Ragland, Christina E., Effects of Osteopathic Manipulative Treatment on the Inflammatory Mediators Related to Asthma. Master of Science (Osteopathic Manipulative Medicine/Clinical Research and Education), May, 2015, 52 pp., 7 tables, 7 figures, bibliography, 50 titles.

The purpose of this study was to explore the impact of OMT on the macroscopic and microscopic measures of asthma. This was accomplished through a repeated measures design, and the asthma quality of life questionnaire was used to assess asthma severity. Inflammatory proteins known as cytokines, fractional exhaled nitric oxide, and spirometry were used to assess for change immediately after the OMT was performed. Although no statistically significant changes were measured, inflammatory cytokines specific to asthma decreased (IL-4, IL-5), while more general inflammatory cytokines increased after OMT (IL-6, CRP). Spirometry showed a slight decrease in FEV1 and FVC after OMT, although this decrease was neither statistically nor clinically significant. These trends illustrate the need for further investigation into the mechanism of OMT and its role in asthma treatment. The inflammatory cascade that drives asthma is complex and other diseases and lifestyle habits also generate and modify inflammation in the body. As such confounding factors such as gastroesophageal reflux disease, obesity, COPD, and cigarette smoking, should be taken into consideration in future studies.

EFFECTS OF OSTEOPATHIC MANIPULATIVE TREATMENT ON THE INFLAMMATORY MEDIATORS RELATED TO ASTHMA

THESIS

Presented to the Graduate Council of the Graduate School of Biomedical Sciences

University of North Texas Health Science Center at Fort Worth

in Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Christina E. Ragland, B.S.

Fort Worth, Texas

May 2015

ACKNOWLEDGEMENTS

Funding for this project was generously provided by the Osteopathic Research Center at the University of North Texas Health Science Center. A special thanks to the many individuals who made this project possible: Tori Como and Perla Gonzalez for analyzing the serum biomarkers, Charlotte Childs, Patricia Hiller, and Sharon Cha, P.A. for allowing me the use of a clinic room and equipment and support while I was in the clinic, and Dr. Leigh Johnson for her assistance in obtaining approval and resources to get this project started. Finally, I would like to thank Drs. Mason, Patterson, O'Bryant, Al-Farra, and Gryczynski for joining time, ideas, and resources to complete this inter-disciplinary, translational project.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

A) Project Summary and Significance

Asthma is a chronic, inflammation-driven respiratory disease that is on the rise in adults, especially in developed nations. Well-defined pharmacologic management exists for asthma, and in small studies, adjunct therapies such as osteopathic manipulative treatment (OMT) have been reported to assist in alleviating symptoms and managing asthma. Osteopathic manipulative treatment involves applying manual techniques to the bones, muscles, and soft tissues of the body. The mechanisms behind OMT is well-theorized on a macroscopic level. OMT utilizes the relationship between anatomic structure and physiologic function. By removing structural restrictions in tissues, treatments help optimize function of those tissues and surrounding organs. On a microscopic level, the mechanism of OMT is less understood and is a current area of investigation.

The focus of this study was to analyze how osteopathic manipulative treatments change the molecular biomarkers that drive the process of asthma. Microscopic changes were measured by analyzing cytokines (inflammatory proteins) with known involvement in the inflammatory response in asthma (interleukins 4, 5, 6, 13 and C-reactive protein). Nitric oxide (NO), an inflammatory marker in the exhaled breath, was also analyzed. At the macroscopic level, spirometry was used to quantify the effect of OMT by measuring lung volumes. Greater understanding of the underlying mechanisms in OMT will allow for better integration of

osteopathic manipulation and traditional asthma management strategies. OMT is particularly valuable as a treatment modality in asthma because no new pharmacologic agent is added, and it can be done in the office, which avoids the issue of patient compliance that is so common with pharmacologic therapies. Additionally, there is potential for future studies to analyze other biomarkers in asthma as well as to translate this idea to study the mechanism of OMT in other diseases.

CHAPTER II

REVIEW OF LITERATURE/BACKGROUND

A) Asthma – Symptoms, Clinical Diagnosis and Management

Asthma is a complex airway disease that accounts for nearly 500,000 hospitalizations annually, and the prevalence of asthma is increasing throughout the United States.[1] Although often considered a childhood disease, asthma is also a major health concern for adults, affecting 8% of US adults and costing $56 billion in medication costs, lost work days and premature deaths according to a 2011 CDC report. In Tarrant County, asthma was reported to be one of the top five health concerns for adults in 2012.[2]

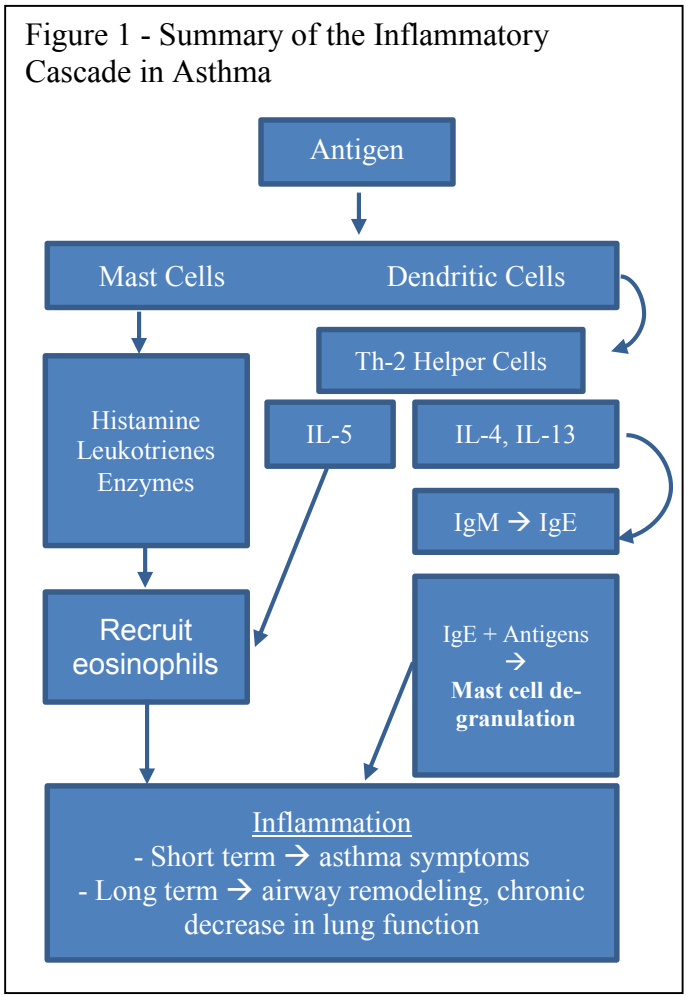
Asthma symptoms are the result of a complex process that begins with exposure to a molecular trigger or some stimuli. Pollen, temperature change, allergens, infections, exercise, perfumes, strong smells, and even hormones are known asthma triggers, and many patients with asthma also experience allergic symptoms and eczema.[3,4] Research shows that individuals with asthma have a genetic predisposition to mount a significant inflammatory response in the airway in contrast to individuals who do not develop asthma symptoms.[5,6] Exposure of the airway tissues to one or more triggers leads to two major pathologic features of asthma: airway inflammation and bronchoconstriction. Cells in the immune system react to the trigger or stimuli and start an inflammatory cascade that leads to increased tone in airway smooth muscle, increased mucus production, and edema in the airway tissue. Together these processes cause

airway obstruction, which is responsible for the clinical symptoms of asthma: wheezing, dyspnea (shortness of breath), chest tightness, cough, and anxiety.

B) Asthma Pathophysiology

Airway inflammation and bronchoconstriction, the two hallmarks of the asthma disease process, are driven by complex interactions between gene expression and environmental factors. The inflammatory cascade (Figure 1) is initiated by a trigger (antigen), for example pollen in the air or a strong perfume. Molecules from the trigger enter the airway and activate mast cells, which leads to direct recruitment of eosinophils and ultimately the release of histamines, leukotrienes, and enzymes. Once recruited eosinophils degranulate and also release toxic enzymes into the surrounding tissue, along with cytokines that serve to augment and continue the inflammatory response.

Figure 1 - Summary of the Inflammatory Cascade in Asthma



Trigger molecules are also bound by dendritic cells in the airway mucosa. Dendritic cells present the trigger molecule (antigen) to other immune cells, activating a specific section of the immune system: the T-helper cells. T-helper cells, specifically type 2 T-helper cells (Th 2),[6,7]

produce cytokines that recruit and activate more eosinophils, leading to eosinophil degranulation and more inflammation.[8,9]

Cytokines are hallmarks of the inflammation process throughout the body. The specific type of cytokine produced depends on the type of immune cell involved, and each subset of T-cells generates a distinct immune response as cytokines activate and alter the function of surrounding cells. The T-helper cell arm of the immune system is made up of two cell types: Th1 and Th2. Th1 cells produce Interleukin-2 (IL-2), IL-18, and INF-gamma (interferon-gamma), while Th2 cells produce IL-4, IL-5, IL-9 and IL-13. [10]

C) Cytokines: IL-4, IL-5, IL-13

By analyzing cytokines that are predominant in asthma, researchers have shown that Th 2 cells are predominantly responsible for the inflammatory response mustered by the immune system in asthma.[6,7] Immune cells found in bronchoalveolar lavage (BAL) samples from asthma patients contained high concentrations of cytokines related to Th 2 cells (IL-4 and IL-5) compared to samples from the control groups.[7] There is also evidence suggesting a genetic link between the IL-4/5/13 locus on chromosome 5 and susceptibility to asthma in humans, further evidence that the Th 2 portion of the immune system is involved in asthma-related inflammation and asthma symptoms.[6] Thus IL-4, IL-5, and IL-13 were chosen for analysis in this study as they are markers of the inflammatory response in asthma. No assay plate with IL-9 was available at the time of this study, so it could not be included in the analysis.

D) IL-6 and C-Reactive Protein

Interluekin-6 (IL-6) and C-reactive protein (CRP) are also involved in asthma pathogenesis. Both are inflammatory proteins that correspond to general inflammation but have also been found to be elevated in asthma.[11,12] IL-6 is readily expressed from pulmonary

epithelial cells after exposure to a trigger and from other immune cells (mast cells, neutrophils, macrophages, dendritic cells). After its release, it binds to receptors on hepatocytes and leukocytes. IL-6 promotes inflammation mediated by Th2 cells by upregulating the production of IL-4. This forms a positive feedback loop, as IL-4 is produced by Th2 cells and upregulates the activation of Th2 cells. Since Th2 cells also produce IL-13, this also results in more IL-13 production, which is responsible for excess mucus secretion. Genetic variations in the IL-6R receptor protein have also been found to increase an individual's risk of developing asthma.[13] While a general marker of inflammation, IL-6 is also very involved in the pathogenesis of asthma.

CRP is primarily made in the liver and is an acute phase reactant meaning it is created early in an inflammatory process. IL-6 also acts on hepatocytes and upregulates CRP production. Also a marker of inflammation, CRP is used as a screening tool clinically, unlike the other biomarkers in this study. CRP is measured when inflammation or infection is suspected based on clinical symptoms. It is sensitive but not specific as CRP can be increased by many different processes such as smoking, cardiovascular disease, type-2 diabetes, and obesity. In cardiovascular disease, CRP has well-established value in predicting mortality and complications.[14] Likewise, CRP is also an indicator of inflammation in asthma. Many studies have shown that high-sensitivity CRP (hs-CRP) is commonly elevated in asthma as compared to levels in healthy controls.[15-17] In addition, individuals reporting nocturnal asthma symptoms and a higher frequency of wheezing were found to have higher levels of CRP, indicating that CRP levels correlate with symptom severity. [18]

E) Exhaled Nitric Oxide

Nitric oxide (NO) is a free radical gas. As such it is highly reactive and found throughout the human cardiovascular and respiratory system. NO is a signal molecule involved in the control of blood flow, both smooth and vascular muscle tone, and neurotransmitter activity. At lower levels, NO causes vasodilation in the vasculature and smooth muscle relaxation. Somewhat paradoxically, at higher levels NO is pro-inflammatory and involved in cytotoxic immune processes.[19]

In the respiratory system, NO is the product of enzymatic oxidation reactions, mediated by nitric oxide synthase (NOS) enzymes. Two major types of NOS are found in airway tissues: inducible (iNOS) and constitutive (cNOS).[19] Constitutive NOS enzymes generate a basal level of NO that fluctuates rapidly but in small amounts in response to sheer stresses, histamines, bradykinins, and acetylcholine. cNOS function is not affected by corticosteroid treatments. In contrast, inducible NOS is activated by pro-inflammatory cytokines in response to allergens, viral infections, and pollutants in the environment. In addition iNOS is responsive to corticosteroid intervention. Oxidative reactions like those mediated by iNOS are often upregulated in inflammatory processes.[20] Due to the significant presence of iNOS in airway tissues and inflammation being a key component of asthma pathophysiology, NO levels in both respiratory epithelium and sputum samples from asthmatic patients are higher than in individuals without asthma.[21]

FeNO is recognized by the American Thoracic Society as an effective and reliable tool that enables clinicians to better manage asthma.[22] Monitoring NO provides information about airway inflammation noninvasively. Prior to the advent of such technology, there was no non-invasive way to effectively measure airway inflammation. Sputum samples, peak expiratory

flow measures, and spirometry continue to provide indirect information about airway function and inflammation, but NO provides a simple, non-invasive, real-time, direct measure of airway inflammation for most asthma patients.[23-25] NO is measured in the exhaled breath through a test called fractional exhaled nitric oxide (FeNO).

Studies show FeNO is age-dependent in children younger than age eleven[26]; however this variability does not persist into adulthood. Another study by Olivieri et. al. showed that men had a slightly higher average FeNO compared to women in non-smoking individuals with normal spirometry values. [27] Cigarette smoking is also shown to effect FeNO, and smokers have lower FeNO values than non-smokers; however, individuals who smoke and have asthma may still have an elevated FeNO. Due to the variability in FeNO, clinical cut points have been recommended by the American Thoracic Society to guide management rather than specific "normal" values.[19,22] In adults FeNO <25 ppb (parts per billion) is considered controlled in an established asthmatic or is an indication that asthma in that patient is not eosinophil-driven in this individual and therefore may not be responsive to corticosteroid therapy. FeNO measurements of >50 ppb in adults indicate asthma driven by eosinophils that will likely be responsive to corticosteroid therapy. FeNO values between these results (25 ppb to 50 ppb) are less informative, and recommendations state to correlate FeNO values in this range with the clinical symptoms to guide treatment decisions. Because of this variability, FeNO is most useful in asthma management in conjunction with other assessments of lung function such as spirometry.

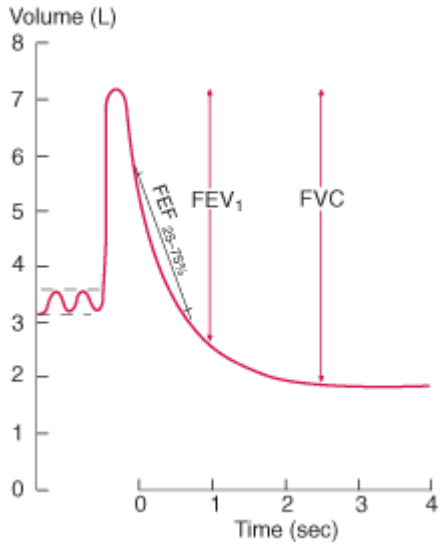
F) Spirometry and Clinical Asthma Management

While asthma is often diagnosed based on symptoms, lung function testing is also used to confirm the diagnosis and establish disease severity. Complete lung function testing is available

and measures lung capacity and volumes in great detail. This testing is very costly and often provides more details than are necessary. Spirometry is a simple, often hand-held test that can be completed in a primary care or specialist's office using a spirometer (shown in Figure 5) that provides more than enough information to assess for asthma. Utilizing a fast, forceful exhalation, a spirometer generates a spirogram (Figure 2). Important values from this data include the volume of air forcibly exhaled in the first second (FEV1) and the forced vital capacity (FVC), the total amount of air expelled during the entire test. The two values are also compared in a ratio, FEV1/FVC.

Figure 2 – Spirogram

Spirogram illustrates the full inhalation and following forced exhalation measured through spirometry. Air volume decreases as air is expelled, quickly at first and then slowing as less air remains in the lungs.[50]



In obstructive lung disorders, the FEV1 is often decreased due to airway narrowing. With smaller diameter airways, forcibly exhaling is more difficult and less air can be expelled in the first second of the test. The FVC volume often remains normal or is slightly increased, thus obstructive lung disorders are present when the FEV1/FVC is <0.80. A second spirometry measurement is often done after the administration of a short-acting bronchodiating agent (SABA) in order to assess for reversibility of the airway obstruction. If FEV1 increases by ≥12% or 200 mL after short acting β-agonist (SABA) administration, the obstruction is said to be reversible. Chronic obstructive pulmonary disease (COPD) is another pulmonary disease that causes airway obstruction; however the degree of obstruction in COPD is less-reversible and

more progressive than in asthma. It is also important to note that individual values for FEV1 and FVC are a function of height, weight, gender, and ethnic background, thus changes in an individual's spirometry values are relevant. However, raw data points cannot be compared between different individuals without being standardized. Validated data representing 100% lung function in height, weight, gender, and ethnicity-matched populations is available and used more commonly than raw volume measurements.[28]

Another measurement now available in a point-of-care device is fractional exhaled nitric oxide (FeNO), and like spirometry, this test can be easily administered in a medical office to evaluate asthma. The test measures the fraction of exhaled nitric oxide in a patient's exhaled breath. This molecule correlates to the amount of inflammation present in the airway at the time of the test. High FeNO values (>50 in adults and >35 in children) indicate asthma that is often uncontrolled and highly responsive to inhaled corticosteroid (ICS) therapy.[22]

Asthma management is step-wise, well-defined, and driven by both patient symptoms and spirometry findings.[9] The mainstays of treatment are inhaled β-agonists, also called bronchodilators, and both oral and inhaled corticosteroids. Bronchodilators alleviate the increased smooth muscle tone, a major component of bronchoconstriction, by delivering a β-agonist agent that rapidly causes smooth muscle relaxation. Most β-agonists are delivered in as a fine powder that is inhaled, which minimizes the systemic delivery of the medication and therefore, minimizes side effects. Corticosteroids break the cycle of ongoing inflammation, which decreases airway edema, smooth muscle tone, and mucus production, and therefore airway constriction.

The first step in asthma management utilizes short-acting bronchodilators (SABA). This class of medications counteracts bronchospasm and is designed to provide rapid symptom relief

during acute-asthma attacks. Commonly, these medicines are known as "rescue inhalers." SABA medications are not intended for daily use, rather they are to be used for emergent symptom relief, and if asthma symptoms occur multiple time per week during the day or night, it is necessary to consider additional therapy.

The next level of asthma management involves adding maintenance therapy, which is designed to reduce the frequency and severity of asthma symptoms. According to the National Asthma Education and Prevention Program, maintenance therapy is indicated if asthma symptoms occur more than twice per week during the day and/or more than four times per month at nighttime.[9] With a short acting β-agonist agent available for short-term control of bronchoconstriction, inhaled corticosteroids (ICS) are the first-choice medications for maintenance therapy as they dramatically reduce airway inflammation. Unlike SABA agents, ICS therapy requires several months to achieve a maximum reduction of inflammation and requires twice daily use in most cases.

If asthma symptoms continue to occur with weekly or daily frequency, a long-acting β-agonist (LABA) medications are also added to an individual's maintenance therapy routine. Like inhaled corticosteroids, LABA medications are delivered through a metered-dose inhaler and require consistent, daily use for several weeks before they are completely efficacious. Unlike ICS long-acting β-agonist medications combat smooth muscle bronchoconstriction, and unlike SABA agents, LABA agents act to maintain airway smooth muscle relaxation throughout the day. Again, the asthma severity, based on symptom frequency and rescue inhaler use, dictates what medication or medications are most appropriate. For more frequent and/or severe asthma symptoms that occur suddenly or within the context of an upper respiratory illness, sometimes a short course of oral steroids is given to decrease the airway inflammation.

Other asthma therapies target specific molecules in the inflammatory cascade, but these therapies are adjunct therapies, used in severe asthma that is refractory to the maintenance therapies listed above. They are often used in conjunction with maintenance therapies. Oral medications that target leukotrienes are available but are most efficacious in controlling symptoms in conjunction with one or more maintenance therapy medication. Recent asthma therapy development has focused on creating antibodies to key inflammatory proteins related to asthma. This medication is an injectable protein that binds to pro-inflammatory proteins. Once bound, the pro-inflammatory proteins cannot interact with immune cells and stimulate further inflammation. The antibody-inflammatory protein unit is then degraded, and the serum level of the pro-inflammatory protein is ultimately lower. Additionally this therapy reduces the expression of pro-inflammatory protein receptors, which will indirectly reduce the tissue's capacity to generate an inflammatory response. Although expensive these therapies have shown promising results for patients with very severe asthma.

G) Osteopathic Manipulative Treatment

In addition to managing asthma with pharmacologic therapies, anecdotal experience and preliminary research shows that osteopathic manipulative treatment (OMT) can be used as adjunctive therapy in the treatment of asthma in children[29] and adults,[30] as well as in other pulmonary disease such as pneumonia.[31] Osteopathic physicians utilize standard of care medications and procedures as well as manual treatments when caring for patients. Manual treatments, known as osteopathic manipulative treatments (OMT), are applied to the bones, muscles, and soft tissues of the body in order to "improve the body's physiologic function and homeostasis that has been altered by somatic dysfunction."[32] Osteopathic manipulative treatments are applicable even in non-musculoskeletal diseases like asthma because of how the

anatomic structure of nerves, blood vessels, muscles, bones and connective tissue impacts physiologic function and vice versa how physiology (and pathology) affects anatomic structures. While inflammation and bronchoconstriction are the microscopic issues that cause asthma, restrictions in the bones and muscles that support the lungs and allow the physical motions of respiration will also hamper breathing. Osteopathic physicians approach patient complaints through five distinct models (shown in Figure 3) as applicable in order to address both the disease process and the patient as completely as possible.[32,33] The biomechanical-structural model, respiratory-circulatory model, and neurologic model are readily addressed by osteopathic manipulative treatments. The metabolic-nutritional model and behavioral models evaluate for and address external factors such as diet, exercise, and stress and their impact on symptoms and disease.

Figure 3 - Five Models of Osteopathic Manipulative Medicine [32]

1. Biomechanical - Structural
2. Respiratory - Circulatory
3. Neurologic
4. Metabolic - Nutritional
5. Behavioral

Osteopathic manipulative treatment (OMT) is the use of manual techniques to "improve physiologic function" as well as to "support homeostasis that has been altered by somatic dysfunction."[32] Somatic dysfunction is defined as "impaired or altered function of related components of the somatic (body framework) system: skeletal, arthrodial, and myofascial structures, and their vascular, lymphatic, and neural elements."[32] In other words, structure and function are interrelated. When a problem occurs in one, the other is affected.

With that in mind, somatic dysfunction related to asthma is likely to be found in the anatomy related to the lungs: the thoracic spine, ribs, thoracoabdominal diaphragm, neck, and head due to the muscle, nerves, and bony components of the respiratory system. OMT can

improve the respiratory function of individuals with asthma by optimizing respiratory mechanics, increasing vascular and lymphatic flow, and restoring balance in the autonomic nervous system. Mechanically, OMT can free restriction in the rib cage, thoracic spine, and surrounding musculature including the thoracoabdominal diaphragm, which improves the motion and efficiency of respiration. As the lungs can expand and contract more easily, there is an increase in compliance and therefore a decrease in the work associated with breathing. While OMT directly to these anatomic structures accomplishes this, OMT to the cervical vertebrae and paravertebral musculature indirectly aid in these effects. The motor innervation of the thoracoabdominal diaphragm is the phrenic nerve from nerve roots C3-5. These nerves exit the spinal cord, through the vertebrae and are in close proximity to paravertebral musculature.

Osteopathic physicians have long known that OMT aids in immunologic defense and lymphatic flow.[33] Like veins, lymphatic channels lack a muscular layer and are dependent on valves, pressure variances with respiration, and skeletal muscles to maintain circulation. Structurally if the chest wall and diaphragms are moving efficiently then lymph will be propelled through the lymphatic channels back to the two large lymphatic ducts at the base of the neck and into venous circulation more efficiently, reducing lymph stasis, edema, and congestion.[33] Recent studies have shown that lymphatic flow is augmented with the use of osteopathic lymphatic pump techniques in a dog model.[34,35] Lymph ducts were canalized and flow rates measured before and during lymphatic pump techniques. A significant increase in lymphatic flow was demonstrated through the thoracic duct during the lymphatic pump techniques.[35] Recently, osteopathic research has begun investigating underlying mechanisms of action of OMT. There is new evidence that inflammatory mediators such as IL1 and IL6 may be altered in individuals

who receive OMT based on laboratory modeling of indirect OMT, [36,37] as well as clinical trials studying OMT in low back pain.[38]

OMT also helps to balance two sides of the autonomic nervous system by removing somatic dysfunction related to sympathetic and parasympathetic nerves.[39] The vagus nerve (cranial nerve X) provides parasympathetic innervation to the respiratory system, causing bronchoconstriction of smooth muscle fibers that line the airways, vasodilation of pulmonary arteries that deliver blood flow, and increased mucus secretion in the airway mucosa. This nerve travels through the internal jugular foramen and exits the skull between the occiput and the first cervical vertebrae, and muscular and/or bony dysfunction can negatively impact function of this nerve as well as local blood flow. OMT directed at this area removes these dysfunctions and optimizes the environment around the nerve, which in turn allows for optimal function of the nerve.

Likewise, OMT directed to the upper thoracic spine, upper thoracic ribs, and surrounding paravertebral musculature impact the sympathetic innervation to the lungs, which comes from the fibers of nerve roots from T2-T7. These nerve fibers help bronchodilate and relax smooth muscle fibers, cause vasoconstriction in the arteries that supply the airway, and decrease glandular secretions. Vasoconstriction and decreased mucus secretions are significant because vasodilation allows for edema to develop in the airways, contributing to airway narrowing. Likewise, less mucus in the airway is advantageous in maintaining airway patency. Balancing the sympathetic and parasympathetic innervation to the bronchial tree and airways has important ramifications in asthmatic patients, such as reduction of airway inflammation, mucous, and bronchodilation. All are main goals of both pharmacologic and manual treatment of asthma.

H) OMT and Asthma: Preliminary Studies

In 2002 Bockenhauer et. al. conducted an evaluation of the immediate effects of osteopathic manipulative procedures on adults with chronic asthma.[30] Ten participants (N = 10) were evaluated using a pretest posttest crossover design. Subjective and objective measures of asthma were made before and after either osteopathic manipulative procedures or the sham procedure. A significant increase in the chest wall excursion was measured after osteopathic manipulative procedures. Study authors concluded that osteopathic manipulative procedures can successfully accomplish chest expansion. Results also indicated that both OMT and the sham groups produced a decrease in peak expiratory flow volume.

The roll of OMT in pediatric asthma patients has also been studied. Guiney et. al. conducted a randomized controlled trial to demonstrate the therapeutic relevance of OMT in pediatric asthma patients.[29] 140 patients between ages 4 and 18 with known, well-controlled asthma were enrolled and randomly assigned to an OMT group or to a sham group. Peak flow measurements were done before and after one treatment. The OMT group showed a statistically significant improvement in peak flow measures, increasing from 7L per minute to 9L per minute, while the sham treatment group showed no improvement.[29]

The Multicenter Osteopathic Pneumonia Study in the Elderly (MOPSE) assessed the effectiveness of OMT as an adjunctive treatment for patients hospitalized with pneumonia.[31,40] Over 400 patients from seven community hospitals across the country were included in the double-blinded, randomized, controlled trial. Participants were randomized into a conventional care only group (CCO), a conventional care plus touch (sham) group (LT), and a conventional care plus OMT group (OMT). A standardized OMT protocol was developed and implemented twice daily for the study duration. Each OMT intervention lasted fifteen minutes. Intention to

treat analysis did not reveal statistical significance between the groups; however, a per protocol analysis revealed a significant difference between the conventional care only and the OMT groups. According to the latter analysis, the group that received OMT in the MOPSE study had shorter hospital stays, received shorter courses of IV antibiotics, and had fewer deaths and cases of respiratory failure. The study concluded that due to the prevalence of pneumonia, the application of OMT in pneumonia merited further study. In contrast to the MOPSE study, the present investigation did not explore the disease process of pneumonia; however, both pneumonia and asthma are disease processes affecting the airways and lungs. The musculoskeletal, neural, and circulatory elements of the respiratory system are affected in both cases, therefore, OMT that was applicable to pneumonia patients was hypothesized to also be useful in the treatment of asthma patients.

I) Hypothesis

In this study, investigators posited that osteopathic manipulative treatment (OMT) would reduce inflammation in the airways of persons with asthma. This would be demonstrated by a decrease in the concentration of inflammatory cytokines associated with asthma and by a decrease in nitric oxide (NO). It was also proposed that OMT would improve the biomechanics of respiration in persons with asthma, thereby improving spirometry measurements.

Specific Hypotheses included:

1. After OMT, serum markers of inflammation (IL-4, IL-5, IL-6, IL-13, and CRP) would decrease.
2. After OMT, spirometry measurements (forced exhaled volume in the first second of the test, FEV1 and forced vital capacity, FVC) would increase.
3. After OMT, fractional exhaled nitric oxide (FeNO) measures would decrease.

4. Changes in inflammatory mediators would be greater in subjects with a higher index of severity, as measured by spirometry values and responses to the Asthma Quality of Life Questionnaire.

CHAPTER III

RESEARCH DESIGN AND METHODOLOGY

In this study, the effect of OMT was evaluated using a repeated measures design in which each participant served as his or her own control. No separate control group was included in this study due to the relatively unexplored look at the mechanism of OMT in the care of asthma. No sham group was included in this study, due to difficulty in creating a sham protocol that mimics OMT but does not have a therapeutic effect. The use of sham protocols and effective shams continue to be an area of investigation and development.

A) Subject Selection

After approval from the UNTHSC's Institutional Review Board (IRB #2013-180), recruitment from the UNTHSC pulmonary clinic began. Potential participants were identified as patients with asthma who were older than 18 years of age. Flyers in the pulmonary clinic advertised for the study, and clinic patients with asthma were given information about the study. If interested, exclusion criteria were discussed and an appointment was made for the individual to return to the clinic to participate if he or she was eligible.

Potential participants were excluded if they had had osteopathic manipulative treatment, chiropractic treatment, chest physiotherapy or other manual medicine to their chest, ribs, thorax or neck in the past 3 months to avoid the possibility of those treatments influencing the response to the OMT study protocol. Additionally, smoking, eating or drinking one hour prior to the study start resulted in exclusion because the measurement of FeNO could have been influenced

by volatile substances in the breath.[22] Individuals found to have poorly controlled asthma upon arrival for the study were excluded in the event that the study pulmonologist determined he or she should not participate. Of the 82 individuals who were contacted about the study, twenty-five (25) were enrolled in the study.

B) Study Protocol

The study session followed the sequence listed in Figure 4. The protocol was explained to the participant, and informed consent and HIPAA forms were signed. The entire study protocol was completed in one session that lasted no more than three hours.

Figure 4 - Summary of Study Events

1. Consent
2. History/Focused Physical Exam
3. Questionnaire (AQLQ)
4. Pre-OMT measurements
   - FeNO
   - Spirometry
   - Blood work
5. OMT protocol
   - Modified MOPSE Protocol (see appendix)
   - 5 minutes to address other somatic dysfunctions
6. Post-OMT measurements
   - FeNO
   - Spirometry

1) History, Focused Physical Exam, and Questionnaire

Each subject received a brief screening exam in order to assess the present control of his/her asthma. In this screening exam, participants were asked questions about his or her respiratory symptoms, allergy symptoms, and use of medications over the past week and month. Based on the frequency of rescue-inhaler usage and of nights per week an individual experiences symptoms, the severity of a patient's asthma was determined.[41] A brief cardiopulmonary physical exam was then performed, specifically evaluating for any signs of pulmonary distress by observation and auscultation of heart and lungs. Physical exam findings, coupled with the symptom information collected, allowed study clinicians to assess the control of an individual's asthma. If a subject was found to be poorly controlled, study clinicians decided the safest course

of action for the subject. If an individual was found to be poorly controlled by study clinicians, he or she was not eligible to continue participating in the study.

The osteopathic respiratory-circulatory exam was also completed at this time. This exam is a screening test and highlights body regions where blood circulation and respiration are not functioning optimally. At this time, subjects also completed the Asthma Quality of Life Questionnaire (AQLQ), a 32-question assessment of the impact of asthma on his or her life over the past two weeks. This validated questionnaire, along with the screening exam and spirometry results, establishes a quantitative measure of each subject's asthma severity at the time of the study.

2) Fractional Exhaled Nitric Oxide

Fractional Exhaled Nitric Oxide (FeNO) was measured using the handheld NIOX- MINO device manufactured in Solna, Sweden by Aerocrine AB. To perform the test, the participant formed a seal with his or her lips around the device's mouthpiece and then followed the prompts on the computer screen to complete the test, which consisted of inhaling and then steadily exhaling into the device mouthpiece. Because asthma severity and symptoms can vary from day-to-day, spirometry and FeNO were always measured on the day of the study session. After OMT was performed, this same procedure was utilized to obtain post-OMT FeNO measurements.

Figure 5 - EasyOne Spirometer (ndd Medical Technologies, Inc.)

3) Spirometry

Spirometry testing was utilized to assess lung function. The handheld EasyOne Spirometer manufactured in Zurich, Switzerland was used (figure 5). The graduate student conducting the protocol received instructions on

how to perform this test and how to coach participants from the clinic's pulmonary technologist prior to the study. When coached, the subject inhaled as deeply as possible and then forcibly exhaled through the spirometer mouthpiece for six seconds. This was repeated until the spirometer reported the test was complete or the maximum number of trials had been reached (no more than five trials). Spirometry was always completed after the FeNO measurement because the repeated, forceful exhalations necessary to complete spirometry testing could irritate the hyperreactive airway in a person in asthma. This could potentially alter the FeNO measurement.[22]

4) Abnormal Values

If the results from FeNO or spirometry were significantly abnormal (repeatedly FeNO greater than 50 or FEV1 less than 50%), study physicians were consulted immediately as this may have indicated that the subject needed further medical evaluation. If necessary, the pulmonologist would evaluate this result and the patient and make recommendations about whether or not the participant should continue. If a participant was not allowed to continue, the pulmonologist was to give him or her instructions about what to do next (i.e. take his/her rescue inhaler, make an appointment with himself or his/her regular doctor, go to the emergency room, etc).

5) Venipuncture and Biomarker Analysis

A ten milliliter (10 mL) blood sample was obtained from each participant both before and after the OMT section of the protocol. All specimens were de-identified prior to being taken to the laboratory for preparation and storage. Samples were allowed to clot for 30 minutes prior to centrifuging. The serum was removed, placed in aliquots, and put into freezer storage until the assays were run. Assays analyzed for levels of interleukins (IL-4, IL-5, IL-6, and IL-13) and C-

reactive protein (CRP) and were run according to the manufacturer's standard procedures (Table 1). Analysis was completed on a SECTOR Image 2400, manufactured by Meso Scale Discoverey. Assay plates were also obtained from Meso Scale Discovery.

Table 1 - Standard Procedures for Blood-based Biomarker Assays.

1. Wash plate with wash buffer. Add sample, calibrator, or control. Seal plate. Incubate with shaking at room temperature.
2. Wash plate with wash buffer. Add detection antibody solution. Seal plate. Incubate with shaking at room temperature.
3. Wash plate with wash buffer. Add read buffer. Read plate.

6) Osteopathic Manipulative Treatment Protocol

A modified version of the techniques utilized in the Multicenter Osteopathic Pneumonia Study in the Elderly (MOPSE) was used as the OMT protocol in this study.[31,40] The pedal lymphatic pump technique, which aims to mobilize lymph flow by treating from the feet rather than the upper body, was omitted for this protocol to maintain the focus of study techniques on body regions directly related to pulmonary mechanics and physiology. OMT directed towards the head, neck, and thorax used in this study are described in the appendix.

Next there was a five minute period during which non-thrust techniques, such as myofascial release, balanced-ligamentous tension, counterstrain, and Still techniques, were used to treat somatic dysfunctions that had not been addressed in the previous sequence. The time spent on additional techniques was limited to less than 5 minutes. No more than 15 minutes were spent on the total OMT intervention.

The OMT protocol was performed by the graduate teaching assistant in the Department of Osteopathic Manipulative Medicine, a fourth year osteopathic medical student who was completing an additional year of training in osteopathic manipulative principles and treatments.

During this year, the student received one-on-one instruction and mentoring with a neuromusculoskeletal medicine (NMM/OMM) physician specialist.

C) Statistical Analysis

Using STPLAN (MD Anderson Cancer Center, University of Texas, Houston, TX), a two-sample t-test was used to estimate the sample size necessary given a normal distribution. Based on data by Schneider et al. in 2013, FeNO results in patients without obstructive airway disease (22.0 ppb ± 16.5 ppb) and in asthmatic patients (42.4 ppb ± 46.4 ppb) were used.[25] Therefore, for statistical significance of 0.05 and 47 subjects in the study, there would be a power of 0.8 to detect a 48.1% difference. Although the 2013 study did not mirror the repeated measures design of this project, this estimation provides an idea of variation associated with measuring FeNO in adults with and without asthma.

CHAPTER IV

RESULTS

A) Demographics

Eighty-two patients with asthma were recruited from the UNTHSC Pulmonary Clinic. A total of twenty-five persons were enrolled in the study, and twenty-four participants completed the protocol. One participant was unable to complete the protocol due to circumstances unrelated to the study protocol. The demographic breakdown of the study cohort is shown in Table 2 and closely mirrors the ethnic diversity of the surrounding community with one exception. Unintentionally, this cohort underrepresented the Hispanic population. In addition more women than men participated in this study. The three men and twenty-one women had a mean age of 49 ± 13 years and an average body mass index (BMI) of 38.5 ± 14.1.

Table 2 - Participant Demographic Information (N=24)

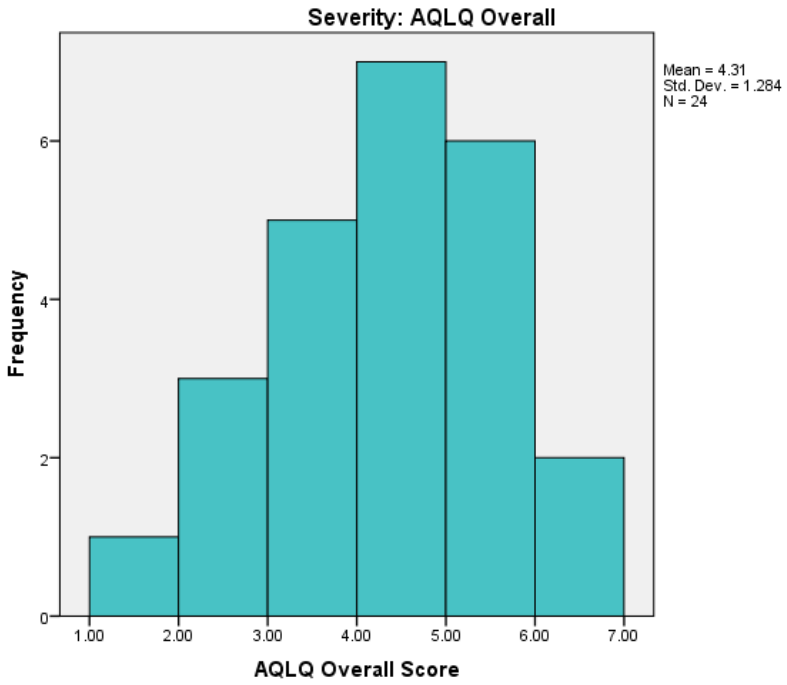
Category	**Absolute Value**	**Percentage**
Male	3	12.5%
Female	21	87.5%
Age (mean ± σ, years)	49 ± 13	---
Body Mass Index (BMI)	38.5 ± 14.1	---
Cigarette Smoking		
Yes	3	12.5%
No	14	58.3%
Former	7	29.2%
Ethnicity		
African American	6	25.0%
Caucasian	15	62.5%
Hispanic	1	4.2%
Asian	2	8.3%

B) AQLQ Survey Responses

Survey responses to the Asthma Quality of Life Questionnaire (AQLQ) provided an estimate of each participant's asthma severity over the two weeks prior to study participation. Data collection was completed between December and May 2014. The mean asthma severity was 4.31± 1.28 out of seven (n=24), where an average score of one represents severe limitation in quality of life due to asthma and seven, the maximum score, represents no limitation due to asthma. Scores ranged from 1.53 to 6.69 over a normal distribution (Figure 6).

Figure 6 - Frequency of AQLQ Scores

Note: A lower score indicates more severe asthma in the two weeks prior to the survey



In addition to the overall severity measure, the AQLQ also evaluated the impact of asthma in subcategories: physical activity limited by asthma (activity limit), the emotional impact caused by asthma (emotional function), asthma symptoms, and the impact of

environmental stimuli. There was no statistical difference between the means of each subcategory (table 3).

Table 3 - Categorical AQLQ Scores

AQLQ Segment	Mean Score	σ
Symptom Score	4.47	1.3
Activity Limit Score	4.40	1.3
Emotional Function	4.07	1.6
Environmental Function	3.73	1.6

C) Serum Inflammatory Markers

Cytokines measured include IL-4, IL-5, IL-6, IL-13, and CRP. IL-13 was entirely below the detection limit in pre-OMT measurements, and only one data point was found to be above the detection limit in post-OMT measurements. Over half of the pre- and post-OMT measurements of IL-4 were below the detection limit. IL-5 demonstrated a slight decrease between the pre-OMT and post-OMT measurements, although it was not statistically significant. In contrast IL-6 and CRP showed a slight increase in concentration after OMT, although again, this change was not statistically significant. Specific results are shown in Table 3.

Table 4 - Comparison of Biomarker Results Before and After OMT

Biomarker	Pre-OMT Mean ± σ	Post-OMT Mean ± σ	N	Trend	p value (student's T test)
IL-4 (pg/ml)	0.0485 ± 0.014	0.0485 ± 0.014	21	Decrease	0.997
IL-5 (pg/ml)	0.696 ± 0.52	0.669 ± 0.52	21	Decrease	0.165
IL-13 (pg/ml)	0.912 ± 0.049	0.881 ± 0.17	3	Decrease	0.817
IL-6 (pg/ml)	1.66 ± 1.15	1.70 ± 1.24	21	Increase	0.547
CRP (µg/ml)	16.7 ± 20.1	17.1 ± 21.7	21	Increase	0.545

D) Lung Function Measures

Spirometry was used to evaluate lung function in each participant both before and after the OMT protocol was performed. Results are summarized in table 5. The average pre-OMT forced exhaled volume in one second (FEV1) was 75.12% ± 20.39% for the cohort, with a range of 41% to 107%. The ratio between FEV1 and forced vital capacity (FVC, FEV1/FVC) is also relevant clinically. Obstructive lung disease is diagnosed when the FEV1/FVC ratio is $\leq$ 0.80. The average individual FEV1/FVC ratio for this study prior to OMT was 0.791 ± 0.069.

In the treatment of asthma, an increase of 200mL or 12% in FEV1 measurement after administration of a SABA or after 2-4 weeks of oral corticosteroid therapy is considered to be a clinically significant change. After OMT the mean individual FEV1/FVC ratio was 0.782 ± 0.064, a decrease of 1.15%. No statistically significant or clinically relevant changes were detected in spirometry measures in this cohort.

Table 5 - Individual Spirometry Values Before and After OMT

	Pre OMT		**Post OMT**	
	Mean	**σ**	**Mean**	**σ**
FEV1 %	75	20	74	19
FVC %	76	21	76	19
FEV1/FVC (individual)	0.792	.064	0.782	0.064

E) Fractional Exhaled Nitric Oxide

The FeNO results for this cohort are summarized in Table 5. FeNO ranged from 7 to 21 ppb with an average measurement of 14 ± 6 ppb before OMT. Significant change in FeNO is defined as a change of at least ten parts per billion ($\geq$10 ppb) for FeNO values less than 50 ppb,

which applies to all data in this study. After OMT, the mean FeNO was 15 ± 7 ppb. This change was neither significant (P = 0.134) nor clinically meaningful.

FeNO values less than 25 ppb are considered low and indicate that asthma is either well-controlled asthma or a person with asthma might not need daily inhaled corticosteroid (ICS) therapy. Based on FeNO measurements taken prior to OMT, all participants in this study had well-controlled asthma on the day of the study.

Table 6 - FeNO Measurements before and after OMT (N = 20)

	Pre OMT	**Post OMT**
Mean (ppb)	14 ± 6	15 ± 7
Range	7 to 24	7 to 28

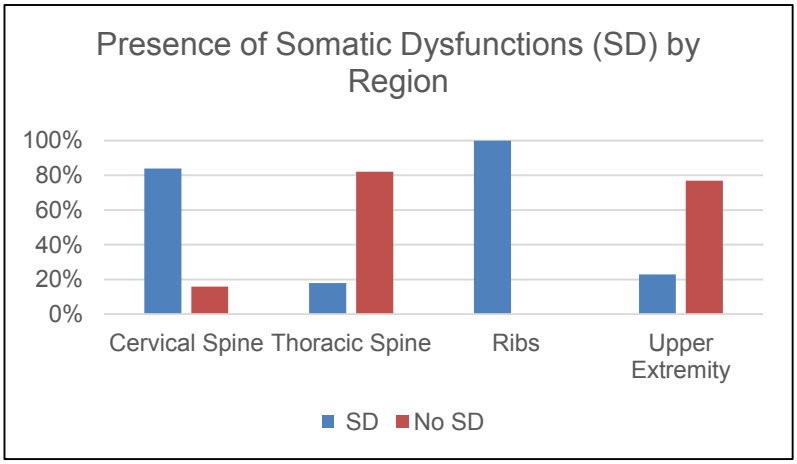
F) Somatic Dysfunction

In the additional time for OMT protocol after the modified MOPSE techniques, somatic dysfunction that did not resolve with the MOPSE protocol was addressed. Data regarding the type of somatic dysfunction was not recorded for two participants. Somatic dysfunction in the ribs occurred in 100% of participants, and cervical spine dysfunction was the second most common region, treated in 84% of participants (figure 7). Conversely, somatic dysfunctions in the thoracic spine and upper extremity were present and treated in less than 18% and 23% of participants respectively.

Figure 7 - Frequency of Somatic Dysfunctions

Regional somatic dysfunctions found and treated during the time for additional treatment (N = 22)

Region	Somatic Dysfunction Present
Cervical Spine	84%
Thoracic Spine	18%
Ribs	100%
Upper Extremity	23%



CHAPTER V

DISCUSSION

A) Biomarkers

IL-13 measures fell below the lower detection limit in this study with the exception of three sample pairs. Although IL-13 is known to be produced in the inflammatory cascade in asthma, this cytokine was not present in abundant amounts in the study cohort. One possible explanation is that the concentration of IL-13 is extremely low in individuals with well-controlled asthma. Spirometry and FeNO measurements corroborate that participants had very well-controlled asthma (Tables 4 and 5). In a less-controlled cohort, it is possible that IL-13 concentrations would be higher and thus within the measurable range. It is also possible that other cytokines are more abundant in well-controlled asthma, and in future studies, other cytokines should be analyzed rather than IL-13.

IL-6 and CRP both increased after OMT, while, IL-4 and IL-5, demonstrated a decrease after OMT. It was anticipated that all of these inflammatory cytokines would decrease after OMT as a sign of decreasing inflammation. Due to the small sample size and large variance among participants, none of these changes was statistically significant. IL-6 and CRP are both general markers of inflammation. It is possible that the slight increase is the result of lymphatic mobilization. The lymphatic circulatory system is responsible for transporting proteins and larger molecules from interstitial spaces to the systemic circulation. Previous in-vivo studies in dogs have shown that lymphatic osteopathic manipulative techniques mobilize lymph flow

during treatment administration.[34] It is possible that increased lymphatic flow returned cytokines such as IL-6 and CRP to the systemic circulation, thereby increasing the concentration of these cytokines in the post-OMT sample. It is also possible that the forcible exhalation of spirometry created transient irritation and thus inflammation in the airways, resulting in mobilization of IL-6 and CRP and thus an increase in the systemic concentration.

If an increase in inflammation were mounting after spirometry, IL-4 and IL-5 would likely increase as well; however, in this cohort, both decreased. IL-4 and IL-5 are produced by Th2 cells, which must be recruited and activated, so it is possible that the increase due to inflammation would be delayed and occur after the post-OMT sample was taken. It is also possible that the decrease in IL-4 and IL-5 illustrate a reduction in asthma-associated inflammation, as these cytokines are most specific for inflammation in the pathophysiology of asthma. Regardless, further studies are necessary to investigate the mechanism of this change.

IL-6 is a unique cytokine with the capacity to act as either a pro-inflammatory molecule or an anti-inflammatory molecule.[13] A previous in-vitro study modeled the effect of indirect OMT on the secretion of inflammatory cytokines and fibroblast cell growth. In-vitro the cohort that received OMT (after a repetitive motion strain was applied) displayed less fibroblast cell growth and reduced IL-6 concentrations immediately after OMT.[36] Authors theorized these molecular changes could be responsible for the clinical effect of indirect OMT. Indirect techniques such as indirect myofascial release and counterstrain were utilized frequently in this study, but IL-6 increased rather than decreased. Because of the complex inflammatory process that influence IL-6, more study is necessary to validate and further investigate this increase.

B) Spirometry

In both the pre-OMT and post-OMT groups, the average FEV1/FVC ratio were close to 0.80 (0.791 ± 0.069 and 0.782 ± 0.064 respectively). A ratio of 0.80 or above is indicative of normal pulmonary function. As the ratio decreases, the obstruction is classified as increasingly severe. The fact that ratios were close to the 0.80 threshold indicates that the participants in this study were a) well-controlled with respect to their asthma and b) relatively homogenous in their asthma severity.

While some spirometry showed some FEV1 values less than 50%, this was discussed with study clinicians and never a reason for a participant to be removed from the study. Low FEV1 (<50%) can be a sign of an active or impending asthma exacerbation. Asthma exacerbations often require the use of SABA medication to maintain airway patency and can also require evaluation by a physician and further treatment if the exacerbation is severe. Each participant's baseline lung function was unknown at the time of the study, thus any FEV1 lower than 50% was assumed to be a potential exacerbation for the participant's safety and discussed with study clinicians. Other factors that can contribute to a low FEV1 include participant effort and coordination. Spirometry depends on the operator's ability to coordinate his or her breath with the directions of the device, and results are also heavily impacted by the individual's effort to fully inhale and exhale during the test. In each instance during the study, a FEV1 below 50% was attributed to body habitus, inadequate effort in performing spirometry, and/or inability to perform an adequate test.

After OMT FEV1, FVC, and FEV1/FVC ratio decreased, although the decrease was neither clinically or statistically significant. In a prior study analyzing the immediate effect of OMT on individuals with asthma, the peak expiratory flow (PEF) decreased in both the group

that received OMT and in the sham group, who received no OMT. The OMT group displayed a larger decrease in the mean PEF compared to the sham group.[30] Follow-up phone calls showed that participants felt more relaxed after OMT, despite the decrease in PEF. The authors hypothesized that because participants were more relaxed, the effort put forth in the post-OMT measurement was less than the pre-OMT measurement. Likewise in the present study, FEV1, FVC, and the ratio of FEV1/FVC decreased after OMT, and participants reported feeling more relaxed. Although PEF and FEV1 are measured in a similar way, studies show one measure cannot be used to predict the other, especially in women and individuals with minimal obstruction.[42] Both spirometry and hand-held peak flow measurements are very effort-dependent, and it is possible that the relaxation experienced after OMT is also in part responsible for the decrease in lung function noted in the current study.

In a 2008 study involving COPD patients and OMT, participants also reported subjective improvement in their breathing after OMT, but objectively, FEV1 and FVC spirometry values decreased, although the decrease was not statistically significant.[43] Likewise, FEV1 and FVC decreased in the current cohort of asthmatics. Other spirometry data in the 2008 study was statistically significant and indicated that individuals experienced more air trapping after OMT. Clinically, this is a detrimental finding as air trapping is part of the pathophysiology of COPD. Although total capacity of the lung increases with air trapping, the increased volume is an increase of reserve volume (RV) rather than functional space where gas exchange can occur. Authors theorized that the increase in air trapping was likely due to a specific treatment technique in the protocol: thoracic pump with activation.[43] The thoracic pump with activation is designed to mobilize lymph through repetitive motion applied to the entire body and coordinated with the participant's breathing. At the end of the technique, the activation step exaggerates the

participant's inhalation. Authors theorize that this step possibly allows a larger volume of air into the lungs than can be immediately exhaled.

Both this study and the Noll 2008 study evidence that what each osteopathic manipulative treatment contributes to the mechanistic process is largely unknown. It is possible that a peak effect exists when treating individuals with asthma and COPD. After a certain number of treatments, no benefit is gained and lung function actually begins to be negatively impacted. It is also possible that some osteopathic manipulative treatments are better at improving lung function than others. Because of the set protocol of this study, no information was collected about individual treatments. Further investigation is needed to explore this area and to further establish trending in spirometry changes after OMT.

C) Fractional Exhale Nitric Oxide

While not statistically significant, FeNO trended upward after OMT (Table 5). Higher levels of FeNO correspond to greater inflammation in the airways; however, all values measured in this study fall at or near the normal/well-controlled range of <25 ppb. A previous study measuring FeNO in healthy individuals during a trapezius muscle stretch showed a statistically significant increase in FeNO after the stretch.[44] However, in the 2010 study, nitric oxide in the exhaled breath was measured throughout the muscle stretch procedure. Measuring the exhaled NO in real time could explain the statistically significant change that was found.

Also, the effect of each OMT technique on the pulmonary system is unstudied. It is possible that techniques have conflicting impacts on the pathophysiology of asthma or that OMT techniques illicit an effect that occurs after the final FeNO measure was performed. Exploring the impact of individual techniques by performing a single technique and utilizing a repeated measures design could provide more insight into the mechanism and potential benefit of each

technique. Additional knowledge can also be gained by altering the study protocol to include multiple sessions of OMT with the repeated measures design. Benefit from multiple sessions of OMT has been established in other studies and is common clinical practice.

D) Change in inflammation in relation to severity

The AQLQ survey provided a snapshot of symptom severity for the two weeks before each participant enrolled in the study. With an average score of 4.31 ± 1.28 out of a maximum score of seven, study participants self-reported that asthma symptoms had significant effect on their quality of life. In addition, participants reported a broad range of effect with scores ranging from as low as 1.55 to as high as 6.69 (out of 7). The survey also included subcategories to measure the impact of symptoms in specific areas of life. Despite allergens and environmental factors commonly reported as triggers, scores in the environmental category were not significantly different than the other categories.

While the survey results show asthma severity (based on self-reported symptoms) varying widely, objective measures of lung function and inflammation illustrate a different story and show less variation. While spirometry show that participants' asthma severity was homogenous, the AQLQ results illustrates a wide variation in participants' perceptions of his or her asthma severity. This highlights that objective measures of asthma severity may not effectively correlate with a patient's perception of his or her asthma control. Current asthma management strategies reflect this phenomenon and are based on symptom frequency and severity in addition to spirometry or peak flow measurements. Even with subjective variation (based on survey results), there were no significant trends noted between subjective severity and changes in inflammatory biomarkers. As discussed above, both spirometry and FeNO results show that asthma in this cohort is well-controlled and well-controlled respectively. In addition,

data collection took place from December to May. Although there were environmental differences such as temperature variations and seasonal pollens and allergens throughout this time period, there was no correlation between the severity measurements and the time at which the study protocol occurred.

E) Confounding Factors

Many participants also reported having other health issues that interact with asthma in complex ways. Two main health concerns that dominated this cohort included obesity (BMI >30) and gastroesophageal reflux disease GERD. Because of the complex interplay of asthma and GERD, obesity, tobacco use, and other respiratory disorders, it is also possible that the actual effect of asthma on quality of life could not be easily differentiated from other ongoing problems. As there was no statistically significant variation in spirometry values or biomarker concentrations, no statistical analysis was performed to assess the effect of confounding factors. There was also insufficient power in the study to allow the cohort to be divided into subgroups according to confounding factors.

Other respiratory disorders, especially chronic obstructive pulmonary disease (COPD) and seasonal allergies, can interfere with asthma pathology and symptoms. COPD can also cause shortness of breath and coughing and also reduces lung function. Both asthma and COPD can reduce the ability of the lung to empty. In a participant with COPD and asthma, it is difficult to know which disease process is causing his or her symptoms. Seasonal allergies lead to increased inflammation and increased mucus production in the nasopharynx and sinuses. Both inflammation and excess mucus contribute to airway irritation and can cause an increase in severity and frequency of asthma symptoms. 16.7% of this cohort had been diagnosed with

COPD in addition to their asthma, and just over half reported experiencing seasonal allergies (54.2%).

Other medical conditions are known to impact asthma symptoms. Gastroesophageal reflux disease (GERD) contributes to a pro-inflammatory environment in the airway when stomach acid refluxes up the esophagus and into the pharynx. Because of the larynx and upper airway are located in close proximity to the esophagus and pharynx, the acid can readily be aspirated and irritate the mucosa. In this way, acid that does reflux can become a trigger for asthma symptoms. In the study cohort, 70.8% of participants had also been diagnosed with GERD, which is significantly higher than the national adult average of 10-20%. The effect of GERD symptoms on asthma symptoms was not investigated during this study; however, with such a large portion of the cohort experiencing both asthma and GERD, the interaction of the two conditions should be considered in future studies.

Cigarette smoking has also been shown to negatively impact individuals with asthma. Although poorly understood, smoking alters the inflammatory process in airways already predisposed to excessive inflammation. Not only does smoking cause lung function to decrease more rapidly in individuals with asthma that also smoke, cigarette smoking also reduces the effectiveness of corticosteroids, a mainstay of asthma treatment.[45] Just over forty percent (40%) were former or current cigarette smokers. In general asthma symptoms are worse and control of symptoms is more difficult in individuals with asthma who also smoke cigarettes.

In addition to cigarette smoking, obesity is an important factor to consider in the discussion of asthma, especially in this study cohort with an average body mass index (BMI) of 38.5. According to a 2010 CDC study, the average BMI for adults in the United States is 28, which is classified as overweight. Body mass index has an inverse correlation with lung

volumes,[46,47] and with increasing BMI, there is a large reduction (3-5%) in functional residual capacity (FRC) and expiratory reserve volume (ERV) per unit of BMI increase between 20 and 30. As BMI increases above 30, the drop in these lung volumes is less severe but continued to decrease approximately 1% for each BMI unit. Functional residual capacity (FRC) is the volume of air remaining in the lungs after a passive exhalation, and expiratory reserve volume is a component of the functional residual capacity. Essentially, the decrease in FRC due to obesity reduces the capacity for exhalation which impacts airway resistance in individuals with an elevated BMI.[46,48] While there is minimal effect from BMI on the volume of air moved in and out during a resting respiratory cycle (known as the tidal volume); however, the work of breathing is increased due to a physical ribcage limitation as well as increased airway resistance. Clinically, spirometry values (FEV1/FVC) are not likely be decreased in obese individuals, but obesity does correlate with increased dyspnea and increased use of bronchodilator medication.[49] Because obesity contributes to asthma symptoms but does not cause significant change in FEV1/FVC, future studies could utilize other markers of expiratory function such as FRC in order to observe the effect of OMT in an asthma cohort with significant obesity.

F) Limitations and Future Steps

This study is an important first step towards understanding how OMT mediates its effect on asthma. Throughout the study and analysis of data, many improvements as well as areas to be explored by new studies were uncovered. Recruitment and study design are areas of specific focus.

A minimum of 50 subjects was necessary to give the study an adequate power (see statistical analysis section). Preliminary analysis of the number of patients presenting to the pulmonology clinic with asthma supported the goal of enrolling at least 50 individuals. The

major limitation in scheduling participants was access to transportation. The length of the study protocol made it infeasible to complete the entire protocol before or after a clinic visit without prior notice as many pulmonology clinic patients relied on inflexible modes of transportation to and from the clinic.

Differences between each subject were accounted for by analyzing the change in each outcome measure. In this way each subject functioned as his or her own control. To control for each subject's unique capacity for pulmonary change, the severity of each subject's asthma was determined through questionnaire responses and by comparing lung function measurements to standardized values based on age, height, weight, and ethnicity.

In future studies, more information about the molecular mechanism of OMT in individuals with asthma could be gained by increasing the number of OMT sessions in the protocol and/or measuring blood-based biomarkers at a later time after the protocol. In real life, OMT is rarely performed once, so a protocol that incorporates multiple sessions would more closely mimic clinical practice. It is also relatively unknown how much time is necessary for biomarkers to change after an OMT session. By measuring these biomarkers several hours or days after a session, the effect of OMT could be better quantified. It is also important to identify other biomarkers that are involved in asthma inflammation. The genetics and pathophysiology of asthma is an exciting area of ongoing investigation. For instance, research now shows that Th 17 cells, in addition to Th 2 cells, participate in airway inflammation in asthma.[13]

Another approach to evaluate OMT's effect on asthma would be to select one treatment technique and conduct the protocol with a single technique. As the microscopic mechanisms are largely unknown and unstudied, it is possible that each technique elicits a distinct effect and when performed together, the combination makes it difficult to assess. The one-technique

approach could be done with either one or multiple sessions of the protocol, although if possible, a multiple session protocol would provide better understanding of how inflammatory markers change over time.

Incorporating a larger study cohort, multiple sessions, and/or focusing on a specific treatment technique would also allow for further exploration of confounding factors. Ideally, a sufficiently large cohort could be subdivided in order to measure the effect of OMT within subsets divided by GERD, obesity, COPD, and cigarette smoking. Measuring this effect would show how each condition is related to and affected by asthma and this intervention. A larger cohort would also capture a wider clinical range of asthma severity. This would allow for examination of the effect of OMT as it is used alongside a large spectrum of pharmacologic asthma treatments.

Table 7 - Areas of Interest for Future Study

Measuring blood-based biomarkers later (hours, days) rather than immediately after OMT
Increase the number of OMT sessions that occur between measuring biomarkers
Sampling asthma patients from other healthcare facilities, cities, and states, thus increasing the variety of subjects and more accurately reflecting the general population that deals with asthma
Including a non-asthmatic control group to investigate how the protocol effects non-asthmatic a sample population
Improve the recruiting strategy. -Obtain a list of all asthma patients within the target population via the EMR if possible -Utilize a dedicated recruiter in the clinic to identify potential participants

G) Conclusion

While asthma continues to be one of the most common childhood diseases, it is also increasing in prevalence in the adult population in developing countries. Driven by a complex cascade of inflammation, asthma is responsible for significant impacts in quality of life. Anecdotally, OMT is one treatment modality that has improved asthma symptoms. In this study, spirometry, FeNO, and blood-based biomarkers showed no statistically significant changes; however, several trends were noted and merit further exploration. General markers of inflammation IL-6 and CRP increased as well as FeNO, while Th 2 specific cytokines (IL-4, IL-5, and IL-13) decreased. FEV1 showed a slight decrease, and FeNO a slight increase. Both results have been noted in previous studies, both changes were neither clinically nor statistically significant. Further study is necessary to explore, validate, and build upon these trends in order to better understand the mechanisms behind OMT in the disease process of asthma.

APPENDIX

List of Standard OMT Protocol Modified from the Multicenter Osteopathic Pneumonia Study in the Elderly (MOPSE) [40]

Technique	Explanation	Description
Thoracic spine soft tissue	Relax the musculature in the thoracic region, improve mechanics of the thoracic cage	Consists of massage, stretching, kneading, and direct inhibitory pressure to the thoracic paravertebral musculature
Rib raising	Improves rib cage motion, helps normalize sympathetic activity to the lungs	With finger pads contacting the rib angles, anterior and lateral traction is gently applied. This raises the rib angles, stimulating the sympathetic chain ganglia that sit just in front of the ribs.
Doming of the diaphragm myofascial release	Reduces fascial tension, improves lymphatic drainage and motion of the major breathing muscle, the diaphragm	Anteriorly, clinician places hand across lower rib cage and places other hand posteriorly across the thoracolumbar junction and ribs 11 and 12. The fascia surrounding the diaphragm is palpated and tested for restriction to motion and ease of motion. To treat, the fascia can be places in either restriction to or ease of motion and held until a release is noted.
Cervical spine soft tissue	Relax the musculature in the cervical region	Consists of massage, stretching, kneading, and direct inhibitory pressure to relax the cervical paravertebral musculature
Suboccipital decompression	Involves traction at the base of the skull, which is considered to release restrictions around the Vagus nerves, theoretically improving nerve function	The clinician places finger pads inferior to the occipital bone, allowing the weight of the head to rest on the finger pads. The clinician then adds light traction and holds until a release is palpated
Thoracic inlet myofascial release	Reduces fascial tension in around structures running through the thoracic inlet, improves lymphatic drainage by freeing restriction around the thoracic duct	The clinician contacts the patient between the first two ribs anteriorly and posteriorly places thumbs along either side of the first thoracic vertebrae. The fascia is then motion-tested to determine the direction of ease and restriction. The tissue is held in either direction until a release is felt.

Thoracic lymphatic pump with activation	Enhances lymphatic circulation, triggers a more-complete expansion of rib cage and thoracic cavity	A combination of rhythmical compressions to the chest wall and the rapid removal of the hands from the chest wall during deep inhalation

REFERENCES

1. Akinbami LMD. NCHS health E-stat: Asthma prevalence, health care use and mortality: United states, 2003-05. [Asthma Epidemiology]. 2010.

2. Community health status assessment: Results report for tarrant county, december 2012. . 2012.

3. Busse WW, Lemanske RF,Jr. Asthma. *N Engl J Med*. 2001;344(5):350-362. doi: 10.1056/NEJM200102013440507 [doi].

4. Holgate ST. The epidemic of allergy and asthma. *Nature*. 1999;402(SUPP):B2-B2-B4. http://www.nature.com.proxy.hsc.unt.edu/nature/journal/v402/n6760supp/pdf/402b002a0.pdf.

5. Longo G, Strinati R, Poli F, Fumi F. Genetic factors in nonspecific bronchial hyperreactivity. an epidemiologic study. *Am J Dis Child*. 1987;141(3):331-334.

6. Marsh DG, Neely JD, Breazeale DR, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science*. 1994;264(5162):1152-1156.

7. Robinson DS, Hamid Q, Ying S, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med*. 1992;326(5):298-304. http://www.nejm.org/doi/full/10.1056/NEJM199201303260504#t=article. doi: 10.1056/NEJM199201303260504.

8. Holgate ST, Polosa R. The mechanisms, diagnosis, and management of severe asthma in adults. *Lancet*. 2006;368(9537):780-793. doi: S0140-6736(06)69288-X [pii].

9. National Asthma Education and Prevention Program. Expert panel report 3 (EPR-3): Guidelines for the diagnosis and management of asthma-summary report 2007. *J Allergy Clin Immunol*. 2007;120(5 Suppl):S94-138. doi: 10.1016/j.jaci.2007.09.043.

10. Lee SY, Kim SJ, Kwon SS, et al. Distribution and cytokine production of CD4 and CD8 T-lymphocyte subsets in patients with acute asthma attacks. *Ann Allergy Asthma Immunol*. 2001;86(6):659-664. doi: 10.1016/S1081-1206(10)62295-8.

11. Yokoyama A, Kohno N, Fujino S, et al. Circulating interleukin-6 levels in patients with bronchial asthma. *Am J Respir Crit Care Med*. 1995;151(5):1354-1358. doi: 10.1164/ajrccm.151.5.7735584 [doi].

12. Pellizzaro AM, Heuertz RM. C-reactive protein levels are elevated in asthma and asthma-like conditions. *Clin Lab Sci*. 2010;23(4):223-227.

13. Rincon M, Irvin CG. Role of IL-6 in asthma and other inflammatory pulmonary diseases. *Int J Biol Sci*. 2012;8(9):1281-1290. doi: 10.7150/ijbs.4874 [doi].

14. Judy Lin M. C-reactive protein . http://emedicine.medscape.com/article/2086909-overview#a30. Updated 2012. Accessed August 8, 2013.

15. Girdhar A, Kumar V, Singh A, Menon B, Vijayan VK. Systemic inflammation and its response to treatment in patients with asthma. *Respir Care*. 2011;56(6):800-805. doi: 10.4187/respcare.00601; 10.4187/respcare.00601.

16. Sahoo RC, Acharya PR, Noushad TH, Anand R, Acharya VK, Sahu KR. A study of high-sensitivity C-reactive protein in bronchial asthma. *Indian J Chest Dis Allied Sci*. 2009;51(4):213-216.

17. Halvani A, Tahghighi F, Nadooshan HH. Evaluation of correlation between airway and serum inflammatory markers in asthmatic patients. *Lung India*. 2012;29(2):143-146. doi: 10.4103/0970-2113.95317; 10.4103/0970-2113.95317.

18. Arif AA, Delclos GL, Colmer-Hamood J. Association between asthma, asthma symptoms and C-reactive protein in US adults: Data from the national health and nutrition examination survey, 1999-2002. *Respirology*. 2007;12(5):675-682. doi: RES1122 [pii].

19. Stewart L, Katial RK. Exhaled nitric oxide. *Immunol Allergy Clin North Am*. 2012;32(3):347-362. doi: 10.1016/j.iac.2012.06.005; 10.1016/j.iac.2012.06.005.

20. Grisham MB, Jourd'Heuil D, Wink DA. Nitric oxide. I. physiological chemistry of nitric oxide and its metabolites:Implications in inflammation. *Am J Physiol*. 1999;276(2 Pt 1):G315-21.

21. Shimoda T, Obase Y, Kishikawa R, Iwanaga T, Miyatake A, Kasayama S. The fractional exhaled nitric oxide and serum high sensitivity C-reactive protein levels in cough variant asthma and typical bronchial asthma. *Allergol Int*. 2013;62(2):251-257. doi: 10.2332/allergolint.12-OA-0515; 10.2332/allergolint.12-OA-0515.

22. Dweik RA, Boggs PB, Erzurum SC, et al. An official ATS clinical practice guideline: Interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med*. 2011;184(5):602-615. doi: 10.1164/rccm.9120-11ST; 10.1164/rccm.9120-11ST.

23. Majid H, Kao C. Utility of exhaled nitric oxide in the diagnosis and management of asthma. *Curr Opin Pulm Med*. 2010;16(1):42-47. doi: 10.1097/MCP.0b013e328332ca46; 10.1097/MCP.0b013e328332ca46.

24. Silkoff PE, Lent AM, Busacker AA, et al. Exhaled nitric oxide identifies the persistent eosinophilic phenotype in severe refractory asthma. *J Allergy Clin Immunol*. 2005;116(6):1249-1255. doi: http://dx.doi.org/10.1016/j.jaci.2005.09.029.

25. Schneider A, Schwarzbach J, Faderl B, Welker L, Karsch-Volk M, Jorres RA. FENO measurement and sputum analysis for diagnosing asthma in clinical practice. *Respir Med*. 2013;107(2):209-216. doi: 10.1016/j.rmed.2012.10.003; 10.1016/j.rmed.2012.10.003.

26. Avital A, Uwyyed K, Berkman N, Bar-Yishay E, Godfrey S, Springer C. Exhaled nitric oxide is age-dependent in asthma. *Pediatr Pulmonol*. 2003;36(5):433-438. doi: 10.1002/ppul.10377.

27. Olivieri M, Talamini G, Corradi M, et al. Reference values for exhaled nitric oxide (reveno) study. *Respir Res*. 2006;7:94. doi: 1465-9921-7-94 [pii].

28. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med*. 1999;159(1):179-187. doi: 10.1164/ajrccm.159.1.9712108 [doi].

29. Guiney PA, Chou R, Vianna A, Lovenheim J. Effects of osteopathic manipulative treatment on pediatric patients with asthma: A randomized controlled trial. *J Am Osteopath Assoc*. 2005;105(1):7-12.

30. Bockenhauer SE, Julliard KN, Lo KS, Huang E, Sheth AM. Quantifiable effects of osteopathic manipulative techniques on patients with chronic asthma. *J Am Osteopath Assoc*. 2002;102(7):371-5; discussion 375.

31. Noll DR, Degenhardt BF, Morley TF, et al. Efficacy of osteopathic manipulation as an adjunctive treatment for hospitalized patients with pneumonia: A randomized controlled trial. *Osteopath Med Prim Care*. 2010;4:2-4732-4-2. doi: 10.1186/1750-4732-4-2; 10.1186/1750-4732-4-2.

32. American Association of Colleges of Osteopathic Medicine, Educational Council on Osteopathic Principles. Glossary of osteopathic terminology. . 2011.

33. Chila AG, ed. *Foundations of osteopathic medicine.* 3rd ed. Baltimore, MD: Lippincott Williams & Wilkins; 2011.

34. Schander A, Downey HF, Hodge LM. Lymphatic pump manipulation mobilizes inflammatory mediators into lymphatic circulation. *Exp Biol Med (Maywood)*. 2012;237(1):58-63. doi: 10.1258/ebm.2011.011220 [doi].

35. Knott EM, Tune JD, Stoll ST, Downey HF. Increased lymphatic flow in the thoracic duct during manipulative intervention. *J Am Osteopath Assoc*. 2005;105(10):447-456.

36. Meltzer KR, Standley PR. Modeled repetitive motion strain and indirect osteopathic manipulative techniques in regulation of human fibroblast proliferation and interleukin secretion. *J Am Osteopath Assoc*. 2007;107(12):527-536.

37. Dodd JG, Good MM, Nguyen TL, Grigg AI, Batia LM, Standley PR. In vitro biophysical strain model for understanding mechanisms of osteopathic manipulative treatment. *J Am Osteopath Assoc*. 2006;106(3):157-166.

38. Licciardone JC, Kearns CM, Hodge LM, Bergamini MV. Associations of cytokine concentrations with key osteopathic lesions and clinical outcomes in patients with nonspecific chronic low back pain: Results from the OSTEOPATHIC trial. *J Am Osteopath Assoc*. 2012;112(9):596-605.

39. Henderson AT, Fisher JF, Blair J, Shea C, Li TS, Bridges KG. Effects of rib raising on the autonomic nervous system: A pilot study using noninvasive biomarkers. *J Am Osteopath Assoc*. 2010;110(6):324-330.

40. Noll DR, Degenhardt BF, Fossum C, Hensel K. Clinical and research protocol for osteopathic manipulative treatment of elderly patients with pneumonia. *J Am Osteopath Assoc*. 2008;108(9):508-516.

41. Bousquet J, Mantzouranis E, Cruz AA, et al. Uniform definition of asthma severity, control, and exacerbations: Document presented for the world health organization consultation on severe asthma. *J Allergy Clin Immunol*. 2010;126(5):926-938. doi: 10.1016/j.jaci.2010.07.019; 10.1016/j.jaci.2010.07.019.

42. Aggarwal AN, Gupta D, Jindal SK. The relationship between FEV1 and peak expiratory flow in patients with airways obstruction is poor. *Chest*. 2006;130(5):1454-1461. doi: 130/5/1454 [pii].

43. Noll DR, Degenhardt BF, Johnson JC, Burt SA. Immediate effects of osteopathic manipulative treatment in elderly patients with chronic obstructive pulmonary disease. *J Am Osteopath Assoc*. 2008;108(5):251-259. doi: 108/5/251 [pii].

44. Kiernan JE. Effects of a manual medicine treatment procedures on nitric oxide release in 23 healthy adults. *J Manipulative Physiol Ther*. 2010;33(1):76-79. doi: 10.1016/j.jmpt.2009.11.005 [doi].

45. Thomson NC, Chaudhuri R, Livingston E. Asthma and cigarette smoking. *Eur Respir J*. 2004;24(5):822-833. doi: 24/5/822 [pii].

46. Jones RL, Nzekwu MU. The effects of body mass index on lung volumes. *Chest*. 2006;130(3):827-833. doi: 10.1378/chest.130.3.827.

47. Nambiar S, Karetzky M, LaRosa J, Compton S, Siddiqui S, Kansagra A. The impact of obesity as measured as body mass index on lung volumes in a multiracial population. *Chest*. 2009;136(4_MeetingAbstracts):120S-a-120S. doi: 10.1378/chest.136.4_MeetingAbstracts.120S-a.

48. Zerah F, Harf A, Perlemuter L, Lorino H, Lorino AM, Atlan G. Effects of obesity on respiratory resistance. *Chest*. 1993;103(5):1470-1476. doi: 10.1378/chest.103.5.1470.

49. Sin DD, Jones RL, Man SF. Obesity is a risk factor for dyspnea but not for airflow obstruction. *Arch Intern Med*. 2002;162(13):1477-1481. doi: ioi10487 [pii].

50. Airflow, lung volumes, and flow-volume loop. The Merck Manual Professional Edition Web site. http://www.merckmanuals.com/professional/pulmonary_disorders/tests_of_pulmonary_function_pft/airflow_lung_volumes_and_flow-volume_loop.html#v912555. Published 2014. Updated 2014. Accessed April 4, 2015.