A preliminary investigation of the effect of the osteopathic lymphatic pump technique on salivary Immunoglobulin A levels in asymptomatic subjects:

A single systems design pilot study

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Declaration

Name of candidate: Heike Ehrlenbach

This Research Project 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

Candidate’s declaration

I confirm that:

• This Thesis/Dissertation/Research Project represents my own work;
• The contribution of supervisors and others to this work was consistent with the Unitec Regulations and 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: 2009-974

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Abbreviations and Terminology

IgA  Immunoglobulin A /Antibody A
S-IgA  Secretory Immunoglobulin A
SC  Secretory Component
pIgR  Poly-Ig receptor, allows mucosal transport of S-IgA
LPT  Lymphatic Pump Technique
OMT  Osteopathic Manipulative Treatment
GALT  Gut Associated Lymphoid Tissue
MALT  Mucosa Associated Lymphoid Tissue
NALT  Nasal Associated Lymphoid Tissue
MLN  Mesenteric Lymph nodes
FVC  Forced vital capacity, a lung capacity measurement
FEV1  Forced expiratory volume in 1 second, a lung capacity measurement
URTI  Upper Respiratory Tract Infection
IL  Interleukin, a protein related to immune response
IV  Intravenous
HIV  Human Immunodeficiency Virus
AIDS  Acquired Immune Deficiency Syndrome, caused by HIV
DO  Doctor of Osteopathy
MD  Doctor of Medicine
SSRD  Single Systems Research Design
ANS  Autonomic Nervous System
SNS  Sympathetic Nervous System
PSNS  Parasympathetic Nervous System
CAUDAD  Towards the feet
CEPHALAD  Towards the head
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SECTION 1 – LITERATURE REVIEW
Introduction to the dissertation

The concept of osteopathic healthcare is based on maintaining and re-establishing homeostasis within and in between body systems to support the body in the activation of its own innate capacity to heal. This philosophy incorporates the belief that unhindered flow of blood and lymphatic fluid is a prerequisite for human health. Osteopathic practitioners aim 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). The osteopathic profession has long been aware of the significance of the lymphatic system in health support and the Lymphatic Pump technique (LPT) is reported to have been utilized by osteopathic practitioners since the late nineteenth century (Hildreth, 1938; Millard, 1922; Parker, 1934; Still, 1899, 1902).

LPT consists of rapid, rhythmic compressions over various lymphatic related tissues of the body and osteopaths refer to thoracic, abdominal, spleen, liver and pedal pump techniques (McMillan, Crow, & Greene, 2004). There are an abundance of osteopathic reviews reporting historical clinical cases supporting the efficacy of LPT (Amalfitano, 1987; Chikly, 2005) and in addition studies of the effect of LPT on lymphatic flow (Knott, Tune, Stoll, & Downey, 2005) or immunological markers in humans (Breithaupt et al., 2001; Jackson et al., 1998; J. W. Measel, Jr., 1982; J. W. Measel & Kafity, 1986; Noll et al., 2004) and animals (Hodge et al., 2010; Hodge et al., 2007; Schander et al., 2008), but to date there have been only three studies investigating clinical outcomes of LPT in humans and animals (Pedrueza, Zhang, Jones, & Hodge, 2010; Sleszynski & Kelso, 1993).

The most active research in the area currently focuses on the reaction of immunological markers to LPT (McMillan et al., 2004). One product of the immune system, immunoglobulin A (IgA), is released from the B lymphocytes into the blood and also, uniquely among immunoglobulin, is secreted in immunologically relevant quantities into saliva, tears, breast milk and respiratory, nasal and vaginal mucosa (Wood, 2006). IgA antibodies are the body’s first line of defense against pathogens that enter via mucosal sites and act by immobilizing micro-organisms or preventing their attachment to mucosal surfaces. Salivary IgA is one of the easiest measurable immune markers and while concentrations are not necessarily an indication of health the literature appears to support the view that increased S-IgA levels may have a positive impact on
human health (Kaufman & Lamster, 2002; Latiff & Kerr, 2007). Decreased S-IgA concentrations are frequently associated with oral as well as systemic diseases. However, individuals with decreased S-IgA levels can present without any symptoms (Brandtzaeg, 2007).

The primary purposes of this thesis were to review literature underpinning LPT and its theoretical and empirical effect on S-IgA in saliva as one output of the immune system and then to investigate the short-term effect of a brief lymphatic pump treatment on salivary S-IgA levels in a homogeneous population of asymptomatic participants. The investigation entails a single systems research design in which a salivary IgA baseline was established over 5 days with subsequent salivary samples before and after a 7 minute thoracic lymphatic pump treatment. S-IgA level as the primary outcome measure was determined via enzyme-linked immunosorbent assay (ELISA).
Osteopathy and Health

Modern osteopathy appears to have its scope of practice limited to the treatment of musculoskeletal problems, yet in the early days of the profession osteopathic practitioners were asked to treat numerous infectious diseases and were also utilized as primary health care providers (Stone, 1999; Ward, 2003). Retrospective data, collected after the 1918 influenza pandemic, suggest that lower morbidity and mortality amongst osteopathically treated patients as compared to those treated with the standard medical care that was available at the time (Kuchera & Kuchera, 1993).

Osteopathic Philosophy and Dysfunction

Osteopathic philosophy insists that for optimal function to be achieved the human body has to be integrated across all its interconnected body systems. In osteopathy, the term “function” implies an ideal structural and physiological state that allows each body component to work correctly and efficiently on its own and in relation to other body components (Kuchera & Kuchera, 1993; Stone, 1999) It is believed by the osteopathic profession that the development of disease is more likely if there is a disturbance in function. Therefore, osteopathic practitioners conclude that the underlying cause of disease is a breakdown of function of one or more body components and thus reestablishing function is the aim of treatment (Ward, 2003).

Osteopathic Dysfunction in relation to the Immune System

Osteopathic practitioners usually address the immune system by assessing and treating the lymphatic system so that restrictions are removed to restore free flow of lymphatic fluid, as the flow rate of lymph fluid within the lymphatic vessels is considered to be related to immune system function (Degenhardt & Kuchera, 1996). Decreased lymphatic flow causes tissue edema and accumulation of waste products and can interfere with cellular activity resulting in cell dysfunction and disease. Increasing lymph flow and drainage of lymph fluid out of tissues optimizes the transport and removal of inflammatory mediators (Degenhardt & Kuchera, 1996; Ward, 2003).
How can Osteopathic treatment influence the Immune System?

Osteopathic practitioners seek to affect the functioning of those 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, neutralization and elimination of immune related cells and are treated either directly or by releasing of restricted tissues surrounding the respective organ (Ward, 2003; Wood, 2006). Structures associated with lymphatic flow such as lymph vessels and lymph nodes are not easily accessible and the osteopathic approach is again centered on treating restrictions in surrounding tissues through which those structures pass such as muscles and fascia. Fascia is a connective tissue that surrounds and connects internal structures throughout the body. Dysfunctional muscle or fascia hinders the movement of lymphatic fluid (Paoletti, 2006). Additionally osteopathic treatment utilizes a wide range of pumping and draining techniques. Osteopaths state that pumping and drainage techniques, applied over immune related tissues, can influence lymphatic flow by altering pressure relationships and providing suction effects (Guyton & Hall, 2000; Ward, 2003).

In a broader sense, osteopathy also asserts that osteopathic intervention influences the immune system by aiming to return the body to a state of homeostasis. Homeostasis can be defined as the optimum physiological and structural balance and equilibrium within the body (St John, 1995). Any challenge to homeostasis triggers a stress response in the body with a subsequent release of stress related chemicals and hormones which affect the immune system in numerous ways (Kuchera & Kuchera, 1993; Stone, 1999; Ward, 2003).
Overview of the Immune system

Our environment contains numerous microorganisms that can cause disease in the human body. The ability of these organisms to evolve, change and enter the human body has to be met by a very adaptive immune system. Additionally, most cells in the body constantly renew themselves and tumor formation, caused by abnormal cell proliferation, has to be monitored and counteracted by the immune system. The enormous diversity of pathogens and the difficulty of destroying pathogens without damaging the host gives an indication of the complexity of the human immune system (Wood, 2006).

The immune system of each individual reflects their genetics and past and present antigen challenges (Roit, Brostoff, & Male, 2001). Immune deficiencies may manifest without any symptoms - as often can be the case in immunoglobulin A deficiency - or may cause minor or major health issues for example in HIV positive individuals that suffer from viral destruction of vital immune cells (Moore & Dalley, 1999; Pilette, Durham, Vaerman, & Sibille, 2004; Wood, 2006).

Challenges to the immune system are met by several levels of defense that are incorporated within either the innate or the cell mediated branches of the immune system (Wood, 2006). The development of topical infections on mucosal surfaces is opposed by the secretory or mucosal part of the innate immune system. Topical infections include those of the oral surfaces and the upper respiratory tract and are met by specialized mucosal cells including those which secrete salivary S-IgA (Woof & Kerr, 2006). The mucosal or secretory division of the immune system aims to neutralize pathogens before they can destroy healthy tissue or penetrate further into the body. Communication between mucosal immune cells and other immune system cells and components ensures further actions on pathogens that threaten to overpower the secretory defense (Roit et al., 2001).
Components of the Immune system

The lymphatic system, a network of lymph vessels, ducts and nodes that is also considered to be a part of the circulatory system, plays a large role in the transport and interaction of immune modulators (Albers et al., 2005; Chikly, 2005; Wood, 2006). The lymphatic organs are classified as primary or secondary. Primary lymphatic organs are the thymus, responsible for the development of T lymphocytes, and the bone marrow where the maturation of B lymphocytes takes place. Secondary lymphatic organs are the spleen, lymph nodes and mucosa associated lymphatic tissue (MALT) including gut associated lymphatic tissue (GALT) and nasal associated lymphatic tissue (NALT) (Roit et al., 2001) Additionally, almost all tissues and organs such as the brain, liver, kidney and lungs contain phagocytic cells. Phagocytes are cells that internalize and then destroy microorganisms (Brandtzaeg, 2007).

The main feature of cell or antibody-mediated immunity is the ability of B lymphocytes to produce antibody-secreting plasma cells with specificity for a certain type of pathogen (antigen). After the initial infection has been eradicated, these cells can remain in the bloodstream as memory cells to protect against future infection from the same organism. In addition antibodies have a considerable half life ranging from ~ 6 days for IgA in secretions to ~ 3 weeks in the case of IgG in serum (Freitas, 2003).

Antibody-mediated immunity is dependent on cellular action where T lymphocytes differentiate into T helper cells (Th). T helper cells promote the production of antibodies and control the ability of B-lymphocytes to express and produce different types of antibody classes. This immune function is known as antibody class switching (Wood, 2006). Protective antibodies can recognize, bind and neutralize antigen and activate immune mechanisms, such as agglutination, activation of complement and antibody dependent cytotoxicity, to destroy and eliminate pathogens. Agglutination decreases the spreading of pathogens by containing them in clumps. This process is made possible by the ability of antibodies to bind to more then one antigenic particle. Larger formations of pathogens are more easily recognized and destroyed. Some classes of antibodies have the ability to activate the complement system, resulting in the release of a complex cascade of molecules that aid in the destruction and clearance of pathogens. Antibodies
can also activate antibody-dependent cell-mediated cytotoxicity (ADDC) where immune cells are attracted to destroy an antigenic cell that is bound to the antibody (Roit et al., 2001; Wood, 2006).

All antibodies have a similar symmetric core structure comprising two heavy chains and two light chains usually depicted in a Y-shape (Figure 1). The heavy chains are linked to each other and to the light chains by disulphide bridges. A hinge region gives the molecule flexibility. The N-terminal domains are responsible for binding antigen and are also known as variable domains or variable regions since their amino acid sequences differ between antibodies to guarantee antigen specificity. The C-terminal domains form the Fc- portion of the molecule, necessary for recognition by the Fc-receptor on macrophages or natural killer cells (Wood, 2006; Woof & Kerr, 2006).

The different functions of antibodies have given rise to five classes of antibodies: IgG, IgM, IgD, IgE and IgA with four subclasses of IgG and two of IgA. Different classes of antibodies can have the same antigen specificity (Pilette et al., 2004; Wood, 2006; Woof & Kerr, 2006).

![Figure 1: Antibody structure (Woof & Kerr, 2006)](image-url)
**Immunoglobulin A (IgA)**

IgA is a major serum antibody and is the main class of antibody found in secretions of mucosal surfaces. The two subclasses of IgA - IgA1 and IgA2 - seem to have similar roles. One major difference between the subclasses lies in the hinge region which is more extended in IgA1 indicating further reach between the two Fab arms of the molecule. Therefore IgA1 may be able to attach concurrently to two antigens that are separated by a considerable distance. IgA2 has greater stability based on increased resistance to certain proteases produced by bacteria (Pilette et al., 2004; Wood, 2006). Secretory IgA (S-IgA) is produced in the form of dimers constituting of two IgA monomers that are linked to one molecule of J (joining) chain and possess an attached secretory piece (Figure 2). The J chain is responsible for transport of IgA into secretions. The secretory piece protects IgA from breakdown by proteolytic enzymes that are present in secretions (Woof & Kerr, 2006).

![Figure 2: Dimeric serum IgA (on left) and Secretory IgA with attached secretory piece (on right) (Woof & Kerr, 2006)](image-url)
Sites of IgA synthesis

Serum IgA is synthesized by bone marrow plasma cells while secretory IgA (S-IgA) is the product of lymphocytes in various lymphoid and non-lymphoid tissues. The highly specialized mucosal immune system tissues such as GALT, MALT and Peyers patches functions largely independent of the systemic immune system (Czerkinsky, Svennerholm, & Quiding, 1991). Following secretion by plasma cells into the mucosa, S-IgA binds to the poly-Ig receptor (pIgR) on mucosal epithelial cells. The pIgR/S-IgA complex is then internalized by the epithelial cells where the poly-Ig receptor undergoes enzymatic cleavage to form the secretory component (SC) attached to the S-IgA molecule (Brandtzaeg, 2007). The secretory component acts as a receptor and transepithelial transporter for S-IgA. The subsequent secretion of S-IgA into the mucosal fluid involves partial proteolysis of the SC where one portion stays complexed to dimeric IgA and the remaining portion of SC is recycled by the epithelial cells. The SC connected to dimeric IgA protects it from proteolysis by microorganisms present on mucosal surfaces (Brandtzaeg, 1995; Mackinnon, 1999).
The secretory IgA (S-IgA) system

The unique ability of secretory immunoglobulin for high antigen binding and their relative resistance to proteolytic destruction enables them to fulfill their role in the specific environment of mucosal surfaces. S-IgA, unlike the other immunoglobulins, has restricted ability to activate defense mechanisms that involve inflammatory processes, therefore preventing chronic inflammation in the mucosa (Brandtzaeg, 2003; Challacombe, Rahman, Jeffery, Davis, & O'Hagan, 1992). However, it has been shown that S-IgA can act as a potent stimulus for eosinophil activation. Eosinophils are involved in pro-inflammatory processes and allergic reactions (Brenner, Shek, & Shepard, 1994; Roit et al., 2001).

The secretion of IgA is dependent on T lymphocyte interaction that initiates the differentiation of B lymphocytes into S-IgA antibody-producing plasma cells and later into memory cells that can provide a quick antibody response to later exposure to the same antigen. Antigen presentation at various lymphoid tissues then results in the migration of antigen-specific salivary IgA secreting plasma cells to the mucosal surfaces. However, there may be a T lymphocyte independent activation of S-IgA producing B lymphocytes (Wood, 2006). This, still not fully understood, process seems to be limited to the peritoneal cavity where macrophages release a substance that attracts B lymphocytes and may be crucial as part of a first line immune defense (Fagarasan & Honjo, 2004; Pilette et al., 2004).

The association between the intestinal and salivary S-IgA response is at present not fully understood and studies investigating those connections show disparities. Although there is evidence that IgA secreting B-lymphocytes migrate from GALT to salivary glands it is now believed that NALT is more important as production site for B-lymphocytes destined for the salivary glands (Brandtzaeg, 2007; Corthesy, 2007).
Saliva as a diagnostic fluid

Saliva analysis is presently used in the diagnosis of hereditary disorders, autoimmune disease, endocrine, infectious and malignant disease as well as for monitoring drug abuse. Saliva collection is non-invasive, pain free, simple, cost-effective for testing large populations and samples can be obtained by individuals without any specialized training. One of the disadvantages in saliva testing is the lack of standardization of the methods for saliva collection (Kaufman & Lamster, 2002; Lakshman, 2007; Mahvash & Satish, 2008).

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 minor salivary glands produce only about 10 percent of the fluid. The daily production of saliva ranges from 0.5 to 1.5 litres (Bokor-Bratic, 2000; Mahvash & Satish, 2008; Teeuw, Bosch, Enno, & Nieuw Amerongen, 2004).

The collection of gland specific saliva is possible however, most routine studies collect whole saliva which 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 are recommended to avoid contamination, however, absorbent collection pads have been known to absorb target molecules leading to false results (Groeschl, 2008; Michishige, 2006). For the calculation of saliva flow rates samples should be timed and weighed (Albers et al., 2005; Brandtzaeg, 2007; Kaufman & Lamster, 2002).

Reported long term storage temperatures for saliva samples range from minus 20 degree to minus 80 degree Celsius (Akimoto et al., 2003; McKune et al., 2005; Michishige, 2006; Pawlow & Jones, 2005) and it is advised to limit storage prior to freezing to a few hours at 4 degree Celsius (Salimetrics, 2009b). While it is unclear how differing storage temperatures may affect salivary S-IgA there is evidence that the length of storage time alters the S-IgA content in saliva.
indicating that S-IgA concentration remains stable for up to 3 months at minus 30 degree Celsius and that increasing storage time then decreases the S-IgA concentration and the variability of the samples (Ng, Koh, Fu, & Chia, 2003).
S- IgA in saliva and its relation to health

While S-IgA concentrations cannot be relied upon as an indicator of optimum immune system function, a number of lines of evidence support the view that there is an association between S-IgA levels and health. Elevated salivary S-IgA levels observed in smokers have been interpreted as a reflection of the protection of the oral mucosa (Norhagen & Engstrom, 1998). Changes in salivary S-IgA have been linked to upper respirator tract infections, respiratory disease, dental caries and salivary S-IgA appears to play a role in the protection against HIV infection. For instance, several exercise related studies associate low levels of S-IgA with an increased incidence of upper respiratory tract infections (URTIs) in elite athletes. While strenuous high intensity exercise is often associated with lower salivary S-IgA and subsequent URTIs (Akimoto et al., 2003; Gleeson, Hall, McDonald, Flanagan, & Clancy, 1999; McKune et al., 2005; Tharp & Barnes, 1990) it was found that moderate, regular exercise can increase salivary S-IgA levels in elderly subjects that have a higher risk of URTI based on their usually lowered salivary S-IgA levels (Akimoto et al., 2003). However, Akimoto (2003) and McKune (2005) did not use a control group.

A study investigating immune markers in frequently ill children found that 94% of the 270 participants had low levels of S-IgA (Markova & Chuvirov, 2007). Frequently ill children (FIC) were characterized as suffering acute respiratory diseases once every month with combined pathologies of the upper airways. The participants in this study were children aged 2-15 years and subsequently the results have to be evaluated under consideration of transient deviations and age related peculiarities of young immune systems (Roit et al., 2001).

In an attempt to investigate the effect of increased S-IgA on upper respiratory tract conditions Kostinov et al. (2006) evaluated the influence of bacterial lysate on the mucosal immunity of HIV infected children. Lysate, a topical immuno-modulator; increased the synthesis of salivary S-IgA and this increase correlated with decreased inflammatory changes in the nasopharyngeal mucosa of the children (Kostinov et al., 2006).

In vitro and in vivo research indicates that S-IgA can inhibit the adherence of bacteria to oral tissues and thereby protect against the development of gingivitis and caries (Teeuw et al., 2004). However, research investigating the relationship between S-IgA and oral health has produced conflicting data and inconsistencies are believed to relate to the fact that some species of oral
bacteria can render S-IgA ineffective (Lamm, 1997). At present it has not been firmly established what role salivary IgA has in the prevention of oral disease (Brandtzaeg, 2007; Teeuw et al., 2004).

In recent years, a specific mucosal immune response against human immunodeficiency virus type 1 (HIV-1) has been evaluated. An HIV-1 epitope recognized by IgA has been identified in HIV seronegative individuals that remained uninfected despite years of exposure to HIV infected partners. Salivary IgA from HIV-1-exposed, persistently seronegative (HEPS) subjects has been shown to neutralize HIV-1 and to block the transport of HIV1 across the epithelial barrier emphasizing the importance of mucosal immunity in the prevention of HIV infection (Bolscher et al., 2002; Devito, 2002; Teeuw et al., 2004).

Secretory S-IgA is also believed to play a, still poorly understood, role in the maintenance of and in the return to local homeostasis and in the modulation and education of the mucosal immunity of the intestines and the airway mucosa (Pilette et al., 2004; Teeuw et al., 2004).
Mechanisms of the protective function of S-IgA in saliva

S-IgA in saliva neutralizes enzymes, toxins and viruses and inhibits the adherence of bacteria to oral surfaces to prevent bacterial penetration of the mucosa. S-IgA neutralizes viruses by inhibiting the viral stages of fusion, attachment, internalization and replication. During its epithelial transport (transcytosis) S-IgA has the ability to bind viral proteins that are present in epithelial cells. This intracellular virus neutralizing ability is believed to be unique to S-IgA. However, the anti-bacterial and anti-viral effects of S-IgA in saliva are mainly derived from in vitro studies (Teeuw et al., 2004). Several bacterial strains have developed strategies to render S-IgA ineffective and this fact may be responsible for some inconsistency amongst studies that investigated the antibacterial action of S-IgA (Michalek & Childers, 1990).

In addition S-IgA fulfills its protective role by interacting with other antibacterial factors in saliva such as lysozyme, salivary peroxidase, cystatins, histatins and agglutinin. In the presence of IgA, possibly due to interactions of its Fc portion, the anti-streptococcal activity of lactoperoxidase is increased. S-IgA also intensifies the ability of lactoferrin to deprive microorganisms of iron and works in synergy with mucins to enhance the clearance of bacteria (Teeuw et al., 2004).

Induction and neuro-endocrine regulation of S IgA in saliva

The response of S-IgA to oral antigen is believed to be generated by two processes. The first process, the local response, is the proliferation and differentiation of lymphoid cells into S-IgA producing B-cells in the submandibular, sublingual and parotid salivary glands following the stimulation by oral antigen. The second process, referred to as the common mucosal system, involves the movement of antigen activated B lymphocytes from MALT via the lymphatic system to the salivary glands where, upon entering the glandular tissue, the B lymphocytes mature under the influence of local T cells into S-IgA 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 (HPA) where the adrenal medulla,
acted upon by sympathetic neurons, presents as a specialized part of the ANS. Activation of these pathways results in elevated levels of cortisol and catecholamines including epinephrine and nor-epinephrine. Immune cells have receptors for these hormones (Bennet-Herbert & Cohen, 1993). SNS related changes in immune response have been observed in the absence of altered cortisol levels suggesting that the SNS plays a greater role in immune alteration than the HPA axis. However, research suggests that glucocorticoids, and the female sex hormones estradiol and progesterone may increase gene expression of pIgR, the secretory component of S-IgA, therefore allowing for increased transport of S-IgA through the epithelial cells into saliva (Teeuw et al., 2004).

The salivary glands are largely under control of the autonomic nervous system (Teeuw et al., 2004). Autonomic stimulation also regulates distant immune cells, located in lymphoid tissues such as MALT, influencing antigen presentation, cell migration and production of antibodies (Bellinger, Lorton, Lubhahn, & Felten, 2001) and may also affect migration of IgA secreting B cells by vasoconstriction of blood vessels (Mackinnon, 1999). The increased S-IgA production that occurs during moderate exercise, which is a sympathetic stimulus, cannot be dampened by blocking alpha or beta adrenergic receptors (Ring et al., 2000; Winzer et al., 1999) and increased saliary IgA was noted in rats, following sympathetic (SNS) and parasympathetic nervous system (PSNS) stimulation of their salivary glands (Proctor & Carpenter, 2002). These findings underline that the sympathetic and parasympathetic branches of the autonomic nervous system do not work in an antagonistic manner and neural control of S-IgA synthesis, secretion and saliva production, although not fully understood, is synergistically influenced by both branches of the autonomic nervous system (Guyton & Hall, 2000; Teeuw et al., 2004).

It is still not clear to what extent increased salivary S-IgA, following ANS stimulation, can be contributed to either an increased translocation of S-IgA across the epithelium of salivary glands or to an up regulated release by B lymphocytes (Bosch, Ring, De Geus, Veerman, & Amerongen, 2002).
Measurement of secretory IgA in saliva

S-IgA in saliva is most commonly measured by immunoassays that employ linking molecules labeled with reagents to detect antibodies or antigen. Immunoassays are economical in the use of reagents and very sensitive to the detection of antibodies (Roit et al., 2001). The enzyme linked immunosorbent assay (ELISA) utilizes an enzyme-linked antibody, bound to a solid support, such as a microtitre plate, that recognizes and binds to the immunoglobulin of interest. Following periods of incubation and rinsing to remove unbound immunoglobulin, a substrate that activates the enzyme is added. Bound antibody is quantified by the color change of the activated enzyme (Mackinnon, 1999).

Salivary IgA is reported either as concentration in microgram per milliliter (μg/ml) or as secretion rate in microgram per minute (μg/min). Normal salivary S-IgA concentration in healthy adults ranges from 79.26 μg/ml to 679.50 μg/ml (Pawlow & Jones, 2005; Salimetrics, 2009a). The salivary S-IgA secretion rate, reported in (μg/min), is suggestive of total S-IgA produced each minute and gives an indication of the availability of S-IgA on the mucosal surfaces (Mackinnon, 1999). The secretion rate is calculated by multiplying salivary S-IgA concentration by salivary flow rate (ml/min). The saliva flow rate is determined by dividing the amount of saliva sample by the time required to collect the sample (min) (Akimoto et al., 2003; McKune et al., 2005). In general it is recommended that salivary flow is not stimulated and that both, the absolute concentration and the secretion rate of S-IgA in saliva is reported (Akimoto et al., 2003; Brandtzaeg, 2007; Groer et al., 1994; Groeschl, 2008; Kaufman & Lamster, 2002; Salimetrics, 2009a).
Influences on secretory S-IgA levels in saliva

Evidence exists that salivary S-IgA can be influenced by everyday activities including diet, exercise and stress levels (Albers et al., 2005). Several other variables may influence the level of antibodies in oral secretions such as sex, age, oral health, circadian influences, the impact of flow rate and protein loss during sample handling. In an ideal study all these factors should be strictly controlled. In practice not all factors can be controlled at the same time. Additionally, IgA deficiency and common variable immunodeficiency are conditions that may influence salivary S-IgA (Albers et al., 2005; Brandtzaeg, 2007):

IgA deficiency

IgA deficiency is a relatively common finding and is usually not of immediate concern (Leman, 2010). It is still a mystery why deficiency of the most copious molecule produced by a normal immune system is usually of such little consequence. However, IgA deficiency is associated with a greater risk of recurrent infections, food sensitization, neoplastic changes and autoimmune disorders (Barka et al., 1995; Koskinen, 1996; Pilette et al., 2004) and adverse reactions to blood transfusions (Eckrich, Mallory, & Sandler, 1993; Latiff & Kerr, 2007; Marwaha, 2006).

IgA deficiency occurs in a primary or a secondary (acquired) form. Primary IgA deficiency with a prevalence of 1/500 to 1/2000 affects mucosal and serum IgA and presents as serum IgA level below 0.06g/L with normal serum IgG and IgM values (Latiff & Kerr, 2007). Discussion exists around the pathogenesis of primary IgA deficiency being either a failure of IgA class switch in B cells (Asano, Kaneko, & Terada, 2004; Brandtzaeg et al., 1999) or an inability of lymphocytes to differentiate into IgA secreting plasma cells (Cunningham-Rundles, 2001). Acquired, or secondary IgA deficiency, can be caused by the effects of drugs or infectious diseases (Latiff & Kerr, 2007) or by bone marrow transplants and blood transfusions from a deficient donor (Chandran, Khetan, Chaudhary, Misra, & Aggarwal, 2006; Marwaha, 2006) and while there have been spontaneous remissions it is considered to be a permanent condition (Latiff & Kerr, 2007).

The reasons why IgA deficient individuals can remain asymptomatic are still not clear. However, the diagnosis of IgA deficiency is based on IgA concentration in serum and not in secretions.
Subsequently it is possible that individuals diagnosed with IgA deficiency may have enough secretory IgA in their mucosal system to offer some protective function (Leman, 2010). Additionally immune compensatory mechanisms may be present as healthy subjects with primary IgA deficiency show an increase in salivary secretory IgM and have a greater susceptibility for infections if their secretory IgM levels are low (Natvig, Johansen, Nordeng, Haraldsen, & Brandtzaeg, 1997). Yet, there is some doubt that secretory IgM grants the same protection then secretory IgA. In IgA deficient subjects the mucosal IgM defense against viruses appears to be less efficient than the IgA response in normal subjects and, following oral vaccination, IgA deficient blood donors were found to contain the poliovirus longer than normal subjects (Cunningham-Rundles, 2001).

*Disorders associated with IgA deficiency*

Regardless of the fact that most IgA deficient individuals are without symptoms, this immunodeficiency has a connection with certain diseases. Salivary S-IgA deficiency is diagnosed - with increasing frequency - in patients that suffer from allergies, recurrent upper respiratory tract infections, gastrointestinal diseases, malignancies and autoimmune diseases (Barka et al., 1995; Sarmiento et al., 2005). The autoimmune disorders most commonly associated with S-IgA deficiency include: systemic lupus erythematosus, rheumatoid arthritis, dermatomyositis, thyroiditis, celiac disease, Addison’s disease, inflammatory bowel disease, Sjoergren’s syndrome, cerebral vasculitis, idiopathic thrombocytopenic purpura, and pernicious anemia (Barka et al., 1995; Cunningham-Rundles, 2001; Leman, 2010).
Common variable immunodeficiency

Common variable immunodeficiency (CVID) is a hereditary defect, affecting either the production of antibody generating plasma cells or the immunoglobulin class switch from IgM to IgG (Blanco-Quiros, Solis-Sanchez, Garrote-Adrados, & Arranz-Sanz, 2006). CVID is related to primary IgA deficiency and both conditions can appear in the same family or evolve from one to the other in the same patient, therefore making differential diagnosis difficult (Espanol, Catala, Hernandez, Caragol, & Bertran, 1996; Vorechovsky et al., 1995). CVID is usually present at birth and clinical symptoms can manifest at any age but often only appear in adults. The symptoms vary greatly in their severity. Initial bacterial respiratory infections are often years later complicated by lymphoid hyperplasia, chronic lung disease and a large range of autoimmune processes (Sneller, 2001).

Treatment of immunodeficiency

For individuals diagnosed with common variable immunodeficiency at present the treatment of choice is Immunoglobulin G (Latiff & Kerr, 2007) therapy. For symptomatic IgA deficient patients the treatment is based on managing the associated diseases. Antibiotics are prescribed to control acute infections. Prophylactic antibiotics may be advised in cases of recurrent respiratory infections. Standard treatment is given for associated allergic or autoimmune disorders. In regard to the potential of anaphylactic reaction to blood transfusion it is recommended that the patient wears a medical alert bracelet. Education and awareness of the effects of hygiene, diet and lifestyle is also of importance (Blanco-Quiros et al., 2006; Latiff & Kerr, 2007; Leman, 2010).
Complementary Therapies and their effect on the immune system

Numerous studies on alternative therapies including spinal manipulation (Brennan et al., 1991), self-hypnosis (Naito et al., 2003), relaxation (Hewson-Bower & Drummond, 1996) and massage have reported increases in immune related cells, among them salivary S-IgA, following the respective intervention. While it goes beyond the scope of this work to discuss all related research we recognize a need to focus on massage therapy as it represents another touch based therapeutic modality and appears to provide a number of clinical outcome studies with symptomatic subjects. Ironson et al.(1996), in a within subject single cohort study on 29 HIV positive participants, associated massage therapy with enhanced cytotoxic capacity of the immune system recorded as an increase in numbers of natural killer (NK) cells. Furthermore, a later randomized controlled study by Diego, Hernandez-Reif, Friedman and Ironson (2001) on 24 HIV positive adolescents, receiving either massage or relaxation therapy, found that the massage group not only displayed higher numbers of NK cells than the relaxation group but also had increased CD4 numbers. CD4, a surface molecule on T cells plays a role in the resistance to viruses and the progression of AIDS is reflected by a reduction of CD4 (Roit et al., 2001). Similar findings are reported by Hernandez-Reif et al. (2005) in a study on woman diagnosed with breast cancer that received either massage therapy, standard cancer therapy or muscle relaxation technique. The massage group showed the greatest increases in NK cells and lymphocytes as compared to the other two groups suggesting a superiority of touch based modalities. The findings of these studies are strengthened by Arroyo-Morales, Olea, Ruiz, & De Dios Luna del Castillo (2009) who noted in their randomized, placebo controlled, single blinded study on 60 healthy subjects that massage therapy decreased the salivary cortisol and increased salivary IgA and total protein concentrations in the treatment group as compared to the sham group, after maximum effort exercise. They concluded that massage therapy may reduce the negative metabolic and immunologic effects of strenuous exercise.

Conversely, a study by Eliska & Eliskova (1995) implies that massage therapy damages the peripheral lymphatic vessels. Massage therapy on men and dogs either with or without lymphedema (a pathological condition where fluid accumulates in the body’s tissues resulting in swelling) resulted, after only 3-5 minutes, in the destruction of lymphatics. The destructive effects were visible in the endothelial lining, the collagen and elastic fibers and also presented as
an accumulation of fibrous material, tissue debris and red blood cells in the walls of the lymphatic vessels. The limitations of these and other related studies are similar and include either small numbers of participants, lack of control group or difficulty in standardizing techniques, especially the pressure of strokes during massage therapy.

**Specifically immune related osteopathic techniques**

Osteopathic treatment addressing the lymphatic system is broadly divided into two categories: those techniques that aim to remove impediments to lymphatic flow and those that promote and enhance the movement of lymph fluid (Ward, 2003). Treatments that aim primarily to reduce blockages to lymphatic flow include Fascia Release treatment and Petrissage whilst treatments that are primarily aimed at improving lymphatic flow consist of Rib Raising, Diaphragm Doming and Lymphatic Pump techniques (Kuchera & Kuchera, 1993; Leman, 2010; Stone, 1999; Ward, 2003). These techniques and their actions are outlined in Appendix D.

Several other techniques, some based on mechanisms that are still disputed or not yet fully understood, claim to influence the immune system. These techniques include Chapman reflex treatment, Craniosacral Therapy and the Galbraith method (Chikly, 2005; Ward, 2003)
The lymphatic pump technique (LPT)

Lymphatic pump treatment consists of a rhythmical pumping action applied to various immune related areas of the body to enhance fluid drainage and flow of lymphatic fluid. Osteopaths utilize LPT to treat infection and edema. Several LPT’s exist including the thoracic pump, abdominal pump, spleenic pump, liver pump and pedal pump (McMillan et al., 2004) (Appendix D). The thoracic LPT utilizes a posterior and caudad force direction to elicit a gliding motion of the ribs around the costo-transverse joints, thereby rhythmically altering the diameter of the ribcage (Sleszynski & Kelso, 1993).

While the processes that enable LPT to enhance lymphatic circulation and protect against infection are not fully understood it is thought that the action of LPT mimics the respiratory effect on lymphatic ducts and nodes in that it changes the positive and negative pressure relationships between abdominal and thoracic cavity thereby producing a suction like effect that encourages flow of lymphatic and venous fluids into the thorax (Galewaler, 1969; McMillan et al., 2004). From here the two main lymph channels - the right and left thoracic duct - return the lymph fluid to the venous circulation (Guyton & Hall, 2000; McMillan et al., 2004).
LPT treatment and its effect on the immune system

Research investigating and validating the immune enhancing effect of LPT is limited. Existing studies focus on three lines of investigation: The effect of LPT on the immune response, on lymphatic flow and on pathological conditions.

Research on the effect of LPT on the immune response includes a small number of randomized controlled studies investigating the effect of LPT on antibody response to vaccination. Breithaupt et al. (2001), in a study on young and elderly populations, receiving daily post influenza vaccination LPT treatment over 4 days noted no significant changes between treatment (n=54) and control group (n=45). However, the authors provided evidence of improved general immunity and clinical outcomes, leading to the conclusion that LPT in conjunction with influenza vaccination may be of value in non-ambulatory patients or in at risk individuals that have had actual exposure to infection.

Jackson et al. (1998) in a study on the effect of LPT on the antibody response to hepatitis B vaccine noted on average higher hepatitis B antibody titers in the treatment group (n=17-19) as compared to the control group (n=17-19) but reported the results of his study as inconclusive because of the small sample size and a wide variation in the antibody response. The outcomes of the Breithaupt (2001) and Jackson (1998) studies have to be considered in the light of unsimilar study populations and differing vaccination and treatment protocols however, they indicate a possible benefit of LPT in conjunction with vaccination.

An older study by Measel (1982) examined LPT 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 peripheral blood. By day 14 the treatment group (n=13), having received twice daily thoracic LPT, had a statistically greater humoral B cell immune response then the control group (n=12). The above study was repeated by the same author without the vaccination protocol in a randomly controlled double blinded manner on 21 medical students and the previous findings were confirmed (J. W. Measel & Kafity, 1986). The serological tests available at the time of these studies raise some doubt as to the reliability of the results and will hinder comparison with future studies on the same topic.
Research of poorer quality, including studies on animals, has looked at the response of immune mediator cells to LPT. Paul, Stomel, Broniak & Williams (1986) in an uncontrolled study on 12 healthy subjects found no effect of LPT on interferon, a class of proteins that protect against viral destruction, whilst Mesina (1998) in a small controlled pilot study noted an increase in the percentage of basophils, white blood cells involved in inflammatory processes, following LPT on 7 healthy male subjects. Numerous factors must be considered in the evaluation of those studies, including differing LPT protocols, small sample size, poor control and the difference in technology employed for performing the cell counts.

Recent research on dogs demonstrated that LPT increases immune related cells in the lymphatic fluid of thoracic and intestinal lymph ducts. The study on 6 dogs by Hodge et al. (2010) found that LPT not only mobilized leukocytes (white blood cells), including macrophages, neutrophils, lymphocytes, T-cells and B-cells from the mesenteric lymph nodes (MLN) into lymph of the mesenteric and thoracic ducts but also increased lymphatic flow rate from 0.5ml/min to 2.7 ml/min. Similar findings are reported by Huff, Schander, King, Downey & Hodge (2010) in a study on twelve dogs that investigated the effect of LPT on mobilization of inflammatory mediators into thoracic and intestinal duct lymph. Comparing baselines, during treatment and post treatment lymph samples and their concentration of various cells that are involved in the inflammatory process showed that the greatest increase had occurred in the concentration of interleukins (IL), especially IL-4, IL-6, IL 8, IL 10, IL 15. These proteins control aspects of the immune response including antibody synthesis. While the results of these studies may provide scientific rational for further research of LPT treatment they have to be interpreted cautiously. Animal research results are not always transferable to humans (Suvorov & Takser, 2008) and, in order to execute LPT on catheterized animals, the technique has to be modified and does not conform with LPT as performed on a human patient in a clinical situation (Knott et al., 2005). Research that evaluates the effect of LPT on lymph flow has been conducted solely on animals. Knott et al. (2005) demonstrated that thoracic and abdominal pump treatment increased lymph flow through the thoracic ducts of five dogs. Similar increases were also reported in the above mentioned study by Hodge (2010) in which flow rate in dogs increased from 0.5 to 2.7 ml/min.
Interestingly, in the study by Hodge (2010), the effects of the lymphatic pump treatment on lymph flow occurred without changes to mean arterial pressure, heart rate or cardiac output supporting the notion that LPT treatment has a primary influence on the lymphatic system.

The effect of LPT on pathological conditions has not been researched extensively. While historic information from the 1918 Spanish influenza pandemic suggests a high success rate of osteopathic treatment (consisting primarily of LPT) reporting a mortality rate of 0.25% for a total of 110120 influenza patients under the care of osteopathic doctors (Smith, 1920) it has to be considered that the data of these uncontrolled studies with their unknown populations was collected retrospectively (Hruby & Hoffman, 2007).

In a more recent study Sleszynski & Kelso (1993) compared thoracic LPT with incentive spirometry in the prevention of postoperative atelectasis, a collapse of the lungs and the most common cause of abdominal postoperative morbidity and mortality (Susini, Sisillo, & Bortone, 1992). Incentive spirometry (IS) is a sustained maximal inspiratory maneuver commonly taught to patients to prevent atelectasis (Ros, Vincent, & Kahn, 1981). In this 1 year randomized researcher blinded trial, cholecystectomy (gall bladder removal) patients received either thoracic LPT (n=21) or IS (n=21) and Atelectasis occurred in 2 (5%) patients of each treatment group, possibly demonstrating that either treatment was equally effective. However, patients treated with thoracic LPT had an earlier recovery from surgery and showed a quicker return to pre-operative values for lung capacity. The main limitation of this study is the lack of a placebo group due to ethical reasons. Other factors that limit the conclusions that can be drawn from this study are the small sample size, the restriction to only one institution and the low atelectasis risk status of the participants.

In the osteopathic profession metastatic cancer is usually regarded as contraindication for certain osteopathic manipulative techniques including LPT. However, a recent controlled study conducted on rats demonstrated that LPT can reduce solid tumors in the lung tissue of rats. Pedrueza, Zhang, Jones, & Hodge (2010) subcutaneously injected laboratory rats with tumors that metastasize to the lungs. Following the injection the rats received four minutes LPT daily for seven days. A control group received no treatment while a sham group was treated with 4 minutes of light touch. The results demonstrated a 30% reduction in pulmonary tumors in the
LPT group compared to sham and control group and, contrary to common believe, there was no distribution of tumor cells from the lungs into other tissues following LPT.
While existing research on LPT appears to suggest that LPT can influence certain aspects of immunity, the transient nature of the benefits of LPT has been noted in several studies, leading to the necessity of further investigation in regards to the decay of immune markers and as to considerations and adjustments in the application protocol of LPT (Hodge et al., 2010; Huff et al., 2010; Knott et al., 2005)
Methodology

Research and its documentation are crucial factors in the growth and acceptance of any profession and the need for experimental research is well recognized within the osteopathic field (Moran, 2005).

While group designs, such as randomized controlled trials, are regarded as the gold standard of experimental research within evidenced based practice, they are often inappropriate for preliminary investigations and the logistics of this type of research requires resources that are not easily available within the osteopathic profession. Group designs are limited in their suitability for certain types of conditions and have been criticized for lacking transferability to individuals by representing only an average of the response to any given intervention (Sanders, 2003).

Single systems research design (SSRD) provides a simple, economical way to evaluate treatment intervention and its effect on individual patients. In single systems research multiple measurements are recorded allowing for a more detailed assessment of the reaction to the treatment. This is particularly suited to osteopathy in its attempt to adept treatment to individual presentation. Single systems research also plays a vital role in building up a body of acceptance and knowledge around a certain intervention to justify further research utilizing more recognized and involved designs (Joshi, 2000).

Immunological research generally requires large resources making single systems design a cost-effective solution particularly for the investigation of emerging or little researched topics.
Research question

Osteopathic practitioners commonly utilize LPT in their clinical practice to improve the functioning of their patients’ immune system although there is limited research to support their claim. Salivary IgA is an immune marker that plays a role in the maintenance of mucosal immunity and it is believed that decreased salivary S-IgA is responsible for the susceptibility to URTI’s. Secretory IgA in saliva is one of the more easily measured outputs of the immune system rendering its investigation suitable for a student project with limited funds. These considerations lead to the following research question: Does the osteopathic lymphatic pump technique (LPT) influence the secretory IgA levels in saliva of healthy subjects?

Conclusion

LPT is a widely used osteopathic technique that is often part of treatment sessions and is therefore financially supported by patients and by the government. It seems imperative to build up the body of knowledge around this technique. Salivary IgA, a major output marker of the immune system, is considered as a promising and worthwhile biological marker. Although the implications of salivary IgA for health and immunity are not yet fully established and understood the importance of salivary S-IgA for the health of the upper respiratory tract in athletes and in the elderly is repeatedly reported in the literature. At present no studies have investigated the influence of LPT on salivary S-IgA. It appears that further research into this topic is warranted and may provide a simple and cost effective means for the prevention and the treatment of infection in humans.

To the author’s knowledge, at the time of submission, the research presented in this thesis may be the first and only single systems design study that investigates immunological markers in response to osteopathic intervention.
References


ABSTRACT

**Background:** The osteopathic lymphatic pump technique (LPT), a treatment that has not been researched extensively, is widely used within the osteopathic profession to improve health in patients. Secretory immunoglobulin A (S-IgA) in saliva is related to mucosal immune system function and high levels of salivary S-IgA have been shown to decrease the incidence of upper respiratory tract infection (URTI). The aim of this pilot study was to determine changes in salivary S-IgA in response to LPT.

**Design:** A single systems research design using a modified A-B-C protocol on eight healthy male participants was used to evaluate the outcome measure defined as change in salivary S-IgA secretion rate (μg/ml) as determined by Enzyme Linked Immuno-Sorbant Assay (ELISA).

**Methods:** Baseline measures of salivary S-IgA were recorded once daily over 5 days. On Day 5 a seven minute thoracic LPT treatment was administered immediately following the pre-treatment measurement. Two post-intervention measurements, at 1 minute post-treatment and at 10 minute post treatment were reported.

**Results:** Visual analysis of the plotted outcome measures showed a short term increase in salivary S-IgA secretion rates following LPT in seven out of eight healthy male subjects. The averaged post-treatment measurements of salivary S-IgA secretion rates were higher when compared to the mean baseline (ES=3.0; p=0.03). Limited data points, lack of control and high variability of data weaken the study and make it difficult to conclude confidently that the intervention caused the results.

**Conclusion:** The results of this study suggest that thoracic LPT may influence the salivary S-IgA levels in healthy males. Further research in this area seems to be warranted and may include a more robust research design with a larger sample size and inclusion of participants that suffer from mucosal immune compromise.

**Key Words:** Immune system, Osteopathic treatment
INTRODUCTION

There is a growing interest in finding and developing techniques that could boost the immune system. Besides the possible therapeutic value in situations of compromised immunity, such studies might further our understanding of the complex interactions between the function of immune parameters and physical therapy.

One such technique, the osteopathic lymphatic pump technique (LPT) is widely used by osteopathic practitioners and is reputed to have a beneficial effect on the immune system by relieving lymphatic stasis and by enhancing the immune response.\textsuperscript{1, 2}

The clinical efficacy of LPT has been reported since the influenza pandemic in 1918. LPT was used to treat victims of the flu epidemic and the mortality amongst those treated fell to 0.25 percent as compared to a 5 percent mortality rate in patients that did not receive the LPT treatment.\textsuperscript{3} More recent research found that LPT may prevent the occurrence of atelectasis following cholecystectomy\textsuperscript{4} and there is evidence that LPT increases immune related cells such as basophils\textsuperscript{5} and B-and-T-cells\textsuperscript{6} in peripheral blood. Other lines of research investigated the effect of LPT on antibody production following antigen exposure or vaccination. Measle\textsuperscript{7} discovered that antibody production in response to introduced antigen such as pneumococcal poly-saccharide was increased in healthy males that received LPT however, some controversy exists in the effect of LPT on antibody production following vaccination. Jackson\textsuperscript{8} reported an enhanced immunological response to Hepatitis B vaccine in elderly individuals while Breithaupt\textsuperscript{1} found no changes in antibody synthesis in response to influenza vaccination in elderly subjects.

The latest evidence from animal studies links LPT to the mobilization of inflammatory mediator cells into the lymphatic ducts,\textsuperscript{9, 10} to increased flow of lymphatic fluid within the thoracic duct\textsuperscript{9, 11} and to a reduction in the size of pulmonary tumors in rats.\textsuperscript{12}

While a number of studies have looked at the effect of LPT on various outputs of the immune system, to date no work has specifically investigated the influence of LPT on the mucosal immune response despite it being the first line of defense of the immune system.\textsuperscript{13, 14} Secretory Immunoglobulin A (S-IgA) in saliva 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.\textsuperscript{15, 16} Low levels of salivary IgA have been reported in connection with dental caries\textsuperscript{15} and may present a risk of an increased susceptibility to upper respiratory tract infections in athletes\textsuperscript{16} and in the elderly.\textsuperscript{17} There appears to be a call to increase the knowledge
base around LPT and its effect on immune markers to support the osteopathic profession in the
development and improvement of suitable treatment for patients with compromised immune
health. 18
Any investigation of the immune response to manual intervention involves extensive methods
requiring monetary resources making it necessary to initiate preliminary trials prior to investing
large amounts into group studies. The aim of this pilot, single systems study was to investigate if
the osteopathic lymphatic pump treatment has any influence on the secretory immunoglobulin A
(S-IgA) in the saliva of healthy male individuals.
METHODS

Design

This pilot study followed a modified A-B-C single systems research design to examine the effect of LPT on the S-IgA levels in the saliva of eight healthy male subjects. Phase A constituted of a five day baseline period with one daily saliva sample collection. During this time no treatment was given. Phase B was a seven minute LPT intervention administered on Day Five immediately after the last baseline saliva sample collection. The duration of the LPT treatment was based on previous studies and on the most common LPT treatment length in a clinical setting.

Phase C consisted of two post intervention saliva samples, one sample immediately after the intervention and one 10 minutes post treatment. The short post-intervention intervals were chosen to minimize the effect of external influences on salivary IgA. Due to pragmatic considerations the post intervention data points had to be restricted to two measurements only. The study was conducted in a room on the premises of Unitec Auckland, Carrington Campus. The research protocol was approved by the Unitec Ethics Committee, Unitec, Mt Albert, New Zealand (Appendix A).

Eligibility Criteria

Subjects were recruited by poster advertisement at the Unitec Campus site. To be eligible for inclusion in the study the participants had to be healthy, male aged between 18 and 40 years and non-smokers.

Male subjects were recruited to avoid hormonal variations of S-IgA during the female menstrual cycle. The lower age limit was determined to prevent legal and ethical under age issues with minors and the upper age limit was established to decrease the likelihood of age related influences on the immune response. The inclusion criteria of non-smoking was specified to limit the influence that smoking has on S-IgA concentrations.

Potential participants were not eligible to partake in the study if they presented with any of the following:

- present oral infections
- a history of cancer
• any autoimmune conditions including recent organ transplants
• intake of medication that affects the immune system such as antibiotics and steroids
• involvement in drug or alcohol abuse
• thorax conditions that are contraindicated for LPT including rib fractures, pacemaker, recent thoracic surgery
• an inability to understand and write the English language

To screen for inclusion and exclusion criteria prospective participants were asked to complete a short questionnaire (Appendix B).

**Procedures**

Data were collected over a 5 day period from 14th – 18th of September 2009. The study protocol was explained to all eligible participants by the study organizer. Each participant received a written information sheet and had the opportunity to ask questions before verbal and written informed consent was obtained (Appendix A).

Participants were asked to refrain from eating, drinking alcohol and exercising, all of which can influence salivary S-IgA levels,\(^ {19,21,26,27}\) one hour prior to each saliva sample collection.

Baseline saliva samples were collected on 5 consecutive days. To minimize circadian influences on S-IgA concentration sample collection took place at the same time each day\(^ {28,29}\). Weight and height of each participant was recorded on day one.

On arrival each participant rinsed their mouth with water and was asked to complete a short questionnaire to determine the presence of factors that may have influenced S-IgA production in the 2 hours prior to saliva sampling (Appendix B).

The saliva collection via passive drooling of non-stimulated whole saliva into pre-weighed 2 ml snap-lock tubes was timed and the collection time was recorded by each participant on a prepared sheet (Appendix C). Each sample was collected in duplicate, labeled and recorded (Appendix C). The filled tubes were weighed and immediately frozen at the recommended storage temperature of minus 20 degree Celsius.\(^ {22}\) On Day Five the final baseline saliva sample, which also acted as the pre-intervention sample, was followed immediately by a 7 minute thoracic LPT treatment administered by a qualified osteopath. Thoracic pump was chosen over other pump techniques as it is applied in close proximity to the salivary glands and has been the most commonly used lymphatic pump technique in previous studies\(^ {1,6}\). The participant was
guided into an adjacent room and asked to recline on a treatment table. To perform the LPT the practitioner’s hands were placed on the clothed torso, bilaterally below the clavicles, with fingers pointing towards the subject’s feet and fanning out across the anterior and lateral aspects of the chest. Rhythmic pumping was applied at a rate of 50 - 60 pumps per minute for duration of seven minutes (Figure 1). Directly after the LPT intervention each participant provided the first post-treatment saliva sample following the saliva sampling routine as outlined above. The participant was then directed to sit quietly while reading a selected textbook chapter. A second post treatment sample was collected following 10 minutes of quiet sitting and reading.

**Figure 2** Thoracic Pump (Jackson et al. 1998)

**Assays**

Salivary S-IgA competitive assay kits were purchased from Salimetrics (Salivary Secretory IgA Indirect Enzyme Immunoassay Kit, Catalog no.1-1602; 96-Well Kit.). Salivary S-IgA concentration (μg/ml) was determined with the ELISA technique which uses a horse radish peroxidase conjugated to goat anti-human S-IgA as the detecting antibody. Laboratory analysis took place at the Unitec Faculty of Social Health Science laboratory. Laboratory conditions and procedures adhered to the recommendations provided with the test kits (www.salimetrics.com). 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
All samples were analyzed in duplicate (Appendix E). Assay analysis and generation of a standard curve ($R^2 = 0.999$) was performed with KC junior software (Bio-Tek Instruments Incorporated, 2002).

**Data Management and Analysis**

Raw data were compiled, checked for errors and manipulated using Microsoft Excel version 2003. Statistical analysis of group data was performed with Statistical Package for the Social Sciences version 15 (SPSS Inc, Chicago, Illinois). Mean and standard deviation for the duplicates of salivary S-IgA concentration was calculated (Appendix E).

Duplicates for Salivary S-IgA concentration were averaged and presented as absolute concentration of salivary S-IgA(μg/ml) and as salivary S-IgA secretion rate (μg/min). The secretion rate reflects the total amount of S-IgA arising on the mucosal surface per time unit and is calculated by multiplying the absolute S-IgA concentration by the saliva flow rate (ml/min). The saliva flow rate is obtained by dividing the amount of saliva in each sample (ml) by the time it took to produce the sample (min)$^{2217}$ (Appendix E).

Data analysis for single systems research design (SSRD) is traditionally based on visual analysis of graphed individual data to depict whether the level or trend in the plotted data during the intervention phase shows a visually detectable difference from the level or trend of the baseline data.$^{19, 23}$ For individual data analysis graphs were constructed displaying S-IgA concentration, flow rates and secretion rates at baseline and following the treatment intervention. Evaluation is by visual inspection to identify trends in the baseline and post intervention phase and to assess changes in level of magnitude between phases.

Group means were compared using analysis of variance (ANOVA) for multiple time points and a paired t-test to compare pre-and post-treatment measures.

To evaluate the clinical significance group and individual effect sizes were calculated and interpreted based on Cohen's “d” formula.$^{24}$ The baseline standard deviation acted as the denominator as is recommended for studies with differing standard deviations between groups$^{25}$ and small numbers of baseline observations.$^{26}$ When calculating effect sizes for SSRD data the
baseline data are treated as a control group and the intervention data acts as the experimental group.23

RESULTS

Eight asymptomatic participants completed the study. The age, weight and height of each participant is shown in Table 1.

Table 1: Participant demographics

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</tr>
<tr>
<td>8</td>
<td>39</td>
<td>70</td>
<td>176</td>
</tr>
</tbody>
</table>

Individual data

The data for salivary S-IgA for each participant’s concentration, flow rates and secretion rates is shown in Figure 1. The normal range of salivary S-IgA concentration is 79.26 to 679.50 μg/ml.27,30 The lines between data points on the individual graphs are omitted to avoid the impression of continuity of the S-IgA data between measurement readings.

Visual inspection of the salivary S-IgA flow rate/concentration graphs for each participant show large variations within and between participants with no discernible trends during baseline or after treatment. All readings for individual participants’ pre- and post- treatment are within expected normal range for asymptomatic adults. An extreme flow rate is noted for participant 3 on Baseline Day 3. All participants showed an immediate post-treatment short-term increase in salivary S-IgA concentration when comparing the Baseline Day 5 to the Post-treatment 1 measurement. However, when the flow rate is taken into account, this pattern is not consistent and salivary S-IgA secretion rates vary between participants.
Visual inspection of the individual salivary S-IgA secretion rates during baseline and intervention phases shows variable baseline with no obvious trend or pattern for participants 1, 3, 5, 6, 7 and 8. For participants 2 and 4 there is a decreasing and an increasing trend respectively across baseline and both post-treatment phases.

The 1 minute post-treatment measurement of the salivary S-IgA secretion rate shows an increase for all participants except one (participant 2). The 10 minute post-treatment measurement shows a further S-IgA secretion rate increase in two participants (3 and 4) while all other participants have a decreased S-IgA secretion rate at 10 minute post-treatment measurement (Figure 1).
Figure 3: Individual salivary S-IgA concentration, flow rate and secretion rate
Column A: saliva flow rate, x salivary S-IgA concentration, 1st y-axis S-IgA concentration (μg/ml), 2nd y-axis saliva flow rate (ml/min). Column B: x represents salivary S-IgA secretion rate, y-axis salivary S-IgA secretion rate values (μg/min)(derived by multiplying S-IgA concentration and saliva flow rate).
Individual effect sizes for salivary S-IgA secretion rate measured as mean for Post Treatment 1 and 2 in relation to Baseline mean ranged from moderate to large in all participants except one (participant 2). The decrease of S-IgA secretion rate for participant 2 is expressed as a negative effect (Table 2).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Baseline mean μg/min</th>
<th>Post-treatment 1+2 mean μg/min</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73.42</td>
<td>124.69</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>154.78</td>
<td>82.56</td>
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<tr>
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<td>348.71</td>
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<tr>
<td>8</td>
<td>144.19</td>
<td>202.30</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Group data

The group data for Baseline, Post-Treatment 1 minute and Post-Treatment 10 minute show means ± 95% confidence interval. The effect size for Baseline to Post-Treatment 1 minute was d=2.1. Mean effect size for baseline to post-treatment 10 minute was d=1.3 (Figure 3).

Because the single systems analysis showed considerable non-systematic day-to-day variability in IgA at baseline, the group baseline data were calculated as the mean of the sum of the individual mean baseline values. There were no differences in salivary S-IgA secretion rate from mean baseline levels when the two post-treatment measurements were considered separately using repeated measures ANOVA. However, because underlying day-to-day variability in S-IgA might also apply to measurements taken 10 minutes apart, a paired t-test between mean baseline and mean post-treatment measurements was also conducted to test this. Post-treatment IgA levels when averaged were significantly higher than baseline levels (Effect size ES = 3.0; p=0.03).
Figure 4: Salivary S-IgA secretion rates μg/min (mean ± 95% CI) NR = normal range of S-IgA

The baseline mean value was derived from all baseline samples collected over five days across 8 participants (=40 samples). The 1 minute and 10 minute post treatment mean values are each derived from 8 participants.
DISCUSSION

Overview

Despite the common use of lymphatic pump technique (LPT) by osteopathic practitioners in the treatment of immune-related conditions, research to validate the therapeutic effect of this technique on immune health is limited. Secreted Salivary Immunoglobulin A (S-IgA) is a relatively easily measured output of the immune system. While it is still unclear to what extent salivary S-IgA can be regarded as an indicator of immune health there is growing evidence that increased levels of salivary S-IgA may represent enhanced mucosal immune function and protection and that low levels of salivary S-IgA can reflect immune compromise. The aim of this pilot study was to observe any short-term influence of the lymphatic pump treatment (LPT) on the secretory immunoglobulin A (S-IgA) levels in the saliva of healthy male participants.

Findings

This study shows an immediate post-treatment increase in salivary S-IgA secretion rate in seven out of eight participants with moderate to large individual effect sizes suggesting there may be a high likelihood of a clinical significance. According to Hopkins, effect sizes of this magnitude are likely to be therapeutically beneficial. True therapeutic benefit would need to be confirmed by comparing changes of this magnitude to changes in symptoms such as infection rates. However, one participant had moderately decreased salivary S-IgA secretion rates for both post-treatment measurements. The salivary S-IgA secretion rates at 10 minutes post-treatment varied, showing a further increase in two participants but a decrease for all other participants, underlining the transience and the individual nature of salivary S-IgA responses commonly observed in studies of salivary S-IgA. Despite inconsistent flow rate patterns, within and across participants, the immediate post treatment S-IgA concentration increased for all participants when compared to the pre-treatment measurement. A comparison of mean group values for saliva S-IgA secretion rate baseline and combined post-treatment was statistically significant. These results, in conjunction with the general lack of studies in this area, may provide a basis for further research utilizing group designs and larger samples.
Interpretation of findings

Whilst the group means show significant increases in salivary S-IgA from pre-treatment to post-treatment, this needs to be interpreted carefully for reasons related to the limitations of this study that are discussed later. The patterns that we observed were not uniform across all participants and highlight the usefulness of looking at individual data in small pilot studies such as this. For instance, participant 2 appeared to have a consistent decrease in salivary S-IgA levels starting from mid baseline and extending to both post-treatment measurements. However, when surveying the reported health data of participant 2, it transpired that he had reported flu like symptoms for each day of the study period suggesting the likelihood of mucosal inflammation. Activation of the mucosal immune response and binding of salivary S-IgA to invading microorganisms decreases the amount of unbound salivary S-IgA and may present one explanation for the results of this participant. However, recent research has also linked salivary IgA, previously regarded as a non-inflammatory immunoglobulin, to the activation of eosinophils in the mucosa. It has been proposed that salivary S-IgA is the principle immunoglobulin associated with mucosal eosinophils in the mediation of inflammatory diseases such as atopic disease and parasitic infection. With these mechanisms in mind, we suggest that the attachment of salivary S-IgA to mucosal eosinophils could provide another explanation for the suppressed salivary S-IgA measurements in this participant. We would recommend that future salivary S-IgA research protocols consider excluding participants with any flu-like symptoms.

To evaluate the observed patterns in this study it is necessary to consider the effect of saliva flow rates on the salivary S-IgA secretion rate. The salivary S-IgA secretion rate is a function of absolute immunoglobulin concentration and saliva flow rate and it represents the total amount of S-IgA available on the mucosal surface and therefore could be considered a more appropriate indicator of mucosal immune health. The need to measure flow rates for the calculation of salivary S-IgA secretion rates has been questioned in earlier studies. Jemmott and Magloire investigated the relationship between academic stress and either salivary S-IgA concentration or S-IgA secretion rate and found both equally influenced by academic stress. At present it is recommended to calculate the salivary S-IgA secretion rate in order to determine if elevated
levels are caused by increased synthesis of salivary S-IgA or by changes in saliva flow rates.\textsuperscript{39, 4320}

To calculate saliva flow rates it is advocated to collect non-stimulated saliva obtained by passive drooling.\textsuperscript{44-46} We suspect that some of the large intra-individual fluctuations in flow rate noted in this study may represent a lack of participant compliance, resulting in the collection of stimulated saliva. The challenges of controlling the flow rate aspect has been recognized in the literature.\textsuperscript{35} Cultural inhibition to spitting and participants not wanting to be observed whilst spitting or drooling makes the control of passive drooling for the collection of non-stimulated saliva difficult.\textsuperscript{36} In this scenario, it may be reasonable to contribute some of the observed fluctuations in salivary S-IgA concentration and secretion rates to alterations in saliva flow rate caused by individual stimulation of saliva flow. This underlines the importance of methodological standardization and possibly supervision during the saliva sampling phase in future studies.

Besides these concerns, the observed immediate post-treatment increases in salivary S-IgA concentration and S-IgA secretion rates indicate that lymphatic pump treatment may exert some influence on the salivary IgA system. The clinical importance of this finding remains speculative. At present there is no recognized minimal clinical important difference (MCID) for salivary S-IgA. However, decreased levels of salivary S-IgA increase the susceptibility for upper respiratory tract infections (URTI). Gleeson\textsuperscript{37} reported that elite swimmers could expect one additional URTI (above their average) when salivary S-IgA levels fell by 6\% while Vernon\textsuperscript{38} noted that for yacht racing athletes a salivary S-IgA level lower than 40\% of their mean healthy S-IgA concentration resulted in a 50\% chance of contracting an URTI within 3 weeks.

In order to understand the biological consequences of a short term evoked increase in salivary S-IgA as observed in this study, long term observational studies including high risk individuals have to be initiated.\textsuperscript{20}

To our knowledge this pilot study is the first investigation into the effect of lymphatic pump technique on salivary S-IgA. We are also not aware of any studies investigating immune markers using single systems research design. Immune markers always show some degree of variation within and between subjects and are affected by a range of subject specific and environmental
factors. Therefore, where possible, placebo controlled randomized cross-over designs are preferred in immune related research. The observed increase in salivary S-IgA from baseline Day 5 to Post treatment 1 in this study is consistent with increases reported in other studies following different types of interventions including meditation, relaxation techniques and touch based therapies such as massage therapy and reiki. However, it is difficult to compare salivary S-IgA studies and raises doubt as to whether the increases are supportive of any change elicited by these interventions. Many of these studies assess the concentration of salivary S-IgA without controlling for the flow rate and therefore neglecting the effect of the intervention on the saliva flow rate. The lack of assay standardization further complicates comparison of salivary S-IgA studies.

In this study, by calculating salivary flow rates, we show that observed increases in S-IgA may be due to changes in flow rate. We were also able to demonstrate that independent of changes in flow rate, in some participants, not only an increase in S-IgA concentration occurred but also an enhanced secretion rate.

The results of our study should be interpreted with caution. The baseline values for salivary S-IgA showed great variability and, for the majority of the participants, the post-treatment increase was only transient. It is possible that these observations are a reflection of the individual biological sensitivity of the salivary S-IgA responses and mirror the inherent difficulty to control the determinants of variability in immune markers. Related studies are not indicative of commonly observed post intervention transience for the salivary S-IgA response since there is a general lack of studies implementing further post-intervention measurements (eg.1 hour post-intervention). However, one unpublished open randomized controlled study by Norton, Saggio & Gilliar (abstract only available) measured increased salivary S-IgA one hour after a 20 minute osteopathic manipulative treatment. For this study no detailed information regarding procedures and methods is available.

While the clinical significance of a transitory increase in salivary S-IgA is currently unknown, future, carefully controlled, studies involving multiple, staggered post intervention samples would add understanding to the decay aspect of the salivary S-IgA response.

We must also emphasize that, while the baseline design of this study allowed us to observe the day to day variations of individual salivary S-IgA it does not give information on salivary S-IgA
changes that occur over hours or minutes. Therefore the observed changes in salivary S-IgA immediately post-treatment could easily be interpreted as part of normal variation. However, the short timeframe between Baseline 5 and First Post-Treatment measurement in conjunction with the substantial changes observed in several participants makes it reasonable to suggest that there may be some influence of LPT on salivary S-IgA. Further study is required.

**Possible Mechanisms of the salivary S-IgA increase following LPT**

The precise mechanisms underlying a LPT- induced increase in salivary S-IgA are speculative. A suction effect of LPT on lymphatic nodes, as suggested by Hodge\(^9\) may extend to the salivary glands resulting in altered fluid flow. Yet, it is unclear to what extent increased fluid movement may influence the complex mechanisms involved in endocytosis and transcytosis of S-IgA across the epithelium of salivary glands. A suction mediated elevation of salivary S-IgA would account for the transience of the S-IgA post-treatment increases observed in this study as the cessation of suction can be expected to result in decreased translocation of S-IgA into saliva.

LPT activated neuroendocrine stimulation may be an explanation for salivary S-IgA elevations and fluctuations that were observed in the post treatment measurements of several participants. Salivary glands are controlled by the sympathetic and the parasympathetic nervous system. These two branches of the autonomic nervous system (ANS) are regulated, amongst other factors, by individual responses to perceived stress or relaxation.\(^{45,46}\)

In this study, the relaxing procedure of the LPT treatment, also bearing in mind the close proximity of the vagus nerve to the treated area could reasonably be expected to have elicited a parasympathetic response.\(^{47}\) Parasympathetic nervous system (PSNS) influence not only generally enhances immune function but also increases mucus production\(^{45}\) possibly providing a mechanism that enhances the translocation of IgA based on the the mucophilic properties of its secretory component (SC).\(^{43}\) Anyhow, it is just as plausible that individual participants perceived LPT, and its associated procedures such as the textbook reading between measurements, as a stressful event. In this case, depending on the individually present level of chronic stress, perceived acute stress and norepinephrine modulation, either an up or down regulation of mucosal immunity takes place.\(^{48,46}\)
To what extent LPT exerts influences on cellular immunity mechanisms of S-IgA synthesis, such as plasma cell activation and immunoglobulin isotype switching is pure conjecture. LPT has shown to mobilize inflammatory mediators, including interleukins IL and IL 6, into the lymphatic ducts of dogs. IL promotes the differentiation of B cells into antibody producing plasma cells while IL 6 increases antibody production by plasma cells. However, these immune functions are usually preceded by antigenic stimulation of B-cells which is unlikely to occur in a study on healthy subjects and research results from animal studies have to be interpreted with caution and their findings cannot always be transferred to humans. It could also be speculated that the bi-directional nature of the immune system leads to mechanisms that interpret an increased lymph flow, following LPT, as an immunological emergency situation that requires the mobilization of cellular immune components.
Limitations of the study

The extent to which a causal relationship exists between the observed increases in salivary S-IgA and the lymphatic pump treatment cannot be determined. To infer causality in a single systems research design a stable, flat baseline must be present, showing no trend of improvement and changes occurring only after the intervention is applied. The fluctuating baselines in this study, though typical for salivary S-IgA, therefore do not allow to conclude a causal relationship between outcomes and intervention and highlight the need for a controlled group design.

Although the immediate post-treatment change in some participants appears convincing, there are, however, alternative explanations challenging the inference that the intervention caused the changes in outcomes in this study. The synthesis and the release of immunoglobulin and the saliva flow rate underlie autonomic nervous system control and are therefore affected by stimulation of the sympathetic and parasympathetic nervous system including factors such as relaxation, stress, and fear. To what extent the individual changes in participants are related to the LPT or to other factors, such as lying in a quiet room and being touched, cannot be determined. However, it is interesting that significant findings resulted over such a short period of time.

Another threat to the internal validity of this study may have unknowingly been introduced by the authors by providing text book reading in between the two post-treatment saliva measurements. It could be speculated that this attempt to create similar post-treatment conditions for all participants actually elicited an individual stress reaction with subsequent variations in salivary S-IgA responses.

This study has not attempted to standardize the pressure of the pumping action during the lymphatic pump treatment and the reliability of the intervention implementation may be questioned.

The modified single systems research design of this study was chosen for two reasons: one, pragmatic considerations based on financial restriction and two, the emerging nature of the topic with no previous studies made it reasonable to investigate individual responses first. Single systems research studies are noted for their innate problems with external validity and one of the most important limitations of this type of design, besides small samples and absence of controls,
is the lack of generalizability. Although we have demonstrated that LPT may influence salivary S-IgA responses in healthy, male subjects of a certain age group the results may not be generalizable to other population groups, e.g. adults with disease, the elderly or children.

There are further considerations related to the single systems research design (SSRD) in this study.

To increase the credibility of SSRD a multiple or non-concurrent baseline approach is recommended as it minimizes the possible influence of extraneous variables. We refrained from staggering baseline and intervention. Conducting baselines non-concurrently increases the risk that the intervention protocol evolves, compromising direct replication of the treatment. Additionally, bearing in mind the sensitivity of salivary S-IgA levels to external factors such as daylight and weather, it seemed more appropriate to attempt to create similar conditions for each participant by refraining from implementing staggered baselines.

Due to the limited number of data points in this study it is not appropriate to apply statistical tests for single-subject data such as Trend-Lines or 2 Standard Deviation Band and our single systems analysis was restricted to visual inspection of data. There is controversy as to the most appropriate method of analysis, however, the agreement between results of statistical tests and visual analysis in single systems research is considered to be high.

While the small number of subjects in our study would clearly invalidate parametric statistics we believe that the preliminary nature of this project justifies the statistical group analysis and we urge the reader to interpret the statistical significant group result and the effect sizes only in regards to their ability in lending strength to the individual results.
Suggestions for further research

As the effect sizes in this study were large between baseline and mean of the two post-treatment measurements further research of this topic seems justified. To avoid the limitations of the present single systems design we would advise a randomized controlled trial. The future study design should include a larger number of participants and preferably a control group receiving a masked and blinded sham treatment. Ideally subjects are maintained under controlled laboratory conditions while treated twice daily and post treatment saliva samples being collected in 10 to 15 minute intervals to investigate the decay of the salivary S-IgA response.

To estimate the sample size required based on effect sizes observed in our single systems study we would assume an effect size of at least 1.8, bearing in mind that we observed a large effect size over a short period of time. Using G*power online analysis based on an alpha error probability of 0.05, to achieve a power (1-β error probability) equal to 0.8 and assuming an effect size of not less than 0.8 a number of 26 subjects per group would be required. However, a more useful way of evaluating the effect of lymphatic pump treatment on salivary S-IgA levels is to evaluate any change in a degree that is clinically meaningful. Therefore future studies should include participants that are adversely affected by low levels of salivary S-IgA such as athletes suffering from recurrent upper respiratory tract infections, symptomatic IgA deficient individuals and frequently ill children.
CONCLUSION

The application of a 7 minute osteopathic lymphatic pump technique appears to increase the salivary S-IgA output in healthy male individuals. Increased salivary S-IgA is thought to be related to enhanced mucosal immune function and decreased salivary S-IgA is understood to be a main factor in the susceptibility of acquiring upper respiratory tract infections. However, the possible biological and clinical significance of the findings of this study is presently unclear. Whilst the results of this study have to be evaluated bearing in mind the limitations of the single systems design we propose that our findings justify further controlled, group investigations.
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SECTION III – APPENDICES
Appendix A

Ethics documentation
Heike Ehrlenbach
1126 Old Mountain Road
RD1
Raglan

1 July 2009

Dear Heike,

Your file number for this application: 2009-974
Title: The Effect of the Osteopathic Lymphatic Pump Technique on one Output of the Human Immune System as measured by Salivary Immunoglobulin A Levels

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: 24 June 2009
Finish date: 24 June 2010

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]

Deborah Rolland
Deputy Chair, UREC

cc: Craig Hilton
Cynthia Almeida


**Participant Information Sheet**

"**The effect of the osteopathic lymphatic pump technique on one output of the human immune system as measured by salivary Immunoglobulin A**"

**About this research**

You are invited to take part in a study undertaken within the 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 are needed between the ages of 18 and 40 years.

**Participants in this project will be asked to:**

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

- Attend five times a 15-20 minute appointment on 5 consecutive days to answer a questionnaire about lifestyle factors such as sleep, exercise, diet and stress levels and to provide a saliva sample on each of these days. You are required to abstain from exercise, food or drink (except for water) 1 hour prior to each appointment.

- On the fifth day the above procedure is followed by a 7 minute lymphatic pump treatment that consists of rhythmic pumping on the upper chest area. The technique is applied by a qualified osteopath. Immediately post treatment and 15 minutes post treatment saliva samples are collected and you are asked to sit quietly in between sample taking.

- Saliva samples are collected in labelled, sterile 2ml vials via passive "drooling" through a plastic straw for 5 minutes or until a certain amount of saliva is collected. The saliva collection vial is held by the participant until the required amount is obtained. The vial is then securely sealed by the researcher and transferred to the deep freeze storage where it will be held until laboratory analysis commences.

- Consent to the research team’s use of the research data in preparing both a research project dissertation and an article for publication.

**The researchers**

The primary researcher is Heike Ehrlenbach
This project is being supervised by Dr Craig Hilton and Dr. Andy Stewart.

**Participation and consent**

You have the right not to participate, or to withdraw from this research project at any time prior to commencement of data analysis. 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 Heike Ehrlenbach.

**Getting help**

Please contact any one of us should you have any queries or require any help with this research project.

Heike Ehrlenbach: heike@raglan.co.nz

Craig Hilton: chilton@unitec.ac.nz

Andy Stewart: astewart@unitec.ac.nz
09 815 4321 x 8384

**Information and concerns**

If you would like further information about the project you can call or email the above addresses. If at any time you are confused or concerned about the research project, you can contact Heike Ehrlenbach, 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: Advocates Network Services Trust, Phone (09) 6235799, (0800)205555, Fax (09)6235798, PO Box 9983, Newmarket, Auckland.*

**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, and a plain English summary will be available to participants and other interested parties. 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: 2009-974**

This study has been approved by the UNITEC Research Ethics Committee from 24-06-2009 to 24-06-2010. 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 one output of the human immune system as measured by salivary Immunoglobulin A

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

Name of Participant: __________________________________________

I have seen the Participant Information Sheet for the project titled ‘The effect of the osteopathic lymphatic pump technique on one output of the human immune system as measured by salivary immunoglobulin A’. I have had the opportunity to read the contents of the information sheet and to discuss the project with Heike Ehrlenbach, and I am satisfied with the explanations I have been given. I understand that the anonymised data from her 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 up until one week after the last data collection 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 understand that I can withdraw from the study at anytime up until one week after the last data collection. 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 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 have had enough time to consider whether I want to take part.

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

The principal researcher for this project is Heike Ehrlenbach.
Contact details: heike@raglan.co.nz

Signature: _____________________________ (participant) Date: ________________

Project explained by Heike Ehrlenbach
Signature: ______________________________ Date: __________________________

The participant should retain a copy of this consent form.

UREC REGISTRATION NUMBER: 2009-974

This study has been approved by the UNITEC Research Ethics Committee from 24-06-2009 to 24-06-2010. 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

Questionnaires
Study Enrolment Questionnaire

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

1. Please list below any medications that you are presently taking
2. Do you have any allergies? □ yes □ no
3. Do you presently suffer from any viral or bacterial infection? □ yes □ no
4. Do you presently have any mouth ulcers, tooth or gum infections? □ yes □ no
5. Did you recently have any dental extractions or dental surgery? □ yes □ no
6. Have you ever been diagnosed with any infectious disease such as AIDS, Hepatitis or Syphilis? □ yes □ no
7. Is there a history of haemophilia in your family? □ yes □ no
8. Have you ever been diagnosed with IgA deficiency? □ yes □ no
9. Have you ever been diagnosed with an autoimmune disorder? □ yes □ no
10. Have you ever been diagnosed with any form of cancer, sickle cell disease, aneurysms, cardiac failure or varicose veins? □ yes □ no
11. Did you recently (within last 2 weeks) return from a long distance flight? □ yes □ no
12. Do you ever experience pain in your legs during exercising? □ yes □ no
13. Have you ever been diagnosed with deep vein thrombosis? □ yes □ no
14. Did you recently undergo any procedure or surgery in the upper body area? □ yes □ no
15. 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 – SOMETIME  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 weightlifting.  
   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

**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.

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<tr>
<th></th>
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<th>2 = Sometimes</th>
<th>3 = Fairly Often</th>
<th>4 = Very Often</th>
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<tbody>
<tr>
<td>1. In the last month, how often have you been upset because of something that happened unexpectedly?</td>
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<tr>
<td>2. In the last month, how often have you felt that you were unable to control important things in your life?</td>
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<td>4</td>
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<tr>
<td>3. In the last month, how often have you felt nervous and “stressed”?</td>
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<tr>
<td>4. In the last month, how often have you felt confident about your ability to handle your personal problems?</td>
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<td>5. In the last month, how often have you felt that things were going your way?</td>
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<td>4</td>
</tr>
<tr>
<td>6. In the last month, how often have you found that you could not cope with all the things that you had to do?</td>
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<td>4</td>
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<tr>
<td>7. In the last month, how often have you been able to control irritations in your life?</td>
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<td>8. In the last month, how often have you felt that you were on top of things?</td>
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<td>4</td>
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<td>9. In the last month, how often have you been angered because of things that were outside of your control?</td>
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<td>4</td>
</tr>
<tr>
<td>10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
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</tbody>
</table>

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 units of alcohol do you drink per week?

Signature:                                                Date:
Daily Pre-Sample Questionnaire

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?
   1 2 3 4 5 6 7 8 9 10 (please circle)

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

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 □
Appendix C

Data Collection Sheets
**Daily Baseline Collection Day 1-4**

**Participant Number:** Weight:  Height:

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<th>Baseline Day 1</th>
<th>Date:</th>
<th>Time:</th>
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<th>Collection time for baseline sample A</th>
<th>Weight of baseline sample A (vial with saliva)</th>
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</thead>
<tbody>
<tr>
<td>Baseline Day 2</td>
<td>Date:</td>
<td>Time:</td>
<td>Collection vial number for baseline sample A</td>
<td>Collection time for baseline sample A</td>
<td>Weight of baseline sample A (vial with saliva)</td>
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<td>Baseline Day 3</td>
<td>Date:</td>
<td>Time:</td>
<td>Collection vial number for baseline sample A</td>
<td>Collection time for baseline sample A</td>
<td>Weight of baseline sample A (vial with saliva)</td>
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<td>Baseline Day 4</td>
<td>Date:</td>
<td>Time:</td>
<td>Collection vial number for baseline sample A</td>
<td>Collection time for baseline sample A</td>
<td>Weight of baseline sample A (vial with saliva)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Collection vial number for baseline sample B</td>
<td>Collection time for baseline sample B</td>
<td>Weight of baseline sample B</td>
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Data Collection Day 5  Pre- and Post-intervention sample data

Participant number:
Date:
Time:

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<th>Time in minutes and seconds</th>
<th>Collection vial number</th>
<th>Collection vial Weight (after saliva collection)</th>
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<td>Collection time of 1st pre-treatment SampleB</td>
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<td>Start time of treatment Session</td>
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<td>Finish time of Treatment Session</td>
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<td>Collection time of 1st post-treatment SampleA</td>
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<td>Collection time of 1st post-treatment SampleB</td>
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<td>Start time of 2nd Post-Treatment sample (10min. post treatment)</td>
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<td>Collection time of 2nd post-treatment SampleA</td>
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<tr>
<td>Collection time of 2nd post-treatment SampleB</td>
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Saliva Sample Vial Codes

B = baseline samples a and b (each day the baseline sample is done in duplicate)
B5a + B5b = last baseline sample also acts as the pre-treatment sample (done in duplicate)
P1a + P1b = 1st post-treatment sample, immediately after treatment (done in duplicate)
P2a + P2b = 2nd post-treatment sample, 10 minutes post treatment (done in duplicate)

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Appendix D

Immune related Osteopathic Techniques
Lymphatic Pump Techniques

All lymphatic pump techniques (LPT) aim to influence pressure gradients between thoracic and abdominal cavity to improve lymphatic fluid flow and to aid tissue decongestion (Hruby & Hoffman, 2007; Ward, 2003). The clinically most commonly used LPT’s are described below.

Thoracic pump

Patient supine, practitioner standing at head of table. Practitioner places hands on patient’s thorax with thenar eminences over pectoralis muscle and fingers spread out facing towards the feet and slightly lateral (Figure 1). A rhythmic pumping action is created by the practitioner by alternating pressure and release. The pumping rate can range from 60-120 times per minute depending on individual tissue response. The patient is asked to continue to breathe normally (Ward, 2003).

Figure D5 Thoracic pump (Ward, 2003)
**Abdominal pump**

Patient supine, practitioner stands at side of table. Practitioner’s palms are placed on the patient’s abdomen with the fingers pointing to the patient’s head (Figure 2). With extended arms and locked elbows a pumping motion is created at a rate of 20-30 times/minute (Ward, 2003).

![Figure D6 Abdominal pump (Ward, 2003)](image1)

**Pedal pump**

Patient supine, practitioner standing at patient’s feet

Practitioner grasps patient’s feet and introduces a rhythmical alternating hyper dorsiflexion to the patient’s feet creating an oscillating pump movement through the longitudinal axis of the patient’s body (Figure 3) (Ward, 2003).

![Figure D7 Pedal pump (Ward, 2003)](image2)
**Liver pump (liver quiver)**

Patient supine, practitioner on right side of patient facing the patient.

Practitioner’s left hand is placed under patient’s lower ribcage while right hand is lies on the abdominal wall below the costal margin (Figure 4). The patient is asked to inhale and exhale deeply and on each exhalation the practitioner employs a vibratory movement of the right hand on the liver (Ward, 2003).

![Liver pump (liver quiver)](image)

Figure D8 Liver pump (liver quiver)(Ward, 2003)

**Spleenic pump**

Patient lying on their right side, practitioner standing behind patient with patients left arm draped over practitioners shoulder. Practitioner hands are placed anterior and posterior on lower thorax (Figure 5). On each patient exhalation the practitioner introduces a vibratory movement through the rib cage. This technique can also be applied over the liver area with the patient lying on their left side (Ward, 2003).

![Spleenic pump](image)

Figure D9 Spleenic pump,( technique here shown on liver)(Ward, 2003)
Other Osteopathic Techniques and their effect on the Immune system.

Rib raising techniques augment sympathetic nervous system (SNS) activity to lymphatic vessels. A similar effect is achieved by inhibiting paraspinal tissues of the trunk. Both techniques stimulate the thoracic sympathetic chain ganglia which are situated near the rib heads. Initial increase in SNS outflow is followed by decreased sympathetic activity resulting in improved lymph flow and drainage of the tissues that receive their sympathetic supply from the treated spinal area (Guyton & Hall, 2000; Kuchera & Kuchera, 1993; Ward, 2003).

Fascia release treatment normalizes tension throughout the connective tissues that form sheets and diaphragms within the body. Lymphatic vessels are closely associated with and have to pass through fascia. The thoracic inlet (a diaphragm formed by fascia in the area of the upper thoracic vertebrae, the first two ribs and the manubrium of the sternum) is of specific importance to lymph flow since both lymphatic ducts have to pass through this area (Paoletti, 2006; Thieme, 2006; Ward, 2003).

Doming of the abdominal diaphragm is a technique that aims to optimize the function of the diaphragm muscle. Efficient contraction and relaxation of the abdominal diaphragm produces pressure gradients between the thoracic and abdominal body cavity. These pressure gradients function as intrinsic pumps supporting the lymphatic circulation. Doming techniques are also applied to the pelvic diaphragm, a muscle sling in the pelvis that provides support for the pelvic organs. Restriction in the pelvic diaphragm can affect fluid flow in lymphatic channels and cause congestion in the lower extremities and the pelvic organs (Kuchera & Kuchera, 1993; Stone, 1999; Ward, 2003).

Petrissage (kneading or squeezing) and Effleurage (stroking) of the extremities, in a distal to proximal (towards the heart) direction, facilitates lymph flow from the appendages to the thorax and to the abdomen. These techniques, in combination with gentle traction, are also employed around the mandible, the cervical and pectoral muscles and on the abdomen, with the aim to enhance tissue drainage.
Appendix E

Raw Data and Transformation Calculations
### Plate read Plate 1 full plate

**Plate: full plate**  
**Date Created:** 14/07/2010 5:27:32 p.m.

#### Raw Data

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#### Transformation Results

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93
Plate Read Plate 2 Half Plate
Plate: 1/2 plate  Date Created: 14/07/2010 5:29:17 p.m.

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Transformation Results

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Calculated Concentrations

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| 128.1198 | 13.30372 | 107.3317 | 100.6869 | 103.9618 | 92.59526 |
| 135.5627 | 133.0372 | 107.3317 | 100.6869 | 103.9618 | 92.59526 |
| 333.269  | 328.8997 | 179.5576 | 160.4789 | 39.31906 | 33.48879 |
| 267.1758 | 212.6367 | 61.99166 | 47.91045 | 39.01221 | 37.50273 |
| 82.32529 | 55.39313 | 44.46212 | 39.31906 | 43.79458 | 47.55716 |

94
Standard Curve

Parameter (y = (A - D) / (1 + (x/C)^B) + D)

A=0.0169  B=-0.6764  C=32.6513  D=1.1145  R-Square = 0.9995
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Mean: 13.04
STDV: 13.13
Appendix F

Journal Publication Information
Guide for Authors

International Journal of Osteopathic Medicine

An official journal of:

• General Osteopathic Council (UK)
• Australian Osteopathic Association
• Ontario Association of Osteopathic Manual Practitioners

Former title: Journal of Osteopathic Medicine

The journal Editors welcome contributions for publication from the following categories: Letters to the Editor, Reviews and Original Articles, Commentaries and Clinical Practice case studies with educational value.

Online Submission

Submission to this journal proceeds totally online.([http://ees.elsevier.com/ijom](http://ees.elsevier.com/ijom)) you will be guided stepwise through the creation and uploading of the various files. The system automatically converts source files to a single Adobe Acrobat PDF version of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail and via the Author's homepage, removing the need for a hard-copy paper trail.

The above represents a very brief outline of this form of submission. It can be advantageous to print this "Guide for Authors" section from the site for reference in the subsequent stages of article preparation.

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, without the written consent of the Publisher.

Types of contributions

Letters to the Editor as is common in biomedical journals the editorial board welcomes critical response to any aspect of the journal. In particular, letters that point out deficiencies and that add to, or further clarify points made in a recently published work, are welcomed. The Editorial Board reserves the right to offer authors of papers the right of rebuttal, which may be published alongside the letter.

Reviews and Original Articles These should be either i) reports of new findings related to osteopathic medicine that are supported by research evidence. These should be original,
previously unpublished works. The report will normally be divided into the following sections: abstract, introduction, materials and methods, results, discussion, conclusion, references. Or ii) critical or systematic review that seeks to summarize or draw conclusions from the established literature on a topic relevant to osteopathic medicine.

Short review The drawing together of present knowledge in a subject area, in order to provide a background for the reader not currently versed in the literature of a particular topic. Shorter in length than and not intended to be as comprehensive as that of the literature review paper. With more emphasis on outlining areas of deficit in the current literature that warrant further investigation.

Research Note Findings of interest arising from a larger study but not the primary aim of the research endeavor, for example short experiments aimed at establishing the reliability of new equipment used in the primary experiment or other incidental findings of interest, arising from, but not the topic of the primary research. Including further clarification of an experimental protocol after addition of further controls, or statistical reassessment of raw data.

Preliminary Findings Presentation of results from pilot studies which may establish a solid basis for further investigations. Format similar to original research report but with more emphasis in discussion of future studies and hypotheses arising from pilot study.

Commentaries Include articles that do not fit into the above criteria as original research. Includes commentary and essays especially in regards to history, philosophy, professional, educational, clinical, ethical, political and legal aspects of osteopathic medicine.

Clinical Practice Authors are encouraged to submit papers in one of the following formats: Case Report, Case Problem, and Evidence in Practice.

Case Reports usually document the management of one patient, with an emphasis on presentations that are unusual, rare or where there was an unexpected response to treatment eg. an unexpected side effect or adverse reaction. Authors may also wish to present a case series where multiple occurrences of a similar phenomenon are documented. Preference will be given to reports that are prospective in their planning and utilise Single System Designs, including objective measures.

The aim of the Case Problem is to provide a more thorough discussion of the differential diagnosis of a clinical problem. The emphasis is on the clinical reasoning and logic employed in the diagnostic process.

The purpose of the Evidence in Practice report is to provide an account of the application of the recognised Evidence Based Medicine process to a real clinical problem. The paper should be written with reference to each of the following five steps: 1. Developing an answerable clinical question. 2. The processes employed in searching the literature for evidence. 3. The appraisal of evidence for usefulness and applicability. 4. Integrating the critical appraisal with existing clinical expertise and with the patient's unique biology, values, and circumstances. 5. Reflect on the process (steps 1-4), evaluating effectiveness, and identifying deficiencies.
Presentation of Typescripts

Your article should be typed on A4 paper, double-spaced with margins of at least 3cm. Number all pages consecutively beginning with the title page.

To facilitate anonymity, the author's names and any reference to their addresses should only appear on the title page. Please check your typescript carefully before you send it off, both for correct content and typographic errors. It is not possible to change the content of accepted typescripts during production.

Papers should be set out as follows, with each section beginning on a separate page:

Title page
To facilitate the peer-review process, two title pages are required. The first should carry just the title of the paper and no information that might identify the author or institution. The second should contain the following information: title of paper; full name(s) and address(es) of author(s) clearly indicating who is the corresponding author; you should give a maximum of four degrees/qualifications for each author and the current relevant appointment only; institutional affiliation; name, address, telephone, fax and e-mail of the corresponding author; source(s) of support in the form of funding and/or equipment.

Keywords
Include three to ten keywords. These should be indexing terms that may be published with the abstract with the aim of increasing the likely accessibility of your paper to potential readers searching the literature. Therefore, ensure keywords are descriptive of the study. Refer to http://www.nlm.nih.gov/mesh/meshhome.html for the MeSH thesaurus.

Abstract
Both qualitative and quantitative research approaches should be accompanied by a structured abstract. Commentaries and Essays may continue to use text based abstracts of no more than 150 words. All original articles should include the following headings in the abstract as appropriate: Background, Objective, Design, Setting, Methods, Subjects, Results, and Conclusions. As an absolute minimum: Objectives, Methods, Results, and Conclusions must be provided for all original articles. Abstracts for reviews of the literature (in particular systematic reviews and meta-analysis) should include the following headings as appropriate: Objectives, Data Sources, Study Selection, Data Extraction, Data Synthesis, Conclusions. Abstracts for Case Studies should include the following headings as appropriate: Background, Objectives, Clinical Features, Intervention and Outcomes, Conclusions.

Text
The text of observational and experimental articles is usually, but not necessarily, divided into sections with the headings; introduction, methods, results, results and discussion. In longer articles, headings should be used only to enhance the readability. Three categories of headings should be used:
• major ones should be typed in capital letter in the centre of the page and underlined
• secondary ones should be typed in lower case (with an initial capital letter) in the left hand margin and underlined
• minor ones typed in lower case and italicised

Do not use 'he', 'his' etc. here the sex of the person is unknown; say 'the patient' etc. Avoid inelegant alternatives such as 'he/she'. Avoid sexist language.

Statement of Competing Interests
When submitting a Research report you will need to consider if you, or any of your co-authors, are an Editor or Editorial Board member of the International Journal of Osteopathic Medicine. If this is the case you will need to include a section, at the end of your manuscript immediately before the reference section, called "Statement of Competing Interests". Example statement, which may require editing, is as follows: {Name of author} is an Editor of the Int J Osteopath Med; {Name of author} is a member of the Editorial Board of the Int J Osteopath Med but was not involved in review or editorial decisions regarding this manuscript.

References
Responsibility for the accuracy of bibliographic citations lies entirely with the Authors.

Citations in the text: Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Avoid using references in the abstract. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either "Unpublished results" or "Personal communication" Citation of a reference as "in press" implies that the item has been accepted for publication.

Text: Indicate references by superscript numbers in the text. The actual Authors can be referred to, but the reference number(s) must always be given.

List: Number the references in the list in the order in which they appear in the text.

Examples:

Reference to a journal publication:


Reference to a book:

Reference to a chapter in an edited book:


Note shortened form for last page number. e.g., 51-9, and that for more than 6 Authors the first 6 should be listed followed by "et al." For further details you are referred to "Uniform Requirements for Manuscripts submitted to Biomedical Journals" (J Am Med Assoc 1997;277:927-934) (see also http://www.nejm.org/general/text/requirements/1.htm)

**Citing and listing of Web references.** As a minimum, the full URL should be given. Any further information, if known (Author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

**Tables, Illustrations and Figures**
A detailed guide on electronic artwork is available on our website: http://www.elsevier.com/artworkinstructions

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**Identifiable clinical photographs must be accompanied by written permission from the patient.**

The text of original research for a quantitative or qualitative study is typically subdivided into the following sections:

**Introduction**
State the purpose of the article. Summarize the rationale for the study or observation. Give only
strictly pertinent references and do not review the subject extensively. Do not include data or conclusions from the work being reported.

**Materials and Methods**
Describe your selection of observational or experimental subjects (including controls). Identify the methods, apparatus (manufacturer's name and address in parenthesis) and procedures in sufficient detail to allow workers to reproduce the results. Give references and brief descriptions for methods that have been published but are not well known; describe new methods and evaluate limitations.

Indicate whether procedures followed were in accordance with the ethical standards of the institution or regional committee responsible for ethical standards. Do not use patient names or initials. Take care to mask the identity of any subjects in illustrative material.

**Results**
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