The Effect of the Osteopathic Thoracic Lymphatic Pump Technique on Salivary Secretory Immunoglobulin A Levels in Adults with Recurrent Upper Respiratory Tract Complaints

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A research project submitted in partial fulfilment of the requirements for the degree of Master of Osteopathy, UNITEC Institute of Technology, 2015
Declaratión

Name of candidate: BLUT, Dominik Patricio Ferdinand

This Research Project entitled
'The effect of the osteopathic thoracic lymphatic pump technique on salivary secretory immunoglobulin A levels in adults with recurrent upper respiratory tract complaints'
is submitted in partial fulfillment of the requirements for the Unitec degree of Master of Osteopathy.
The regulations for the degree are set out in the Masters of Osteopathy Programme Schedule and are elaborated in the course handbook.

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• The contribution of supervisors and others to this work was consistent with the Unitec Policies.
• Research for this work has been conducted in accordance with the Unitec Research Ethics Committee Policy and Procedures and has fulfilled any requirements set for this project by the Unitec Research Ethics Committee.
Research Ethics Committee Approval Number: 2011-1236

Candidate Signature: Date: 15. June 2015

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<tr>
<td>ANS</td>
<td>Autonomic Nervous System</td>
</tr>
<tr>
<td>B cells</td>
<td>B lymphocytes (WBC) that mostly mature into Effector B cells.</td>
</tr>
<tr>
<td>Effector B cells</td>
<td>Plasma cells (WBC) that produce Ig, including IgA.</td>
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<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
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<td>ES</td>
<td>Effect Size</td>
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<td>GALT</td>
<td>Gut Associated Lymphoid Tissue</td>
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<tr>
<td>J-chain</td>
<td>A polypeptide that joins dimeric IgA together with the SP</td>
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<tr>
<td>Ig</td>
<td>Immunoglobulin / Antibody</td>
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<td>IgA</td>
<td>Immunoglobulin A / Antibody A</td>
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<td>IgA1</td>
<td>Monomeric IgA that is mainly found in serum</td>
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<tr>
<td>IgA2</td>
<td>Dimeric IgA that is mainly found in secretions</td>
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<tr>
<td>LPT</td>
<td>Lymphatic Pump Technique, a subcategory of OMT</td>
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<tr>
<td>MALT</td>
<td>Mucosa Associated Lymphoid Tissue</td>
</tr>
<tr>
<td>OMT</td>
<td>Osteopathic Manipulative Treatment</td>
</tr>
<tr>
<td>OU</td>
<td>Oral Ulceration</td>
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<tr>
<td>pIgA</td>
<td>Polymeric Immunoglobulin A</td>
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<td>pIgR</td>
<td>Poly Ig Receptor, allows transcytosis of dimeric IgA across epithelium</td>
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<td>T cells</td>
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<td>TLP</td>
<td>Thoracic Lymphatic Pump. Categorised under LPT</td>
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<td>URTI</td>
<td>Upper Respiratory Tract Infection</td>
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<tr>
<td>WBC</td>
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**Manuscript**

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SECTION 1 - LITERATURE REVIEW
1.0 Introduction

Osteopathic healthcare is based on manual therapeutic attempts to help the body maintain and reestablish homeostasis and healing. This approach incorporates the broad understanding that unhindered flow of blood and lymphatic fluid is a prerequisite for human health. Osteopathic practitioners claim to locate and correct structural or functional changes in the tissues of the human body that may impair the free flow of blood and lymph (Degenhardt & Kuchera, 1996). Osteopaths have used osteopathic lymphatic pump techniques (LPT) as a treatment tool since the late nineteenth century (Still, 1899, 1902). Lymphatic Pump Techniques are rhythmic techniques that attempt, over certain areas of the lymphatic system such as the thorax, liver, spleen, abdomen, and feet, to improve lymphatic flow locally as well as systematically. The osteopathic thoracic lymphatic pump (TLP) is a technique that is performed on the thorax and has been used by osteopaths claiming to improve lymphatic flow (Amalfatino, 1987; Chikly, 2005; Kuchera & Kuchera, 1994a, b; Ward, 2003b). One possible consequence of these techniques is to increase immunoglobulin A (IgA) levels in secretions such as saliva and mucous membranes to protect the body against pathogens. People who have relatively decreased levels of salivary secretory IgA (Sal SIgA) commonly exhibit recurrent upper respiratory tract infection (URTI) and or oral ulceration (OU) (Benjamini, Coico, & Sunshine, 2000; Brandtzaeg, 2007; Hanson, Bjokander, & Oxelius, 1983; Paul, 2003). Two recent research studies (Ehrlenbach, 2011; Saggio, Docimo, Pilc, Norton, & Gilliar, 2011) suggest that Sal SIgA following TLP in a healthy study population may increase slightly over a short period of time. Ehrlenbach (2011) reported a short-term increase of Sal SIgA concentrations immediately post-TLP treatment in all eight participants and a short-term increase of Sal SIgA secretion rate immediately post-TLP treatment in 7 of 8 participants. The immediate post-treatment short-term increase of Sal SIgA secretion rate showed a large effect size (ES) of Cohen’s $d = 3.0$ ($p=0.03$) with an average increase of 136%. Though not controlled, these data indicate a strong correlation between TLP and post-intervention increase of Sal SIgA secretion rate. Saggio, Docimo, Pilc, Norton, and Gilliar (2011) investigated the impact of Osteopathic Manipulative Treatment (OMT), consisting of a combination of three osteopathic techniques including TLP, on Sal SIgA levels by measuring Sal SIgA concentrations 60 minutes post-intervention in a stressed population ($n=25$). Participants were second-year osteopathic medical students at New York College of Osteopathic Medicine who were scheduled to take their national board examination (Comprehensive Osteopathic Medical Licensing Examination - USA Level 1) two to three weeks after their participation in the study. Osteopathic Manipulative Treatment resulted in a significant ($F,5.92; p<0.025$) increase (average increase of 139%) in post-intervention Sal SIgA levels compared with the control group, who sat quietly and relaxed in a separate area for 20 minutes (average increase of 32%).
1.1 Aim

The aim of this research thesis is to investigate the claims and theory behind osteopathic lymphatic pump techniques and a possible effect on immune health, in this case measured by changes in Sal SIgA. The thesis is presented in three sections, literature review, manuscript and appendices. The first section comprises a review of the literature surrounding the human immune system emphasising the physiology and salival secretion of immunoglobulin A, its links with health indicators, and the effect of osteopathy on immune function.

The second section describes a study undertaken to establish to what extent the osteopathic TLP technique affects immediate and short-term Sal SIgA in a repeated case series of four adult males who report recurrent URTI and OU but are otherwise healthy. In order to improve the possibility of detecting any small changes that might occur following this technique, this population was targeted because of the likelihood of a suppressed mucosal immune system. Another aim was to observe whether this population displayed levels of salivary SIgA below the normal reference range.

Ethics documentation, research questionnaires and additional data not reported in the manuscript are presented in the third section.
2.0 Overview of the Human Immune System

Our environment contains numerous microorganisms that can cause disease in the human body. The human immune system is a complex system that is concerned with the defence of the host, primarily against the threat of disease caused by infectious organisms, termed pathogens. Immunity essentially refers to all mechanisms used by the body as protection against environmental agents that are foreign to the body and it may be innate or acquired.

2.1 Innate Immunity

Innate immunity describes pre-existing mechanisms and elements that are designed to prevent infection by pathogens or to mount an immediate defence against the infectious agent. There are physical, chemical and biochemical barriers with which an individual is born, including body surfaces and internal components, such as skin, mucous membranes, the cough reflex, and particular immune cells (Benjamini et al., 2000). These elements present effective barriers to environmental agents and are always present to protect the body from challenges by foreign invaders. They are called innate because they are present before infection, although the amount of some components may increase following infection (Wood, 2006).

2.2 Acquired Immunity

Acquired immunity, also known as the adaptive immune system, is a more specialised form of immunity. It supplements the protection provided by innate immunity and is acquired by the initial contact with the invader, also termed antigen, triggering a chain of events that leads to activation of certain cells. When pathogens enter the human body, antibodies, also called immunoglobulins (Ig), recognise the specific antigens and trigger the immune system to produce antibodies specific to that antigen. The basic antibody molecule is depicted as a Y-shaped structure consisting of four protein subunits, two longer identical subunits called heavy chains linked to each other, which are linked to two shorter identical subunits called light chains, by disulphide bridges (Wood, 2006). Antibodies are divided into five major classes, IgA, IgD, IgE, IgG, and IgM, each of which has several unique biological properties. All antibodies have common structural features, which enable them to recognise and bind specifically to a unique structural entity on an antigen and perform a common biological function after combining with the antigen. Acquired immunity can be induced against thousands of natural and synthetic compounds.
There are two major types of cells that participate in acquired immunity. B lymphocytes or B cells, named after their origin in the bone marrow and T lymphocytes or T cells, named for their differentiation in the thymus. Both B cells and T cells are responsible for their specificity exhibited by the acquired immune response. B cells synthesise and secrete immunoglobulins, with specificity against the antigen, into the bloodstream. This process is termed antibody-mediated or humoral immunity (Benjamini et al., 2000). T cells, which also exhibit specificity against the antigen, by virtue of their T cell receptors, do not make antibodies but perform various effector functions when antigen-presenting cells come into contact with them within secondary lymphoid organs. Unlike B cells, each T cell, bearing many identical antigen receptors, circulates directly to the site of the antigen and performs its function when interacting directly with it. This process is termed cell-mediated immunity (Benjamini et al., 2000).

Lymphocyte maturation, differentiation and proliferation all take place in the lymphatic organs. The primary (central) lymphatic organs, such as the thymus and the bone marrow, are those in which the maturation of T cells and B cells into antigen-recognising lymphocytes occurs. From there, mature B and T cells migrate through the bloodstream to the secondary (peripheral) lymphatic organs, where the antigen-driven proliferation and differentiation take place (Benjamini et al., 2000). Secondary lymphatic organs include mainly the mucosa-associated lymphoid tissue (MALT), particularly the Peyer's patches of the terminal ileum as well as other parts of gut-associated lymphoid tissue (GALT), such as the numerous isolated lymphoid follicles and the appendix. Other sites include the lymph nodes, the spleen and the tonsils (Brandtzaeg, 2007).

2.3 Immunoglobulin A

Immunoglobulin A (IgA) is the predominant antibody in mucosal secretions and acts with innate mucosal defences to provide the first line of defence against pathogens and antigens presented at the mucosa. Reduced secretion of IgA may indicate immunosuppression (Gleeson & Pyne, 2000; Gleeson et al., 1999; Gleeson, Pyne, & Callister, 2003; Hagewald, Bernimoulin, Kottgen, & Kage, 2002; Moreira, Delgado, Moreira, & Haahela, 2009; Neville, Gleeson, & Folland, 2008; Pyne et al., 2000; Tewu, Bosch, Enno, Veerman, & Amerongen, 2004); IgA levels and secretion rate are linked with health indicators (Chikly, 2005; Hanson et al., 1983; Rossen, Butler, & Waldman, 1970). This section provides an overview of IgA physiology, including structure, production, secretion, induction and regulation; diagnosis and monitoring of IgA; as well as consequences of deficiency and its relation to health. In the human body IgA is present as serum IgA and as secretory IgA (SIgA), the latter of which can be found as the main antibody in various external secretions such as saliva (Sal
SIgA), mucus in the intestinal and respiratory tracts, gastric fluid, sweat and breast milk. Two IgA heavy constant region (C\(\alpha\)) genes are present, giving rise to two IgA subclasses (Snoeck, Peters, & Cox, 2005, Wood, 2006). Immunoglobulin A1 is monomeric (basic Ig structure of one four-chain unit), and is the main constituent of IgA found in serum. Monomeric IgA is produced by B cells in the bone marrow as well as in some lymphoid organs. IgA2 is mainly found in mucosal secretions and has a dimeric form consisting of two Ig molecules (two four-chain units) joined by an additional polypeptide termed 'J-chain' and an additional protein called the secretory piece (Paul, 2003; Wood, 2006). Immunoglobulin A1 and A2 appear to have the same functions (Wood, 2006).

### 2.3.1 Immunoglobulin A Production, Synthesis and Secretion

The IgA and J-chain are produced by T cell dependent B cells in organized germinal centres of MALT such as Peyer's patches. The high prevalence of SIgA on mucosal surfaces is a result of cooperation between polymeric IgA (plgA) producing plasma cells and mucosal epithelial cells that express an immunoglobulin receptor called polymeric Ig receptor (plgR). After plgA is released by nearby activated plasma cells, it diffuses through the stroma and binds to plgR. The receptor-mediated transcytosis of dimeric IgA across the epithelium is shown in Figure 1. This process results in transportation of IgA across mucosal epithelial cells and its cleavage from plgR for release into external secretions as SIgA, while the transcytosis helps to protect the IgA from breakdown by proteolytic enzymes that are found in secretions (Benjamini et al., 2000; Snoeck et al., 2005; Wood, 2006). Thus SIgA can survive in the gastrointestinal tract and provide protection against microbes that multiply in body secretions (Wood, 2006). SIgA is of importance in the primary immunological defence against local infections and its protective effect is thought to be due to its ability to prevent the invading organism from attaching, replicating and penetrating the epithelial surface (Benjamini et al., 2000; Latiff & Kerr, 2007; Wood, 2006).

![Figure 1: Secretion of dimeric IgA and receptor mediated transcytosis across the epithelium (Wood, 2006).](image-url)
2.3.2 Immunoglobulin A Deficiency

Disorders in the development and differentiation of T cells, B cells (and antibody), synthesis of their products or interactions between them may lead to immune deficiencies that range in clinical severity from mild to fatal (Benjamini et al., 2000). Immune deficiencies are divided into two major categories: primary, which may be hereditary or acquired, in which the deficiency is the cause of the disease; and secondary, in which the immune deficiency is a result of other diseases, infection, drugs, age or malnutrition (Benjamini et al., 2000). Primary immune deficiencies are mainly inherited, can be very mild to severe and are categorised based on clinical presentation, roughly corresponding to the arm of the immune system malfunctioning (Wood, 2006).

Patients with IgA deficiency either have a complete absence, termed selective IgA deficiency, or present with significantly low serum levels of IgA, partial IgA deficiency. Selective IgA deficiency is defined as an undetectable serum IgA level at a value ≤5mg/dL (≤0.05 g/L). Partial IgA deficiency refers to detectable but decreased IgA serum levels that are more than 2 standard deviations below age-adjusted means (Cochino, Popescu, & Gherghina, 2011; Latiff & Kerr, 2007). The most common primary immunoglobulin deficiency is selective IgA deficiency with an incidence of approximately 1 in 500 in the Western World (Benjamini et al., 2000; Latiff & Kerr, 2007). Although selective IgA deficiency can be primary (i.e. genetically determined) or secondary (due to the effects of drugs or diseases), primary IgA deficiency appears to be the most common (Latiff & Kerr, 2007). Latiff and Kerr (2007) report that partial IgA deficiency is a relatively common finding in laboratories that routinely measure immunoglobulins with approximately 1 in 35 samples tested as IgA deficient (IgA<0.06 g/L; adult reference range 0.8-4.0 g/L) and approximately 1 in 100 tested as selective IgA deficient. Primary IgA deficiency appears to be associated with decreased release of IgA by B cells due to a defect of terminal lymphocyte differentiation (Latiff & Kerr, 2007).

Many diseases have been reported in association with IgA deficiency, particularly autoimmune diseases (Latiff & Kerr, 2007). The most common association is with coeliac disease (Latiff & Kerr, 2007). There is an established association between coeliac disease and oral ulceration, commonly caused by recurrent aphthous stomatitis (Field & Allan, 2003). IgA deficient patients with symptoms predominantly suffer from recurrent OU (Field & Allan, 2003; Porter & Scully, 1993), gastrointestinal and sinopulmonary infections, particularly URTI (Brandtzaeg, 2007; Cochino et al., 2011; Hanson et al., 1983; Latiff & Kerr, 2007; Paul, 2003).
2.4 Secretory Immunoglobulin A in Saliva

As an early line of defence in the oral cavity, Salivary Secretory Immunoglobulin A has the same function as SlgA in other parts of the body. Salivary SlgA can specifically neutralise enzymes, toxins and viruses and Sal SlgA inhibits the adherence of bacteria to oral surfaces to prevent bacterial penetration of the mucosa. It neutralises viruses by inhibiting the viral stages of fusion, attachment, internalisation and replication (Benjamini et al., 2000; Wood, 2006).

2.4.1 Induction and Regulation of Salivary Secretory Immunoglobulin A

Induction and regulation of Sal SlgA is complicated. The local response involves the proliferation and differentiation of lymphoid cells into SlgA-producing B cells in the submandibular, sublingual and parotid salivary glands. The second response, referred to as the common mucosal system, involves the movement of antigen-activated T cells from MALT via the lymphatic system to the salivary glands where, upon entering the glandular tissue, the B cells mature under the influence of local T cells into SlgA-producing plasma cells (Teeuw et al., 2004). Both processes underlie neuro-endocrine influences governed by the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal axis where the adrenal medulla, acted upon by sympathetic neurons, presents as a specialised part of the ANS. Activation of these pathways results in elevated levels of cortisol and catecholamines (epinephrine, norepinephrine), both able to bind with hormone receptors on immune cells (Bennet-Herbert & Cohen, 1993). This sympathetic stimulation not only regulates the induction and regulation but simultaneously regulates distant immune cells located in lymphoid tissue such as MALT, influencing antigen presentation, cell migration and antibody production (Elenkov, Wilder, Chrousos, & Vizi, 2000) and may also affect migration of IgA-secreting B cells by vasoconstriction of blood vessels (Mackinnon, 1999). The increased SlgA production that occurs with moderate exercise, a sympathetic stimulus, cannot be dampened by blocking alpha- or beta-adrenergic receptors (Ring et al., 2000). This finding implies mechanisms of sympathetic nervous system action that are not mediated through classical receptor pathways. Further evidence suggests synergistic actions of sympathetic and parasympathetic stimuli. Proctor and Carpenter (2007) demonstrated that both sympathetic and parasympathetic stimuli of salivary glands in rats increased salivary secretion of SlgA and appeared to be regulated through transcytosis of the epithelial plgR-containing vesicles. Proctor, Carpenter, and Garrett (2000) showed that one week of sympathectomy in rats reduced parasympathetically-mediated secretion of SlgA, a non-storage product mediated by plgR, and this appeared to be related to decreased routing of plgR to the basolateral membranes of epithelial cells. The same study showed that one week of pre-ganglionic sympathectomy and parasympathetic nerve stimulation in rats increased
stored protein secretion but not IgA secretion into saliva. These findings underline that neural control of SlgA synthesis, secretion and saliva production is synergistically influenced by both parts of the ANS (Guyton & Hall, 2000; Teeuw et al., 2004).

**2.4.2 Measurement of Secretory Immunoglobulin A in Saliva**

Saliva analysis is presently used in the diagnosis of hereditary disorders, such as immune deficiencies, autoimmune disease, endocrine, infectious and malignant diseases as well as for monitoring drug abuse. Saliva collection is a non-invasive, pain free, simple, cost-effective method for testing large populations. Moreover, individuals without any specialised training can obtain samples. Whole saliva is produced by the three major salivary glands (parotid, submandibular and sublingual gland) and by the minor salivary glands that are present throughout the mouth. The collection of gland specific saliva is possible, however most routine studies collect whole saliva that is either stimulated via spitting or the use of citric acid, or non-stimulated by passive drooling (Mahvash & Satish, 2008). Saliva collection via spitting and drooling may prove to be problematic for reasons of social barriers, particularly in elderly individuals and the use of citric acid can interfere with immunoassay analysis as it decreases the pH of saliva (Groeschl, 2008). Commercially available collection devices, such as absorbent collection pads are recommended to avoid cross contamination between subjects, however, they have been shown to absorb target molecules leading to falsely decreased measurement results (Groeschl, 2008).

Immunoglobulin in secretions is most commonly measured by immunoassays that employ linking molecules labelled with reagents to detect antibodies or antigen. Immunoassays are economical in the use of reagents and very sensitive to the detection of antibodies (Roit, Brostoff, & Male, 2001). The enzyme-linked immunosorbent assay (ELISA) utilises an enzyme-linked antibody, bound to a solid support, such as a microtitre plate, that recognises and binds to the immunoglobulin of interest. Following periods of incubation and rinsing off any unbound immunoglobulin, a substrate that activates the enzyme is added. Bound antibody is quantified by the colour change of the activated enzyme (Mackinnon, 1999). The secretion rate is suggestive of absolute SlgA produced each minute indicating the availability of SlgA on the mucosal surfaces (Mackinnon, 1999). The salivary flow rate is determined by weighing and timing saliva samples and dividing the amount of saliva sample (ml) by the time required to collect the sample (min) (Salimetrics, 2009b). In general it is recommended that both the absolute concentration and secretion rate of Sal SlgA are reported (Akimoto et al., 2003; Brandtzaeg, 2007; Groer et al., 1994; Groeschl, 2008; Kaufman & Lamster, 2002; Salimetrics, 2009b).
2.4.3 Variability of Secretory IgA in Saliva

Salimetrics (2009a) define normal salivary SIgA concentrations in healthy adults ranging from about 79 µg/ml to about 679 µg/ml. Jafarzadeh et al. (2008), define normal Sal SIgA levels in healthy individuals as above about 43 µg/ml at age 1-10 years, about 82 µg/ml at age 11-20, about 94 µg/ml at age 21-30, about 98 µg/ml at age 31-40, about 106 µg/ml at age 41-50, reaching a peak of about 113 µg/ml at age 51-60 and significantly decreasing to about 93 at age 61-70. Even though Sal SIgA is a popular choice of mucosal immune marker for research studies, due to its cost-effectiveness and ease of measurement, it is difficult to standardize quantifications of salivary SIgA due to many methodological problems with sample collection, processing and storage, even for studies performed by the same laboratory (Brandtzaeg, 2007). Salivary SIgA levels vary between studies depending on the methodology of saliva collection, for example the choice of salivary gland and type of collection device, or the type of test, for example single radial immunodiffusion or ELISA. Differences in SIgA levels in the saliva from different glands have been observed, with the highest levels found in the minor salivary glands (Brandtzaeg, 2007; Crawford, Taubman, & Smith, 1975). It is important to be aware of the impact of salivary flow rate on salivary SIgA concentration, which may partly explain differences among studies (Brandtzaeg, 2007).

2.4.4 Influences on Salivary Secretory Immunoglobulin A

There are several possible influences on Sal SIgA, including age, circadian effects, diet, exercise, smoking, stress and chewing. Sal SIgA exhibits age-related changes and begins to decrease during the lifetime of individuals, with an average onset age of over 60 years (Jafarzadeh et al., 2008). It also shows diurnal rhythm, decreasing from the highest levels in morning to the lowest in the evening (Hucklebridge, Clow, & Evans, 1998; Li & Gleeson, 2004). Dimitriou, Sharp, & Doherty (2002) suggest a significant circadian variation in Sal SIgA concentration (18.69% decrease from am to pm), saliva flow rate (48.39% increase am to pm) and secretion rate (32.11% increase am to pm) in well trained swimmers. There is evidence that levels of Sal SIgA can be additionally influenced by everyday activities including diet (Albers et al., 2005; Bishop & Gleeson, 2009; Gleeson, Bishop, Oliveira, & Tauler, 2011; Gleeson, Nieman, & Pedersen, 2004; Hao, Dong, Huang, & Wu, 2011), exercise (Bishop & Gleeson, 2009; Gleeson & Pyne, 2000; Gleeson et al., 1995, 2003; Kientrou et al., 2002; Nieman, 1994, 2000) and stress levels (Bennet-Herbert & Cohen, 1993; Jemmot & McClelland, 1989; Volkmann & Weekes, 2006). In general diet, along with age, is one of the major exogenous factors modulating individual immunocompetence (Albers et al., 2005) and dietary deficiencies of protein and micronutrients such as iron, zinc and vitamins A, E, B6 and B12 are associated with immune dysfunction (Gleeson et al., 2004). Gleeson, Bishop, Oliveira, and Tauler (2011) showed a clinically and statistically...
significant increase, with a large ES of 5.6 ($p=0.03$) in Sal S IgA secretion after 8 and 16 weeks of probiotic supplementation versus placebo in highly active winter athletes. A review by Gleeson, Pyne, and Callister (2003) notes that regular moderate intensity exercise can have a positive long-term effect on Sal S IgA concentrations, irrespective of age or fitness level, with studies showing 15-57% increases in IgA concentrations from pre- to post-exercise. In contrast to the increases in IgA with moderate exercise, exercise at high intensities results in immediate and longer-term (1 – 4 hours) 20 – 60% decreases in Sal S IgA concentration, with a full recovery of Sal S IgA concentrations by 24 hours after exhaustive exercise (Gleeson et al., 2003; Moreira et al., 2009). Smokers are observed to have elevated Sal S IgA levels, which have been interpreted as a reflection of oral mucosal protection (Norhagen & Engstrom, 1998). Opposingly, passive smoking in young children of both sexes has shown to decrease Sal S IgA levels (Avsar, Darka, Bodrumlu, & Bek, 2009). As for exercise and smoking, the effect of stress on IgA levels is not clear cut. Acute psychological stress has been shown to enhance Sal IgA levels (Allgrove, Gomes, Hough, & Gleeson, 2008), whereas chronic stress has been found to suppress Sal S IgA (Jemmot & McClelland, 1989; Volkmann & Weekes, 2006). Finally, research has shown that chewing stimulates epithelial cell transcytosis of IgA and increases secretion of S IgA into saliva (Proctor & Carpenter, 2001). Therefore, chewing is commonly used to stimulate saliva fluid volume and consistent instructions regarding chewing need to be delivered to participants prior to saliva sample collection (Brandtzaeg, 2007).

2.5 Salivary Secretory Immunoglobulin A and its Relation to Health

The complexity of the immune system with its numerous specific and important components makes it impossible to recognise one specific marker for immune health but individual markers are commonly used to reflect the function of a specific component of the immune system. As described earlier, IgA is commonly used as an immune marker for mucosal immunity (Brandtzaeg, 2007; Saluja, Kale, & Hallikerimath, 2012), as it exhibits an important protective function against pathogens. This raises the question about the extent that Sal S IgA levels indicate or affect health.

Numerous studies have attempted to link decreased Sal S IgA concentrations to a variety of oral as well as systemic diseases, including the relationship of low Sal S IgA levels to dental caries, periodontal disease, mucous membrane diseases, chronic sialadenitis, Sjögren's disease, non-Hodgkin's malignant B cell lymphoma, celiac disease, human immunodeficiency virus (HIV) infection and AIDS (Benjamini et al., 2000; Brandtzaeg, 2007; Hanson et al., 1983; Paul, 2003). Reviews of such reports have been published but the
relation between low Sal SIgA levels and the above-mentioned diseases remains unclear (Brandtzaeg, 2007). There are several important variables influencing the levels of total IgA and other specific antibodies in oral secretions. These include difficulties with reproducibility and standardization of immunoassays, protein loss during sample handling and impact of flow rate (Brandtzaeg, 2007). In addition, a number of biological phenomena induced by mucosal antigen exposure are poorly defined in experimental animals and still more obscure in humans. Salivary SIgA is also believed to play a still poorly understood role in maintenance and return to local homeostasis as well as in modulation and education of the mucosal immunity of the intestines and airways (Teeuw et al., 2004).

In addition to its association with specific conditions, a number of lines of evidence support the view that there is an association between Sal SIgA and general immune health (Chikly, 2005; Hanson et al., 1983; Rossen et al., 1970). For example, Volkmann and Weekes (2006) investigated the relationship between stress and health in young adults (n=34) identified by stress and health inventories and alterations in Sal SIgA and Sal cortisol concentration during a baseline (low-stress) session and during an examination (high-stress) session. Similarly, low basal SIgA and high basal cortisol has been linked with increased infection rates. Markova and Chuvirov (2007) report that 94% of 270 2 – 15 year old frequently ill children with monthly acute respiratory disease and combined pathology of the upper airway show low levels of Sal SIgA. Information about the selection of participants and their demographics is not available. The childrens’ low IgA levels could be related to factors other than frequent illness. For example, the selection of children may have been biased towards those with lower socio-economic status. Selection of participants from lower socio-economic status might have resulted in a large number of participants exposed to cold or damp housing or exposure to passive smoke, which could both have contributed to suppressed IgA levels. Therefore, it is difficult to assess the importance of infection in its influence of IgA levels.

Salivary SIgA has been shown to be a useful diagnostic marker for monitoring exercise-induced mucosal immunosuppression in high-performance athletes (Gleeson & Pyne, 2000; Gleeson et al., 1999; Pyne et al., 2000). For instance, several studies associate exercise-induced low levels of Sal SIgA with an increased incidence of URTI in elite athletes (Gleeson, & Pyne, 2000; Gleeson et al., 1999, 2003; Hagewald et al., 2002; Hanson et al., 1983; Klentrou et al., 2002; Moreira et al., 2009; Neville et al., 2008; Nieman, 1994; Pyne et al., 2000; Tseuuw et al., 2004). Neville, Gleeson, and Folland (2008) examined the relationship between Sal SIgA and URTI in a cohort of elite professional yacht racing athletes (n=38) in the United States over 50 weeks of training by collecting weekly unstimulated
saliva samples at rest (38 hours post-exercise, consistent time of day) together with clinically confirmed URTI, training load and perceived fatigue rating. On a group basis, a significant 28%, reduction in Sal SIgA occurred during 3-week pre-URTI episodes (p<0.005), and returned to baseline by 2 weeks after a URTI. A reduction in Sal SIgA of more than 40% of an athlete’s mean healthy Sal SIgA concentration indicated a one in two chance of contracting an URTI within 3 weeks when an athlete did not have or was not recovering from URTI. Typical decline in an individual’s relative Sal SIgA concentration over the 3 weeks pre-URTI appears to predict URTI risk, with a magnitude of the decrease related to the risk of URTI, independent of the absolute SIgA concentration (Neville et al., 2008).

Salivary SIgA has also been used to monitor responses to acute psychological stressors. Acute stress results in elevated Sal SIgA levels, in contrast to chronic stressors which induce Sal SIgA suppression. In an attempt to investigate the effect of increased Sal SIgA on URTI, Kostinov, Suloeva, Tarasova, and Lukushkina (2006) evaluated the influence of bacterial lysate on the mucosal immunity of HIV infected children. This lysate, a topical immunomodulator, increased the synthesis of Sal SIgA in correlation with decreased inflammatory changes in the nasopharyngeal mucosa of the children (Kostinov, Suloeva, Tarasova, & Lukushkina, 2006).

There is also good evidence that Sal SIgA has a protective role against the development of allergy (Böttcher, Häggström, Björgstén, & Jenmalm, 2002). Additionally, in vitro and in vivo research indicate that Sal SIgA can inhibit the adherence of bacteria to oral tissues and thereby protect against the development of gingivitis and caries (Teeuw et al., 2004). However, epidemiological research investigating the relationship between Sal SIgA and dental caries has produced conflicting data and inconsistencies are believed to relate to the fact that some species of bacteria can render Sal SIgA ineffective (Lamm, 1997). For instance, decreased levels of Sal SIgA are associated with dental caries, however studies that show significantly higher levels of Sal SIgA in children with caries hypothesise that increased levels of Sal SIgA may reflect a past exposure of the host to cariogenic microorganisms (Thaweboon, Thaweboon, Nakornchai, & Jitmaitree, 2008).

For the most part, studies support the view that increased IgA levels have a positive impact on health, while decreased IgA concentrations are frequently associated with oral as well as systemic diseases. The potential role of manual therapies, in particular osteopathy, in modifying Sal SIgA levels, and therefore perhaps improving human immune health, by assisting lymphatic drainage is still unknown.
3.0 Osteopathy and Immune Health

Osteopathic healthcare is a form of drug-free, non-invasive manual therapy attempting to help the body maintain and reestablish homeostasis and healing. This part of the literature review provides a short overview on the field of osteopathy and commonly utilised techniques for immune stimulation. Further, the features of osteopathic lymphatic pump techniques are discussed, with focus on the thoracic lymphatic pump, to understand how and to what extent TLP might influence Sal SIgA levels.

In New Zealand osteopaths do not work as fully licensed physicians and modern osteopathy appears to have its scope of practice primarily specialised to the treatment of neuro-musculoskeletal problems (OCNZ, 2013). Yet in the early days of the profession osteopathic practitioners were asked to treat numerous infectious diseases and were also utilised as primary health providers with full medical practice rights (Kuchera and Kuchera, 1994a; Still, 1899, 1902; Ward, 2003). Osteopathic practitioners seek to optimise structures that are considered to be directly or indirectly related to immune function. Organs such as liver, thymus, spleen and the intestines, play an important role in the synthesis, neutralisation and elimination of immune related cells and are treated by osteopaths who claim that this treatment can improve their function either directly or by releasing restricted tissues surrounding the respective organ (Ward, 2003; Wood, 2006). Structures related to lymphatic flow such as lymph vessels and lymph nodes are not easily accessible by manually-applied hand contact and the osteopathic approach is centred on treating restrictions, a resistance or impedement to movement, in surrounding connective tissue, such as muscles and fascia (myofascia), through which those structures pass (Paoletti, 2006; Ward, 2003). Fascia is connective tissue that surrounds and connects internal structures throughout the body.

Therefore, distorted function of myofascia is believed to hinder the movement of lymphatic fluid (Degenhardt & Kuchera, 1996; Kuchera & Kuchera, 1994a; Paoletti, 2006). Decreased lymphatic flow causes tissue accumulation of waste products and can interfere with cellular activity resulting in cell dysfunction and disease (Degenhardt & Kuchera, 1996). Increasing lymph flow and drainage of lymph fluid out of tissues optimises the filtering of fluid and the transport and removal of inflammatory mediators from sites of dysfunction (Degenhardt & Kuchera, 1996; Ward, 2003). Additionally, osteopathic treatment utilises a wide range of pumping and draining techniques.
3.1 Lymphatic Pump Techniques

Lymphatic flow relies on the production of pressure gradients between abdominal and thoracic regions, produced by the action of the abdominal diaphragm to direct lymph through the thoracic duct towards the vena subclavia and inferior vena cava. Osteopathic medicine emerged as the first system of healthcare to develop specific manual techniques related to the lymphatic system (Chikly, 2005). Today, osteopaths use Osteopathic Manipulative Treatment (OMT) techniques for lymphatic stimulation, in particular a group of manipulations knowns as Lymphatic Pump Techniques (LPTs) which are designed to augment abdominal-thoracic pressure gradients that develop during normal respiration (Ward, 2003). Some techniques are claimed to influence negative intrathoracic pressure of the thorax, while others are said to increase abdominal pressure. Techniques can be rhythmic or continuous and LPTs include the thoracic lymphatic pump, the liver, splenic, pancreatic, abdominal and pedal pumps and pectoral traction. The increased lymph flow that is believed to occur from these treatments is thought to accelerate removal of cellular waste, toxins, and bacteria from the interstitial fluid and also reduce oedema (Ward, 2003).

3.2 Thoracic Lymphatic Pump Techniques

TLP techniques are designed to affect the intrathoracic pressure gradients that are thought to augment thoracic range of motion and expiratory efficacy, while at the same time affecting lymphatic flow through the thoracic duct, rib cage mobility and associated ANS centres of sympathetic ganglions which take part in the autonomic regulation of salivary glands and intrathoracic organs (Kuchera & Kuchera, 1994b). Commonly TLP techniques are clinically indicated as initial treatments for clearing the thoracic duct region to improve lymphatic flow and for patients with chronic obstructive pulmonary disease, upper and lower respiratory infections, mastitis, or swollen upper extremities or for postsurgical reduction of respiratory volume (Ward, 2003).

3.2.1 The Thoracic Lymphatic Pump Without Activation

Osteopathic textbooks (Kuchera & Kuchera, 1994a, 1995b; Paoletti, 2006; Ward, 2003) and research studies (Noll, Degenhardt, Johnson, & Burt, 2008; Noll, Johnson, Baer, & Snider, 2009) describe two different types of TLP, the TLP Without Activation and the TLP With Activation. The TLP Without Activation technique is performed with the patient in the supine position and the practitioner standing at the head of the table, placing his or her hands on the thoracic wall with the thenar eminence of each hand over the pectoralis muscle just below the clavicles (Fig. 2). The fingers are spread and angled towards the patient’s body to evenly distribute pressure across the chest wall. The TLP Without Activation consists of a rhythmic
pumping action of alternating pressure and release on the patient’s chest at a rate of 110-120 times/min, while the patient is asked to continue breathing as usual (Kuchera & Kuchera, 1994b; Noll et al., 2009; Ward, 2003). The terminology 'Without Activation' indicates that there is no recoil, in this case no sudden release of the hands away from the chest cage, as applied during TLP With Activation. Recoil techniques are techniques used in OMT that initiate recoil of certain body tissues by a sudden release of the operator’s hands in relation to the patient’s body. The recoil is thought to cause a stimulation of the underlying tissues. Osteopathic literature indicates that recoil techniques should not be used in patients with certain respiratory conditions that have hyperreactive and or bronchospasmic characteristics (e.g. asthma, COPD), as it might further stimulate these characteristics (Kuchera & Kuchera, 1994a, 1994b; Noll et al., 2008; Ward, 2003). Therefore, TLP Without Activation is the primary choice of technique in symptomatic patients but is also commonly used as a preventative and to manage healthy individuals who commonly suffer from respiratory tract infections, such as URTI.

Figure 2: Miller's Thoracic Lymphatic Pump (Channel & Mason, 2009)

3.2.2 Mechanisms of TLP in Relation to Salivary Secretory Immunoglobulin A
The immediate and short-term increase in Sal SIgA after manual intervention such as TLP and other types of LPT is most plausibly explained by a combination of different types of manual stimulation to the lymphatic system and the ANS. Firstly, the rhythmic pressure of the upper chest with TLP could cause an increase in lymphatic flow of the thoracic duct due to the pumping mechanism of the technique and due to the decreased resistance to lymphatic flow secondary to an increase of mobility of the upper chest. Secondly, the rhythmic mobilisation of the upper chest, which connects posteriorly to the upper thoracic spine, is also likely to induce an immediate and short-term stimulation of the sympathetic preganglionic nerves in the upper thoracic segments of T1-3 that synapse in the superior
cervical ganglion with postganglionic neurons that release norepinephrine (Proctor & Carpenter, 2007). Norepinephrine binds to β-adrenergic receptors on the acinar and ductal cells of the adrenal glands leading to an increase in salivary gland secretion and a synergistic secretion of SIgA. Additionally, this stimulation of the sympathetic nervous system might induce an indirect effect on salivary glands and SIgA secretion by vasoconstriction of the blood vessels that supply the salivary glands (Proctor & Carpenter, 2007; Ward, 2003b).

3.3 Effect of OMT on the Immune System Within Evidence-Based Practice

A great number of papers within the osteopathic historical literature claim that OMT, especially techniques that involve lymphatic pumping, are efficacious for enhancing immune response (Castlio & Ferris-Swift, 1932, 1934; Galbreath, 1929; Smith 1920; Whiting, 1910). Unfortunately data from these uncontrolled studies were based on retrospective clinical reports. Therefore, it is unclear what population these results can be generalized to. Similarly, because patient histories, diagnoses and interventions are not standardised or systematically recorded, it is difficult to establish what factors influenced the beneficial immune effects. Even some of the studies state promising results, there is often a lack of proof of the significance of their results. For example, in 1934, Castlio and Ferris-Swift examined the effects of splenic pump on patients with acute infectious disease by taking blood as a baseline sample before treatment and at two points following treatment, varying from 5 minutes to 2 hours. It was reported that 73% of patients had an increase in total leukocyte counts and in 84% of patients had an increase in mature neutrophils, as measured by a shift to the right of the Arneth index. There was also an increase in the opsonic index, agglutination, and bacteriolytic properties of the serum in patients following treatment (Castlio & Ferris-Swift, 1934). Castlio and Ferris-Swift (1934) concluded that the increased leukocyte count was a result of contraction of the spleen and expulsion of its contained leukocytes. They also believed that the decreased erythrocyte count was related to an increased destruction of red blood cells (Castlio & Ferris-Swift, 1934). Noll, Johnson, & Brooks (2008) revisited Castlio and Ferris-Swift's experiment by contemporary nonparametric statistical methods and confirmed a modest post-treatment increase in leukocytes, a decrease in erythrocytes, a decrease in the Arneth index, and an increase in reticulocytes. However, laboratory testing times and the number of splenic compressions used in treatment varied independently (Noll et al., 2008). A further limitation is that participants were most likely not blinded to the blood draw times, as double-blinding was uncommon during that era and not mentioned by the authors. Therefore the key problem is that the lack of blinding and the inconsistent study procedure lowers the internal validity of the study and its ability to draw conclusions about the effect of lymphatic pump technique.
3.4 Thoracic Lymphatic Pump Techniques Within Evidence-Based Practice (Table 1)

There are few studies investigating links between manual therapeutic techniques such as TLP and immune health. The TLP has shown to have a positive effect on immune response by increased levels of B cells and T cells after vaccination (Jackson et al., 1998; Measel, 1982) and without antigenic challenges (Measel & Kafity, 1986), as well as an immune enhancing effect on health in a post-operative clinical setting (Sleszynski & Kelso, 1993), while one controlled study has shown to decrease pulmonary tumours in laboratory rats following TLP (Pedrueza, Zhang, Jones, & Hodge, 2010).

Measel (1982) examined TLP and the immune response of male medical students (n=25) after vaccination with pneumococcal polysaccharide. Two serological tests, bacterial agglutination and passive agglutination were employed to measure the impact on B cell and T cell components in the blood. By day 14 the treatment group (n=13), having received twice daily TLP, had a statistically greater humoral B cell immune response than the control group (n=12). The conclusion was that TLP had a positive effect on B cell and T cell components of the human immune system as measured in peripheral blood. In accordance with these results, Jackson et al. (1998) demonstrated that TLP and splenic pumping enhanced antibody production on hepatitis B vaccination. The experimental subjects (n=20) received TLP and splenic pump treatments three times per week for two weeks after each vaccination. Subjects were given the vaccinations at 0, 5, and 25 weeks. The control group received the vaccinations but no OMT. Resultant serum antibody levels were measured by enzyme immunoassay. Fifty percent of the subjects in the treatment group achieved protective antibody titers (> or = 10 mIU/mL) by week 13 compared with an average titer of 374 mIU/mL. Positive antibody responses were measured in 16% of the control group, with average titers of 96 mIU/mL. The results were presented as further evidence that TLP and LPT enhance immune response. Breithaupt et al. (2001) investigated whether TLP after FluShield vaccination would enhance the production of anti-influenza antibodies. Their particular interest was in the immune response of elderly populations, as they have an increased risk from influenza and achieve lower levels of immunity on influenza vaccination. Two healthy test populations, elderly adults and young adults, were randomly divided into non-treated controls and TLP-treated subjects. Individuals performing the antibody analysis were blinded until assay series were completed. Their results suggest that TLP in conjunction with influenza vaccination does not enhance immunisation against influenza in otherwise healthy and active populations (Breithaupt et al., 2001)
Other studies have investigated the baseline effects of lymphatic pumping by itself without antigenic challenges such as vaccines or bacterial antigens. In an uncontrolled study abstract, Measel and Kafity (1986) reported that TLP increased total white blood cell (WBC) counts, decreased lymphocyte counts and increased the relative percentages of T cells and B cells. They reported an average increase by 31.5% from 7460 erythrocytes per µL to 9810 per µL. The B cell component increased from 5.07% to 9.25% of white blood cell while T cell component rose from 73.2% to 80.9%. The TLP may also play a role in recovery from surgery. Sleszynski and Kelso (1993) conducted a one-year randomized researcher-blinded trial of TLP in patients who had undergone low-risk cholecystectomy. Half of the subjects received TLP, while the other half received incentive spirometry. Incentive spirometry is a breathing exercise designed to help patients take long, deep breaths in order to decrease the chance of developing breathing problems after surgery. Atelectasis, the collapse or closure of the lung resulting in reduced or absent gas exchange, occurred in two patients in each group. However, patients treated with TLP had earlier recovery and quicker return to preoperative values of forced vital capacity than did those treated with incentive spirometry. The outcome of this study indicates a possible immune enhancing effect of TLP on health in a post-operative clinical setting.

There is also some evidence from animal studies of the effect of TLP in cancer amelioration. Pedrueza, Zhang, Jones, and Hodge (2010) subcutaneously injected laboratory rats with tumours that metastasise to the lungs. Following the injection the rats received four minutes TLP daily for seven days. A control group received no treatment while a sham group was treated with four minutes of light touch. The results demonstrated a 30% reduction in pulmonary tumours in the TLP group compared with the control or sham group and there was no distribution of tumour cells from the lungs into other tissues following TLP. Additionally, TLP produced increased WBC numbers in lungs with tumours, which the authors suggested may have played a role in preventing tumors from spreading. However, recent research suggests that tumours in animals and humans most likely metastasise through the fusion of a white blood cell and a cancer cell to form a genetic hybrid (Lazova et al., 2013). There are some questions about the ability to generalise these results to human. It is questionable if the operators were able to perform the TLP technique on small subjects like rats with the same characteristics as the earlier described technique used on humans. Their study design and results reflect a rat specific thoracic lymphatic pump technique but should not be compared to the osteopathic TLP performed on humans, as for example hand positions on subjects must have been different between the two TLP techniques. Further the difference in anatomical and physiological properties between rats and humans makes it difficult to reflect their results to humans.
<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Population Characteristics</th>
<th>Intervention</th>
<th>Timeframe and Measures (per participant)</th>
<th>Sample and Data Analysis</th>
<th>Results</th>
</tr>
</thead>
</table>
| Breithaupt et al. (2001) | 36 young adults, 61 elderly adults | Randomization into:  
Experimental group (n=32 elderly and N=18 young adults):  
- FluShield vaccination  
- 5 min. TLP with 50 rpm daily for 4 days post-vaccination  
Control group (n=29 elderly and n=18 young adults):  
- No TLP | 4 weeks  
Blood samples to measure positive antibodies to vaccination:  
- 1 baseline  
- 1 post-intervention sample at average at 30 days post-vaccination | Serological analysis:  
- HIA  
- ELISA  
Data Analysis:  
- Mixed analysis of variance  
- Chi-square tests | Elderly group:  
- Increase in antibody production in 35% of TLP group  
- Increase in antibody production on vaccination in 30% of control group  
- 72% of positive responders with twofold or fourfold antibody increase  
Young group:  
- Increase in antibody production in 69% of TLP group  
- Increase in antibody production on vaccination in 67% of control group  
- 72% of positive responders with eightfold or greater antibody increase |
| Jackson et al. (1998) | 20 participants | Treatment group (n=20):  
- Vaccination at 0, 5, 25 weeks  
- 3 x per week for two weeks protocol of OMT after each vaccination:  
> TLP  
> Splenic pumping  
Control group:  
- Vaccination at 0, 5, 25 weeks  
- No OMT | 27 weeks  
Blood samples to measure positive antibodies to vaccination | Serological analysis:  
- ELISA | Treatment group:  
- 50% with protective antibody titers by week 13 with an average titer of 374 mIU/ml  
Control group:  
- 16% with protective antibody titers with an average titer of 96 mIU/ml |
| Measel (1982) | 25 male medical students | Randomization into:  
Treatment group (n=13):  
- Vaccination with pneumococcal polysaccharide  
- 5 min. TLP twice daily for 1 week  
Control group:  
- Vaccination with pneumococcal polysaccharide  
- No TLP | 14 days  
Two serological tests:  
- Bacterial agglutination  
- Passive agglutination | - Serum analysis assayed by agglutination  
- Split-plot, mixed model, analyses of variance | - Increase in humoral B cell response in some of the bacterial strains |
3.5 Effect of TLP on Salivary SIgA Within Evidence-Based Practice (Table 2)

In addition to the limited amount of research in the field of TLP within evidence-based practice, there are also very few studies investigating and evaluating the immune enhancing effects of TLP on Sal SIgA. Two recent studies have investigated the effect of TLP on salivary SIgA levels, and concluded positive effects (ES = 2.1 - 3.0) of TLP in isolation or part of osteopathic treatment on salivary SIgA (Ehrlenbach, 2011; Saggio et al., 2011). Saggio et al. (2011) investigated the impact of OMT on Sal SIgA levels in a stressed population of osteopathic medical students. Twenty-five second-year osteopathic medical students were randomly assigned to an experimental group (n=12) or a control group (n=13). The experimental group received a 20-minute OMT protocol consisting of five minutes of occipitoatlantal release, five minutes of rib raising and 10 minutes of TLP. The experimental group exhibited a 139% average increase (ES = 2.1) in post-intervention Sal SIgA levels which was significantly (p<0.025) greater than the 32% average increase noted in the control group. The study by Saggio et al. (2011) demonstrates the positive effect of OMT on Sal SIgA levels in a stressed population and the authors suggest possible therapeutic preventative and protective effects of OMT on healthy and hospitalised patients, especially when experiencing high levels of emotional or physiological stress and when being at risk of acquiring URTI. A possible limitation of their study is that participants in the experimental group were treated by 1 of 12 operators. Multiple operators increase the chance of inter-rater variability by differences in the application of treatment and therefore possible differences in treatment outcomes. On the other hand a positive treatment effect with multiple operators indicates a positive inter-rater reliability. Inter-rater variability and reliability has not been reported by Saggio et al. (2011). While control group participants were asked to rest calmly while sitting, experimental group participants were asked to remain seated or supine after the 20-minute OMT application and rest quietly for one hour, before a second saliva sample was collected. Differences in resting position of participants in the experimental group and also differences of resting duration in between the groups could have influenced lymphatic flow and Sal SIgA levels, resulting in relatively lower levels in the experimental group. Unfortunately, the study was unable to determine which OMT technique or combination of the three techniques was responsible for the observed increase in Sal SIgA.

Another small cohort study by Ehrlenbach (2011) used a single system research design with a modified A-B-C protocol on eight healthy male participants to measure changes in Sal SIgA secretion rate in response to a seven-minute treatment of TLP. Baseline measures were recorded once daily over 5 days. On Day 5 the single pre- intervention measure was immediately followed by the intervention. Two post- intervention measurements, at one-minute post- treatment and at 10-minute post- treatment were reported. An immediate post-
treatment short-term increase of Sal SIgA concentration was identified in all eight participants and an immediate post- treatment short-term increase of Sal SIgA secretion rate was reported in 7 out of 8 participants with a large effect size of 3.0 (Cohen's $d$ formula; $p=0.03$) with an average increase of 136% indicating a strong correlation between TLP and increase in immediate post- intervention and short-term Sal SIgA secretion rate. On day 5 participants provided the first post-treatment saliva sample directly after TLP. The participants were then guided into an adjacent room and directed to sit quietly while reading a selected textbook chapter, before 10 minutes later a second saliva sample was collected. The transfer to an adjacent room between the first and second saliva sample on day 5 might have, most likely positively, influenced lymphatic flow and Sal SIgA levels. Further the study is limited by following a modified A-B-C design, providing only a small number of measures. Baseline data were collected at five measurement points, whereas post-treatment changes in Sal SIgA were assessed at only two measurement points, immediately and 10 minutes following treatment. Therefore, the observed post-treatment changes may have resulted from chance variation. The absence of a control group is another potential concern. Because the study wasn't controlled, cause and effect links between treatment and the immune changes are difficult to establish with certainty.

In addition to limitations arising from study design factors, the mechanism of changes observed by Ehrlenbach (2011) are not clear. An effect by the production of new IgA can be excluded, as specific production and transportation of SIgA to the site of the saliva collection, the salivary glands, would have taken several days. Most likely the increase in Sal SIgA levels must have resulted from an increased release of Sal SIgA in the oral cavity.

Apart from the osteopathic reviews that claim efficacy of LPT and TLP in historical clinical cases (Amalfitano, 1987; Chikly, 2005; Degenhardt & Kuchera, 1996) and the often claimed efficacy of TLP in osteopathic textbooks (Channel & Mason, 2009; Kuchera & Kuchera, 1994a, 1994b; Ward, 2006) as well as the effect observed on immunological markers in humans (Breithaupt et al., 2001; Ehrlenbach, 2011;; Jackson et al., 1998; Measel & Kafity, 1986; Measel, 1982; Saggio et al., 2011), to date, no studies have investigated the clinical outcome of TLP in healthy humans complaining of recurrent URTI and OU, which might be indicative of suppressed levels of SIgA.
**4.0 Conclusion**

Osteopaths have used lymphatic pump techniques for over 100 years and osteopathic literature claims their efficacy at increasing lymphatic flow and their importance in the treatment and prevention of disease. The results of Saggio et al. (2011) and Ehrlenbach (2011) indicate a possible effect of TLP on Sal SIgA. The study outlined in the second part of this thesis is linked to that of the 2011 Ehrlenbach study, where a short-term increase in Sal SIgA concentration post-treatment was identified in all eight participants. Ehrlenbach's study included a five-day baseline with a single saliva sample on each day, as well as only two saliva samples pre-intervention. The small number of data points limited the study, as it is difficult to identify possible pre-intervention fluctuations of Sal SIgA. However, the data are promising enough to follow up on with an increased number of data points to observe and discuss possible mechanisms by which TLP affects immediate and short-term Sal SIgA. Future studies would need to include a control group and to greatly increase the size of the study before any conclusion could be made regarding cause and effect.

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**Table 2: Effect of TLP on Salivary SIgA Within Evidence-Based Practice**

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Population Characteristics</th>
<th>Intervention</th>
<th>Timeframe and Measures (per participant)</th>
<th>Sample and Data Analysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ehrlenbach (2011)</td>
<td>8 healthy male participants - 21-39 years</td>
<td>7 min. TLP</td>
<td>5 days - Total of 5 baseline saliva samples (1 sample daily for 5 consecutive days) - Total of 2 post-intervention saliva samples on day 5 (1 min. and 10 min. post-intervention)</td>
<td>- ELISA of Sal SIgA - Comparison of group means (ANOVA) - Comparison of pre- and post-intervention measures (Paired t-test) - Evaluation of clinical significance (Cohen's d formula)</td>
<td>- 136% average increase (ES=3.0, p=0.03) in Sal SIgA</td>
</tr>
<tr>
<td>Saggio et al. (2011)</td>
<td>25 healthy second year osteopathic medical students: - 12 men - 13 women - 18-40 years - high stress levels</td>
<td>Randomization into: Experimental group (n=12): - 20 min. OMT protocol: - 5 min. occipitoatlantal release - 5 min. rib raising - 10 min. TLP Control group: - 20 min. sit and relax</td>
<td>Single day Experimental group: - 1 baseline saliva sample - 1 saliva sample 60 min. post-intervention Control group: - 1 baseline saliva sample - 1 saliva sample 60 min. after sit and relax</td>
<td>- ELISA of Sal SIgA - 2 x 2 analysis of variance (ANOVA)</td>
<td>- 139% average increase (ES= 2.1) in Sal SIgA levels Control group: - 32% average increase in Sal SIgA levels Interaction between group and time: - p=0.027</td>
</tr>
</tbody>
</table>
5.0 Thesis Study

The aim of the research study completed as part of this thesis was to use Ehrlenbach’s approach by replicating that study but to include an increased number of baseline and follow-up data points that assess Sal SIgA variability over the short time-frame that effects were reported. In this investigation, on a single day, multiple data points pre-, peri-, and post-intervention will be collected to get a better understanding of possible fluctuations of Sal SIgA pre-intervention and of how the Sal SIgA secretion rate responds to the intervention. Furthermore, healthy male adults who report symptoms of recurrent OU and or URTI will be selected since these symptoms have been previously associated with relative IgA deficiency. It is understood that there are many factors involved in OU/URTI but the presence of recurrent episodes of OU/URTI in otherwise healthy individuals suggests a suppressed mucosal immunity in that individual. Thus, this study has the potential to provide preliminary data regarding the clinical applicability of the TLP technique. This study will focus on how the osteopathic TLP technique affects immediate and short-term Sal SIgA secretion rate in four healthy male adults complaining of recurrent OU and or URTI. A single case study design was deemed most suitable for investigating these aims.

Research and its documentation are crucial factors in the growth and acceptance of any profession and the need for experimental research is well recognised within the osteopathic profession (Moran, 2005). Group designs, such as randomised controlled trials, are regarded as the gold standard of experimental research within evidence based practice, they are often inappropriate for preliminary investigations and the logistics of this type of research requires sources that are not easily available within the osteopathic profession. Group designs are limited in their suitability for certain types of conditions and have been criticised for lacking transferability to individuals by representing only an average of the response to any given intervention (Sanders, 2003). Immunologic research generally requires large resources making single case studies a cost effective solution particularly for the investigation of emerging or little researched topics.

This research project will consist of four individual single case studies designed to identify the immediate and short-term effect of a single intervention of TLP on Sal SIgA concentration (µg/mL) and secretion rates (µg/min) in healthy male subjects complaining of recurrent OU and URTI. The presence of URTI and OU will be self-reported identified via a short questionnaire and not based on a clinical diagnosis. The intervention will imply a single standardised treatment of seven-minute TLP Without Activation. Multiple data points (Fig. 3) will be collected on the day of the intervention to get a better understanding of when the secretion of Sal SIgA responds to the intervention. Pre- (sample 1-4) and post-intervention
(sample 6-9), four saliva samples are collected at 10 minute intervals. The last pre-intervention sample collection (sample 4) is immediately followed by the intervention. During the intervention one saliva sample (sample 5) is collected to identify possible immediate effects. The first post-intervention sample collection (sample 6) starts immediately after the intervention. Saliva samples will be collected by oral swab and the collection time is limited to one minute per sample. Samples will be analysed with a SlgA competitive ELISA kit and assays will be analysed with Biotek software Gen5.

Figure 3: Methodology for saliva sample collection.
6.0 References


Note: The manuscript presented here is intended for submission to the *International Journal of Osteopathic Medicine* (IJOM) but rather than the referencing style specified in the IJOM guidelines for authors, the referencing style follows the American Psychological Association (“APA”). Elsevier’s initiative ‘Your Paper, Your Way’ ([www.elsevier.com/yourpaperyourway](http://www.elsevier.com/yourpaperyourway)) now permits manuscripts submitted using other referencing formats and APA was selected because it is easier to follow authors’ names in the text and it matches that required for the Literature Review.
Title

The Effect of the Osteopathic Thoracic Lymphatic Pump Technique on Salivary Secretory Immunoglobulin A Levels in Adults with Recurrent Upper Respiratory Tract Complaints
Abstract

Background:
Low levels of salivary SlgA have been associated with upper respiratory tract infection, oral ulceration and systemic diseases. Osteopathic Manipulative Treatment has been shown to have a positive effect on salivary SlgA levels.

Objective:
This single case study aimed to determine changes in salivary SlgA concentration and secretion rate in response to a single 7-minute intervention of the osteopathic thoracic lymphatic pump in four male subjects who indicated suppressed mucosal immunity identified by recurrent upper respiratory tract infection and oral ulceration.

Methods:
On a single day a total of nine saliva samples were collected per participant via oral swab, including four samples pre-, one peri- and four post-intervention. Participant results were analysed individually with group statistics reported.

Results:
At baseline all participants showed suppressed levels of salivary SlgA concentrations with an average of 58.03 µg/mL (<79.26 µg/mL). Post-intervention Salivary SlgA concentrations increased in three participants [Effect size (ES) = 1.74 - 2.71]. On average salivary SlgA concentrations increased to 72.73 µg/mL (25.3%). Salivary SlgA secretion rates showed no clear pattern of change (ES = -1.82 to 1.96). On average salivary SlgA secretion rates increased from 23.8 µg/min to 24.4 µg/min (5.9%). The mean effect size for salivary SlgA secretion rates baseline to post-intervention was d=0.05.

Conclusion:
This study showed some indication of an overall trend for an increase in salivary SlgA concentration. Changes in salivary SlgA secretion rate were inconsistent and the therapeutic benefit of the thoracic lymphatic pump in participants with a suppressed mucosal immune function remains unclear.

Keywords: Antibody; Aphthous Ulcer, Recurrent; Marker, Biological; Manual Immunomodulatory Therapy; Osteopathy; Serology; System, Immune
1.0 Introduction

Secretory immunoglobulin A (SIgA) is the major immunoglobulin secreted by the mucosal system (Wood, 2006) and is found in gastrointestinal fluids, mucosal fluids of the respiratory tract, saliva and other mucosal fluids (Wood, 2006). Secretory IgA provides primary immunological protection from pathogenic organisms by preventing attachment, penetration, replication, and colonization of such organisms on the epithelial surface (Latiff & Kerr, 2007) and by interaction with other antibacterial factors in saliva (Jackson et al., 1998). Secretory IgA in saliva (salivary SIgA) is a relatively easily measured product of the mucosal immune system and is considered to be a major factor in determining the resistance to mucous membrane infection (AbouEl-Yazeed, Taha, Elshehaby, & Salem, 2009; Gleeson, Pyne, & Callister, 2003).

While salivary SIgA concentrations are not necessarily an indication of health, the literature appears to support an association between IgA and health outcomes (Chikly, 2005; Rossen, Butler, & Waldman, 1970). Notably, low SIgA has often been associated with infections (Gleeson & Pyne, 2000; Gleeson et al., 1999, 2003; Hagewald, Bernimoulin, Kottgen, & Kage, 2002; Hanson, Bjokander, & Oxelius, 1983; Klentrou, Cieslak, MacNeil, Vintinner, & Plyley, 2002; Moreira, Delgado, Moreira, & Haahtela, 2009; Neville, Gleeson, & Folland, 2008; Nieman, 1994; Pyne et al., 2000; Teeuw, Bosch, Enno, Veerman, & Amerongen, 2004), impaired stress response (Saggio, Docimo, Pilc, Norton, & Gilliar, 2011; Volkmann & Weekes, 2006), and systemic diseases (Benjamini, Coico, & Sunshine, 2000; Brandtzaeg, 2007; Hanson et al., 1983; Latiff & Kerr, 2007; Paul, 2003).

The effects of low levels of salivary SIgA in precipitating infection, in particular recurrent upper respiratory tract infection (URTI) have been widely studied (Gleeson & Pyne, 2000; Gleeson et al., 1995, 1999, 2003; Hagewald et al., 2002; Jemmot & McClelland, 1989; Teeuw et al., 2004). Increased incidence of URTI during periods of chronic stress has been associated with suppression of salivary SIgA (Jemmot & McClelland, 1989; Volkmann & Weekes, 2006). Volkmann and Weekes (2006) assessed differences in stress and anxiety markers and upper respiratory tract infection (URTI) prevalence in 34 young adults during a low stress (summer holiday) period compared to a high-stress (examination) period. Greater stress, anxiety and more prevalent URTI symptoms were observed in the high-stress period, compared to low-stress period. A greater difference in URTI prevalence was noted in those with SIgA below or cortisol above the median levels, suggesting an impaired immune response to stress associated with low IgA or elevated cortisol.
The increased incidence of URTI has also been associated with exercise-induced low levels of salivary SIgA in elite athletes (Gleeson & Pyne, 2000; Gleeson et al., 1999, 2003; Moreira et al., 2009; Neville et al., 2008; Nieman, 1994; Pyne et al., 2000). Neville, Gleeson, and Folland (2008) examined the relationship between salivary SIgA and URTI in a cohort of elite professional yacht racing athletes (n=38) over 50 weeks of training by collecting weekly (38 hours post-exercise, consistent time of day) unstimulated saliva samples at rest together with clinically confirmed URTI, training load and perceived fatigue rating. On a group basis a significant (28%, p<0.005) reduction in salivary SIgA occurred during the three weeks prior to the URTI episodes and returned to baseline by two weeks after the URTIs. A salivary SIgA value lower than 40% of their mean healthy salivary SIgA concentration indicated a one in two chance of contracting an URTI within three weeks when an athlete did not have or was not recovering from an URTI. Therefore, a decline in salivary SIgA levels have been associated with an increased risk of URTI and this increase was directly proportional to the magnitude of the decline independent of the absolute salivary SIgA concentration.

Low levels of salivary SIgA have also been linked to an increased incidence of systemic diseases. One study investigating immune markers in frequently ill children, aged 2 – 15 years, with monthly acute (6 – 15 times/year) respiratory disease and combined pathology of the upper airway found that 94% of the 270 participants had decreased levels of salivary SIgA (Markova & Chuvirov, 2007). In addition, numerous reviews address the relationship between SIgA and a variety of oral as well as systemic diseases, including dental caries, periodontal disease, mucous membrane diseases, chronic sialadenitis, Sjögren's disease, non-Hodgkin's malignant B cell lymphoma, celiac disease, HIV infection and AIDS (AbouEl-Yazeed et al., 2009; Brandtzaeg, 2007; Paul, 2003). Unfortunately these reviews are inconclusive, mainly due to the number of other important variables influencing IgA levels, such as impact of flow rate, protein loss during sample handling, difficulties with reproducibility and standardization of immunoassays, and uncontrolled admixture of serum-derived monomeric IgA and IgG to the samples (Brandtzaeg, 2007; Gleeson et al., 1995).

Reports within the osteopathic literature claim that Osteopathic Manipulative Treatment (OMT), especially lymphatic pump techniques (LPT) are efficacious for enhancing immune response (Chikly, 2005; Smith, 1920; Whiting, 1910). One such technique, the osteopathic thoracic lymphatic pump (TLP) is widely used by osteopathic practitioners and is reputed to have a beneficial effect on the immune system. The TLP was developed by Miller in 1920 for relieving lymphatic stasis, enhancing immune response, and treating infections (Miller, 1923; 1926). Many osteopaths have claimed this technique improves lymphatic flow by clearing the thoracic duct in patients with respiratory infections, mastitis, or swollen upper extremities.
They also have used it for postsurgical reduction of respiratory volume (Kuchera & Kuchera, 1994; Ward, 2003)

Several studies confirm a beneficial immune response from TLP. Measel (1982) reported that subjects (n=13) who received TLP had a statistically greater humoral B cell response and increased levels of antibodies against pneumococcal polysaccharide two weeks post-vaccination than a control group (n=12). In accordance with these results, Jackson et al. (1998) demonstrated in their study that TLP and splenic pumping enhanced antibody production following hepatitis B vaccination. Measel and Kafity (1986) reported that TLP increased total white blood cell counts, increased the relative percentages of T cells and B cells and decreased lymphocyte counts.

More recently, two studies have investigated the effect of TLP on salivary SlgA levels, and concluded positive effects of TLP in isolation or part of osteopathic treatment on SlgA (Ehrlenbach, 2011; Saggio et al., 2011). Saggio, Docimo, Pilc, Norton, and Gilliar (2011) investigated the impact of OMT, consisting of a combination of three osteopathic techniques including TLP, on salivary SlgA levels in a stressed population of 25 osteopathic medical students. OMT resulted in a 139% (ES = 2.1) short-term increase in post-intervention salivary SlgA levels, compared with a 32% increase in the control group (p=0.027 for interaction between group and time).

The results of Ehrlenbach (2011), in a research project carried out while a Master of Osteopathy student at Unitec Institute of Technology, also supported the efficacy of TLP for improving salivary SlgA secretion by demonstrating an immediate post-treatment short-term increase of salivary SlgA concentration in all eight participants. Further she demonstrated an immediate post-treatment short-term increase of salivary SlgA secretion rate (SR) in 7 out of 8 participants with a large effect size of 3.0 (Cohen's d formula; p=0.03) indicating a strong correlation between TLP and increased levels of salivary SlgA secretion rates.

Because of a lack of data points in previous studies, the time-frame of short-term changes in salivary SlgA following TLP are unclear. The objective of this single case study design aimed to determine changes in salivary SlgA concentration and secretion rate in response to a single 7-minute intervention of the osteopathic thoracic lymphatic pump. This investigation was conducted in four male subjects who indicated suppressed mucosal immunity identified by recurrent upper respiratory tract infection and oral ulceration. This group was selected to help maximise any changes that might occur, since these individuals might be more likely to have depressed SlgA levels from which a therapeutic benefit might result.
2.0 Methods & Materials

2.1 Design

This report consists of four single case studies with observational measures designed to identify how a single standardised treatment protocol of TLP influences immediate and short-term SIgA concentrations (µg/mL) and secretion rates (µg/min) in saliva of healthy males with compromised mucosal immunity identified by recurrent URTI and OU.

The study was conducted in a research facility at an osteopathic student clinic. The research protocol was approved by the Unitec Research Ethics Committee (UREC), Unitec Auckland, New Zealand, UREC registration number: 2011-1236.

2.2 Participants

Recruitment of participants was via posters and general announcements to groups based in and around the Mt Albert campus of Unitec, Institute of Technology. To be eligible for inclusion in the study, participants were healthy, male non-smokers aged between 18 and 40 years. Individuals with recurrent URTI and or OU were targeted via a questionnaire to increase the chance of selecting participants with relatively low levels of salivary SIgA and the first eligible males to respond were recruited. The presence of URTI and or OU were self-reported and not established by clinical diagnosis. Exclusively male subjects were recruited to avoid hormonal variations of salivary SIgA during the female menstrual cycle (Albers et al., 2005). The inclusion criterion of non-smoking was specified to limit the influence that smoking has on salivary SIgA concentrations (Norhagen & Engstrom, 1998).

Potential participants were excluded if they met any of the following criteria:

- a present disease or infection
- intake of medication that affects the immune system such as antibiotics or steroids
- Intake of ≥21 standard drinks/week of alcohol or drug abuse
- diagnosis of any immunosuppressive syndrome, autoimmune condition including recent organ transplant
- a history of cancer, radiation therapy or chemotherapy in the past three years
- a contraindication for TLP including rib fractures, osteoporosis, a pacemaker or recent thoracic surgery
2.3 Procedure

The study protocol was explained to all eligible participants and each participant received a hard copy and an emailed information sheet prior to the day of sample collection. Participants were asked to refrain from eating, chewing (e.g. gum), drinking and exercising for two hours prior to the saliva sample collection, all of which can influence salivary SIgA levels.

Data collection was completed in a single day on 28 May 2012. On arrival the study protocol was reviewed, verbal and written consent was obtained and all participants completed a short questionnaire to determine the presence of factors that may have influenced SIgA production 24-hours prior to saliva sampling. Participants wore comfortable loose clothing consisting of a pair of shorts and a t-shirt to prevent any tight garment possibly interfering with lymphatic flow. Each participant rinsed their mouth with filtered natural water after their weight and height were recorded. Participants were reminded to refrain from casual conversation or other forms of communication not relevant to safety or to the procedure during the saliva collection. Finally each participant was asked to position themself in the supine position on one of the two identically adjusted treatment tables.

2.3.1 Saliva Sampling and Analysis

Prior to the intervention, four saliva samples were collected at 10-minute intervals with the last sample immediately followed by the intervention, during which one saliva sample was collected to measure immediate effects. Following the intervention, four saliva samples were collected at 10-minute intervals with the first sample collected immediately after the intervention. Saliva sample collection was conducted as recommended by Salimetrics guidelines. Non-stimulated saliva samples were collected via pre-weighed oral swabs made of an inert polymer shaped into a 30 x 10 mm cylindrical roll, and immediately stored into 2 ml snap-lock tubes, which were also pre-weighed and labeled. Oral swabs were placed under each participant's tongue for one minute targeting collection of saliva produced mainly from the sublingual glands. Each saliva sample was carefully collected and handled with a new pair of standard safety gloves to prevent droplet contamination, reweighed and recorded a second time before being stored in a snap-lock bag on crushed ice inside a closed cool box. After collecting each participant's set of nine saliva samples, samples were securely sealed inside a snap-lock bag, labelled and frozen at the recommended storage temperature of -20°C.

One Salimetrics salivary SIgA competitive enzyme-linked immunosorbent assay (ELISA) kit was purchased from Stratech Scientific APAC, Australia (Salimetrics Salivary Secretory IgA...
Indirect Enzyme Immunoassay Kit, Item No.1-1602; 96-Well Kit). Salivary SIgA concentration (µg/mL) was determined with the ELISA technique, which uses a horseradish peroxidase enzyme conjugated to goat anti-human SIgA as the detecting antibody. Laboratory analysis took place at the Unitec School of Health Science laboratory. Laboratory conditions and procedures adhered to the recommendations provided with the test kits (Salimetrics salivary SIgA ELISA Kit Manual).

Saliva samples were thawed on crushed ice, vortexed and centrifuged at 1500 x g (=3000rpm) for 15 minutes. The saliva volume was established by weight, assuming a specific gravity of one. All samples were analyzed in duplicate. Assay analysis and generation of a standard curve ($R^2 = 0.999$) was performed with Gen5 (BioTek Instruments Inc., 2002).

2.3.2 Intervention

The intervention consisted of a single standardised OMT protocol of seven-minute TLP without activation. The terminology 'without activation' indicates that there is no initiation of a recoil, meaning the operator does not initiate a sudden release of the hands away from the chest cage, as is done with different variations of TLP. The TLP was performed by one qualified registered New Zealand Osteopath, with the patient supine and the operator standing at the head of the table, placing both hands on the anterior thoracic wall with the thenar eminence of each hand over the pectoralis muscles just below the clavicles. Fingers were spread and angled towards the patient's body to distribute pressure across the chest wall. The TLP consisted of a continuous rhythmic pumping action of alternating pressure and release on the patient's chest at a rate of about 110-120 repetitions per minute (figure 1). The rate was estimated by the operator and was slightly alien depending on the patient's own natural recoil. The patient continued breathing at his usual comfortable rate.

Figure 1: Intervention of the osteopathic thoracic lymphatic pump (TLP) performed by a qualified registered New Zealand Osteopath.
2.4 Data Management and Analysis

Raw data were compiled and manipulated using Microsoft Excel version 2003. Immunoglobulin A concentrations and secretion rates at all nine test points were plotted for each individual. Visual inspection of graphs for each participant were undertaken using a slightly modified Conservative Dual Criteria method (CDC; Cohen, Feinstein, Masuda, & Vowles, 2014) with changes in variability assessed from a 2 standard deviation (SD) margin plotted either side of the mean of pre-treatment (baseline) measurements (Cashman, Mortenson, & Gilbart, 2014). Effect sizes were calculated for each individual using the difference between the mean pre-treatment and post-treatment measurements divided by the SD of the pre-treatment measurements over the four participants.

3.0 Results

Four men between the ages of 22 and 25 years who fulfilled the inclusion criteria participated in the study. All participants were second-year students in the Master of Osteopathy Program at the Department of Osteopathy of Unitec Institute of Technology Auckland, New Zealand and complained of both recurrent URTI (2-4/year) and OU (2-6/year). Their characteristics are shown in Table 1.

The normal range of SIgA concentration is defined by Salimetrics Salivary Research as 79.26 to 679.50 µg/mL (Salimetrics, 2009). As hypothesized at baseline all four participants showed suppressed levels of salivary SIgA concentrations (<79.26 µg/mL), with an average of 58.03 µg/mL.

The data for each participant's salivary SIgA concentration and salivary SIgA secretion rates pre-, peri- and post-intervention of TLP are shown in Figure 2. Visual inspection of the salivary SIgA concentration and secretion rate graphs for each participant shows that only two of the three participants had any peri- or post-treatment IgA concentration or secretion rates that fell outside a 2 SD range of the mean of their pre-treatment measures. For Participant 1, this was a single measurement 10 minutes following treatment. For Participant 3, measurements during and immediately following treatment were both elevated beyond 2 SD of measurements taken at baseline. A possible change in trend was noted for Participant 1, whereby a gradual increase in IgA that occurred prior to treatment appeared to be attenuated during and following treatment.
Baseline to post-intervention, three of four participants showed increased levels of salivary SIgA (Figure 3), with Participant 1 concentrations increasing from a baseline average of 58.3 µg/mL to 74.1 µg/mL across all post-intervention measures, Participant 2 from 53.5 µg/mL to 71.9 µg/mL, Participant 3 from 49.7 µg/mL to 74.3 µg/mL (ES=1.74-2.71). Participant 4 showed no change in concentrations from 70.6 µg/mL at baseline. On average salivary SIgA concentrations increased from 58.03 µg/mL before the intervention to 72.73 µg/mL after the intervention (25.3%). The mean effect size for salivary SIgA concentrations pre-intervention to post-intervention was d=1.62.

When SIgA was expressed as a secretion rate, no clear pattern was evident. Two participants increased and two participants decreased their salivary SIgA secretion rates baseline to post-intervention (ES=-1.82-1.96). On average salivary SIgA secretion rates increased from 23.8 µg/min to 24.4 µg/min (5.9%) The mean effect size for salivary SIgA secretion rates pre-intervention to post-intervention was d=0.05. During the intervention salivary SIgA concentrations were higher than baseline with an average of 75.19 µg/mL, while salivary SIgA secretion rates were lower with an average of 22.34 µg/min.

**Table 1: Participant characteristics**

<table>
<thead>
<tr>
<th>Participant</th>
<th>Age</th>
<th>Body Mass Index</th>
<th>Upper Respiratory Tract Infection (times/year)</th>
<th>Oral Ulceration (times/year)</th>
<th>Vigorous Exercise &gt;20min (times/week)</th>
<th>Perceived Stress Scale (maximum = 40)</th>
<th>Alcohol (standard drinks/week)</th>
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<td>1</td>
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<td>2</td>
<td>2</td>
<td>3+</td>
<td>19</td>
<td>5</td>
</tr>
</tbody>
</table>
Figure 2: Test points of individual salivary SlgA concentration [SlgA] (µg/mL) and salivary secretion rates SlgA SR (µg/min) in four participants tested before (Test Points 1-4), during (Test Point 5) and after (Test Points 6-9) an intervention with the osteopathic thoracic lymphatic pump (P1 = Participant 1, P2 = Participant 2, P3 = Participant 3, P4 = Participant 4). The solid horizontal line represents the mean of the pre-intervention test points (1-4), with dotted lines showing 2 standard deviations above and below. The sloped line shows a fitted linear regression curve of the pre-intervention test points (1-4) extrapolated to later test points.
Figure 3: Individual data point values of salivary SIgA concentrations [SIgA] (µg/mL) (A) and salivary secretion rates SIgA SR (µg/min) (B) in four participants tested before and after osteopathic thoracic lymphatic pump. Numbers above bars are effect sizes calculated as the difference between average baseline and post-treatment measurements divided by the standard deviation of the pre-treatment measurements for all four participants. Dashed line for [SIgA] indicates the lower reference range.
4.0 Discussion

The primary aim of this single case design was to determine if a single intervention of the osteopathic thoracic lymphatic pump (TLP) would cause an immediate or short-term increase in salivary SlgA levels. Immediate and short-term effects of TLP on Sal SlgA were investigated because it was hypothesized that the neural machnisms underpinning the effect would occur during and immediately after treatment. I hypothesized that participants with recurrent URTI and OU would have decreased levels of salivary SlgA and that a single intervention of TLP would cause a short-term increase in salivary SlgA levels. In this preliminary investigation, I have not demonstrated any consistent changes. Two participants, One and Three, presented with increased short-term salivary SlgA levels and large effect sizes, whereas two participants, Two and Four, did not. Because of the short time-frame for observed responses, any changes in salivary SlgA levels are likely to be associated with an increased release of salivary SlgA, rather than an increase in IgA production, as the time frame was too short in order to produce IgA.

These results do not concur with those of two recent similar studies, which both noted increases in salivary SlgA levels with large effect sizes. Saggio et al. (2011) showed OMT increased short-term salivary SlgA levels significantly (F, 5.92; \( p < 0.025 \)) in 11 out of 12 participants with an average increase of 139%. The control group showed an increase in 8 out of 13 participants with an average increase of 32%. Ehrlenbach (2011) demonstrated an immediate post-treatment short-term increase of salivary SlgA concentration in all eight participants and an immediate post-treatment short-term increase of salivary SlgA secretion rate in 7 out of 8 participants with a large effect size of \( d = 3.0 \) (Cohen's \( d \) formula; \( p = 0.03 \)) with an average increase of 136% indicating a strong correlation between TLP and post-intervention increase of salivary SlgA secretion rate. Increases in the current study are far less and less consistent amongst the four participants. Here, large short-term post-treatment increases of salivary SlgA concentration of around 25% were observed. Short-term post-treatment increases of salivary SlgA secretion rate are noted in only two of four participants.

The reasons for differences between past studies and the current findings are unclear. It is important to be aware of the impact of salivary flow rate on salivary SlgA, which has previously been suggested to explain differences among previous studies (Brandtzæg, 2007). In the present study, both salivary SlgA concentrations and secretion rates (corrected for changes in salivary flow) have been reported and changes are inconsistent for both measures, particularly for secretion rate. In the Ehrlenbach (2011) study, increases were consistent for both measure, whereas Saggio et al. (2011) reported only SlgA concentration
and not secretion rate. Therefore, differences in outcome measures would not appear to explain the differences from past studies in IgA changes noted here.

I observed large effect sizes for Participant One and Three, which are likely to indicate important clinical change. The large effect sizes for Participant One and Three suggest there may be a high likelihood of a clinical significance and a therapeutic benefit of TLP for some individuals. Although SIgA concentration increased for Participant Two following TLP, there was no increase when concentration was adjusted for mucosal salivary secretion, and salivary SIgA secretion was reduced following TLP. One possible reason why Participants Two and Four were different from One and Three might be that Participants One and Three participated in vigorous exercise more than three times a week, while Participants Two and Four only participated one to two times a week in vigorous exercise. Perhaps TLP is less effective in people whose reduced IgA is a consequence of frequent and intense exercise. This could be because chronic vigorous exercise may increase the release of salivary SIgA and therefore deplete salivary SIgA storage. The build up of IgA in people undergoing prolonged intense exercise may be more difficult to alleviate via acute changes in release of IgA brought about by a single TLP intervention. No information about the exercise levels in either of the previous studies was reported so it is difficult to tell if these patterns emerged previously.

Another difference in the design of the present study which might help explain differences in results is the timing of outcome measures. In order to improve on the design of Ehrlenbach’s 2011 study, where a small number of data points limited identifying possible fluctuations of baseline SIgA in response to TLP, an increased number of baseline, intervention and follow-up data points were used to assess SIgA variability here. The present study increased the data points measuring changes in salivary SIgA up to 30 minutes post-TLP and were administered at the same frequency as the baseline measures to allow an equivalent pre- and post- intervention comparison of immediate and short-term effects, whereas Ehrlenbach limited data points to 10 minutes post-TLP. Saggio et al. (2011) only used two data points, one at baseline and one 60 minutes post-TLP.

This study observed whether participants with recurrent URTI and OU had decreased levels of salivary SIgA. Individuals with recurrent URTI and or OU were targeted to increase the chance of selecting participants with relatively low levels of salivary SIgA. All four participants, who were selected due to recurrent URTI and OU, presented with suppressed salivary IgA concentrations. Regardless of the increase in salivary IgA levels, mean post-treatment salivary SIgA did not attain levels within the normal range for any participant. The
suppressed levels of salivary SIgA in all four participants supports the hypothesis that individuals with recurrent URTI and OU have decreased levels of salivary SIgA. The deliberate selection of individuals with impaired immune function possibly created additional variation in design compared with the previous studies of Ehrlenbach (2011) and Saggio et al. (2011) and may also be a reason for the differences in results noted.

Attributing a cause and effect relationship between the treatment and changes in SIgA in this study is limited by small sample size and an inability to control for many factors that influence SIgA concentration and secretion. Secreted salivary IgA is a relatively easily measured output of the immune system. Nevertheless, many variables can influence results, such as methodological problems with sample handling, storage and processing, as well as many other factors such as stress, infection, diet and exercise. Due to many factors known to influence salivary SIgA, it is vital to maximise standardisation of variables. These factors may also explain differences noted here compared with previous studies. Saliva secretion might have been influenced by the presence of oral swabs as the swabs were fully soaked with saliva before the one minute allowed for sample collection elapsed.

It is still unclear to what extent salivary SIgA can be regarded as an indicator of immune health; however, there is growing evidence that increased levels of salivary SIgA may represent enhanced mucosal immune function and protection. Moreover, low levels of salivary SIgA can reflect some sort of immune suppression.

Despite the common use of TLP by osteopathic practitioners in the treatment of patients with immune suppressed conditions, research to validate the therapeutic effect of this technique on immune health is limited. Further investigation of the effects of Osteopathic Manipulative Treatment techniques on salivary SIgA is therefore warranted. Salivary SIgA as a simple and cost-effective immune marker for mucosal immunity is a commonly studied immune marker in research projects. Even though there are many methodological problems with sample collection, processing and storage, which impede standardisation of normal quantifications in humans, there is strong and growing evidence of the association of salivary SIgA with various immune health indicators.
5.0 Conclusion

This study was designed as a single case study exploratory investigation to examine the feasibility and possible efficacy of a novel approach to managing immune conditions in some individuals. There may be some indication of an overall trend for an increase in salivary SIgA levels with a single application of TLP that might have been detected with a larger sample size or possibly with a greater number of Post-TLP data points. The limited resources allotted for this preliminary study did not allow for either.

Despite two previous studies which reported sizeable effects of TLP on salivary SIgA levels, here I have not shown a consistent therapeutic benefit of TLP in participants with a suppressed mucosal immune function.

At this stage, it is unclear why some participants appeared to respond to treatment whilst others did not.

6.0 Conflict of Interest

None.
7.0 References


SECTION 3 - APPENDICES
Appendix A - Ethics Documentation
Dominik Blut
25 Fairleigh Ave
Mt Albert
Auckland 1025

26.1.2012

Dear Dominik,

Your file number for this application: 2011-1236
Title: The effect of the osteopathic lymphatic pump technique on short-term salivary immunoglobulin A secretion rate - Multiple case studies

Your application for ethics approval has been reviewed by the Unitec Research Ethics Committee (UREC) and has been approved for the following period:

Start date: 12.1.12
Finish date: 12.1.13

Please note that:

1. The above dates must be referred to on the information AND consent forms given to all participants.

2. You must inform UREC, in advance, of any ethically-relevant deviation in the project. This may require additional approval.

You may now commence your research according to the protocols approved by UREC. We wish you every success with your project.

Yours sincerely,

[Signature]

Scott Wilson
Deputy Chair, UREC

cc: Craig Hilton
Cynthia Almeida
PARTICIPANT INFORMATION SHEET

The effect of the osteopathic lymphatic pump technique on short-term salivary immunoglobulin A secretion rate

About this research
You are invited to take part in a study undertaken as part of a Master of Osteopathy Degree at Unitec NZ. This research investigates the effect of a widely used osteopathic technique on antibody levels in human saliva. Healthy males between the ages of 18 and 40 years who have experienced recurrent infections such as upper respiratory tract infections/colds or mouth ulcers are needed.

Participants in this project will be asked to:

• Attend a brief appointment to:
  o Ensure that the inclusion and exclusion criteria are met, and that they are eligible for the project.
  o Sign a consent form.
  o Fill out a questionnaire about any previous illnesses or diseases.

• Attend an arranged 120-minute appointment on a single day to answer a questionnaire about lifestyle factors such as sleep, exercise, diet and stress levels, and provide a total of 9 samples of saliva. Four samples will be collected prior to a 7-minute thoracic lymphatic pump treatment on the upper chest, one during the treatment and four after the treatment. You are required to abstain from alcohol for 24 hours prior to this appointment and from exercise, food or other drinks (except for water) for 1 hour prior to this appointment.

• Saliva samples are collected with Oral Swab, sterile non-toxic, inert polymer shaped into a 30 mm x 10 mm cylinder. The sterile Oral Swab is removed from outer packaging and placed into proper mouth location for 1 minute to insure that it is saturated with saliva.

The researchers
The primary researcher is Dominik Blut, post-graduate student in Osteopathy (Master of Osteopathy) This project is being supervised by Drs Craig Hilton and Catherine Bacon.

Participation and consent
You have the right to choose not to participate, or to withdraw from this research project at any time prior to commencement of data analysis and up to 2 weeks after the date of collection of your samples. This can be done by emailing us, phoning us, or telling us when we contact you that you do not want to participate. You also have the right to access your own records on request.

Any data collected will be kept in an anonymised format to allow for future re-analysis. This data may be re-analysed in future student studies. Appropriate research and ethic approval will be sought prior to the use of data collected in this study being made available in additional studies.

If you’re interested in participating please complete a consent form (attached) for this project and return it to Dominik Blut.
Information and concerns

If you would like further information about the project or have any concerns, please feel free to contact either Dominik Blut or Dr Craig Hilton.

Dominik Blut  dominik.blut@gmail.com
Dr. Craig Hilton: chilton@unitec.ac.nz

If you would like further information about the project, please feel free to contact Dominik Blut or, the primary researcher, on the details above.

If you have any concerns about the way in which the research is being conducted, you can contact the following:

Health Advocates Trust, Freephone: 0800 555 050, P.O. Box 9983, Newmarket, Auckland.
Access Ability, Phone: 09 262 5370, Fax: 09 262 5371, P.O. Box 23-725, Papatoetoe, Auckland.

Māori participants

Please be aware that the collected samples of your saliva cannot be returned.

Confidentiality

Your confidentiality and anonymity will be protected in the following ways:

• Information and data collected from you during this research will be labelled with an identification number for the purpose of anonymously comparing your data.
• All computer records will be accessible solely by passwords held only by the researchers.
• Any data derived from the research will be anonymous and your identity will be kept confidential.

A copy of the final report will be available at the Unitec NZ library. You will also be given a summary of your own personal data and general findings. Summaries and recommendations may be published in research journals.

Finally, we would like to extend our appreciation and thanks to you for your valuable contribution to this research.

UREC REGISTRATION NUMBER: 2011-1236

This study has been approved by the UNITEC Research Ethics Committee from 12.01.12 to 12.01.13. If you have any complaints or reservations about the ethical conduct of this research, you may contact the Committee through the UREC Secretary (ph: 09 815-4321 ext 7248). Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.
PARTICIPANT CONSENT FORM

The effect of the osteopathic lymphatic pump technique on short-term salivary immunoglobulin A secretion rate

This research project investigates the effect of an osteopathic technique on salivary antibody A levels. The research is undertaken by Dominik Blut, a Master of Osteopathy student at Unitec, New Zealand and will be supervised by Dr Craig Hilton and Dr Catherine Bacon.

Name of Participant: __________________________

I have seen the Participant Information Sheet for people taking part in the project titled ‘The effect of the osteopathic lymphatic pump technique on short-term salivary immunoglobulin A secretion rate’. I have had the opportunity to read the contents of the information sheet and to discuss the project with Dominik Blut, and I am satisfied with the explanations I have been given. I understand that the anonymised data from the project will be held indefinitely for the purposes of future analysis and research. I understand that taking part in this project is voluntary (my choice) and that I may withdraw from the project at any time prior to commencement of data analysis and this will in no way affect my access to the services provided by Unitec NZ, or the Unitec Osteopathic Clinic.

I understand that I can withdraw from the study if, for any reason, I want to do so.

• I have had enough time to consider whether I want to take part.
• I understand that I can withdraw from the study at anytime up until the date of the last data collection session.
• I understand that my participation in this project is confidential, and no material that could identify me will be used in any reports of this project.

I also understand that the collected samples of my saliva will not be returned to me.

• I acknowledge that any materials collected during the study will be stored securely so that only the researchers may access them. I understand that my data collection records will be made available on request. I understand that any material collected will made anonymous and kept indefinitely to enable future re-analysis.

I know whom to contact if I have any questions or concerns about the project.

The principal researcher for this project is Dominik Blut.
Contact details: dominik_blut@yahoo.de

Signature: __________________________ Date: / / (participant) (dd/mm/yyyy)

Signature: __________________________ Date: / / (Dominik Blut) (dd/mm/yyyy)

The participant should retain a copy of this consent form.

UREC REGISTRATION NUMBER: 2011-1236
This study has been approved by the UNITEC Research Ethics Committee from 12.01.12 to 12.01.13. If you have any complaints or reservations about the ethical conduct of this research, you may contact the Committee through the UREC Secretary (ph: 09 815-4321 ext 7248). Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.
Appendix B - Advertisement
Research Participants Needed

Do you suffer from recurrent mouth ulcers or upper respiratory tract infection/colds??

We are investigating the effect of an osteopathic technique on the levels of a specific antibody in the human saliva.

Healthy, non-smoking males with recurrent upper respiratory tract infection/colds and/or mouth ulcers aged between 18 and 40 years are required.

If you are interested in participating, you will be asked to:
• Complete a questionnaire about lifestyle factors that may influence saliva antibodies such as diet, sleep, stress levels and exercise. This will identify whether you are able to participate in the study.
• If you are available you will be asked to attend our laboratory for approximately 120 minutes and provide about 9 saliva samples.
• During this visit, you will receive a free treatment with a rhythmic pumping technique that will be applied to your upper chest by a qualified osteopath for a duration of 7 minutes.

Contact:
Dominik Blut Master of Osteopathy student
Phone: 0220959250
Email: dominik_blut@yahoo.de

..or get a participant information sheet by the receptionist!!

UREC REGISTRATION NUMBER: 2011-1236
This study has been approved by the UNITEC Research Ethics Committee from 12.01.12 to 12.01.13. If you have any complaints or reservations about the ethical conduct of this research, you may contact the Committee through the UREC Secretary (ph: 09 815-4321 ext 7248). Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.
Appendix C - Questionnaires
Study Enrolment Questionnaire

Name: 
Address: 
Date of birth: 
Ethnicity: 
Phone number: 
Email: 
Male □  Female □  Smoker: □ yes  □ no
Weight:  
Height: 

1. Please list below any medications that you are presently taking:

........................................................................................................................................

2. Do you suffer from any medical condition?
   □ yes  □ no

3. Are you presently receiving any medical treatment?
   □ yes  □ no

4. Do you have any allergies?
   □ yes  □ no

5. Do you suffer from common viral or bacterial infection, such as upper respiratory infections, colds?
   □ yes  □ no  If yes, please state how often per year: _____x/per year

3. Do you presently suffer from any viral or bacterial infection?
   □ yes  □ no

6. Do you suffer from common mouth ulcers/sores, tooth or gum infections?
   □ yes  □ no  If yes, please state how often per year: _____x/per year

7. Do you presently have any mouth ulcers, tooth or gum infections?
   □ yes  □ no

8. Did you recently have any dental extractions or dental surgery?
   □ yes  □ no

9. Have you ever been diagnosed with any infectious disease such as AIDS, Hepatitis or Syphilis?
   □ yes  □ no

10. Is there a history of haemophilia in your family?
    □ yes  □ no

11. Have you ever been diagnosed with IgA deficiency?
    □ yes  □ no

12. Have you ever been diagnosed with an autoimmune disorder?
    □ yes  □ no

13. Have you ever been diagnosed with any form of cancer, sickle cell disease, aneurysms, cardiac failure or varicose veins?
    □ yes  □ no

14. Did you recently (within last 2 weeks) return from a long distance flight?
    □ yes  □ no

15. Do you ever experience pain in your legs during exercising?
    □ yes  □ no

16. Have you ever been diagnosed with deep vein thrombosis?
    □ yes  □ no

17. Did you recently undergo any procedure or surgery in the upper body area?
    □ yes  □ no

18. Do you suffer from any condition/illness for which you are currently receiving treatment?
    □ yes  □ no
The following questions aim to establish lifestyle factors that are known to have an influence on salivary antibodies.

Please answer as indicated.

Listed below are a series of statements about people’s exercise habits. Please circle the number that reflects how often you could make the following statements:

1 - NEVER  2 - SOMETIMES  3 - USUALLY  4 - ALWAYS

1. I engage in physical exercise on a daily basis.
   1 2 3 4

2. I engage in one/more of the following forms of exercise: walking, jogging/running or weight lifting.
   1 2 3 4

3. I exercise more than three days per week.
   1 2 3 4

4. How often do you take part in vigorous exercise (sufficient to make you slightly breathless and makes your heart beat faster) which lasts for 20 minutes or more? (please circle)
   A occasionally or never
   B once or twice a week
   C three times a week or more

Listed below are some questions regarding your diet. Please answer as indicated.

1. Are you currently dieting to reduce your weight?
   □ yes  □ no

2. Do you regularly take any dietary supplements?
   □ yes  □ no
   If yes, please list them here:
   ................................................................................

3. How many standard drinks of alcohol do you drink per week?
   ................................................

Examples for standard drinks:
• 1 small bottle of beer (330ml) with 4% Alc.  ≈ 1 standard drink
• 1 small bottle of beer (330ml) with 5% Alc.  ≈ 1,3 standard drinks
• 1 glass of red or white wine (100ml) with 14% Alc.  ≈ 1 standard drink
• 1 bottle of red or white wine (750ml) with 14% Alc.  ≈ 8 standard drinks
• 1 shot of spirits (30ml) with 37,5% Alc.  ≈ 1 standard drink
• 1 bottle of spirits (500ml) with 37,5% Alc.  ≈ 16 standard drinks
Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate by circling how often you felt or thought a certain way.

0 = Never  1 = Almost Never  2 = Sometimes  3 = Fairly Often  4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly? .................................................. 0 1 2 3 4

2. In the last month, how often have you felt that you were unable to control the important things in your life? .................................................. 0 1 2 3 4

3. In the last month, how often have you felt nervous and “stressed”? .................................................. 0 1 2 3 4

4. In the last month, how often have you felt confident about your ability to handle your personal problems? .................................................. 0 1 2 3 4

5. In the last month, how often have you felt that things were going your way? .................................................. 0 1 2 3 4

6. In the last month, how often have you found that you could not cope with all the things that you had to do? .................................................. 0 1 2 3 4

7. In the last month, how often have you been able to control irritations in your life? .................................................. 0 1 2 3 4

8. In the last month, how often have you felt that you were on top of things? .................................................. 0 1 2 3 4

9. In the last month, how often have you been angered because of things that were outside of your control? .................................................. 0 1 2 3 4

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them? .................................................. 0 1 2 3 4

Date:  __________ / __________ / __________

(dd/mm/yyyy)

Signature: ________________________________
Pre-Sample Collection Questionnaire

Date: ____________________________________________

Name: __________________________________________

This questionnaire aims to determine factors that may have influenced your salivary antibody levels in the past 24 hours. Please answer the following questions as indicated.

1. Did you engage in any exercise in the last 24 hours (sufficient to make you slightly breathless and make your heart beat faster for more than 20 minutes)?
   Yes ☐ No ☐
   If you answered yes, please indicate time: ________________________________

2. Did you drink any alcohol or coffee in the last 24 hours?
   Yes ☐ No ☐
   If you answered yes, please indicate amount and time ________________________________

3. Did you have any food, drink (other than water) in the last hour?
   Yes ☐ No ☐
   If you answered yes, please indicate type and amount: ________________________________

4. How many hours do you usually sleep per night? ________________________________

5. How many hours did you sleep last night? ________________________________

6. How would you rate your average stress level in the last 24 hours on a scale from 0 to 10? (please circle)
   1 2 3 4 5 6 7 8 9 10 (1=minimal, 10=maximal)

7. How would you rate your stress level at this moment on a scale from 0 to 10? (please circle)
   1 2 3 4 5 6 7 8 9 10 (1=minimal, 10=maximal)

8. Did you have any symptoms of a bacterial or viral infection in the past 24 hours? (temperature, sore throat, cough, runny nose)
   Yes ☐ No ☐

Signature: ____________________________________________
Appendix D - Sample Collection Sheets
Participant Nr.:

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Appendix E - Plate Layout & Data Matrix
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Individual data point values of salivary SIgA concentrations [IgA] (µg/mL) in four participants tested before (test points 1-4), during (test point 5) and after (test points 6-9) an intervention with the osteopathic thoracic lymphatic pump.

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Individual data point values of mean salivary SIgA concentrations [IgA] (µg/mL) of four participants before (MeanPre) and after (MeanPost) an intervention with the osteopathic thoracic lymphatic pump.
Appendix H - Journal Publication Information
http://www.journals.elsevier.com/international-journal-of-osteopathic-medicine/