone

ELISA- Enzyme linked immunosorbent assay

GALTs- Gut-associated lymphoid tissues

HPA axis- Hypothalamus pituitary adrenal axis

Ig- Immunoglobulin

PC- Plasma cell

pIgA- Polymeric immunoglobulin A

pIgR- Polymeric immunoglobulin receptor

PVN- Paraventricular nucleus

sIgA- Secretory immunoglobulin A

TMB- Tetramethylbenzidine

URTI- Upper respiratory tract infection

WHO- World Health Organization

ABSTRACT

Introduction: Within the South African population there is an increase in the percentage of elderly individuals. In Census 2014, 8.4% of the population was ≥ 60 years compared to 5.4% in Census 2007. Chronic disease, illness, stress and disability increase in incidence with advancing age. Increased physical activity or exercise has been shown to play an important role in reducing these negative effects of ageing. However, there is no research examining the effects of exercise on markers of immunity and stress in older persons residing in aged care facilities in South Africa.

Purpose of the study: To determine the effect of a 1) 12 week, group exercise programme on salivary biomarkers of mucosal immunity (secretory(s)IgA) and the hypothalamus-pituitary-adrenal axis (HPA-axis) [(Cortisol and Dehydroepiandrosterone (DHEA)] and 2) training twice versus three times /week on these biomarkers.

Methodology: This was a pre-test-post-test experimental design measuring salivary biomarkers in 95 older individuals aged between 60-86 years training: twice/week n = 40; three times/week n = 45, from five aged care facilities in the eThekwini region of KwaZulu-Natal, South Africa. Over-night, fasted sIgA secretion rate, cortisol and DHEA were measured pre and post a 12 week group exercise programme that included progressive balance, strength and endurance training. Pre and post exercise salivary biomarker data for between and within group differences were analysed using univariate analysis of variance. Significance was set at $P \leq 0.05$.Cohen's *d* effect sizes were also calculated.

Results: There was a significant increase in salivary cortisol in the group training three times/week (P=0.01) that exhibited a small to moderate effect size (Cohen's $d = 0.42$). Increases in sIgA secretion rate approached significance in both groups with small to moderate effect sizes (twice/week P=0.07, Cohen's $d = 0.44$; three times/week P=0.09, Cohen's $d = 0.34$). Although there was no statistical significant increase for DHEA the effect sizes (twice/week Cohen's $d = 0.22$; three times/week Cohen's $d = 0.15$) indicated that was a practical effect.

Conclusions: Twelve weeks of training twice or three times/week had a low to moderate effect on salivary cortisol, sIgA and DHEA. The increase in cortisol was not pathological and suggested an adaptive response in the HPA-axis to training three times/week. The practically significant increase in sIgA and DHEA suggest enhanced mucosal immunity allowing for better immune defence in the URT in the elderly. The increase in DHEA shows promise that long term exercise has the potential to increase DHEA levels in the elderly further enhancing their mucosal immunity. It is therefore recommended that group training using balance, endurance and resistance training, either twice or three times/week over 12 weeks, be introduced in aged care facilities.

CHAPTER 1: INTRODUCTION

1.1 Background

The percentage of elderly individuals in South Africa is increasing. The mid-year South African population estimates highlighted that approximately 8.4% (4.54 million) of the population was ≥ 60 years in 2014 when compared to the Census 2007 figure of 5.4% (2.61 million) (Statistics South Africa, 2014). By 2025 more than one South African in ten will be 60 years or older (5.23 million individuals) (Ramklass et al., 2010). Illness, stress, disability and chronic disease increase in incidence with advancing age placing an unprecedented health care burden on the population. The increase in life expectancy has been associated with physical and mental well- being challenges such as loneliness and psychological distress (Chipps and Jarvis, 2015). Population ageing has become a global phenomenon and the world's older population is growing at a rapid rate in the less developed countries than the developed countries (Joubert and Bradshaw, 2006).

In their report on the health of older persons, Joubert and Bradshaw (2004) stated that less priority was generally given to older person's health, chronic care and geriatric services. In particular, exercise and physical activity have been shown to play an important role in reducing the negative effects of ageing and improving quality of life (Joubert and Bradshaw, 2004). The World Health Organisation recognizes the importance of physical activity and exercise for the elderly. They promote active ageing as the process of optimizing opportunities for health, participation and security in order to enhance quality of life as people age (World Health Organisation, 2002). According to the American College of Sports Medicine (2006), physical activity is recognized as one of the important determinants for the improvement of life expectancy and quality of life. Older people residing in aged care facilities are, due to their care needs, primarily dependent on others in order to realise their quality of life (Van Malderen et al., 2013).

Kohut and Senchina (2004) stated that one of the many benefits of exercise especially for the elderly is that it is non-invasive and can be conducted in any kind of environment. Bean et al. (2004) further added that exercise also has important health benefits for most chronic diseases associated with ageing including heart disease, stroke, arthritis, diabetes, osteoporosis, peripheral vascular disease and pulmonary disorders. Other documented advantages of physical activity in the elderly are loss of body fat, increase in muscular mass and flexibility (Teixeira et al., 2008). According to Xu et al. (2006) exercising regularly greatly assists in slowing down or reversing some deterioration of muscular function that is generally associated with ageing. Emery et al. (2005) stated that via neuroendocrine regulation, exercise accelerated wound healing in the elderly. They postulated that exercise might contribute to blood flow to the skin and increased oxygen skin tension therefore enhancing the wound healing rate (Emery et al., 2005). Exercise has been shown to increase mobility, strength, balance, and endurance in the elderly (Eyigor et al., 2007). Romeo et al. (2010), reported that studies have indicated that regular moderate-intensity exercise assists in lowering infection risk and autoimmune diseases in the elderly. Kohut et al. (2005) looked at the influence of exercise interventions on psychosocial parameters in older adults and concluded that exercise improved depression levels. A follow up to their study showed that both the control and exercise group had reduced depressive symptoms (Phillips et al., 2007). A study conducted by Sakuragi and Sugiyama (2006) on a four week daily walking programme resulted in subjects having a reduction in anxiety and depression levels, an improvement in self-esteem as well as a better tolerance to stress (Teixeira et al., 2008). Despite the known benefits of physical activity and exercise for the elderly and the advocacy of the World Health Organisation there are limited resources dedicated to specialized physical activity and exercise programmes for the elderly in South Africa.

According to Joubert and Bradshaw (2004), South Africa has shown an increased awareness of the need for healthy ageing. To obtain a balanced healthy ageing programme, the guidelines and management need to be implemented at the service delivery level. Multiple social, psychological and biological factors, commonly referred to as the health triangle model, can influence the mental health of a person. As

suggested by this model, a person is only healthy if all three factors exist cohesively in a person's life (Alli and Maharaj, 2013). In South Africa there are no known scientifically evaluated exercise or physical activity programmes for older persons residing in aged care facilities. Specifically, related to the present study, the effects of physical activity or exercise on immune and stress related salivary biomarkers in these individuals has not been determined. In addition, the dose response of these salivary biomarkers relating to training frequency (twice /week versus three times/week) is unknown. Data relating to the effects of exercise on immune and stress related biomarkers as well as frequency of exercise sessions to obtain health benefits in older persons are important for the planning and delivery of such programmes in aged care facilities.

1.2 Purpose of Study

The purpose of this study was to determine the effect of a 1) 12 week, group exercise programme performed by older persons ≥ 60 years living in aged care facilities on salivary biomarkers of mucosal immunity (secretory(s)IgA) and the hypothalamus-pituitary-adrenal axis (HPA-axis) [Cortisol and Dehydroepiandrosterone (DHEA)] and 2) training twice versus three times /week on these biomarkers.

1.3 Objectives

1. sIgA was measured pre and post a 12 week, group exercise programme, to determine the effects of exercise on mucosal immunity in older persons living in aged care facilities.

2. Salivary cortisol and DHEA were measured pre and post a 12 week, group exercise programme to determine the effects of exercise on HPA axis activation in older persons living in aged care facilities.

3. The pre and post exercise salivary sIgA, cortisol and DHEA levels were compared between groups training twice versus three times/ week to determine the frequency "dose" of exercise for benefits in mucosal immunity and the stress response in older persons living in aged care facilities.

1.4 Hypotheses

1. A 12 week, group exercise programme will increase mucosal immunity (increase sIgA) in older persons aged ≥ 60 years living in aged care facilities.
2. A 12 week, group exercise programme will enhance HPA axis activation (decrease salivary cortisol and increase DHEA) in older persons aged ≥ 60 years living in aged care facilities.
3. Training twice versus three times a week over a 12 week, group exercise programme will result in different effects on mucosal immunity (sIgA) in older persons aged ≥ 60 years living in aged care facilities.
4. Training twice versus three times a week over a 12 week, group exercise programme will result in different effects on HPA axis activation (salivary cortisol and salivary DHEA) in older persons aged ≥ 60 years living in aged care facilities.

The structure of the outline of the dissertation is as follows:

Chapter 2 presents a review of the literature.

Chapter 3 is the original research manuscript that will be submitted to an accredited journal for publication.

Chapter 4 is the conclusion of the overall dissertation together with recommendations for future study, the references and appendices.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Biological markers or biomarkers are measureable products or substances that are used as indicators of the biological state and to objectively determine the body's physiological or pathological processes (Palacios et al., 2015). Biomarkers are very useful in sport, exercise and physical activity research as they are important parameters to assess the impact of exercise on different systems, organs and tissues (Palacios et al., 2015). The measurement of biomarkers in saliva for determining immune and hormonal status has become popular over the past two decades as this is a non-invasive and a stress-free alternative to blood (Papacosta and Nassis, 2011). The composition of saliva includes hormones, peptides, electrolytes, mucus, antibacterial compounds and various enzymes. Saliva also contains detectable steroid hormones, specifically testosterone, dehydroepiandrosterone (DHEA) and cortisol as well as markers of mucosal immunity which includes the immunoglobulin secretory IgA (sIgA) (Papacosta and Nassis, 2011). Michalke et al. (2015) stated that for many years saliva has been used in clinical and forensic toxicology to study normal human metabolism as well as for determining the function of salivary glands and hormonal status. This review will discuss saliva and the salivary biomarkers cortisol, DHEA and sIgA, and the impact ageing and exercise has on these biomarkers in the elderly living in aged care facilities.

2.1.1 Physiology of Saliva

Saliva is composed of 98% water, is a colourless and dilute liquid with a density between 1002 and 1012 g/L with a pH around 6.64 (Kreusser et al., 2008). Salivary glands are exocrine glands containing ducts that produce and secrete a large volume of fluid containing organic and inorganic components to the outside of the body. The excretory units or acini are formed by groups of primary acinar cells and form a sac-like lumen and drains into small ducts (Teeuw et al., 2004). Within the oral cavity there are three major pairs of salivary glands (Figure 1) and more than 600 minor mucus

glands. The contribution of the total unstimulated saliva secretion of each pair of glands is as follows: submaxillary glands contribute ~65%; parotid glands ~23%; sublingual glands ~5% and the minor mucus glands contribute ~8% (Farnaud et al., 2010). The total plasma volume that is translocated everyday through the salivary glands is around 20% or ~750ml. In a healthy human, the saliva secretion is estimated to range from 750 to 1500 ml/day (Chicharo et al., 1998, Bishop and Gleeson, 2009).

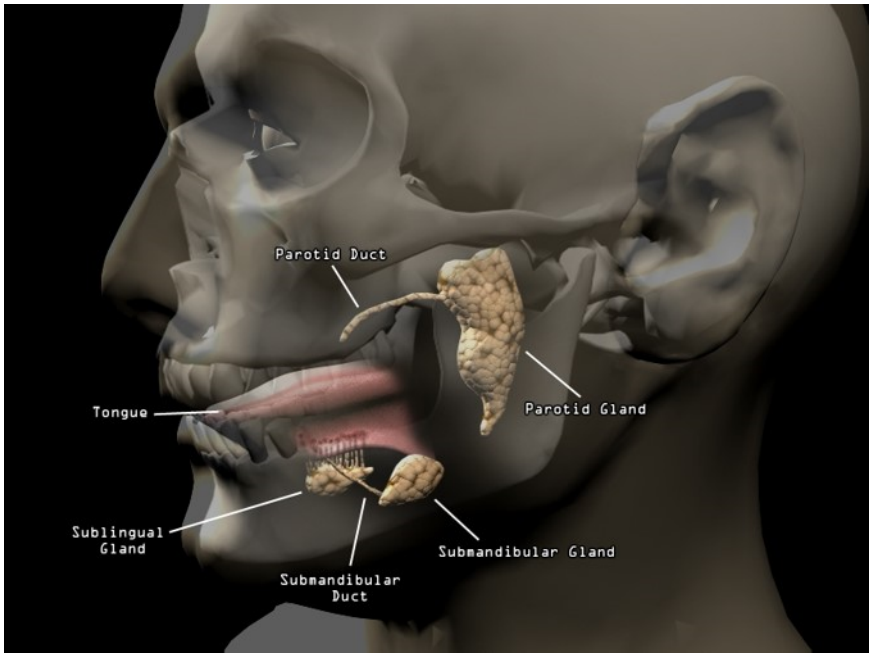

Figure 1: Diagrammatic representation of the position of the various salivary glands (Salimetrics, 2015)

Both the parasympathetic and sympathetic nerves innervate the salivary glands. Each gland contains lobules that are rich in blood vessels and nerves. They enter the glands at the hilum and diverge into branches. The parotid glands are supplied by arterial capillaries originating from the carotid artery. The sublingual glands are supplied by branches of the sublingual and submental arteries. The facial and lingual arteries supply the submandibular gland (Chicharo et al., 1998).

2.1.2 Secretion and Regulation of Saliva

According to Chicharo et al. (1998), the autonomic nervous system is the primary regulator of salivary secretion and indirectly regulates the salivary flow rate. Baum (1987) found that the salivary flow rate was highly dependent on the type of autonomic receptor that was activated and this could affect the composition of saliva. Vasodilation of the capillaries supplying the salivary glands seems to be induced by parasympathetic cholinergic nerve stimulation. This stimulation increases blood flow to the glands thereby increasing the saliva secretion rate. Saliva secretion from the submandibular and parotid glands is regulated by the stimulation of the parasympathetic nerves (Baum, 1987). The main stimulus for increased saliva secretion is by parasympathetic innervation. The sympathetic adrenergic nervous system stimulates saliva secretion from the sublingual and minor mucus glands (Baum, 1987). Studies performed by Pilardeau et al. (1992) demonstrated that strenuous exercise increases sympathetic activation and therefore may play a role in reducing saliva flow rate and modifying the components of saliva.

2.1.3 Saliva and the Immune System

There are various salivary defence mechanisms which includes locally produced immunoglobulins, lysozyme, mucins and a range of antimicrobial peptides (Farnaud et al., 2010). According to Mestecky (1993), saliva has been regarded as a convenient and a representative system to study acquired secretory immunity as the salivary glands form a common effector site for IgA-secreting plasma cells. The IgA secreting cells all migrate to the salivary glands and are activated at various mucosal induction sites where antigen presentation takes place. The immune system is made up of a network of cells and molecules that have specialized roles in defending an organism against infection. This system is divided into two parts as determined by the speed and specificity of the reaction, viz. innate immunity and acquired or adaptive immunity (Parkin and Cohen, 2001). Delves and Roitt (2000) stated that the extent of the innate response remained the same even though the infectious agent has been detected

many times before. The response in acquired immunity however, improves when there is repeated exposure to an infection.

2.1.3.1 Acquired Immunity

Acquired immunity is the trade mark of the immune system of higher animals and its responses involves the propagation of the antigen-specific reactions of the T and B lymphocytes. This reaction occurs when the antigen-specific surface receptors on the T and B lymphocytes bind to the antigen. Antigen presenting cells (APCs) are important in the induction of acquired immunity responses as they expose the antigen to the lymphocytes and work together in response to the antigen (Delves and Roitt, 2000). According to Phillips et al. (2007) the decline in the efficiency of the immune system with age leads to thymus atrophy, a drop in naive T cell number as well as a lowered B cell antibody production in response to an antigen (Buford and Willoughby, 2008).

Major components of acquired immunity are the various classes and sub-classes of immunoglobulins that are present in the blood as well as mucosal tissue and secretions. The most abundant class of immunoglobulins synthesized in the human body is immunoglobulin A (IgA). Daily, approximately 66mg of IgA/kg of body weight is produced as compared to 34 mg of IgG and only 7.9 mg of IgM (Yoo and Morrison, 2005). The serum concentrations of IgD and IgE are 0-0.4 mg/ml and 10-400 ng/ml, respectively (Harlow and Lane, 1988). In humans, IgA exists as two isotypes i.e. IgA1 and IgA2. IgA1 is predominant in serum making up approximately 90% and only 10% is IgA2. In mucosal secretions IgA2 can be as high as 50%.The source of serum IgA is plasma cells in the bone marrow and the source of sIgA are the plasma cells in the lamina propria (Yoo and Morrison, 2005).

2.1.3.2 Acquired Immune Responses

Acquired immunity drives targeted effector responses in two stages. In the first stage, once the antigen is presented to the T or B antigen-specific cell it is recognized and this leads to cell priming, activation and differentiation which normally occurs in the lymphoid tissue. The effector response takes place in the second stage. This occurs either due to activated T cells leaving the lymphoid tissue and converging to the site of infection or by the release of antibody from the activated B cells via blood and tissue fluids and eventually the infection site (Parkin and Cohen, 2001).

B and T cells develop from primordial cells which are found within the bone marrow. While the B cells reside within the marrow for their development, the T cells travel to the thymus early in its development stage as thymocytes. In both cell types the production of the antigen-specific receptors results from a unique process of random rearrangement and splicing of multiple DNA segments that code for these receptor binding sites. This is called the complementarity-determining regions (CDR) (Parkin and Cohen, 2001).

According to Arstila et al.,(1999) during the early development of the cells before exposure to the antigen this rearrangement of genes happens. This rearrangement contributes to the production of a repertoire of over 10^8 T-cell receptors and 10^{10} antibody specificities. This is sufficient to cover a diversity of pathogens likely to be encountered in life.

2.1.3.3 Secretory IgA Synthesis and Function

According to Moldt (2014), research has shown that most of the activated B cells are found within the mucosae and exocrine glands. Here the antibody-secreting plasma cells (PCs) produce polymeric IgA (pIgA), which consists of dimers and some trimers of IgA. In humans, the mucosal IgA-producing PCs synthesize a J-chain which is critical for the interaction with polymeric Ig receptor (pIgR). The pIgR/pIgA/J chain complex is stabilized by disulphide bonds between the Cα2 domain of the antibody

and domain 5 of the receptor. This enables transport to the mucosal lumen which is supported by pIgR (Figure 2) (Strugnell and Wijburg, 2010).

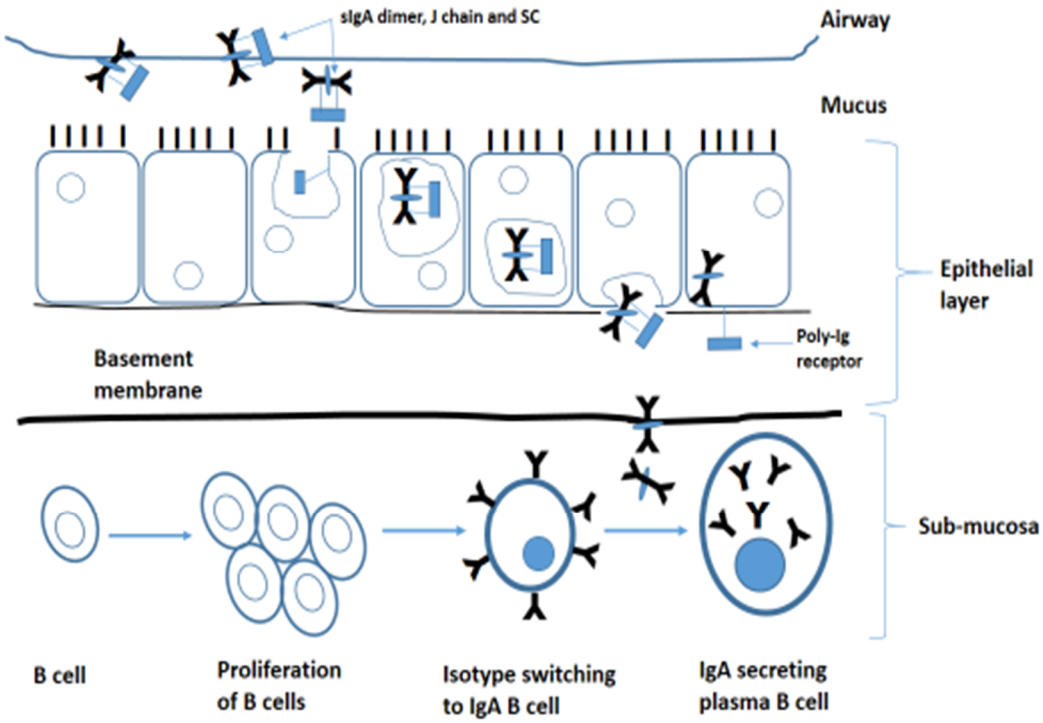

Figure 2: Synthesis of sIgA and transport across the epithelium adapted from Janeway et al. (2001) and Salvi and Holgate (1999)

Studies have indicated that transportation of this complex is internalized and actively transcytosed within vesicles through the epithelial cells and directed to the apical surface where the extracellular portion of the pIgR is proteolytically cleaved. This cleaved fragment stays disulphide bonded to pIgA, forming the newly generated sIgA that is released at the mucosal surface (Moldt et al., 2014). Kaetzel (2005) established that the pIgR that was unbound was transcytosed to the lumen and was released as free secretory component (SC). Research has shown that where serum IgA is mainly monomeric, sIgA originates from two different cell types to form a multi-polypeptide complex (Figure 3) (Moldt et al., 2014).

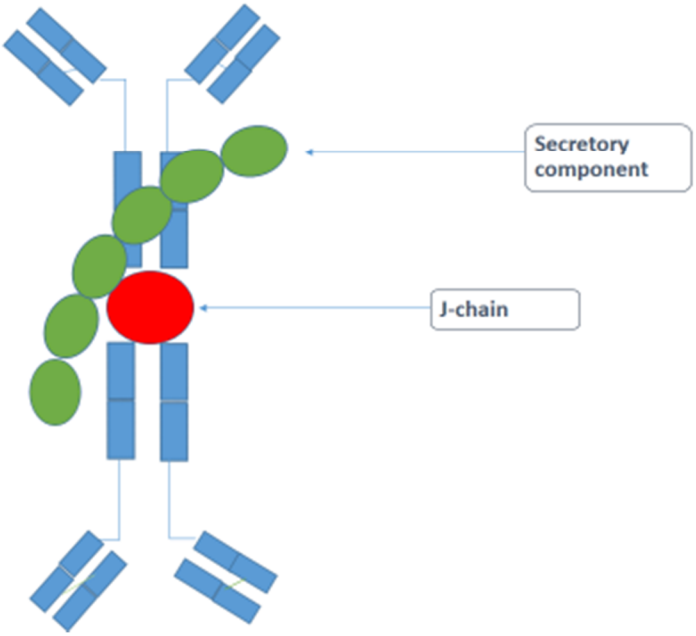

Figure 3: Structure of sIgA adapted from Janeway et al. (2001)

In their review on sIgA's complex roles in immunity, Mantis et al. (2011) stated that even though sIgA is primarily located within an external environment, it has to fight microbial infections through mechanisms that are different from those used by antibodies in systemic sections. They further stated that sIgA acts mainly via receptor blockade, steric interruption and/or immune exclusion. Research over the years has indicated that there is increasing evidence that sIgA influences the composition of intestinal microbiota, promotes the uptake and delivery of antigens from intestinal lumen to dendritic cells subsets located in gut-associated lymphoid tissues (GALTs) and influences inflammatory responses that are usually associated with the uptake of highly pathogenic bacteria and potentially allergenic allergens (Mantis et al., 2011). According to Yoo and Morrison (2005), sIgA plays a crucial role in providing protection at mucosal surfaces and is effective in neutralizing bacterial toxins and inhibits the binding of viruses to cell surface receptors. Studies have shown that sIgA's inhibitory action occurs from the initial steps of the infection process (Mantis et al., 2011).

According to Bishop and Gleeson (2009) the secretion rate is said to be the most important measure of immune defence as it represents the actual amount of IgA available on mucosal surfaces.

2.1.3.4 Effects of Ageing and Exercise on the Immune System

Research has shown that the functioning of the immune system decreases with advancing age thereby increasing the risk of infections especially in the upper respiratory tract (Fujihashi and McGhee, 2004). Mackinnon and Hooper (1994) stated that effective functioning of the immune system in the upper gastrointestinal and respiratory tracts depends on the absolute sIgA concentration and salivary flow rate. Miletic et al. (1996) found that saliva flow and sIgA secretions rates were much lower in the elderly than in the young which was associated with upper respiratory tract infections (URTI). URTIs are the most commonly occurring infectious diseases in the elderly and comprises of the common cold, corona viruses and rhinoviruses (Nieman et al., 2010).

Studies have indicated that regular light to moderate exercise improves immune function and lowers the risk of URTI (Nieman, 2012). Studies conducted on the elderly over a 12 month exercise period showed that regular moderate exercise increased sIgA levels thereby improving mucosal immunity. The mean sIgA concentration increased significantly from 24.7 µg/ml at baseline to 33.8 µg/ml at 12 months. The mean salivary flow rate had a slight increase from 1.17 ml/min at baseline to 1.35 ml/min at 12 months. There was a notable increase in the mean sIgA secretion rate at both 4 and 12 months as compared to baseline which was 29.5, 33.8 and 46.5 µg/min respectively (Akimoto et al., 2003). Nieman (1994) described the relationship between exercise workload and susceptibility to URTI as a J-shaped curve. This model suggests that when engaged in moderate activity the immune system is enhanced above sedentary levels, while prolonged high intensity exercise suppresses the immune system (Gleeson, 2007). Epidemiological evidence shows that regular moderate physical activity has been associated with decreased infection incidence.

Studies conducted by Matthews et al. (2002) showed that ~2 hours of regular moderate exercise per day lowered the risk of picking up a URTI by 29% as compared to leading a sedentary lifestyle. An investigation to determine the effect of 6 months of moderate aerobic training on sIgA concentration in elderly men was conducted by Akbarpour et al. (2011). Subjects were divided into 2 groups which were the experimental and control group. The experimental group performed moderate aerobic exercise 3x/week for 6 months. The control group did not perform any regular exercise. The results of this study concurred with literature, where there was a significant increase in sIgA concentration post exercise. The control group did not have a significant improvement in sIgA concentrations post exercise. The researchers concluded that 6 months of aerobic training can be very effective in delaying immune system ageing. They also suggested that to improve cellular immune system performance in the elderly, moderate aerobic exercise should be undertaken for more than 3 months as this will also increase their quality of life (Akbarpour et al., 2011).

Sloan et al. (2013), investigated the effects of a walking programme on sIgA in postmenopausal women over a 16 week study intervention period, which comprised of an exercise group and a control group. Their study measured sIgA, its secretion rate and salivary flow rate at rest immediately before acute maximal exercise and at rest 5 minutes following the graded maximal exercise bout. The control group were informed to continue their usual daily activities. The exercise session was a 30 minute brisk walk at a prescribed moderate aerobic exercise intensity. At the end of the intervention period there was a significant increase in the sIgA resting secretion rate ($P<0.05$) in the exercise group. The absolute concentrations of sIgA in both groups remained unchanged (Sloan et al., 2013).

The literature discussed above indicates that light to moderate intensity exercise increases sIgA levels, enhancing mucosal immunity. This has been shown to be beneficial in the elderly as it reduces the rate of URTIs. Immunity, exercise and ageing are strongly associated with alterations in HPA axis activity. These relationships will be discussed in detail below.

2.1.4 The Hypothalamic-Pituitary Adrenal Axis: Cortisol and Dehydroepiandrosterone (DHEA)

A moderate response to stress is adaptive for human survival (Mura et al., 2014). The hypothalamic-pituitary-adrenal (HPA) axis is essential for the homeostasis of the immune system and it is pivotal in the body's neuroendocrine response to stress. The HPA axis consists of the paraventricular nucleus (PVN) in the hypothalamus, anterior pituitary gland and the adrenal glands. The HPA axis plays a vital role in the stress response and its activation is represented by the secretion of glucocorticoids (Mura et al., 2014). The HPA axis is also associated with many immune mediated diseases and is regulated by the central nervous system (CNS) (Bauer, 2005) and (Webster et al., 2002). According to Finsterwald and Alberini (2014) the activation of these pathways leads to a fast physical and psychological response and during adaptive conditions it produces long-term positive modifications in the brain, which allows humans to efficiently deal again with similar stress. Therefore, a moderate level of stress has a positive effect on coping mechanisms (Mura et al., 2014). Wilcox et al. (2014) stated that cortisol and DHEA are considered to be essential markers of the HPA and play an important role in its regulation activity. According to Heaney et al. (2013) severe stress in the aged has been linked with a significant increase in the cortisol/DHEA ratio. This has been discussed in greater detail below.

2.1.4.1 Physiology of Cortisol

Cortisol is a catabolic hormone produced in the adrenal glands which is released into the blood and via passive diffusion moves into saliva and the epithelial cells (Figure 4). According to Kirschbaum and Hellhammer (1994) up to 95% of secreted cortisol is bound to large proteins and carried via blood throughout the body while saliva contains the low molecular weight unbound cortisol. The unbound cortisol is thought to be biologically active as it comes to the target tissue and forms the reaction of glucocorticoidal effects (Ljubijankic et al., 2008). Salivary cortisol is therefore an "easy-to-assess" measure of the unbound 'free' cortisol fraction (Kirschbaum and

Hellhammer, 1994). It is involved in a number of functions which includes response to stress, vascular activity, energy metabolism, inflammatory and immune response (Heaney et al., 2012).The use of saliva in stress research especially for determining cortisol levels in saliva, is commonly used as it allows for the assessment of the stress hormone without inducing additional stress via venepuncture (Michalke et al., 2015). Research carried out by Ljubijankic et al. (2008) on the daily fluctuation of cortisol, in saliva and blood showed that there is a high correlation of cortisol levels between saliva and blood. Tunn et al. (1992) determined that the correlation coefficients between salivary cortisol and blood ranged between r =.71 (patients on alpha-cholinergic medication) to r =.96 (healthy elderly patients), determining 50.4-86.4% of the total variance, when they simultaneously measured cortisol in saliva and blood following administration of exogenous cortisol (Kirschbaum and Hellhammer, 1994). The results clearly indicate that salivary cortisol assessment is closely correlated with cortisol levels in blood (Kirschbaum and Hellhammer, 1994).

Ljubijankic et al. (2008) stated that most studies found that the amount of salivary cortisol is linked with the amount of cortisol in blood, however the absolute concentration found in saliva is lower than that in blood. Cortisol levels in humans are higher in the morning and decline during the day (Gardner et al., 2013). Several studies have shown that basal cortisol levels are similar in elderly and young persons, whereas others have shown increases or decreases with age. This has therefore lead to a controversy regarding age-related basal cortisol production in humans (Ahn et al., 2007). According to Kumari et al. (2010) studies have shown cortisol secretion to be etiological in the development of various conditions especially seen in the elderly, and these include heart disease, osteoporosis, cognitive decline and frailty. According to Wilcox et al. (2014) research has shown that the mean cortisol levels show an initial rise after awakening. This is known as the cortisol awakening response (CAR), which is followed by a drop in cortisol during the rest of the day.

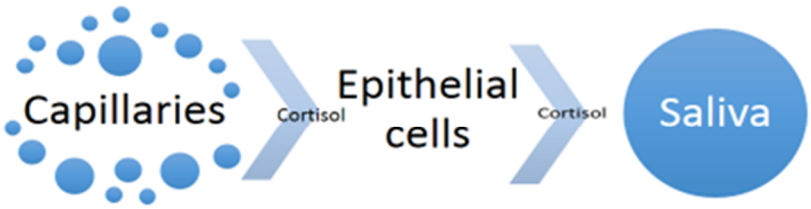

Figure 4: Movement of unbound cortisol from capillaries into epithelial cells and saliva via passive diffusion

2.1.4.2 Effects of Exercise on Salivary Cortisol

Studies have shown that cortisol levels increases during high intensity physical activity. Cortisol levels and exercise intensity are directly proportional (Papacosta and Nassis, 2011). The use of salivary cortisol has been considered a fine indicator of its response to exercise as its levels are not changed by flow rate variations as it diffuses freely through the salivary gland (Gatti and De Palo, 2011).

Alghadir et al., (2015) reported that participants with reduced physical activity levels demonstrate a greater cortisol response to a bout of exercise than those who regularly engage in physical activity. They conducted a study assessing the effects of a four week aerobic training programme on salivary cortisol in young healthy persons. They proposed that this form of moderate intensity exercise may be suitable for most sedentary and elderly subjects. Each subject participated in the exercise training programme 3x/week for four weeks which was treadmill walking and comprised of 3 phases which were warm-up, active and cool down. The exercise regime was

approximately an hour in duration. The saliva samples were taken during a rest day which was 24 hours after training in mid-morning, following an overnight fast (Alghadir et al., 2015). Their results showed that salivary cortisol concentrations significantly increased in the post exercise recovery samples compared to the pre-training values. Their findings also showed that the increase in lactate at the end of exercise could also increase cortisol levels during recovery.

Inagawa et al. (2012) conducted a study on 40-60 year olds where they wanted to establish the effects of two types of exercise on sIgA and cortisol secretion. The subjects were divided in two groups which included a fair proportion of both age groups. The exercise intervention was carried out on one day. One group performed moderate treadmill training and the second group performed abdominal breathing supervised by an instructor. Both groups performed their respective exercises for 30 min with the treadmill group exercising at a speed of 6 km/h. Salivary cortisol and sIgA levels were measured in all subjects pre and post exercise. The results showed that salivary cortisol levels in all subjects that completed both the exercise programmes had decreased. In the abdominal group, the average cortisol measured before exercise in the 60s age group was 0.104 ± 0.01μg/dl and after exercise it was 0.080 ± 0.01μg/dl. In the treadmill group, average cortisol in the 60s age group before exercise was 0.122 ± 0.01μg/dl and after exercise it was 0.086 ± 0.0μg/dl (Inagawa et al., 2012). The researchers concluded that the intensity of these recreational exercises were not stressful but had a relaxing effect on the subjects and should be useful in preventing URTI in the elderly and those with disabilities (Inagawa et al., 2012).

2.1.4.3 Properties of DHEA and its Relation to Cortisol

DHEA is an anabolic hormone which is a precursor to sex hormones and has been proposed to be anti-ageing and immune enhancing (Chahal and Drake, 2007). It is secreted by the adrenal cortex and works opposite to cortisol at both the peripheral and central levels (Wilcox et al., 2014). DHEA moves via passive diffusion from blood into saliva (Figure 5). The correlation between serum DHEA and salivary DHEA is very

high (r = 0.86, P<0.001) as established by Ahn et al. (2007) in their study conducted on a Korean population. According to Phillips et al. (2007), human production of DHEA declines with age. This process is called adrenopause (Phillips, 2007). Riechman et al. (2003) stated that DHEA concentration is the highest in the third decade of life. The study conducted by Ahn et al. (2007) also found that the salivary cortisol and DHEA ratio changed sharply with ageing. However, their results indicated that salivary cortisol levels did not significantly change with age. According to Maes et al. (1994), the increased ratio of cortisol to DHEA is used as an ageing biomarker. Buford and Willoughby (2008) reported that although it is not the primary source, an increased cortisol to DHEA ratio appears to be a contributing factor to the age-related decline in immune function. Phillips et al. (2007) stated that adrenopause has been shown to occur at almost the same rate in both males and females. Maximum production of DHEA in humans is at ages 20-30 years and gradually declines thereafter such that by 70 years of age, DHEA levels can be as low as 10% of that seen in the young (Orentreich et al., 1992). The study on the Korean population was carried out over various age groups and it was found that adrenopause was most likely to occur in the 50s age group amongst Koreans (Ahn et al., 2007). In females, several studies have reported a link between decreased DHEA levels and low physical activity level, elevated blood lipids, reduced insulin sensitivity, reduced lean body mass and increased body fat (Riechman et al., 2003). In men, lower concentration of DHEA is associated with cardiovascular disease and diabetes (Riechman et al., 2003).

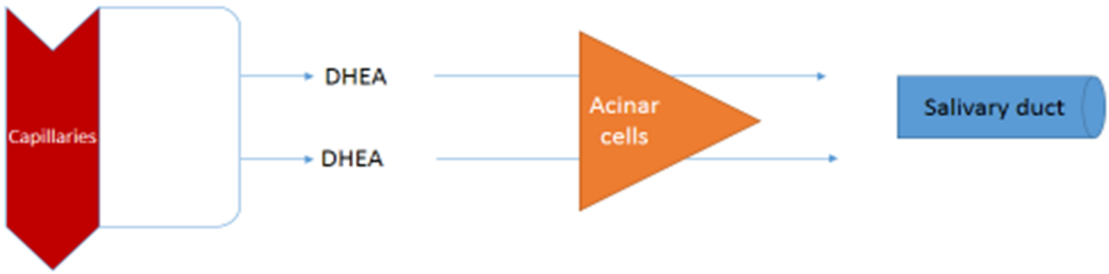

Figure 5: Movement of DHEA from capillaries into saliva via passive diffusion

2.1.4.4 Effects of Exercise on DHEA

The literature indicates that DHEA levels increase after acute exercise. A study conducted on post-menopausal women by Kemmler et al. (2003) demonstrated that there was an increase in DHEA levels following a high impact physical exercise session. A review on the impact of DHEA and cortisol on immune function in ageing by Buford and Willoughby (2008) stated that DHEA also improved physical performance, reduced infection rates and it enhanced wound-healing. A study by de Gonzalvo-Calvo et al. (2012), evaluating the effects of long-term training on the endocrine biomarker profile in elderly men reported that higher DHEA levels were associated with long-term exercise. They had two groups which each comprised of 13 men older than 65 years and who maintained a certain measure of independence. One group (SE) comprised of sedentary subjects without a history of regular physical activity and the other group had a history of long-term training since adulthood (TE). The DHEA levels were significantly higher (46%) in the TE group than the SE group. Their findings concurs with literature that DHEA levels have been shown to increase in more active elderly subjects (de Gonzalvo-Calvo et al., 2012).

A study to investigate the resting measure of DHEA and its response and recovery to acute exercise in both older male and female subjects of varying exercise training status was conducted by Heaney et al. (2013). Subjects aged between 66-70 years who were either sedentary, moderately active or endurance trained, undertook an acute bout of exercise which was a submaximal treadmill test. The testing was conducted over 14 consecutive days. Their results indicated that there was a significant increase in DHEA levels immediately post exercise. The findings of their study suggested that older adults, irrespective of their training status, are able to produce a DHEA response to exercise (Heaney et al., 2013).

Lee et al. (2004) investigated the effects of a three times a week, 10 month aerobic fitness intervention on the immunological and psychosocial parameters in older people. Their study showed that there were improvements in depression levels among the exercise group as compared to the control group. They also noted that there were

immunological improvements in the aerobic fitness group. The subjects were reported to have experienced fewer days of URTI symptoms compared to the sedentary control group (Lee et al., 2004). In their review on stress and exercise, Phillips et al. (2007) proposed that exercise be used as an intervention to protect against changes in the neuroendocrine system with ageing and improve immunity in older adults. Literature has indicated that while prolonged exhaustive exercise is linked to increased secretion of stress hormones and impaired immune function, prolonged moderate exercise has direct and positive psychosocial and immunomodulatory effects (Barbour and Blumenthal, 2005, Kohut and Senchina, 2004).

2.1.4.5 Effects of Ageing on the Salivary Biomarkers

The decline in the efficiency of the immune response with age has been termed immunosenescence and there are also age related changes to the HPA axis, which is the key effector to the stress response (Phillips et al., 2007). Alterations to immune function in the aged has been associated with T-cell subset counts and function, age-associated changes in surface molecule expression, changes in intracellular signalling, increased rates of apoptosis and decreased proliferative capacity (Romeo et al., 2010). Fujihashi and McGhee (2004) reported in their review on mucosal immunity and its tolerance in the elderly, that the severity and mortality caused by infectious pathogens invading mucosal surfaces is greatly increased in the elderly. Since immunosenescence has been documented, there has been an increased awareness on the positive impact of regular moderate physical activity on immune function amongst the elderly (Romeo et al., 2010). According to McEwen (1998), ageing in the healthy has been linked with increased activation of the HPA axis thereby indicating that psychosocial interventions could be useful in reducing chronic stress and immune function. Fries et al. (2005) established that chronic stress could also lead to alternate defects in HPA axis function. A study conducted by Traustadottir et al. (2005) to investigate whether ageing is associated with greater HPA axis reactivity to psychological stress and the effect of exercise on these parameters showed that among unfit women, ageing is associated with greater HPA axis reactivity to psychological stress. The older fit women had a blunted cortisol response to

psychological stress. This study was conducted on 3 groups of women viz. young unfit women, older unfit women and old fit women. Each group underwent the Matt Stress Reactivity Protocol (Traustadottir et al., 2003) which is made up of a battery of stressors in a set order. The results indicated that the older unfit women had a significantly greater total cortisol response than the young unfit women. The older fit women had a significantly lower total cortisol response than the age-matched unfit women (Traustadottir et al., 2005). The researchers concluded that higher aerobic fitness among older women can decrease age-related changes in threshold sensitivity to stress (Traustadottir et al., 2005).

Teixeira et al. (2008), conducted a study to evaluate the changes in functional fitness, mood states and sIgA levels on the elderly after a 19 week exercise training programme found that there was an improvement in the mood states in the exercising group as their results showed that they were statistically significantly less depressed (P=.001), exhibited lower tension levels (P=0.013), less fatigue (P<.0001) and more vigour (P=.004). On the other hand the sedentary control group did not have improved mood states, their confusion increased and they had decreased vigour levels (Teixeira et al., 2008). The results for sIgA secretion rate increased significantly in the exercise group post intervention (P=0.01) and there were no changes in the control group. The sIgA concentration increased in the exercising group and decreased in the control group. The changes however did not reach statistical significance in both groups (Teixeira et al., 2008). The functional fitness improved in all components in the exercise group after the exercise programme, where significant differences were recorded for aerobic endurance (P=.003). The researchers of this study concluded that their exercise programme was long enough to stimulate important adaptations in the elderly especially whole body strength and improved aerobic capacity. They further stated that exercise could have important benefits at the psychological level in the elderly. These benefits include enhancing mood states, preventing their deterioration with time and leading to improved quality of life. This study focused on the positive aspects of exercise on physical, psychological and mucosal immune factors in the elderly (Teixeira et al., 2008). In their review on the Psychoneuroendocrinology of Ageing, Epel et al. (2007) stated that the elderly are more often exposed to chronic

stress compared to younger adults and there is evidence that the elderly are less likely to recover from the physiological consequences of such stressors. According to Wolkowitz and Reus (2000) anxiety and chronic stress have been linked to low levels of DHEA. Chronic stress can also lead to additional shortfalls in the HPA axis such as blunted diurnal rhythm (Epel et al., 2007).

2.2 Conclusion

The endocrine system, by modification of the anabolic and catabolic processes, plays a major role in the physiological adaptation to exercise training (McMurray and Hackney, 2000). Salivary biomarkers has been shown to be a useful and pain-free alternate assessment tool compared to blood. This is beneficial especially with the elderly subjects as saliva collection is a non-invasive process. The HPA axis salivary biomarkers assessed in this study have been shown to have very high correlation to that found in blood and the results represent not only local immune but also the systemic HPA axis response. The literature indicates that low moderate intensity exercise increases sIgA levels and DHEA while cortisol levels should decrease thereby enhancing quality of life and ensuring a decrease in UTRIs in the elderly.

CHAPTER 3: SCIENTIFIC PUBLICATION

The following paper will be submitted to the European Review of Aging and Physical Activity for publication:

Effects of group exercise on salivary biomarkers of mucosal immunity and hypothalamic-pituitary adrenal axis activation in older persons living in aged care facilities

This study was undertaken as a result of limited research available on the effects of exercise, in particular the weekly frequency of exercise, on salivary biomarkers of mucosal immunity and hypothalamic-pituitary adrenal axis activation in older individuals living in aged care facilities in South Africa.

Abstract

Introduction: Ageing is a constant multi-faceted process of biological changes which includes various immunological alterations described by increased susceptibility to infectious and autoimmune diseases (Romeo et al., 2010). Chronic disease, illness, stress and disability increase in incidence with advancing age. Mucosal immunity decreases with increasing age leading to incidences of Upper Respiratory Tract Infections (URTIs). Increased ageing leads to increased cortisol production and this mediates a negative cortisol feedback on the hypothalamic pituitary adrenal (HPA) axis leading to many age-related diseases (Gupta and Morley, 2014). Increased physical activity and exercise have been shown to play important roles in reducing the effects of ageing, enhancing health and quality of life.

Purpose: To determine the effect of a 1) 12 week, group exercise programme on salivary biomarkers of mucosal immunity (secretory(s)IgA) and the hypothalamic-pituitary-adrenal axis (HPA axis) (Cortisol and Dehydroepiandrosterone (DHEA) and 2) training two (2x) versus three (3x) times a week on these biomarkers.

Methodology: Pre-test-post-test experimental design measuring salivary biomarkers in 95 (training: twice/week n = 40; three times/week n = 45) older individuals (72.4 ± 6.7 years) from five aged care facilities. Over-night, fasted sIgA secretion rate, cortisol and DHEA were measured pre and post a 12 week group exercise programme that included progressive balance, strength and endurance training. Pre and post exercise salivary biomarker data for between and within group differences were analysed using univariate analysis of variance. Significance was set at P≤ 0.05. Cohen's *d* effect sizes were also calculated.

Results: There was a significant increase in cortisol in the group training 3x/week (P=0.01) that exhibited a small to moderate effect size (Cohen's *d* = 0.42).There was no significant change in cortisol in the group training 2x/week (P=0.38) with an effect size (Cohen's *d* = 0.17). Increases in sIgA secretion rate approached significance in both groups with small to moderate effect sizes (twice/week P=0.07, Cohen's *d* = 0.44; three times/week P=0.09, Cohen's *d* = 0.34). Increases in DHEA had no statistical significance for both groups and the effect sizes (twice/week Cohen's *d* = 0.22; three times/week Cohen's *d* = 0.15) indicated that were a practical effect.

Conclusions: Twelve weeks of training twice or three times/week had a low to moderate effect on salivary cortisol, sIgA and DHEA in the elderly. The increase in cortisol was not pathological and suggested an adaptive response in the HPA-axis to training three times/week. The practically significant increases in sIgA and DHEA suggest enhanced mucosal immunity allowing for better immune defence in the URT in the elderly. The increase in DHEA shows promise that long term exercise has the potential to increase DHEA levels in the elderly thereby further enhancing their mucosal immunity. It is therefore recommended that group training using balance, endurance and resistance training, either twice or three times/week over 12 weeks, be introduced in aged care facilities.

Introduction

Studies have shown that the average life expectancy within developed countries is increasing at a rate of two years per decade. It has been estimated that by 2020 one in five of the population will be ≥ 65 years and older (Phillips et al., 2007). Although there is an increase in the average life span, the incidence of infectious and chronic disease associated with ageing also increases together with reductions in mobility, strength, endurance and coordination (O'Donnell et al., 2006).

An increase in age leads to increased susceptibility to infectious diseases especially Upper Respiratory Tract Infections (URTIs) (influenza and the common cold) (Phillips et al., 2007). According to Globerson and Effros (2000), the mortality due to infections accounts for almost 1 in 7 deaths amongst those aged over 85 years. An important factor for the increased susceptibility is that mucosal immune function decreases with increasing age (Sakamoto et al., 2009). However, Sakamoto et al. (2005) examined the effects of exercise on sIgA in subjects over 65 years old and showed that low intensity exercise rapidly improved sIgA levels.

Salivary sIgA has a vital function in the human mucosal immune system. It is the dominant antibody in the area and is the first line of defence against pathogens in the airways (Sloan et al., 2013). Secretory IgA has been shown to prevent the invasion of pathogens by preventing their replication and blocking their movement into the mucosal epithelium (Cunningham-Rundles, 2001). According to Sakamoto et al. (2009) the regulation and secretion of sIgA is not only controlled by sensitization by an antigen but also strongly controlled by the neuroendocrine system, via exercise or stress.

The Hypothalamic-Pituitary-Adrenal (HPA) axis, as demonstrated by its influence on dehydroepiandrosterone (DHEA) and cortisol release into the circulation, is one of several pathways that undergoes age-related alterations (Phillips et al., 2007) and also contributes to biological ageing (Gardner et al., 2013). Epel et al. (2007), concluded

that the relative imbalance of the anabolic to catabolic hormone activity is the principal pattern with aging. They further stated that this could to some extent, be responsible for most of the age related psychiatric and medical diseases. Bauer (2005) and Butcher and Lord (2004) reported that ageing resulted in a reduced ability to produce immune enhancing dehydroepiandrosterone (DHEA) but an increased production of the immunosuppressive cortisol. Cortisol can be immunosuppressive if it is chronically high (Phillips et al., 2007). DHEA production is optimum in humans between 20-30 years of age and gradually declines, such that by the seventh decade its levels could be as low as 10% compared to that of a young adult.

The use of physical activity as a form of therapy for the elderly has received significant scientific attention as it is effective, easy to implement, cost efficient, can be carried out in home or clinical settings and is logistically advantageous over other therapies (Senchina and Kohut, 2007). Traustadottir et al. (2005) suggested that exercise training may be an effective method of modifying some of the neuroendocrine changes associated with ageing such as increased cortisol production. de Gonzalvo-Calvo et al. (2012) argued that long-term training could improve the inflammatory-endocrine imbalance associated with frailty, disease, functional decline and mortality in elderly men. Specifically, they found that long term training in elderly men with a history of practicing regular physical activity resulted in an increase in DHEA (de Gonzalvo-Calvo et al., 2012). Kohut et al. (2005), reported that there is evidence indicating that cortisol might provide protection from viral infection after exposure to intense physical stress.

Romeo et al. (2010) reported that due to immunosenescence, there has been an increase in the interest of the benefits of regular moderate physical activity on mucosal immune function in the elderly. According to Goldhammer et al. (2005) numerous studies have shown that there is an inverse relationship between high intensity exercise and immune response, while moderate intensity exercise enhances immune function. Kostka et al. (2000) reported that retrospective and prospective studies carried out to examine the relationship between physical activity and URTI in the

elderly aged 66-84 years, showed that the greatest protection from URTI was associated with a specific level of physical activity.This level of physical activity has been reported to be equivalent to jogging 7 km/day or walking 90 min/day at a speed of about 19-20 min/km (Kostka et al., 2000).

The purpose of the study was to determine the effect of a 12 week group exercise programme on salivary biomarkers of mucosal immunity and the HPA axis and the effect of training twice versus three times a week on these biomarkers. The literature has shown that exercise has been found to be beneficial in the elderly. In most studies the training frequency used was three times a week. However, based on our experience, the literature and the feasibility of conducting training sessions three times a week in an aged care facility, we were interested in the frequency dose response and whether benefits would also be achieved with training twice a week. We hypothesised that a structured exercise programme conducted three times a week in old age homes would be more beneficial to the elderly than training twice a week.

Methods

Study Participants

Sampling Strategy

A listing of all aged care facilities located within a 20-30km radius of the Durban CBD in KwaZulu-Natal, South Africa, was obtained from the Department of Social Development. Five aged care facilities were randomly selected. Saturation sampling was used at each facility to enable voluntary participation in accordance with the study's inclusion/exclusion criteria. Given the sampling strategy, no formal sample size was calculated at the start of the study. There are no results from previous studies in this context to guide the sample size. However, in accordance with the performance of group exercises a conservative number of not more than 20 participants was considered effective for monitoring changes during group exercise sessions (Armitage-Johnson, 1994).

One hundred individuals aged between 60 and 86 years from five aged care facilities within the eThekwini Municipality were recruited for the study. Participants had to be independent in their activities of daily living and non-practitioners in a physical activity programme for at least 3 months. The participants were subjected to a medical history and physical examination directed at identifying cardiac risk factors, signs/symptoms upon exertion, and contra-indications to exercise testing/training and physical limitations. This screening and clearance for participation in the study was conducted by an exercise medicine physician. Exclusion criteria included those undergoing hormone supplementation, those participating in other clinical trials or research projects, those with any disease or condition that excluded participation in a physical activity programme, and those who were judged unable based on a medical assessment. Reasons for non-compliance of eligible participants were insufficient saliva samples produced, they did not attend 80% of the sessions, sputum produced instead of saliva and some participants were on medication that resulted in dry mouth thereby preventing saliva production.

The eligible participants at each site were randomized to either TRAIN2 (supervised exercise session; 2days/ week) or TRAIN3 (supervised exercise session; 3days/ week) for a 12 week period. Fifty-one complete sets of data (Group 1: N=41; Group 2: N=46) were available for analysis upon conclusion of the study. The demographic data for the study groups are presented in Table 2. Written informed consent was obtained from all study participants.

This study was approved as a sub-study of BE251/11 from the Biomedical Research Ethics Committee (BREC), University of KwaZulu-Natal. The title of the main study was: Group exercise and its relation to perceived health status, functional fitness, immune and hormonal status of older persons living in aged care facilities within the eThekwini Municipality. The BREC approval number for this study was BEO79/14.

Baseline and Post-Testing

Testing was conducted at each of the respective aged care facilities at baseline 48 hours before the first and then 48 hours after the final exercise session. Study participants were asked to avoid heavy physical activity, alcohol, smoking, food and caffeine containing food or beverages for 10 hours prior to testing. Food ingestion was permitted if chronic medications needed to be taken, provided that there was no food consumption two hours prior to testing. Individuals were identified if they deviated from this requirement but were not excluded from participation in the study. Study participants on chronic medication/s were requested to continue as per the prescription dictate.

Saliva collection

One week before saliva collection, the participants were provided with the salivary collection procedure instructing them on the brushing of teeth and intake of food and drink on the morning of the collection (Appendix A). Saliva samples were collected between 7:30 and 8:30, approximately 90 minutes after waking. All the participants adhered to the following standardized saliva collection instructions (Salimetrics, 2010). The participants should 1) not eat a major meal (breakfast) within 60 minutes of sample collection, 2) not brush their teeth prior to sample collection (this may cause the gums to bleed causing blood contamination of the saliva), 3) avoid dairy products for 20 minutes before sample collection, 4) avoid foods with high sugar or acidity, or high caffeine content, immediately before sample collection (these have all been shown to impact on the saliva pH altering assay results), 5) rinse their mouths with water to remove food residue before sample collection, and swallow to increase hydration, and 6) wait at least 10 minutes after rinsing before collecting saliva to avoid sample dilution.

The saliva samples were collected via unstimulated passive drool over a period of five minutes. The participants were seated and were asked to lean slightly forward, tilt their heads forward and accumulate saliva in the floor of the mouth for a minute, which was

then swallowed. There was a four minute collection period where the participants dribbled saliva through a specific saliva collection aid (SCA) (Salimetrics, State College, PA) into a pre-weighed polypropylene cryovial. After collection the cryovial (Salimetrics, State College, PA) was weighed to determine salivary flow rate. Samples were placed on dry ice immediately and kept frozen until reaching the laboratory (Naidoo et al., 2012).

The samples were stored at -80^{o}C in a bio-freezer in the Department of Chemical Pathology, University of KwaZulu-Natal until further analysis. Concentration of salivary immunoglobulin A (sIgA) and salivary cortisol and DHEA, were determined by immunoassay using ELISA kits (Salimetrics, State College, PA).

Saliva biomarker analysis

Cortisol

All samples were assayed for salivary cortisol using a highly-sensitive enzyme immunoassay (Salimetrics, State College,PA). A microtitre plate was coated with monoclonal antibodies to cortisol. Cortisol in standards and unknowns competed with cortisol linked to horseradish peroxidase for the antibody binding sites. The unbound components were washed away after incubation. The bound cortisol peroxidase was measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). The reaction produced a blue colour. Optical density was read on a standard plate reader at 450nm. Each assay used 25 μl of saliva (for singlet determinations), had a lower limit of sensitivity of 0.007 μl/dl, range of sensitivity from 0.007 to 1.8 μg/dl, and average intra-and inter-assay coefficients of variation of less than 5 and 10%, respectively.

DHEA

All samples were assayed for salivary DHEA using a highly-sensitive enzyme immunoassay (Salimetrics, State College,PA). A microtitre plate was coated with rabbit antibodies to DHEA. DHEA in standards and unknowns competed with DHEA linked to horseradish peroxidase for the antibody binding sites. Unbound components were washed away after incubation. The bound DHEA peroxidase is measured by the reaction of the peroxidase enzyme on the substrate TMB. A blue colour was produced. Optical density was read on a standard plate reader at 450nm. The intra-assay precision was determined from the mean of 12 replicates each of high, medium and low concentrations. The Coefficients of Variation (%) ranged from 5.3-5.8. The inter-assay precision was determined from the mean of average duplicates for 12 separate runs of high and low concentrations. The Coefficient of Variation (%) ranged from 7.9-8.5.

SIgA

All samples were assayed for salivary SIgA using a highly-sensitive enzyme immunoassay (Salimetrics, State College,PA). A constant amount of goat anti-human SIgA conjugated to horseradish peroxidase was added to tubes containing specific dilutions of standards of saliva. The antibody conjugate bound to the SIgA in the standard or saliva samples. The amount of free antibody that remained was inversely proportional to the amount of SIgA that was present. After incubation and mixing an equal solution from each tube was added in duplicate to the microtitre plate coated with human SIgA. The unbound antibody conjugate bound to the SIgA on the plate. After incubation the unbound components were washed away. The bound conjugate was measured by the reaction of peroxidase enzyme on the substrate TMB. The reaction produced a blue colour. Optical density was read on a standard plate reader at 450nm. The intra-assay precision was determined from the mean of 10 replicates each of high, medium and low concentrations. The Coefficient of Variation (%) ranged

from 4.49-6.99. The inter-assay precision was determined from the mean average duplicates for 8 separate runs of high and low concentrations. The Coefficient of Variation (%) ranged from 8.65-8.93.

To control for the impact of secretion rate on concentration of the sIgA, salivary secretion rate (μg/min) of these parameters were calculated by multiplying the concentration of these parameters (μg/ml) by saliva flow rate (ml/min), which was calculated by dividing the total volume of saliva obtained in each sample (ml) by the time taken to produce the saliva sample (4 minutes) (Sloan et al., 2013).

Aged care facility programme co-ordination

The exercise programme was co-ordinated at four of the aged care facilities by postgraduate students (Biokineticists) who had a background in exercise physiology, exercise prescription and implementation and were registered for a Masters degree in the College of Health Sciences. A research assistant (registered physiotherapist) complying with the stipulated criteria was employed for the purpose of the exercise programme co-ordination at the fifth facility as a Masters Student could not be identified. The students/research assistant were first trained in the procedures and processes of data collection. This included demonstrations and training in conducting interviews, implementation of the exercise programme and associated exercise testing procedures. In addition, the demonstrations/training sessions were video recorded and a copy of the DVD was given to each student/research assistant for ease of reference towards maintaining consistency.

Exercise intervention

Randomization of the participants into the two exercise frequency groups was performed after assessment of baseline data. The exercise intervention comprised of a combined programme of endurance, strength and balance training (Table I). The

exercise program intensity and volume were progressed every four weeks. These sessions were monitored and supervised by trained health professionals as discussed earlier.

Resting values of heart rate and blood pressure were recorded prior to the start of each exercise session to monitor the wellbeing of the participants. These same variables were measured and recorded at the end of each session to monitor recovery from exercise. Each exercise session began with a warm-up and ended with a cool-down. Balance training was included during the warm-up that was followed by the endurance and then strength training.

The warm-up was dynamic and included joint mobility and balance exercises. Balance training involved dynamic semi-tandem and tandem stances. The endurance training comprised of four stations that included drills that involved step-up and over aerobic steps, weaving between cones, walking between cones and walking with high knees/heel kicks. This format was suitable given the space restrictions at each facility. This component was progressed by increasing time and step height (Table I). The resistance training was performed using free-weights and resistance bands, involving large muscle groups and engagement of core muscles. The progressions included increases in resistance of both the free-weights and resistance bands (Table I). The cool-down involved slow walking to assist and static stretching of the major muscle groups.

Rating of perceived exertion (Category Ratio (CR) 0-10 scale) was used during each component of the exercise session to ensure the stipulated exercise intensity was maintained. According to Day et al. (2004), the CR-10 RPE scale has become a standard method to evaluate perceived exertion in exercise testing, training and rehabilitation and has been validated against objective markers of exercise intensity. The intensity of the entire session was recorded at the end of the session using the CR scale to calculate Sessional RPE (Foster et al., 2001). Participants were provided

with an explanation of the rating scale prior to the program inception, to ensure understanding and maintenance of exercise intensity. The instructions were based on those recommended by Borg (1998). Subjects were asked to rate the total amount of exertion that they felt. They were asked to concentrate on the total feeling of exertion and not to focus on any one factor/component of training.

Table I: Exercise Intervention Programme

Activity	**Intensity (CR scale)**	**Duration**	**Resistance**
Phase 1: weeks 1-4			
Warm-up	2 (Easy)	10 min	
Endurance Exercise	3 (Moderate)	2.5 min x 4 (10 min)	
Resistance Exercises	3 (Moderate)	10 stations (x10; 1 set)	1) Body weight 2) Dumbells(1-2kg), 3) Theraband (red)
Cool-down	2 (Easy)	5 min	
Phase 2: weeks 5-8			
Warm-up	2 (Easy)	10 min	
Endurance Exercise	4 (Sort of hard)	3.5 min x 4 (14 min)	
Resistance Exercise	4 (Sort of hard)	10 stations (x10; 2 sets)	1) Body weight 2) Dumbells (3-4kg) 3) Theraband (blue)
Cool-down	2 (Easy)	5 min	
Phase 3: weeks 9-12			

Warm-up	2 (Easy)	10 min	
Endurance Exercise	4 (Sort of hard)	5 min x 4 (20 min)	
Resistance Exercise	4 (Sort of hard)	10 stations (x10; 2sets)	1) Body weight 2) Dumbells (3-4kg) 3) Theraband (blue)
Cool-down	2 (Easy)	5 min	

Data Management and Analysis

A database was created supported by Epi-Info. Data for sIgA, cortisol and DHEA was double entered independently to minimize error. Data was checked and cleaned prior to analysis. The Shapiro-Wilk algorithm analysis indicated that all variables demonstrated a normal distribution. All measured variables are presented as mean (standard deviation) for baseline and post-testing. Percentage change from baseline to post-test was also calculated for all variables. Pre and post exercise salivary biomarker data for between and within group differences were analysed using univariate analysis of variance. Cohen's *d* effect sizes (ES) and 95% confidence intervals (CI) were also calculated. Magnitudes of the standardized effects were interpreted using thresholds of < 0.2, 0.2, 0.6 and 1.2 (Hopkins et al., 2009). These values correspond to trivial, small, moderate and large ES, respectively (Hopkins et al., 2009). Statistical significance was set at $P \leq 0.05$. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 22.0. (Armonk, NY: IBM Corp.)

RESULTS

There were no significant differences between pre and post exercise values within or between groups for their demographic and physical characteristics (Table II).The effect sizes were all trivial.

Table II: Demographic analysis of participants training twice (2x) (TRAIN2) a week (n=41) vs. training three times (3x) (TRAIN3) a week (n=46)

Parameter	Training Frequency	Mean (SD)	Cohens ES (d)	95% CI	Significance P
Age (y) **Pre**	2x/week	72.34 (7.41)	0.07	-0.36,0.50	0.23
Post		72.85(7.37)			
Height (m) **Pre**		1.55 (0.09)	-0.11	-0.54, 0.32	0.41
Post		1.54 (0.09)			
Weight (kg) **Pre**		68.66 (16.27)	0.03	-0.40, 0.46	0.48
Post		69.13 (16.43)			
BMI ($kg.m^2$) **Pre**		28.59 (5.89)	0.07	-0.37, 0.50	0.14
Post		28.99 (5.95)			

Age (y) **Pre**	3x/week	71.93 (7.44)	0.01	-0.39, 0.42	0.63
Post		72.04 (7.48)			
Height (m) **Pre**		1.57 (0.09)	0	-0.41, 0.41	0.21
Post		1.57 (0.10)			
Parameter	**Training Frequency**	**Mean (SD)**	**Cohens ES (d)**	**95% CI**	**Significance P**
Weight (kg) **Pre**		68.35 (15.92)	-0.00	-0.41, 0.41	0.95
Post		68.31 (15.56)			
BMI (kg.m^2) **Pre**		27.64 (5.54)	-0.01	-0.42, 0.40	0.88
Post		27.60 (5.39)			

Salivary Biomarkers

The primary questions in this study were related to whether 12 weeks of exercise training would alter salivary cortisol, DHEA and sIgA secretion rate and whether training frequency had an impact on these changes. The statistical (P values) and practical (Effect Sizes: Cohen's *d*) significance are discussed for salivary flow rate as well as the salivary biomarkers.

Salivary Flow Rate

There was a statistically significant (P = 0.03) increase in salivary flow rate for TRAIN2. There was no change in salivary flow rate for TRAIN3 (P = 0.49) (Table III). Cohen's effect size value for TRAIN2 *(d=0.31)* and TRAIN3 *(d=0.13)*, suggested a small and trivial practical significance, respectively. For percent change there was no statistically significant difference (P = 0.25) between the flow rate for TRAIN2 [32.91 (85.49) %] and TRAIN3 [78.38 (223.77) %].

Salivary DHEA

The increase in salivary DHEA for TRAIN2 was approaching significance (P=0.06). There was no change in salivary DHEA for TRAIN3 (P=0.31) (Table III). Cohen's effect size for TRAIN2 *(d=0.22)* and TRAIN3 *(d=0.15)*, suggested a small and trivial practical significance, respectively. For percentage change there was no statistically significant difference (P=0.68) between the DHEA for TRAIN2 [51.54 (89.97) %] and TRAIN3 [62.87 (131.05) %]. There were no differences between groups at any of the time-points.

Salivary Cortisol

There was a statistically significant (P=0.01) increase in salivary cortisol for TRAIN3 (Figure 6). There was no change in salivary cortisol for TRAIN2 (P=0.38). Cohen's effect size value for TRAIN2 (*d*=0.17) and TRAIN3 (*d*=0.43), suggested a small and moderate practical significance, respectively. For percentage change there was no statistically significant difference (P=0.52) between the salivary cortisol for TRAIN2 [36.55 (97.88) %] and TRAIN3 [49.03 (77.87) %].

Salivary sIgA Secretion Rate

The increase in salivary sIgA was approaching significance (P=0.07) for TRAIN2. There was no change in salivary sIgA for TRAIN3 (P=0.09) (Table III). Cohen's effect size value for TRAIN2 (*d*=0.44) and TRAIN3 (*d*=0.34), suggested a moderate and small/moderate practical significance, respectively. For percentage change there was no statistically significant difference (P=0.39) between the salivary sIgA for TRAIN2 [64.18 (160.95) %] and TRAIN3 [91.52 (209.93) %].

Table III: Overall analysis of participants' saliva flow rate, DHEA and sIgA secretion rate during training twice (2x) a week vs. training three times (3x) a week pre and post exercise intervention

Parameter	Training frequency	N	Mean (SD)	Cohens ES *d*	95% CI	Significance P
Saliva Flow Rate Pre	2	40	0.39 (0.30)	0.31	-0.13,0.75	0.03*
Saliva Flow Rate Post	2	40	0.49 (0.35)			
% change			32.91 (85.49)			0.25
Saliva Flow Rate Pre	3	45	0.46 (0.30)	0.13	-0.28,0.54	0.49
Saliva Flow Rate Post	3	45	0.50 (0.31)			
% change			78.38 (223.77)			
DHEA Pre	2	33	76.31 (106.46)	0.22	-0.26,0.71	0.06
DHEA Post	2	33	104.78 (147.95)			
% change			51.48 (89.97)			0.68
DHEA Pre	3	41	56.93 (47.55)	0.15	-0.28,0.58	0.31
DHEA Post	3	41	63.33 (36.46)			
% change			62.87 (131.05)			
sIgA Sec Rate Pre	2	36	23.95 (17.67)	0.44	-0.03,0.91	0.07
sIgA Sec Rate Post	2	36	39.32 (46.11)			
% change			64.18 (160.95)			0.39
sIgA Sec Rate Pre	3	37	17.78 (14.69)	0.34	-0.12,0.80	0.09
sIgA Sec Rate Post	3	37	26.19 (31.57)			
% change			91.52 (209.93)			

* P<0.05

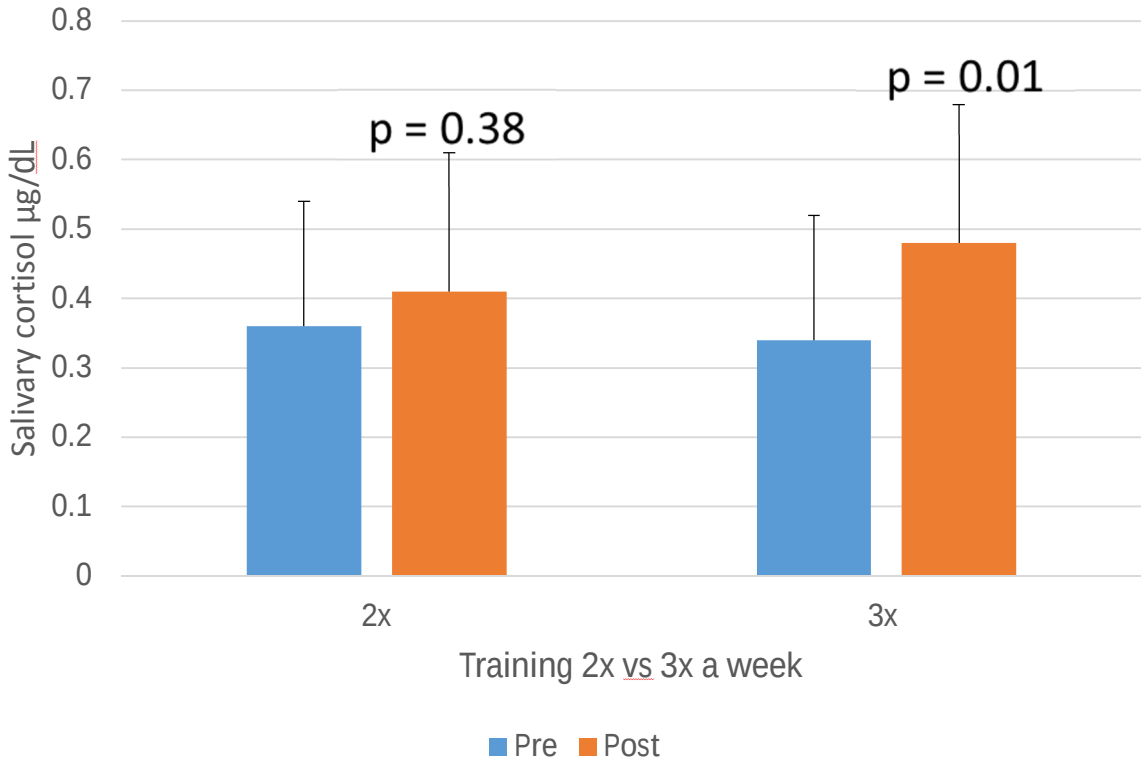

Figure 6: Salivary cortisol analysis of participants training twice (2x) a week vs. three times (3x) a week showing the P values pre and post exercise

DISCUSSION

The main finding of this study was that a group exercise programme performed three times per week significantly increased the baseline salivary cortisol of elderly individuals. In addition, training two or three times a week resulted in increases in salivary DHEA, sIgA and cortisol that were of small to moderate practical significance based on the calculation and interpretation of effect sizes (Hopkins et al., 2009).

The result of the present study is similar to that of Mura et al. (2014) who also reported a significant increase in cortisol in elderly subjects at the conclusion of a 12 week exercise programme. Similarly to Mura et al. (2014), it is proposed that the significantly higher salivary cortisol levels post exercise could be an adaptation of the HPA axis to training three times a week as the level is not pathological, remaining within the normal ranges of 15.5 ± 0.8 nmol/L (range, 10.2-27.3) as determined by Laudat et al. (1988). Heaney et al. (2013) stated that when the elderly were exposed to severe stress, exercise could be one of the ways to protect them from an increased cortisol:DHEA ratio. Increasing age leads to a decline in DHEA production, a process termed adrenopause. Cortisol production does not change with age and this leads to an increased level of cortisol than DHEA in the elderly (Phillips et al., 2007). According to several studies the increased cortisol:DHEA ratio has been linked to frailty (Heaney et al., 2012). These authors reported that elderly participants with low cortisol levels during the day demonstrated the worst performance on the Berg Balance Scale and lower handgrip strength (Heaney et al., 2012). This indicated a correlation between physical functioning and levels of cortisol during ageing (Mura et al., 2014). Based on Seyle's original theories on stress and distress, Mura et al. (2014) deduced that if a strong stress can bring about extremely high levels of cortisol, then moderate stress could be regarded as adaptive thereby stimulating a moderate but not excessive cortisol level.

Cortisol restores homeostasis of the body after exposure to exercise and this is vital in promoting protein synthesis that is necessary for the adaptation process in response to exercise (Hackney and Walz, 2013). The current exercise programme may have reset baseline cortisol levels thereby enhancing all the positive effects of cortisol on the body “at rest” such as promoting gluconeogenesis. In their review on certain biomarkers of physical activity and exercise, Palacios et al. (2015) reported the benefits of cortisol as opposed to being regarded as the stress hormone only. They stated that cortisol counters insulin effects, where via the stimulation of gluconeogenesis, cortisol promotes high blood glucose levels. The expression of enzymes that are critical for gluconeogenesis is activated by the presence of cortisol, thereby increasing glucose production. According to Giordano et al. (2012), cortisol is also responsible for stimulating glycogen synthesis in the liver, thereby decreasing the net blood sugar levels. Cortisol has therefore been found to carefully control the level of blood glucose. This is beneficial in an exercise programme in the elderly especially if they are suffering from chronic diseases such as diabetes.

Palacios et al. (2015), highlighted other metabolic functions of cortisol, one of which is the regulation of a correct pH of extracellular liquid. Cortisol regulates the action of the cellular sodium-potassium pumps in order to reach an ion equilibrium after a destabilizing event (Giordano et al., 2012). The increase in baseline cortisol levels in this study could have been associated with increased Na^+, K^+ pump enzyme (ATPase) activity in the sarcolemma of muscle in the elderly subjects as an adaptation to the exercise intervention. Studies indicated that the elevation in cortisol levels during exercise promotes epinephrine formation, thereby activating Na^+, K^+ pumps (Hackney and Walz, 2013). Related to the impact of exercise on cortisol, Traustadó´ttir et al. (2004), showed that elderly women who did aerobic exercise in the form of walking on the treadmill had a slower reduction in cortisol levels compared to their control group, during the cool-down period, immediately after the exercise. Therefore from the evidence cited above, the high levels of cortisol seem to indicate a positive impact on the health of the elderly living in the old aged homes. The results of the study also indicated that the increased cortisol levels post exercise was not found to be immunosuppressive and will be discussed in detail below.

The results of this study indicated that salivary sIgA secretion rates approached significance with both the groups having small to moderate effect sizes which is in keeping with results from other similar studies, despite the significant increase in cortisol. Numerous studies have shown that moderate intensity exercise increases the salivary sIgA secretion rate. Shimizu et al. (2007) found from their study that the elderly who exercised daily at moderate intensity had a higher salivary sIgA secretion rate compared to those whom led sedentary lives. They further stated chronic moderate physical activity constructively modified immune function. Studies conducted by Akimoto et al. (2003) concluded that sIgA concentration and secretion rates significantly increased after their 12 month exercise programme on the aged.

The data obtained for DHEA indicated that for training twice a week post exercise the levels were approaching significance with a P value of 0.06 and there was a small effect size (*d*=.22). Training three times a week had no change post exercise. This could have resulted from the high cortisol:DHEA ratio as is commonly seen in the elderly due to adrenopause. The cortisol levels post exercise were significantly higher than DHEA. Our findings are in keeping with literature that also indicated that an increase in DHEA approached significance or was not statistically significant (Aldred et al., 2009), with exercise. A study on the effects of a single bout of exercise on postmenopausal women conducted by Kemmler et al. (2003) reported a 10% increase in DHEA immediately after exercise. However, Aldred et al. (2009) established that DHEA levels did not significantly increase in their study on the elderly, after a bout of low moderate intensity exercise and they concluded that a number of studies presented contradictory results for the effects of exercise on DHEA (Aldred et al., 2009).

The results of this study indicate that training twice and three times a week was beneficial for the elderly as the effect sizes of sIgA for both training frequencies showed practical significance. We have determined that training twice a week has greater benefits for mucosal immunity, as it resulted in a trend for an increase in DHEA which is also immune enhancing, while there was no trend for cortisol increasing. We can therefore say that in theory training twice a week, the cortisol:DHEA ratio is expected to drop and therefore has greater immunity benefits on this aspect of health

but there is no proof that this holds true when looking at functional or physical abilities of the elderly. Most research studies have designed their training programmes using a training frequency of three times a week. Although there was no significant percentage change in the sIgA secretion rate between TRAIN2 and TRAIN3, there was a slightly higher percentage in the TRAIN3 group. This is in accordance with literature where moderate intensity exercise performed three times a week increases mucosal immunity. Importantly, to the best of the author's knowledge this is the first study to compare mucosal immunity and HPA axis salivary biomarkers in response to different training frequencies in the elderly living in aged care facilities.

Study Limitations

Considering the age of the individuals under study, participant attrition may be due to ill health or mortality. This could have affected the reliability of the results obtained due to the sample size decreasing mid-way through the exercise programme as the participants had to complete the full twelve week exercise intervention in order for their saliva samples to be tested. Only institutionalized older persons participated in the study and this posed a restriction as it lead to a non-diverse group of participants. There was also a restriction in obtaining more participants that were medically fit to complete the twelve week exercise intervention. A longer exercise programme may have been more beneficial in obtaining better results especially for DHEA. Cortisol samples should be collected immediately after awakening, 30 minutes after awakening and in the evening (Wilcox et al., 2014). This will give us the diurnal cortisol levels. A recommendation will be to conduct a similar study but with cortisol collected during these times of the day to get a true reflection of the cortisol levels. Cortisol levels are highest immediately after waking and the samples in this study were collected at that time. This could have influenced the results obtained in this study where cortisol levels were significantly increased post exercise. An extension of such a study to evaluate the relationship between increased cortisol and blood glucose levels is also recommended for future studies. Since the participants in this study were in a fasting state when saliva collection was done, a possible reason for their increase in cortisol

post exercise could be due to their blood glucose levels possibly being low (Tabata et al., 1991). During fasting when blood glucose levels are low, cortisol is responsible for ensuring a glucose basal concentration by activating gluconeogenesis (Giordano et al., 2012).

CONCLUSION

In South Africa there is limited research on older persons living in aged care facilities. The findings from this study will help in the development of an effective exercise programme for implementation at these facilities. This will encourage the older persons living there to improve their health, thereby reducing the burden of chronic diseases and promote active ageing. The training frequency of both twice and three times a week had small to medium effect sizes on salivary sIgA secretion rate which enhances immune function in the elderly. Training twice/ week resulted in a trend for an increase in DHEA which is also immune enhancing and with long term implementation could lead to a decreased cortisol:DHEA ratio. This may help prevent the incidence of illnesses such as the common cold and flu and thereby allow them to lead better quality lives. A similar study may be replicated in the future using community dwelling older persons as participants. This will enable us to draw comparisons on the effects of the lifestyle and living environment on mucosal immunity and HPA axis activation, of the aged that are community dwelling or living in aged care facilities.

CHAPTER 4: CONCLUSION

Statistics South Africa (2014) reported that a changing population age structure over the last decade has led to an increase in the proportion in elderly people in South Africa. Population ageing raises a serious health concern as there is an increase in most chronic diseases such respiratory illness, diabetes and cardiovascular diseases with ageing (Alli and Maharaj, 2013). Frail people are vulnerable to stress and therefore it has been postulated that increased activity of the HPA axis may accelerate their decline (Gupta and Morley, 2014). Participating in regular physical activity has been shown to be an effective treatment of depression in the elderly (Phillips et al., 2007). During the last few years there has been an increasing interest in biomarkers which has been directed at evaluating health-related aspects that can be controlled by regular physical activity (Palacios et al., 2015).

The use of salivary biomarkers has become preferential to blood as saliva is non-invasive and pain-free to collect, and in addition, has a high correlation to blood. The results of this study demonstrated that after a 12 week exercise programme there was a practical increase in their sIgA levels for both groups as the effect size for sIgA showed practical significance. DHEA approached significance in training twice/week. The increase in DHEA levels could have reduced the cortisol:DHEA ratio. A high ratio is typically associated with ageing. The long term and regular use of such an exercise programme could lead to possible increases in DHEA concentrations (de Gonzalvo-Calvo et al., 2012). Based on these findings we can conclude that training twice/week may also have benefits for mucosal immunity. The significant increase in sIgA in both groups concurs with the numerous studies carried out as well as one of the aims of this study, indicating that moderate intensity exercise increases sIgA levels in the elderly. This finding indicates that such an exercise programme has the potential to improve mucosal immunity in the elderly population living in aged care facilities. There was a significant and practical increase in cortisol after training three times/week. Although our aim on achieving decreased cortisol levels post exercise was not met, the significant increase in cortisol has been thought to be an adaptation to stressful events and circumstances associated with living in old age facilities. We have concluded that this can have positive physiological bearings on the health of the

elderly as it was not found to be immunosuppressive and did not conform to most of the literature.

Recommendations for further research could include the collection of cortisol three times a day pre and post a structured exercise programme, to give a more accurate reading of the levels of cortisol over a period of time. An exercise programme as such will be highly beneficial to the elderly living in aged care facilities as it has been found to be effective in improving mucosal immunity in the elderly. It is recommended that a twelve week structured exercise programme, training twice/week, be implemented as part of an old age home activity for the residents. This recommendation is made based on the findings that DHEA especially, showed promise for increasing and over a long period of time the DHEA levels could improve thereby adding to the enhancement of the mucosal immunity. This programme or one similar should be implemented in all or most aged care facilities in the eThekwini Municipality and other provinces within South Africa. This programme can be adapted to become part of the daily activity in aged care facilities. Such a programme may lead to increased quality of life, decrease in chronic and URTI diseases, lead to better mobility and decrease in frailty.

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APPENDICES

Appendix A: Pre-saliva collection instructions

Saliva sample to be collected between 8:30 and 9:30, approximately 90 minutes after waking.

1. The participants should not eat breakfast within 60 minutes of sample collection
2. DO NOT brush their teeth before sample collection
3. Avoid dairy products for 20 minutes before sample collection
4. Avoid foods with high sugar, acidity, high caffeine content immediately before sample collection
5. Participants should rinse their mouth with water to remove food residue before sample collection
6. Participants should wait at least 10 minutes after rinsing before collecting saliva to avoid dilution of the sample

Appendix B: Intervention

DATA RECORDING SHEET 2.1 - DATA COLLECTED PRIOR TO EACH EXERCISE SESSION

STUDY ID:

DOB: (DD/MM/YYYY)_____ GENDER: _____ AGE:

DATE	SESSION NO.	DIETARY INTAKE	FLUID INTAKE	SLEEP PATTERNS	MEDICATION

DATA RECORDING SHEET 2.2

STUDY ID:

DOB: (DD/MM/YYYY)_____ GENDER: _____ AGE: __

MEASUREMENTS

DATE	RESTING HEART RATE (BPM) - BEFORE EXERCISE	
	RESTING BP (MMHG) – BEFORE EXERCISE	
	RESTING HEART RATE (BPM) – AFTER EXERCISE	
	RESTING BP (MMHG) – AFTER EXERCISE	

DATE	RESTING HEART RATE (BPM) - BEFORE EXERCISE	
	RESTING BP (MMHG) – BEFORE EXERCISE	
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DATE	RESTING HEART RATE (BPM) - BEFORE EXERCISE	
	RESTING BP (MMHG) – BEFORE EXERCISE	
	RESTING HEART RATE (BPM) – AFTER EXERCISE	
	RESTING BP (MMHG) – AFTER EXERCISE	

Appendix C: Exercise Programme

Phase 1: Weeks 1-4 (Group 1 - 3x/ week; Group 2 - 2x/ week)

ACTIVITY	REPS	SETS	RESISTANCE	INTENSITY (RPE)	WORK	REST	TOTAL DURATION
WARM-UP				2			10 min
1. Fwd walk, turn							2 min
Dynamic stretching: See Appendix E1							
2.Sit to stand	10	1					
3. Seated shoulder abduction (double side arm raise)	10	1					
4. Seated ankle 4 way	10	1					
5.Semi-tandem walk (stride balance)	1min	1					
6.Circle walking	1min	1					
7.Tandem walk (heel- toe)	1min	1					
8.Seated neck flexion	10	1					

9.Seated neck rotation	10	1					
ENDURANCE EXERCISE See Appendix E2				3	3.5min /station	1min/ station	18 min
ACTIVITY	REPS	SETS	RESISTANCE	INTENSITY (RPE)	WORK	REST	TOTAL DURATION
STRENGTH EX See Appendix E3				3			
1.Ball Squat (slide)	10	1	Body weight	3			
2. Wall push-up	10	1	Body weight	3			
3. Hip ext (thigh extend)	10	1	Theraband (yellow/red)	3			
4. Seated bicep curl	10	1	1-2 kg	3			
5. Calf raises	10	1	Body weight	3			
6. Seated Tricepext	10	1	1-2 kg	3			
7. Seated shoulder lateral raises	10	1	Theraband (yellow/red)	3			

8. Supine arm/leg march	10	1	No weight				
9. Seated rowing	10	1	Theraband (yellow/red)	3			
10. Bridging	10	1	No weight				

ACTIVITY	REPS	SETS	RESISTANCE	INTENSITY (RPE)	WORK	REST	TOTAL DURATION
COOL-DOWN				1-2			5min
1. Fwd walk, turn							2 min
Static stretching See Appendix E4							
1. Neck/ shoulder/chest	15s	1					
2. Seated rhomboid	15s						
2. Seated triceps	15s	1					
3. Lats/oblique	15s	1					
4. Hamstring	30s	1					
5. Gastroc	15s	1					

Phase 2: Weeks 5-8 (Frequency: Group 1 – 3x/ Week; Group 2 – 2x/ Week)

ACTIVITY	REPS	SETS	RESISTANCE	INTENSITY (RPE)	WORK	REST	TOTAL DURATION
WARM-UP				2			10 min
1. Fwd walk, turn							2 min
Dynamic stretching: See Appendix C1)							
2.Sit to stand	10	2					
3. Seated shoulder abduction (double side arm raise)	10	2					
4. Seated ankle 4 way	10	2					
5.Semi-tandem walk (stride balance)	1min	2					
6.Circle walking	1min	2					
7.Tandem walk (heel- toe)	1min	2					
8.Seated neck flexion	10	2					
9.Seated neck rotation	10	2					

ENDURANCE EXERCISE See Appendix C2				4	3.5min /station	1min/ station	18 min
STRENGTH EX See Appendix F3				4			22 min
1. Ball Squat (slide)	10	2	Body weight	4			
2. Wall push-up	10	2	Body weight	4			
3. Hip ext (thigh extend)	10	2	Theraband (green/blue)	4			
4. Seated bicep curl	10	2	3-4 kg	4			
5. Calf raises	10	2	Body weight	4			
6. Seated Triceps ext	10	2	3-4 kg	4			
7. Seated shoulder lateral raises	10	2	Theraband (green/blue)	4			
8. Arm/leg march in sitting	10	2	No weight				
9. Seated rowing	10	2	Theraband (green/blue)	4			
10. Seated crunches	10	2	Theraband (green/blue)				

ACTIVITY	REPS	SETS	RESISTANCE	INTENSITY (RPE)	WORK	REST	TOTAL DURATION
COOL-DOWN				2			5min
1. Fwd walk, turn							2 min
Static stretching See Appendix C4							
1. Neck/ shoulder/chest	15s	1					
2. Seated rhomboid	15s						
2. Seated triceps	15s	1					
3. Lats/oblique	15s	1					
4. Hamstring	30s	1					
5. Gastroc	15s	1					

Phase 3: Weeks 9-12 (Frequency: Group A – 3x/ Week; Group B – 2x/ Week)

ACTIVITY	REPS	SETS	RESISTANCE	INTENSITY (RPE)	WORK	REST	TOTAL DURATION
WARM-UP				2			10 min
1. Fwd walk, turn							2 min
Dynamic stretching:							

See Appendix C1							
2.Sit to stand	10	2					
3. Seated shoulder abduction (double side arm raise)	10	2					
4. Seated ankle 4 way	10	2					
5.Semi-tandem walk (stride balance)	1min	2					
6.Circle walking	1min	2					
7.Tandem walk (heel- toe)	1min	2					
8.Seated neck flexion	10	2					
9.Seated neck rotation	10	2					
ENDURANCE EXERCISE See Appendix C2				4	5 min /station	1min/ station	24 min
STRENGTH EX See Appendix C3				4			22 min
1. Ball Squat (slide)	10	2	Body weight	4			

2. Wall push-up	10	2	Body weight	4			
3. Hip ext (thigh extend)	10	2	Theraband (green/blue)	4			
4. Seated bicep curl	10	2	3-4 kg	4			
5. Calf raises	10	2	Body weight	4			
6. Seated Triceps ext	10	2	3-4 kg	4			
7. Seated shoulder lateral raises	10	2	Theraband (green/blue)	4			
8. Arm/leg march in sitting	10	2	No weight				
9. Seated rowing	10	2	Theraband (green/blue)	4			
10. Seated crunches	10	2	Theraband (green/blue)				

ACTIVITY	REPS	SETS	RESISTANCE	INTENSITY (RPE)	WORK	REST	TOTAL DURATION
COOL-DOWN				2			5min
1. Fwd walk, turn							2 min
Static stretching See Appendix C4							
1. Neck/	15s	1					

shoulder/chest							
2. Seated rhomboid	15s						
2. Seated triceps	15s	1					
3. Lats/oblique	15s	1					
4. Hamstring	30s	1					
5. Gastroc	15s	1					

Appendix C1: Warm-up Exercises

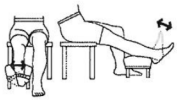
Exercise Program For: Date:2013/02/07
Warm-up Page:1

Sit to stand

• Begin by sitting on the front half of a chair, feet shoulder width apart.
• Stand up with a straight back to full upright position.
• Sit back down.
• Repeat.

Perform 1 set of 10 Repetitions, M,W,F.

Ankle 4 way



• Sit with good posture, one leg straight out.
• Move foot up (toward shin), then down (pointing toes).
• Move foot to left, then to right.
• Repeat 5x.
• Perform 5x with other leg out.

Perform 1 set of 10 Repetitions, M,W,F.

Walking

• Walk around a circle at a comfortable pace.
• Maintain good posture.
• Walk for 1 min..

Perform 1 set of 1 Minute, M,W,F.

Neck forward bend

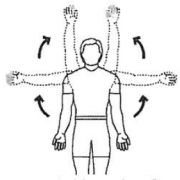
• Sit with good posture, back supported, head facing forward.
• Move chin down to chest.
• Return to start position.
• Repeat.

Special Instructions:

Move in painfree range.

Perform 1 set of 10 Repetitions, M,W,F.

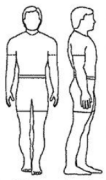
Double side arm raise



• Sit with good posture, arms at side, palms forward.
• Lift arms out and upward above head as shown.
• Return to start position.

Perform 1 set of 10 Repetitions, M,W,F.

Standing stride balance



• Stand with good posture, feet in contact with heel of left foot in line with big toe of right.
• Step forward with heel of right foot in line with bog toe of left, keeping feet in contact.
• Walk for 1 min.
• .

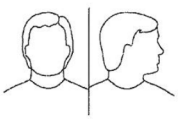
Perform 1 set of 1 Minute, M,W,F.

Heel toe walk

• Begin by standing as shown (left foot in front, in line with right, heel and toe in contact).
• Step forward with right foot, placing it in line with left foot.
• Continue to step, placing left foot in front of right.
• Repeat sequence for 1 min..

Perform 1 set of 1 Minute, M,W,F.

Neck twist



• Sit with good posture, back supported, head facing forward.
• Turn head to right, return to start.
• Turn head to left, return to start
• Repeat.

Special Instructions:

Stay in painfree range.

Perform 1 set of 10 Repetitions, M,W,F.

Appendix C2: Endurance Exercises

EXERCISE 1 – WEAVING BETWEEN CONES

- Participants weave between 6 cones set approximately 1 meter apart.
- Participants continue to weave until they are requested to stop.

EXERCISE 2 – STEP UP AND OVER

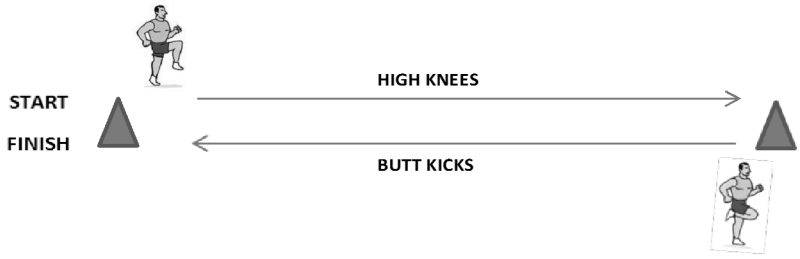
- Participants step up and over 5 aerobic steps whilst walking. The aerobic steps are placed 1 meter apart. The start is designated by a cone, placed 1 metre before the first step.

EXERCISE 3 – SHUTTLE WALK

- 6 cones are placed 1 meter apart. Cone 1 is the start (designated by a different colour). Participants are instructed to walk to cone 2 and return to the start, then to cone 3 and return to the start, then to cone 4 and return to the start, then to cone 5 and return to the start and finally cone 6 and back to the start. This sequence is continued until the participant is requested to stop.

EXERCISE 4 – HIGH KNEES/BUTT KICKS

- Two cones are placed approximately 6 meters apart. Participants proceed to cone 2 whilst performing high knees and return to cone 1 performing butt kicks. This sequence is continued until the participant is requested to stop.



PROGRESSION

Phase 1 – Weeks 1 to 4

Five participants are placed at each of the four exercise stations .Participants perform the designated exercise continuously for 3½ minutes. A rest of 1 minute is given after the 2½ min work period. Participants move on to the next exercise station.

Intensity: RPE = 3

Phase 2 – Weeks 5 to 8

Five participants are placed at each of the four exercise stations .Participants perform the designated exercise continuously for 3½ minutes per exercise station. A rest of 1 minute is given after each exercise station is completed .

Intensity: RPE = 4

Phase 3 – Weeks 9 to 12

Five participants are placed at each of the four exercise stations .Participants perform the designated exercise continuously for 5 minutes per exercise station. A rest of 1 minute is given after each exercise station is completed. Participants move on to the final two exercise stations.

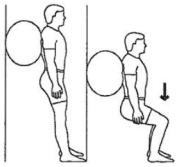
Intensity: RPE = 4

Appendix C3: Strength Exercise

Exercise Program For: Date:2013/02/08

Resistance exercises (weeks 1- 4) Page:1

Ball 90 wall slide



- Place ball between back and wall, feet shoulder width apart.
- Slowly bend knees to 60-90 degrees.
- Keep knees behind line of toes during bend.
- Return to standing position.
- Repeat.

Special Instructions:

Maintain proper low back posture.

Perform 1 set of 10 Repetitions, M,W,F.

Use Ball.

Elastic thigh extend

- Stand with good posture alongside a wall.
- Use the wall for balance and support.
- Attach elastic to secure object at knee level in front.
- Loop elastic around thigh just above knee.
- Stand, facing toward the pull.
- Extend leg backward, keeping knee straiight.
- Return to start position and repeat.

Special Instructions:

Keep knee slightly bent on leg that you are standing on.

Perform 1 set of 10 Repetitions, M,W,F.

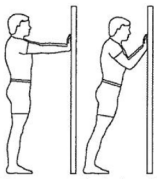
Use yellow/red Elastic.

Double heel raise

- Stand with good posture, using chair for balance.
- Raise up on toes, through full range.
- Return to start position and repeat.

Perform 1 set of 10 Repetitions, M,W,F.

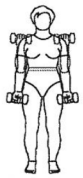
Wall push up



- Stand facing wall, 30-45 cm away, feet shoulder width apart.
- Place hands slightly wider than shoulder width on wall at shoulder height.
- Slowly bend elbows, bringing face and chest to wall.
- Push back up to start position and repeat.

Perform 1 set of 10 Repetitions, M,W,F.

Double DB biceps curl



- Sit with good posture.
- Begin with arms at side, elbows straight, palms up, weights in hand.
- Bend elbows upward.
- Return to starting position.

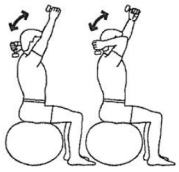
Special Instructions:

Keep elbows close to sides through entire movement.

Perform 1 set of 10 Repetitions, M,W,F.

Use 1-2 Kilograms.

DB Triceps lift on ball



- Sit on chair with good posture, back supported, weights in hands.
- Raise arms as shown, with elbows bent.
- Straighten one elbow, return to start.
- Straighten other elbow, return to start.
- Alternate arms.

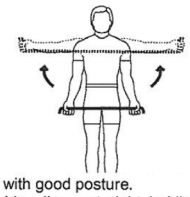
Special Instructions:

Maintain proper low back position.

Perform 1 set of 10 Repetitions, M,W,F.

Use 1-2 Kilograms.

Tubing double arm raise

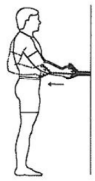


• Sit forward on chair with good posture.
• Begin with arms at side, elbows straight, holding elastic which is beneath thighs, palms forward.
• Raise arms upward, out to side to shoulder height.
• Return to starting position.

Perform 1 set of 10 Repetitions, M,W,F.

Use yellow/red Elastic.

Close elbow rows

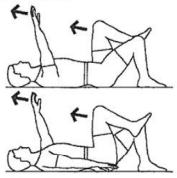


• Sit with good posture.
• Secure elastic at waist level.
• Hold elastic in hands with arms extended.
• Pull back, bending elbows and squeezing shoulder blades together, keeping elbows close to sides.
• Return to start position and repeat.

Perform 1 set of 10 Repetitions, M,W,F.

Use yellow/red Elastic.

Supine marching arm salute



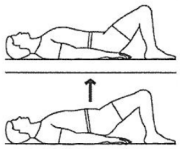
• Lie on back with knees bent, low back in neutral.
• Tighten abdominal muscles.
• Raise left leg and right arm off floor as shown.
• Return to start position.
• Repeat with right leg and left arm.

Special Instructions:

Maintain neutral spine without twisting or rotating hips. Move in smooth and controlled movements.

Perform 1 set of 10 Repetitions, M,W,F.

Bridging



• Lie on back with knees bent.
• Tighten abdominal muscles.
• Lift buttocks off floor, maintaining neutral spine.
• Return to start position.

Special Instructions:

Maintain neutral spine.

Perform 1 set of 10 Repetitions, M,W,F.

Appendix C4: Cool-down Exercises

Exercise Program For: Date:2013/02/0ε
Cool down stretches Page:1

Rhomboid stretch

- Sit with good posture, back supported.
- Bring left arm across in front of body as shown.
- Hold elbow with right arm.
- Gently pull across chest until a stretch is felt in the back of shoulder.
- Repeat with other arm

Perform 1 set of 1 Repetition, M,W,F.

Hold exercise for 15 Seconds.

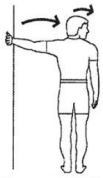
Triceps stretch

- Sit with good posture, back supported.
- Lift arms overhead.
- Bend elbow of one arm.
- With other arm, slowly push down on ebow, keeping elbow bent.
- Repeat with other arm.

Perform 1 set of 1 Repetition, M,W,F.

Hold exercise for 15 Seconds.

Plexus stretch



- Stand with good posture, left arm on wall, hand backward as shown, feet shoulder width apart.
- Slowly turn body outward until as stretch is felt across chest.
- Slowly turn neck to right until a stretch is felt down the front of arm.
- Repeat on other side.

Perform 1 set of 1 Repetition, M,W,F.

Hold exercise for 15 Seconds.

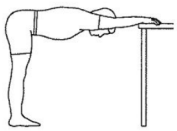
Sidebend stretch

- Stand with good posture, feet shoulder width apart.
- Raise right arm overhead behind head, holding with left arm.
- Bend knees slightly to provide better balance.
- Pull arm as you bend trunk to left.
- Repeat with other side.

Perform 1 set of 1 Repetition, M,W,F.

Hold exercise for 15 Seconds.

Bent 90 ham stretch

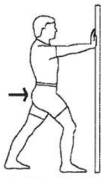


- Stand in front of table with feet shoulder width apart.
- Place hands on table.
- Bend at hips and tighten the muscles in fronts of thighs, keeping the knees straight.
- Keep low back straight.

Perform 1 set of 2 Repetitions, M,W,F.

Hold exercise for 15 Seconds.

Runner stretch



- Stand facing wall, hands on wall, elbows straight.
- Step forward with foot of one leg, bending knee and leaning hips toward wall.
- Keep rear leg straight with heel on floor.
- Keep feet facing forward.
- Repeat on other side.

Perform 1 set of 1 Repetition, M,W,F.

Hold exercise for 15 Seconds.

Appendix D: Borg's Rating of Perceived Exertion Scale

Rating of Perceived Exertion

Rating	Verbal Anchor
0	Rest
1	Very easy
2	Easy
3	Moderate
4	Sort of hard
5	Hard
6	
7	Very hard
8	Very, very hard
9	Near maximal
10	Maximal

Appendix E: Ethical Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

26 February 2014

Ms Prathna Dudhrajh
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4133
dudhrajhp@ukzn.ac.za

PROTOCOL: Effects of group exercise on mucosal immunity and hypothalamic-pituitary adrenal axis activation in older persons living in aged care facilities within the eThekwini Municipality. REF: BE079/14

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 07 November 2013. The Committee has noted that this is a **sub-study of BE251/11.**

The conditions have now been met and the study is given full ethics approval and may begin as from 28 February 2014.

This approval is valid for one year from **28 February 2014.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

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

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

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

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on **08 April 2014.**

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

Yours sincerely

Professor D.R Wassenaar
Chair: Biomedical Research Ethics Committee

cc: Supervisor: Ramklasss@ukzn.ac.za

APPENDIX F: Saliva Data collection sheet

PRE-TEST							POST-TEST					
CODE	DRY WEIGHT (g)	WET WEIGHT (g)	TIME (min)	DHEA pg/ml	CORTISOL ug/dl	IgA ug/ml	CODE	DRY WEIGHT (g)	WET WEIGHT (g)	TIME (min)	DHEA pg/ml	CORTISOL ug/dl
1-TOR-DUB	3.31	3.83	4	12.57	0.44	27.45	103-TOR-DUB	3.32	4.33	4	16.26	0.46
2-RAY-MOOA	3.31	3.36	4	142.35	0.25	187.21	131-RAY-MOO	3.31	7.11	4	181.13	0.56
3-TOR-GUE	3.31	4.24	4	139.38	0.46	52	107-TOR-GUE	3.31	3.07	4	186.12	1.05
4-RAY-DHUN	3.31	4.96	4	25.67	0.63	43.69	112-RAY-DHU	3.29	4.61	4	108.29	2.58
5-ABH-GOVP	3.31	4.4	4	31.76	0.19	66.24	139-ABH-GOV	3.31	3.76	4	35.75	0.12
6-TOR-HAR	3.3	5.04	4	59.13	0.51	62.13	106-TOR-HAR	3.32	8.18	4	80.87	0.86
7-MARY-SHER	3.3	3.76	4	29.29	0.32	127.26	167-MARY-SH	3.32	5.51	4	55.13	0.84
8-TOR-MAR	3.28	5.92	4	181.13	0.59	96.54	101-TOR-MAR	3.32	5.57	4	113.74	0.49
9-TOR-PILL	3.31	4.58	4	n/a	n/a	n/a					n/a	n/a
10-TOR-MUD	3.31	3.47	6	n/a	n/a	n/a					n/a	n/a
11-TOR-BAR	3.31	3.76	5	140.22	0.54	37.35	104-TOR-BAR	3.33	4.38	4	93	0.57
12-TOR-HOB	3.3	7.05	4	18.26	0.11	23.41	151-TOR-HOB	3.32	8.11	4	35.6	0.25
13-TOR-MOO	3.3	7.26	4	44.93	0.82	7.39	152-TOR-MOO	3.32	8.67	4	91.84	1.31
14-TOR-ADA	3.3	4.48	4	40.01	0.33	23.48	111-TOR-ADA	3.32	6.77	4	36.04	0.62
15-TOR-BEH	3.31	4.12	4	39.54	0.29	41.76	110-TOR-BEH	3.32	6.19	4	28.05	0.5
16-TOR-GRO	3.3	0	5	n/a	n/a	n/a	109-TOR-GRO	3.32	5.17	4	n/a	n/a
17-TOR-ALD	3.3	3.92	4	193.58	0.23	65.44	108-TOR-AND	3.32	7.37	4	28.95	0.49
19-TOR-CUYT	3.28	4.3	4	108.96	0.67	48.08	105-TOR-CUY	3.32	9.53	4		1.33
20-TOR-LANG	3.31	NO SALIV	5				102-TOR-LAN	3.32	NO SALIVA	4		
21-RAY-CHE	3.32	5.33	4		0.27	81.09	114-RAY-CHE	3.32	5.65	4	300.36	0.54
22-RAY-TIM	3.29	4.54	4	23.99	0.29	129.77	116-RAY-TIM	3.31	4.72	4	41.45	0.52
23-RAY-LEE	3.33	3.79	6	26.54	0.23	305.3	115-RAY-LEE	3.29	4.34	4	77.8	0.32
24-RAY-KUM	3.3	5.01	4	48.77	0.26	119.21	123-RAY-KUM	3.32	5.43	4	48.94	0.34
25-RAY-PILLR	3.31	4.26	4	93.81	0.35	18.88	129-RAY-PILL	3.32	4.45	4	133.28	0.64
26-ABH-CHET	3.32	5.54	4	43.76	0.23	18.84	150-ABH-CHE	3.31	5.4	4	38.4	0.2
27-RAY-SURA	3.3	7.41	4	34.29	0.26	16.46	113-RAY-SUR	3.29	5.16	4	42.97	0.32
28-RAY-SLAU	3.32	5.86	4	87.71	0.34	33.4	130-RAY-SLAU	3.32	4.37	4	85.94	0.47
29-RAY-PILLM	3.32	5.7	4	30.48	0.36	69.83	117-RAY-PILL	3.3	5.5	4	23.36	0.55
30-RAY-GEN	3.32	4	4	<10.20	0.27	67.83	125-RAY-GEN	3.32	4.79	4	<10.20	0.4
31-RAY-NAIK	3.3	5.24	4	43.37	0.52	94.46	118-RAY-NAID	3.31	4.72	4	89.31	0.59

32-RAY-PACH	3.31	5.22	4	36.32	0.51	10.34	121-RAY-PACH	3.3	5.53	4	<10.20	0.2
33-RAY-RUIT	3.33	3.89	4	31.85	0.33	461.94	124-RAY-RUIT	3.31	4.64	4	17.05	0.42
34-RAY-VIRR	3.32	3.7	4	26.63	0.37	77.72	119-RAY-VIRA	3.29	4.52	4	40.41	0.54
35-RAY-SATH	3.32	4.12	4	10.24	0.35	74.41	153-RAY-SADA	3.31	4.25	4	20.43	0.5
36-RAY-GOVL	3.32	4.47	4	12.66	0.18	15.78	128-RAY-GOVI	3.33	4.72	4	26.2	0.21
37-RAY-RAM	3.32	4.29	4	10.75	0.23	31.55	126-RAY-RAM	3.29	5.15	4	<10.20	0.23
38-RAY-MOOI	3.32	7.19	4	35.96	0.56	<2.50	120-RAY-MOO	3.29	6.88	4	<10.20	0.12
39-RAY-GANG	3.32	7.57	4	<10.20	0.19	22.81	122-RAY-GANG	3.31	7.23	4	<10.20	0.3
40-RAY-GOVK	3.31	6.58	4	26.52	0.19	10.34	127-RAY-GOVI	3.3	7.06	4	43.63	0.3
41-ABH-APP	3.3	4.37	4	32.19	0.24	22.13	142-ABH-APPL	3.31	4.45	4	44.02	0.16
42-ABH-NAIST	3.33	3.67	4	92.36	1.29	90.91	138-ABH-NAID	3.32	3.69	4	138.09	0.73
43-ABH-JOS	3.31	3.54	4	26.2	0.51	>600.00 insufficient sample to redo	137-ABH-JOSE	3.29	3.66	4	<10.20	0.66
44-ABH-MOO	3.32	6.97	4	70	0.2	66.51	141-ABH-MOO	3.29	6.96	4	95	0.21
45-ABH-BIS	3.29	5.04	4	18	0.24	17.78	133-ABH-BISM	3.31	4.65	4	33	0.2
46-ABH-RAM	3.32	4.02	4	18	0.13	34.7	146-ABH-RAM	3.31	3.79	4	20	0.14
47-ABH-VAN	3.3	5.32	4	21	0.27	8.58	140-ABH-VANI	3.29	6.13	4	32	0.14
48-ABH-CHE	3.32	4.04	4	160	0.26	55.69	147-ABH-CHET	3.29	3.89	4	158	0.52
49-ABH-SINGS	3.31	7.18	4	21	0.18	<2.50	143-ABH-SING	3.32	4.01	4	68	0.28
50-ABH-REDD	3.32	3.47	4	66	0.21	8.29	154-ABH-REDI	3.32	5.31	4	37	0.24
51-ABH-PILL	3.32	6.53	4	42	0.2	16.44	144-ABH-PILLT	3.29	6.48	4	80	0.29
52-ABH-GOVK	3.32	5.65	4	420	0.33	412.95	145-ABH-GOV	3.29	4.73	4	270	0.62
53-ABH-DEEP	3.31	5.44	4	78	0.12	19.65	136-ABH-DEEI	3.31	6.26	4	66	0.21
54-ABH-REDD	3.32	6.54	4	15	0.1	<2.50	132-ABH-REDI	3.32	6.05	4	22	0.25
55-ABH-MAHA	3.32	4.06	4	n/a	n/a	n/a					n/a	n/a
56-ABH-NAISA	3.32	3.67	6	210	0.42	407.21	148-ABH-NAIS	3.32	5.25	4	80	0.19
57-ABH-GOVS	3.31	0	6	n/a	n/a	n/a					n/a	n/a

58-ABH-SAN	3.32	6.12	4	68	0.22	<2.50	149-ABH-SUNI	3.31	6.97	4	31	0.24
59-ABH-SINGH	3.31	4.6	4	n/a	n/a	n/a	134-ABH-SING	3.31	NO SALIVA	6	n/a	n/a
60-ABH-RUGC	3.3	3.61	4	158	0.24	68.21	135-ABH-RAGI	3.2	3.82	4	68	0.42
61-CLAY-HARI	3.32	5.15	4	66	0.4	<2.50	178-CLAY-HAR	3.32	4.46	4	14	0.36
62-CLAY-BEEH	3.32	3.62	4	72	1.1	>600.00 insufficient sample to redo	187-CLAY-BEE	3.31	3.66	4	26	0.14
63-CLAY-NAIS	3.32	3.75	4	150	0.61	372.9	181-CLAY-NAI	3.32	3.48	4	216	1.52
64-CLAY-GOV	3.33	5.24	4	48	0.1	15.36	174-CLAY-GOV	3.32	5.09	4	86	0.19
65-CLAY-HAM	3.31	4.82	4	66	0.54	9.93	186-CLAY-HAN	3.32	4.29	4	21	0.17
66-CLAY-MOC	3.32	4.68	4	<10.2	0.2	115.09	180-CLAY-MO	3.29	4.62	4	23.43	0.26
67-CLAY-NAIV	3.32	6.47	4	13.52	0.13	14.51	179-CLAY-NAI	3.31	4.71	4	31.08	0.13
68-CLAY-PADA	3.32	4.33	4	63.34	0.38	75.78	182-CLAY-PAD	3.31	4.59	4	53.29	0.24
69-CLAY-SING	3.31	6.14	4	54.67	0.4	70.27	177-CLAY-SIN	3.31	5.64	4	90.49	0.4
70-CLAY-LUTC	3.31	4.03	4	241.84	0.67	97.01	176-CLAY-GOV	3.31	4.18	4	53.79	0.31
71-CLAY-BANS	3.32	4.14	6	15.43	0.85	104.98	173-CLAY-BAN	3.32	4.37	4	89.76	1.19
72-CLAY-PATH	3.31	3.43	5	20.4	0.5	133.85	172-CLAY-PAT	3.32	3.79	4	115.94	1.12
73-CLAY-MAIS	3.32	7.07	4	83.28	0.88	11.73	169-CLAY-MAI	3.32	7.25	4	38.23	0.22
74-CLAY-MOC	3.32	7.62	4	73.99	0.15	21.87	188-CLAY-MO	3.31	7.04	4	73.99	0.17
75-CLAY-PUCK	3.32	6.97	4	61.54	0.35	24.64	171-CLAY-PUC	3.32	6.18	4	26.61	0.24
76-CLAY-DEOI	3.31	3.93	4	51.68	0.38	82.81	170-CLAY-DEO	3.32	4.21	4	28.93	0.2
77-CLAY-SEWI	3.32	6.09	4	38.75	0.18	28.8	185-CLAY-SEW	3.31	6.07	4	53.54	0.3
78-CLAY-SEWI	3.31	5.08	4	15.76	0.17	54.31	183-CLAY-SEW	3.32	5.36	4	58.17	0.21
79-CLAY-GAN	3.33	4.48	4	77	0.4	46.38	184-CLAY-GAN	3.29	4.67	4	95.11	0.44
80-CLAY-MAH	3.3	4.95	4	46.46	0.12	57.49	175-CLAY-MAI	3.32	5.15	4	46.46	0.17
82-MARY-NAI	3.32	3.91	4	129.39	0.4	346.04	159-MARY-NA	3.29	3.48	4	1586.8	0.16

83-MARY-GOV	3.32	3.68	4	n/a	n/a	n/a					n/a	n/a
84-MARY-BIJC	3.32	5.58	4	<10.2	0.33	69.22	156-MARY-BIJ	3.31	4.51	4	28.06	0.3
85-MARY-MU	3.31	5.12	4	14.21	0.13	35.55	164-MARY-MU	3.31	6.37	4	32.94	0.26
86-MARY-PILL	3.32	3.79	6	603.93	0.1	430.89	158-MARY-PIL	3.31	4.48	4	65.9	0.57
87-MARY-MAI	3.32	5.2	4	17.7	0.16	16.69	161-MARY-MA	3.3	5.78	4	35.53	0.22
88-MARY-MO	3.31	4.71	4	n/a	n/a	n/a					n/a	n/a
89-MARY-MU	3.32	5.16	4	17.9	0.38	55.2	157-MARK-MU	3.31	4.83	4	17.29	0.38
90-MARY-MU	3.32	6.97	4	87.04	0.24	24.63	165-MARY-MU	3.32	7.45	4	31.81	0.23
91-MARY-VAN	3.31	6.15	4	31.44	0.14	11.24	155-MARY-VA	3.32	4.86	4	17.36	0.35
93-MARY-PRE	3.32	3.38	6	didn't use sample	0.08 (did 1:2 dilution)	111.26	168-MARY-PR	3.32	3.66	4	644.27 (did a 1:2 dilution)	0.13
94-MARY-SITH	3.32	6.64	4	74.8	0.28	60.86	166-MARY-SIT	3.32	6.07	4	142.48	0.47
96-MARY-ROC	3.3	5.11	4	17.36	0.22	38.4	162-MARY-RO	3.31	5.85	4	35.83	0.25
98-MARY-GAI	3.32	5.02	4	22.37	0.06	13.17	160-MARY-GA	3.32	3.95	4	65.19	0.24
99-MARY-SMI	3.31	4.22	4	37.48	0.31	94.14	163-MARY-SM	3.31	4.03	4	49.59	0.67
100-MARY-CA	3.32	7.62	4	n/a	n/a	n/a					n/a	n/a